THE DEEP PHAEOPIGMENTS MAXIMUM IN THE OCEAN: REALITY OR ILLUSION?

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ABSTRACT. The vertical distribution of phaeopigments in the ocean is traditionally interpreted as the result of the balance between two processes: (1) Grazing by herbivores (= production) and (2) photo-oxidation by excess of light (= degradation). If this interpretation is still correct for coastal and surface waters (*e.g.* spring bloom of phytoplankton and upwelled waters) there is today some evidence that the permanent (or seasonal) deep phaeopigment maximum layer in the bottom of the stratified euphotic zone (tropical and subtropical oceans) is largely overestimated. The artifact would be due to the presence of chlorophyll b which interferes with phaeopigments in the widely and routinely used acid fluorometric method for determination of Chla . The high concentration of Chlb relative to Chla at depth (and only at depth) suggests the existence, and probably the dominance, of shade adapted green algae (eukaryotes) in the deep chlorophyll maximum layer of the stratified euphotic zones. Recent direct observations and counting of cells support this hypothesis. The example in this paper shows that although they are irreplaceable, universal and simple techniques must be used with due considerations of their properties and limits when they are applied in structures where the conditions are expected to be very different from those where the method has been developed.

1.   INTRODUCTION

Measurements of photosynthetic pigments have played an important role in studies of primary production in lakes and oceans. Among these pigments, chlorophyll a (Chla) was specially investigated because it is both the primary photosynthetic pigment in all oxygen evolving photosynthetic organisms (the other algal chlorophylls are considered as "accessory" or "secondary" photosynthetic pigments) and the most abundant : although Chla represents only about 1 % of the dry weight of a phytoplankton cell, in quite variable proportion (Strickland, 1965; Shuter, 1979) the concentration of Chla was and is still the best and the most practical chemical indicator of phytoplankton

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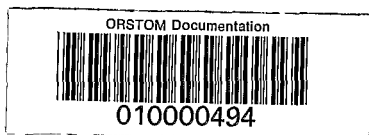
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biomass in natural samples (Cullen, 1982).

Yentsch (1965) and various authors cited by him have presented evidence that decomposition products of chlorophyll a can be present in natural-population extracts of phytoplankton, and one of the classical problems in the field of phytoplankton ecology has been to differentiate between "true" or "active" Chla and its degradation products in senescent phytoplankton and detritus.

The sequence of decomposition of Chla follows the pathways showed in Figure 1. Chla may lose the phytol (chlorophyllide a), only magnesium (Phaeophytin a or magnesium and phytol (phaeophorbide a). Prepared Chla extracts may easily be converted to magnesium-free products by the addition of acids and phytol may be removed by an enzyme, chlorophyllase. But since Chla and chlorophyllide a have identical visible absorption and fluorescence spectra, addition of acid only indicates whether the extract contains magnesium free products (phaeophorbide a and phaeophytin a) i.e. the phaeopigments.
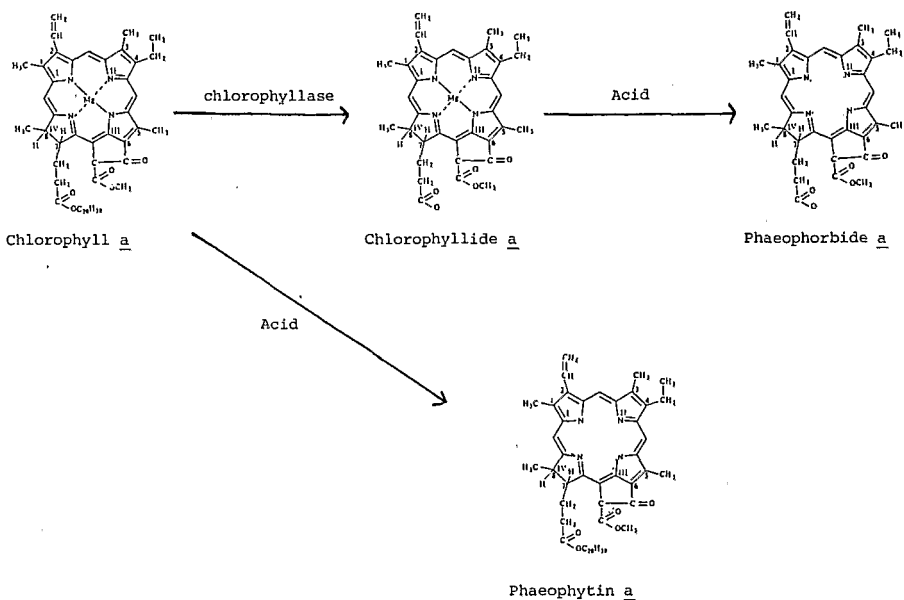

