International Council for the
Exploration of the Sea

C.M.1980/L:57
Biological Oceanography Committee

REPORT OF THE WORKING GROUP ON PRIMARY PRODUCTION METHODOLOGY

Texel, 23-25 June 1980

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REPORT OF THE WORKING GROUP ON PRIMARY PRODUCTION METHODOLOGY

The group met at Texel (Netherlands) on 23-25 June 1980 . The representation was as given in annex I .

The Chairman welcomed the participants and thanked Dr W.Gieskes for hosting the meeting. The participants were invited to introduce themselves and give a brief account on their activities in the field of phytoplankton production research.

The terms of reference given in the resolution of the Council establishing the Working Group (C.Res.1978/2:8) were then reviewed :

(a) to develop a detailed proposal for measuring phytoplankton and primary production according to the ^{14}C method in the northwest and northeast Atlantic , including the US and Canadian Shelf, the North Sea , and the Baltic Sea. Both in situ and incubator techniques should be evaluated , intercalibrated , and , if possible, standardized ; and

(b) to investigate possible realistic alternatives to the ^{14}C method .

In this context , the suggestion given by the former Chairman of the WG in his letter of 7 February 1979 to the members of the Group was emphasized :

"(...) although many "primary production" measurements are made each year, we still lack a satisfactory operational definition of primary production such that we cannot evaluate alternative methods according to the criterion of their fidelity to some accepted absolute standard (...)"

It was agreed that the meeting could not reasonably begin with the first topic without an introductory discussion of the operational definition problem. Therefore, a short outline was given by the Chairman of the processes that are assumed to take place in the environment and that are also relevant to primary production experiments (see figure in annex II) :

- uptake phenomena : - gross photosynthesis
  - dark uptake (anaplerotic reactions ,e.g. Wood-Werkman)
- losses : - phytoplanktonic respiration and photorespiration
  - excretion of dissolved organic matter by the phytoplankton
  - "natural" mortality of the phytoplankton
  - grazing mortality of the phytoplankton

Recycling processes , bacterial consumption of produced DOM and bacterial dark uptake were also mentioned.

Thus the question arose whether "primary production" refers to gross photosynthesis , net production sensu strictu (i.e. gross minus algal respiration), net production sensu lato (i.e. the latter minus excretion minus mortality) or to other definitions . With respect to the present state of uncertainty , it was agreed that results should be presented in a more objective way ,i.e. :

- referring to ^{14}C fixation rather than to some kind of production
- specifying the phase concerned (particulate or dissolved)
- specifying the period concerned (light-day or 24 h -day)

Among the points then discussed , respiration and excretion have deserved most of the attention :

respiration and internal recycling problems

The problem of net versus gross production estimations was discussed as well as the problem of the unbalanced carbon budgets due to often observed differences between community respiration and autotrophic production : evidence was presented that on some occasions the latter was too low to account for the heterotrophic processes measured in some environments as well as the exchanges at their boundaries . Whether it is the gross primary production figures that are underestimated , the respiration figures that are overestimated or other organic carbon pathways that have been overlooked is still a problem. Some authors are accumulating evidence that phytoplankton itself could be responsible for a much larger part of the total respiration than previously assumed . However, this kind of conclusion strongly depends on the methods used. In this context , incubation length could play a determining role . Indeed, the pattern of ^{14}C flows through the various compartments (algal, bacterial, etc.) of the system will vary with time. Region ,time of the year and community composition can play an important role as well . At the present time , the respiration problem appears far from being settled. Therefore , this problem should deserve much attention in the future since accurate estimates of respiration of phytoplankton as well as of heterotrophic organisms are necessary for a correct understanding of ecosystem functioning and interpretation of primary production measurements.

excretion of dissolved organic matter

The point was raised whether or not the phenomenon exists at all : it could for instance be an arte fact caused by filtration. But many participants provided evidence that excretion is a real mechanism however important variations of PER have been recorded. The excretion could account for a significant fraction of

total primary production. It is nowadays recognized that this flux of organic matter can be of great importance next to the particulate matter flux through the food web. Methodological problems can arise since heterotrophic consumption can take place in the bottle during the incubation period. Labelling kinetics studies have been providing a mean to estimate this consumption rate and hence to provide a correction factor. Of course, the longer the incubation lasts the more difficult becomes the interpretation of the results.