Figure 1. The principal steps in the decomposition of chlorophyll a. Phaeopigments = phaeophytin + phaeophorbide.

Accordingly a simple acidification step has been introduced to both the spectrophotometric (Lorenzen, 1967; Marker, 1972) and fluorometric (Yentsch and Menzel, 1963; Holm Hansen et al, 1965) techniques to help correct this source of error. In most works, it is the simple, rapid and sensitive fluorometric acidification technique which is used (Lorenzen and Jeffrey, 1980).

2. VERTICAL DISTRIBUTION OF PHAEOPIGMENTS AND THE CLASSICAL INTERPRETATION

A considerable amount of data exists on Chla and phaeopigment vertical distribution. In the permanent thermally stratified waters of the tropical ocean and in the seasonally stratified waters of the subtropical and temperate ocean, the vertical distribution of Chla and phaeopigments are closely related and show a typical pattern (Yentsch, 1965; Lorenzen, 1967; Soo-Hoo and Kiefer, 1982a and Figure 2):
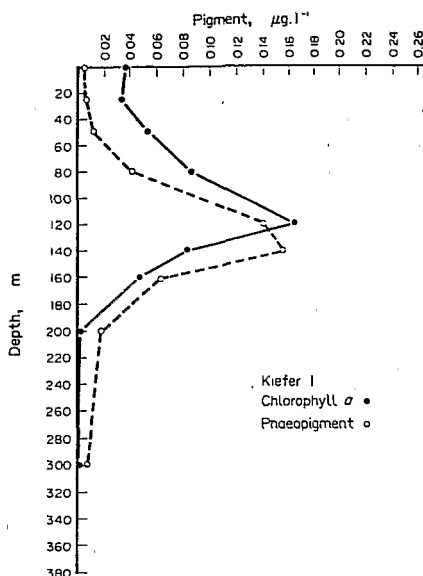

Figure 2. Vertical distribution, typical of open-ocean waters, of chlorophyll a and phaeopigments. Data are for 29°00'00"N and 122°31'12"W. (From Soo Hoo and Kiefer, 1982).

At the surface, and in the warm, nutrient-poor mixed layer, values of Chla are low but phaeopigments are found at lower concentrations. Deeper down, in the deep chlorophyll maximum (DCM) generally located in the thermocline at the top of the nitracline (see the concept of Typical Tropical Structure by Herbland and Voituriez, 1979; Cullen, 1982) phaeopigments concentration increases, the maximum being reached systematically at a slightly deeper level than that of DCM. This phaeopigments decline but less than Chla. As a result the more or less constant percentage of phaeopigment in the mixed layer increases with depth.

2.1. How to explain such a wide-spread distribution?

Although the phaeopigments formation may result from a number pathways Chla degradation (bacterial and viral degradation, prolonged darkness, according to Yentsch, 1965, and nutrient deficiency according to Wolken *et al*, 1955) it is generally recognized that the most frequent fate of phytoplankton is to be grazed by herbivores, so that the primary source of chlorophyll break-down products in natural waters is in fecal materials. Currie, (1962); Lorenzen, (1967); Nemoto, (1968, 1972); Daley, (1973) and Jeffrey, (1974), all demonstrated that phaeopigments are abundant in the guts and fecal pellets of herbivorous zooplankton. Shuman and Lorenzen (1975) found a stochiometric relationship between the ingestion of chlorophyll by grazing *Calanus* sp and the egestion of chlorophyll and phaeophorbide in fecal material.

It has also been known for a long time that phaeopigment quality is related to light : significant losses of phaeopigments were noted during photodegradation experiments by Lorenzen (1967), Moreth and Yentsch, 1970, Shuman (1978). The change in phaeopigment concentration follows a first-order decay kinetics that implies loss is proportional to the amount of radiation irrespective of exposure time.

The idea that the vertical distribution of phaeopigments represents a net result of grazing (= production of phaeopigments) and photooxidation (= destruction of phaeopigments) on short term scales in the water column was proposed by Yentsch (1965) and confirmed by Moreth and Yentsch (1970) and Lorenzen (1976).

In the former studies, most emphasis has been on copepods grazing. More recently, two papers have extended the concept to evaluate the role of microzooplankton grazing : Soo-Hoo and Kiefer (1982a, 1982b) considering the extreme small size of phaeopigment-containing particles suggested two pathways : a large particle pathway and small-particle pathway (see Figure 3a and its legend). Because feces of macrozooplankton are not quantitatively sampled by water bottles, patterns in vertical distribution of phaeopigments would result from processes affecting small particles.

In the same way, and more recently, Welschmeyer and Lorenzen (1985) constructed a model describing the dynamic budget of Chla and phaeopigments within the euphotic zone of two different oceanic areas (Figure 3b). The model, based on field measurements of the vertical distribution of pigments, the vertical flux of solar radiation through the water column, experimental photodegradation experiments and downwards flux of pigments provided estimates of phytoplankton growth rates and microzooplankton grazing intensity. For the North and South central Pacific gyre phytoplankton growth rates averaged 0.2 d^{-1} and 95 % of daily grazing was due to microzooplankton herbivores, and the model suggests that grazing and growth are in balance.
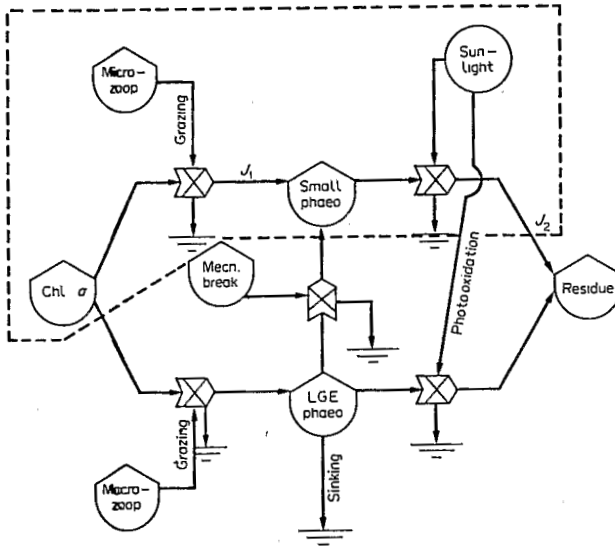
Figure 3a. **Schematic representation of the major pathways of** chlorophyll _a_ **degradation in the upper ocean. The part enclosed by** the dashed lines describes the pathway attributed to microzooplankton grazing. **The scheme is presented** in the energy circuit language of Odum (1971).
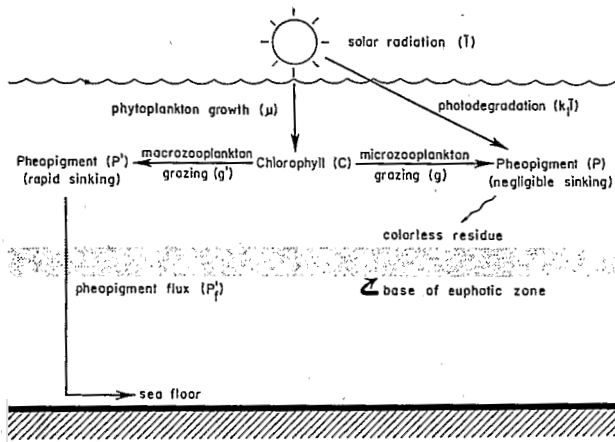

Figure 3b. **Simplified diagram showing the dominant processes** affecting the concentrations of chlorophyll and phaeopigments in the euphotic zone. (From Welschmeyer and Lorenzen, 1985).

To summarize, the vertical distributions of phaeopigments in the
oceans and especially those in the stratified euphotic layer does not
seem to raise a problem : *in situ* determinations, *in vitro* production
and degradation experiments and models give, all together a coherent
picture which agrees with the commonly accepted dynamics of the
planktonic ecosystem. However, we shall see that there is to day some
evidence that the deep phaeopigments maximum (DPM) is largely
overestimated.