Other problems have been discussed during the meeting :

problems related to the ampoules

The various ways the ampoule or the solution it contains can interfere with the labelling experiments were reviewed :

- risks caused by toxic impurities
- other radioactive substances than inorganic ^{14}C
- release of silica from the glass wall of the ampoules (stimulating effect)

signification of dark bottle results

The various phenomena that could occur in the dark were reviewed : next to anaplerotic fixation (autotrophs + heterotrophs) there are non-biological mechanisms such as adsorption on particles . The abundance and nature of this particulate matter will influence the ratio of biological versus non-biological uptake . The non-biological uptake is known from e.g. time zero determinations.

size fractionation

Fractionation studies were recognized to be important with respect to the understanding of the pattern of ^{14}C fluxes -in the light and in the dark- through the planktonic microcosm and also with respect to the suitability of given size classes for transfer to higher trophic levels .

incubation length

Already mentioned with respect to respiration and excretion measurements , this topic was also discussed in relation to the possible mortality of algae. Moreover , evidence has been provided that such effects simultaneously depend on bottle size and characteristics of the sampled ecosystem . Therefore , the question was raised whether one should prefer short incubations ($\lesssim$ 6 h) to long ones (e.g. half day or 24 h) or not. It has been known for a long time that the sum of short incubations results is in most cases higher than the result from one single incubation covering the same period.

It has been suggested however that this is because short time incubations give results that are closer to gross production than to net production. Short incubations have advantages such as saving ship time or minimizing artefacts (e.g. keeping bottles into position whereas phytoplankton circulates). The results can readily be converted to "daily" - i.e. the daylight period - production figures using a model based on two biologically significant parameters of the photosynthesis-light relationship : $P^B_m = p_{max}$ / chlorophyll a (i.e. the assimilation ratio) and the slope α (i.e. the productivity index).Of course, from an ecological point of view , it remains that it is very important to know the production during a 24-hour period .

Elaboration of a detailed proposal for measuring primary production according to the ^{14}C method

The "Recommendations on methods for marine biological studies in the Baltic Sea" - elaborated by the organization of the Baltic Marine Biologists (BMB) which has made strong efforts to unify the methods in their area - have provided the Working Group with useful guidelines for a discussion of a proposal.

A first version for part of this proposal is given in annex III . The WG was not able to complete this task within the time at its disposal and agreed to continue partly by corresponding and to suggest that the task would be completed during a new session of the Group (recommendation nr 1).

Discussion of alternatives to the ^{14}C method

Again, due to lack of time , this topic has not been discussed during this first meeting of the WG. It was also suggested to have this problem examined at a new session (recommendation nr 1).

RECOMMENDATIONS

1. It is recommended that the Working Group on the Methodology of Primary Production would meet next year in order to examine the following points :
   - finalizing the proposal for measuring primary production according to the ^{14}C method
   - discussing fundamental aspects related to the operational definition problem : due to the complexity of the underlying phenomena, "primary production" results cannot be understood without simultaneously paying attention to other physiological processes such as respiration and growth or to variables such as biomass and active chlorophyll.
   - discussing alternatives to the ^{14}C method : the presence of specialists in the following fields is needed : ^{15}N labelling , CO_2 equilibria in seawater, pigment fluorescence, autoradiography , particle counting and identification, etc. . The WG recommends these specialists should be made aware of these needs so that member countries could encourage their participation in future sessions.
2. It is recommended that , with respect to the latter point , the Marine Chemistry WG of the ICES should be invited to report to the WG on the Methodology of Primary Production , as soon as possible and preferably before its next session, on the techniques and concepts related to the following alternatives to the ^{14}C method : - oxygen changes in water
   - pH , CO_2 , alcalinity changes in water
   - nutrients changes in water
3. (a)It is recommended that , given the problems that have frequently been mentioned with respect to labelled impurities or toxic substances in ^{14}C ampoules , users be either invited to preferably purchase their ampoules from the ^{14}C Agency in Copenhagen or send ten of their home-made ampoules to the Agency for intercalibration. Conversely, ampoules could be sent on request from the Agency to users who wish to do the intercalibration themselves.

   (b)It is recommended that ICES distribute a description of the techniques applied at the ^{14}C Agency for standardization and specific details on the liquid scintillation counting methodology .

4. It is recommended that primary production researchers should be encouraged to - specify the computation used for the calculation of their results
   - indicate what are the relative contributions of the different determinations to their result , namely :
     - carbon fixation in the particulate fraction , in the light
     - " " " " " " " " dark
     - " " " " dissolved " " " light
     - " " " " " " " " dark
     - non-biological fixation ("time zero" determination)
     - respiration (measured or presumed)

   in order to facilitate the work of researchers who have other interpretations or enable other interpretations in the future whenever this becomes necessary.
5. It is recommended that those data would be eventually stored in a data bank but that ICES should not collect them before the WG has established their degree of comparability.