3.   TOWARDS A NEW INTERPRETATION

3.1. Interference with chlorophyll b (Chlb)

It has been known for a long time that the fluorometric acidification
techniques and specially those using filter instrument (the most
commonly used) are affected to some degree by the presence of the
other chlorophylls: Chlb and Chlc. Loftus and Carpenter (1971) noted
that Chlb showed an acidification factor less than 1 (*i.e.*
fluorescence of Chlb increases after acidification) and when Chlb is
present in the extract, the decrease of fluorescence after
acidification due to the phaeophytinisation of Chla is counteracted by
the increase of fluorescence due to the formation of phaeophytin b.
This results in a slight underestimation of Chla and calculation of
significant quantities of phaeopigments even if none are present! The
errors as a function of Chlc/Chla ratios are less severe than those
associated with Chlb/Chla ratios.
      In spite of the warning and the re-examination of the method by
different authors (Holm-Hansen and Rieman, 1978; Gibbs 1979, Coveney
1982) the interference with Chlb was generally neglected because it
was thought that Chlb concentrations are insignificant in the sea,
particularly in the truly oceanic waters. In a study devoted to the
presence of Chlb using the HPLC technique in the eastern North Pacific
Ocean, Lorenzen (1981) found that although the pigment was detected in
72 % of samples, Chl(b)/Chl(a) ratios were less than 0.09 in 95 %
samples. With such ratios the Chla underestimation is less than 5 %
and the artificial phaeopigment /Chla ratio is less than 0.06. From
the analysis of all the available data on Chlb distribution and
relative abundance to Chla SOO-HOO and Kiefer (1982a) concluded :
"until more data are reported as concentrations determined by
separation techniques, evaluation of fluorometrically determined
phaeopigments and Chla remains a consideration".
      However, Jeffrey (1976) early, demonstrated (with a thin layer
chromatography technique), the presence of Chlb in significant
proportion (ratios Chlb/Chla 0.5 at 100 m depths) in the Central North
Pacific Gyre and Gieskes *et al.* (1978) found high ratios (0.2 to 0.56)
for samples collected in the deep chlorophyll maximum layer of the
Tropical North Atlantic. Today, more data exist and particularly on
the vertical axis. Firstly, Neveux and De Billy (1986), using a
modified spectrofluorometric method, have studied the distribution of

Chl_a, Chl_b, Chl_c of the Indian Ocean where oligotrophic conditions
characterized most of the area. Vertical profiles showed typical DCM
located at depth of 1 % of surface light. These DCM often were
associated with high Chl_b/Chl_a ratio. More over, *spectrofluorometry
showed no significant increase in the percentage of phaeopigments a in
the vicinity of the DCM, whereas such an increase was observed when
simultaneously using the traditional fluorometric method* :
overestimations as much as 300 % of the value computed by
spectrofluorometry were observed. The artifact was partially
attributed to the relative abundance of Chl_b in the DCM.

To the contrary, in the upwelling region, an underestimation of
phaeopigments concentration was observed, but always less than 50 %.
This was related to the relative abundance of Chl_c which tends to
underestimate phaeopigments (Loftus and Carpenter, 1971; Neveux,
1976).

Secondly, using reverse phase HPLC, Gieskes and Kraay (1986) have
made the same observations in the stratified waters of the tropical
Atlantic Ocean where a permanent DCM occurs in the bottom of the
euphotic zone : At the depth of the DCM layer, Chl_b concentration was
considerable (Chl_b/Chl_a ratios near 0.7) while near the surface only
traces were found. They only detected traces of phaeoplytin a and
phaeophorbide a with HPLC methodology in the deep samples and they
concluded : "the abundance of phaeopigments measured in the
conventional way near the bottom of the euphotic zone can therefore

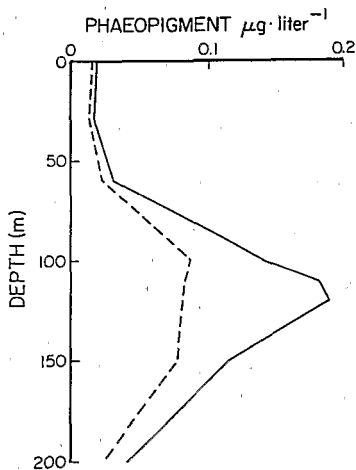Figure 4. Vertical profile of phaeopigment concentration in
tropical waters South of Hawaii. Composite profile for four
stations (n = 45). Solid line: phaeopigments as estimated in the
fluorometer; dashed lines: phaeopigment concentrations after
correcting for chlorophyll b concentration in the sample. (From
Vernet and Lorenzen, 1987).

not simply be used as an indicator of the presence of detritus or of
grazing. *It may just be an artifact of the method used for its
measurement reflecting the abundance of Chl b containing
phytoplankton"*.

Finally, and very recently Vernet and Lorenzen (1987), in a
paper specially devoted to the problem of interference with Chlb,
found consistently high concentrations of Chlb at the bottom of the
euphotic zone in the tropical North Pacific Ocean (100-200 m) *but it
was not detected in several samples of the mixed layer (0-60 m)*. The
discrepancy between the phaeopigments concentration measured in the
water column, by fluorometry and its Chlb corrected value (Figure 4)
is most pronounced between 100 and 150 m, at the depth of
phaeopigments maximum. If the Chlb correction, in that case, does not
remove the feature, it decreases the overall concentration of
phaeopigments suspended in the euphotic zone and flattens the deep
phaeopigments maximum (DPM).

3.2. Field observations of Chlb containing algae

Now that there is some evidence that the DPM is largely overestimated
by the presence of a Chlb rich layer, the question is : Are there
field observations of vertical distribution of algae containing Chlb
that support the pigment distribution? Chlb is an accessory pigment of
algae belonging to the Chlorophyceae, Prasinophyceae and Euglenophycea
and is not found in other algal groups. (Jeffrey, 1976). These classes
form the commonly designated "green algae". Although Allen (1961)
listed green algae in records of Pacific phytoplankton, they were
often regarded as being coastal and estuarine species and not present
in truly oceanic waters (Butcher, 1959).

In fact, the absence of green algae in significant proportion in
the stratified euphotic layer may be attributed to a lack of
appropriate methodology : most of the studies devoted to the vertical
distribution of phytoplankton have used the Utermöhl *settling technique*
with an inverted microscope on *preserved samples*.

Recently, it has been noted that much of the photosynthetic
activity and Chla of water samples (especially in oligotrophic waters)
is contained in very small cells (less than few microns) which have
remained largely ignored because they are so difficult to identify and
count (Fogg, 1986). Because of their fragility, they are also
selectively destroyed by the use of preservatives (Booth, 1987) : for
example, even with the best known of preservatives (glutaraldehyde),
Murphy and Haugen (1985) found that 15-20 % of the cells in
prasinophytes (= green algae) cultures disintegrated immediately and
another 35-40 % were lost within 8 days! It is only with the general
and recent use of the epifluorescence microscopy - initially designed
to count stained bacteria (Hobbie *et al.*, 1977) that it became evident
that these very small phytoplankters (*i.e.* picoplankton and small
nannoplankton) make a significant contribution to total phytoplankton
(see for example LI *et al.* 1983; Platt *et al.* 1983).

Although the studies of the *in situ* size distribution of
"Phaeopigments"-containing particles (that is Chlb - containing algae)