ANNEX I : LIST OF PARTICIPANTS AND ADDRESSES

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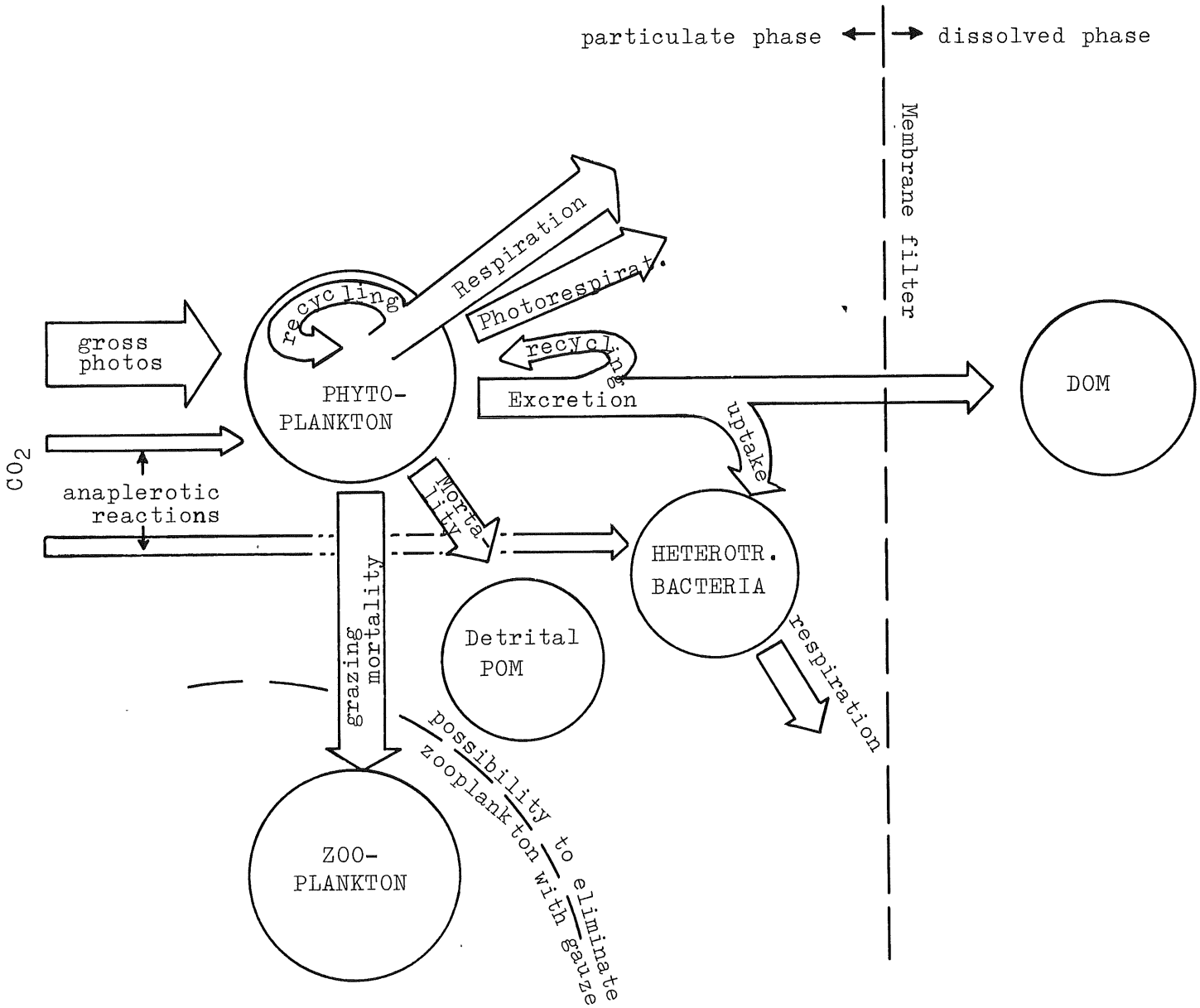
ANNEX II



Tentative representation of the major pools and fluxes pertinent to a labelling experiment.