are less numerous than those of Chla containing particles, there is
evidence that the small size categories dominate : Soo-Hoo and Kiefer
(1982a) reported that "phaeopigments" passed through the 5 μm filter
and were more usually found in particles smaller than those containing
Chla. Herbland *et al*. (1985) found the same result in the whole
equatorial Atlantic Ocean : the percentage of "Phaeopigments" was
larger in the < 2 and < 1 fm fraction and the < 1 μm "Phaeopigments"
showed a typical vertical pattern with a well defined maximum in the
nitracline, slightly deeper than the Chla maximum. Are there recent
reports of direct observations suggesting the presence, in significant
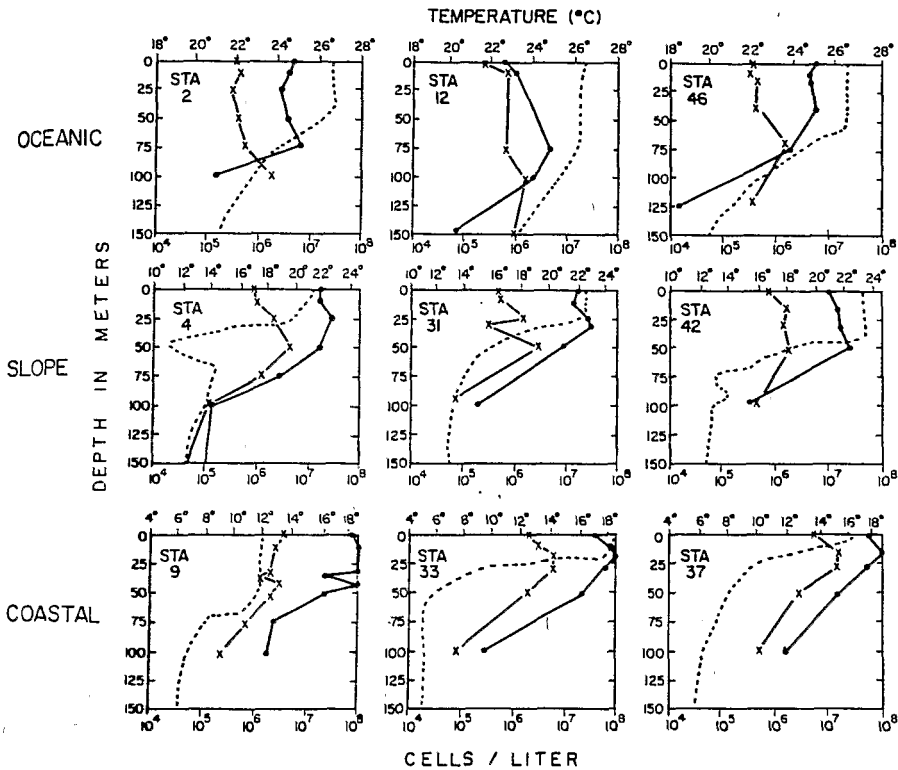proportions of small "green algae" in the vicinity of the DPM?

Figure 5. Comparison of the vertical distributions of cyanobacteria
(o) and eucaryotes (x) in vertically stable water columns. (From
Murphy and Haugen, 1985).

Murphy and Haugen (1985) compared the vertical distribution and abundance of procaryotic chroococcoid cyanobacteria and eucaryotic phototrophic ultraplankton at 50 stations in the North Atlantic. In the isothermal layer of the vertically stable waters, cyanobacteria outnumbered eucaryotes by roughly an order of magnitude (Figure 5). They reached a maximum at the thermocline at about the 1 % light level, and their abundance decreased rapidly below that. In contrast, the eucaryotic assemblage continued to increase for another 25 m (the maximum is at about the 0,5 % light level) and decreased less rapidly thereafter. Thus in the thermocline and below the eucaryotes equaled or exceeded the cyanobacteria. Glover *et al.* (1985) found similar results for the Sargasso Sea (Figure 6). Moreover, Glover *et al.*
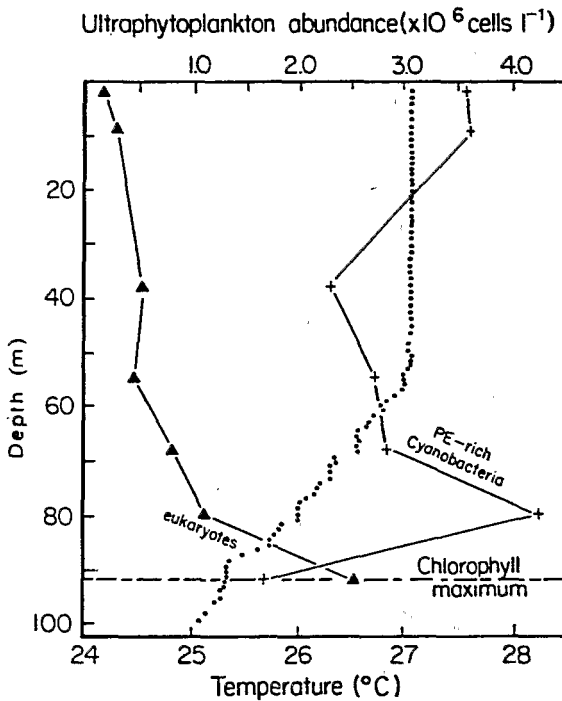
Figure 6. Depth profiles of phycoerythrin(PE)-rich *Synechococcus* (+), eucaryotic ultraplankters (Δ) and temperature (○). Data were collected from the Sargasso Sea at 34°49'N, 66°20.6'W in August 1983. Pump water was immediately filtered at <125 mm Hg through a 3-μm Nuclepore filter. The filtrate was passed through a 0.2-μm Nuclepore filter and cells were classified as either *Synechococcus* or eukaryotes. (From Glover *et al.*, 1985).

(1986), using photosynthesis and growth experiments with variable light quality and quantity found that eucaryotic ultraplankters have greater photosynthetic and growth efficiencies than *Synechococcus* in the dim-blue violet light occurring at the bottom of the euphotic layer (Figure 7).

Thus, the candidates for the validation of Chl<u>b</u> distribution do exist, but they have been largely ignored because their smallness and fragility require new techniques of identification and counting. It remains to be unambiguously demonstrated that these small dominant eucaryotes are Chl<u>b</u> containing algae.
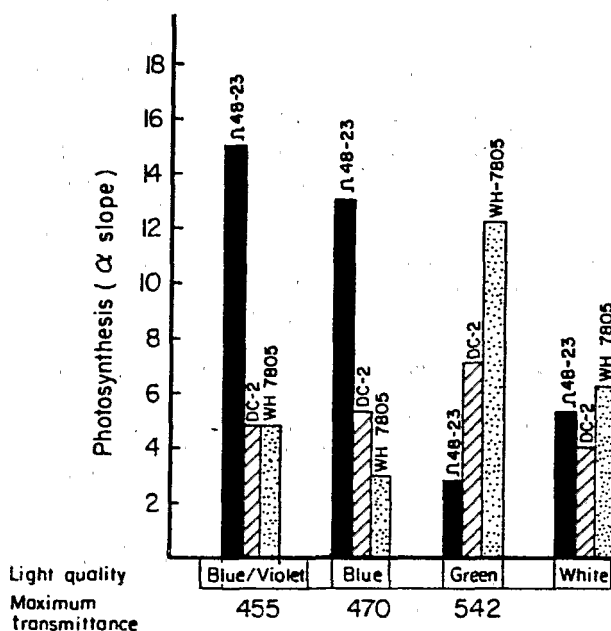
Figure 7. Photosynthetic efficiencies in different light qualities for an ultraplanktonic prasinophyte clone Ω48-23 (□). *Synechococcus* clone (DC-2 (□) and *Synechococcus* clone WH 7805 (□). Batch cultures were grown at 20°C, in 14/10 h light/dark cycles in blue-violet light at 1.6×10^{15} quanta $cm^{-2}s^{-1}$. In 'log-phase', we determine chlorophyll <u>a</u> concentrations[24] and photosynthetic rates[25] at four rate-limiting fluxes of blue-violet, blue, green or white light. (From Glover et al., 1986).

4. CONCLUSION

For the non-specialist, the "story of phaeopigments" may appear
somewhat tedious, of limited interest, and irrelevant to the problem
of biological-physical interactions in the ocean. If I concede that
the matter is an affair of specialist I think on the opposite that
this example concerns a general feature, with important ecological
implications, including physical-biological interactions.

4.1. DPM or Chl$\underline{b}$ layer : a general feature in the ocean

Relevante and Gilmartin (1973) and Shulenberger (1978) showed that the
DCM crosses the Pacific Ocean at 28°N and that it is quasi-permanent
between 40°N and 25°S in the eastern part of the Pacific. Similar
observations have been made in the whole equatorial Atlantic and in
the eastern part of the tropical Atlantic (Gieskes and Kraay, 1986;
Herbland, 1983 and Herbland et al. 1985).
    Accordingly, there is large amount of evidence that the DCM is a
permanent feature in tropical and subtropical ocean and, if not fully
explained, it is considered as a biological consequence of the
presence of a thermocline in the euphotic zone (see Cullen, 1982).
Although the vertical distribution of phaeopigments is less documented
in the literature, it can be inferred from the available data that the
DCM and DPM are often if not always, associated in the stratified
euphotic zone (Figure 2). In consequence, the problem deals with about
40 % of the world ocean (or more if we take into account the seasonal
thermocline in temperate waters). And if we are wrong, we are wrong at
a large scale!