ANNEX III : PROPOSAL FOR MEASURING PRIMARY PRODUCTION ACCORDING TO THE ^{14}C METHOD (in progress)

1. Preparation of 14C solution

1.1. Ampoules can be purchased from the International Agency for ^{14}C Determination in Copenhagen. Otherwise , the solution used in the production studies should be prepared from $Ba^{14}CO_3$ transferred to $NaH^{14}CO_3$ in a closed evacuated system by acidification of the $Ba^{14}CO_3$ and absorption of the evolved $^{14}CO_2$ in a NaOH solution.

1.2. The active solution should be diluted by freshly prepared double glass distilled water.

1.3. The pH of the solution should be adjusted to 9.5-10.0 . The pH range is chosen to minimize loss of ^{14}C during storage and handling of the solution and will not effect either partial pressure of CO_2 or the photosynthesis of algal samples in sea water.

1.4. Only high grade (p.a.) chemicals should be used for preparation of the ^{14}C solution.

2. Standardization of ^{14}C solution in the ampoules

2.1. The liquid scintillation counting should be preferably used as a basis for computation of the absolute radioactivity. Alternatively, 10 ampoules should be sent for standardization to the International Agency for ^{14}C Determination.

2.2. The activity of the $NaH^{14}CO_3$ solution and the algae or filters should be measured by the same counting technique to allow the direct comparison of the two activities .

3. Samplers and bottles

3.1. Non-transparent and non-toxic sampling devices are recommended . Experimental bottles should be thoroughly cleaned and dried at min. 120°C before use. Diluted HCl is recommended as cleaning agent followed by fresh or distilled water. Chromic acid must not be used.

3.2. Bottle size should be comprized between 50 and 100 ml for standardization

purposes. The bottles should be made of high-quality hard glass (e.g. Jena).

3.3. Before the start of the experiment the bottles should be rinsed with water from the appropriate sample . The bottles should be filled up to the neck , leaving a little air in the bottles. The stoppers of the bottles should always be tightly closed in order to avoid loss of ^{14}C during the experiment.

4. Concentration of ^{14}C solution

4.1. The ^{14}C solution should be added to the experimental bottles in such concentration that statistically sufficient estimations of the radioactivity fixed by photosynthesis in the different fractions of the sample (dissolved and particulate) can be obtained . However, it is also important not to disturb the CO_2 equilibrium in the water sample by adding too much $NaH^{14}CO_3$ solution.

5. Dark fixation of carbon

The dark fixation of carbon is not directly related to photosynthetic production . It should be reported separately from the light bottle . Whenever possible a "t_o" value should also be made available in order to be able to discriminate between biological and non-biological phenomena taking place in the dark. One dark bottle should be used from each depth, as often as found necessary to obtain statistically reliable results.

6. Sampling depths

Sampling depths should be selected so as to give an adequate vertical production curve.

6.1. It is recommended to select the sampling depths from those standard oceanographic depths which are the closest possible to depths at which 95 , 75, 50 ,30,10,3 and 1 % of the surface light are found. The light depths should be measured with a quantameter whenever possible , or a submarine photometer , with the photocell covered by a green filter (e.g. VG 9 , 2 mm , Schott and Genossen, Mainz) and a diffusing filter. Secchi disc values might also be used in order to calculate the incubation depths, provided an appropriate calibration by one of the above mentioned instruments has taken place.

6.2. "Light intensity" (irradiance) should be expressed in quanta $cm^{-2}sec^{-1}$ or Joules . Irradiance measurements (if possible by quantameter) should be performed within the range 400-700 nm.

7. here comes a paragraph on the concepts whereon particular incubation techniques and strategies are based

- e.g. care should be taken that the determination of the parameters of the photosynthesis-light relationship -normalized to chlorophyll a - namely α ,the slope at low light intensity and P_m^B ,the rate at optimal light intensity,could be made on curves drawn from the above mentionned samples

- e.g. when should irradiance be measured ? § 6.2. should be more explicit

8. Incubation methods

8.1. In situ measurements

The in situ technique for measuring primary production is today the best available approximation of the production in space and time. Considering the various phenomena that can take place into a bottle and the resulting interpretation difficulties , the incubation time should not be too long. For standardization purposes it should be comprized between 4 and 6 hours.

8.2. Using this technique,samples are placed in an incubator under surface temperature and exposed to full daylight. The light level for the samples is controlled by colored filters matching the sea water color. The depths chosen for sampling from the different levels should be determined according to § 6.1. The technique can be used on cruises and should be calibrated against in situ measurements.

8.3 Artificial light incubators

9. Filter procedure

10. Total CO_2 concentration in water

11. Calculation of carbon fixation