4.2. Physical - biological interactions : ecological consequences

It stands to reason that the vision of how the planktonic ecosystem in
tropical and subtropical waters works will be different according to
whether the DPM is actually a layer of detritus or a layer of active
ultraplankton, perfectly adapted to the physical-chemical conditions
that prevail at this level.
    For example, light absorbed by Chl$\underline{b}$ can promote photosynthesis at
this depth : deep penetrating light in blue oceanic waters is near 480
nm (Jerlov, 1978 ; Morel and Prieur, 1977) ; it agrees well with in
vivo absorption (470 nm : Yentsch and Guillard, 1969) and corrected
excitation fluorescence peaks (486 nm : Neveux, 1982) of Chl$\underline{b}$.
    The recent discovery of a deep Chl$\underline{b}$ layer reinforces the concept
of two superimposed layers of phytoplankton living in the stratified
euphotic zone (Venrick, 1982) with different dominating pigments,
different mean size and growth rates (Gieskes and Kraay, 1986) and
probably different nutrients requirements (Fogg, 1986). It gives a new
argument for the presence of a "shade flora" (Sournia, 1982), and the
existence of closed interactions between physical forcing, (light
spectrum) and biological response (pigments).
    On the other hand, it implies that recent estimates of
microzooplankton grazing from pigment budgets (Soo-Hoo and Kiefer,

1982a, Welschmeyer and Lorenzen, 1985) would be lower than previously believed for certain parts of the ocean, *i.e.* where a thermally stratified water column exists.)

Obviously, further investigations, with a new vision and new techniques, are necessary to complete the picture of a component of the ecosystem that may be responsible for most of the energy transformations and recycling of material taking place in the oceans (Fogg, 1986).

Three concluding remarks

1)   The interference with Chl b was neglected during 20 years principally *because corrections found in surface samples were applied to deep samples.* If comparison and extrapolation are two techniques which are widely used in the scientific approach, this example shows that they must be applied with care. One would make today the same mistake if we should consider that the existence of phaeopigments is doubtful in the old upwelled waters or after a spring bloom of diatoms in temperate waters. The concepts of Lorenzen (1967) relating the amount of phaeopigments to zooplankton grazing are still valid. It is only the generalization to any environmental conditions which is questionable.

2)   Another cause of error is less the lack of a suitable method to detect the presence of Chl b (as early as 1976 Jeffrey drew the attention upon the problem and wrote that thin layer chromatography was used in her laboratory as a routine method for field samples) - than the availability of a rapid, sensible and cheap method for measuring Chl a. The attractive "easy to use" of the routine fluorometric technique has prevented the extension of more sophisticated and appropriate methods, more especially they give a smaller number of samples (quantity versus quality). However, a few profiles of precisely known different pigments in representative vertical structures far surpasses data obtained by some hundred profiles by the routine technique. Although, they are irreplaceable the *universal and simple methods should be used with due considerations of their properties and limits.* The controversy with the ^{14}C method is an other important example of the necessity of such a caution.

3)   Finally, for about 20 years (1965-1985) there was a kind of consensus (the word in vogue is "paradigm") on the phaeopigments status in the ocean: *in situ* observations, laboratory experiments and models agreed, even in case where it appears today that the presence of phaeopigments is doubtful! However, some observations (unpublished data of the author), were not, or hardly taken into account by the previous concepts : for example : how to explain the simultaneously short term increases of Chl a and "phaeopigments" in certain low-lighted tamised cultures of natural phytoplankton of a deep chlorophyll maximum ? Why use photo-oxidation properties of *in vivo* phaeopigments as an explanation for their vertical *in situ*

distribution and forget the same properties of *in vivo* chlorophyll ?
These two inconsistencies are well explained by the new
interpretation. Let us bet that now the old paradigm is attacked, many
other unpublished contradictions will appear in the literature.

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