

NOTE

The effect of food on the respiration rates of *Daphnia magna* using a flow-through system*

CLAIRE SCHMOKER¹ and SANTIAGO HERNÁNDEZ-LEÓN²

¹ Institut de Zoologie, Université de Liège, 22 Quai Van Beneden, B-4020 Liège, Belgium.

² Biological Oceanography Laboratory, Facultad de Ciencias del Mar, Universidad de Las Palmas de Gran Canaria, Campus Universitario de Tafira, 35017 Las Palmas de G.C., Canary Islands, Spain. E-mail: shernandez@dbio.ulpgc.es

SUMMARY: Respiration rates and gut fluorescence of the cladoceran *Daphnia magna* were studied using a flow-through system. This open system has the advantage of introducing food or producing a starvation effect during the course of the experiment. Severe variations in respiratory rates were observed in relation to the presence or absence of food, indicating short-term variability. Organisms kept starved or at low food for a long period (15-20 h) responded to a sudden increase in food by increasing their respiration rates three- to four-fold in parallel with their gut content. A significant relationship between gut fluorescence and respiration rates was observed, suggesting that feeding and the related swimming activity were responsible for the observed metabolic variability.

Key words: zooplankton, metabolism, feeding.

RESUMEN: EL EFECTO DEL ALIMENTO SOBRE LAS TASAS DE RESPIRACIÓN DE *DAPHNIA MAGNA* MEDIANTE EL USO DE UN SISTEMA ABIERTO. – Se han estudiado las tasas de respiración y la fluorescencia del tracto digestivo del cladócer *Daphnia magna* mediante el uso de un respirómetro de sistema abierto. Este sistema tiene la ventaja de poder introducir alimento o bien producir un efecto de inanición durante el experimento. Se observaron variaciones importantes de las tasas de respiración en relación con la presencia o ausencia de alimento, indicando una variabilidad a corto plazo. Los organismos mantenidos en inanición o a baja concentración de alimento durante un largo periodo (15-20 h) respondieron a un repentino aumento del alimento, incrementando tres o cuatro veces sus tasas de respiración en paralelo con el contenido del tracto digestivo. Se observó una relación significativa entre la fluorescencia del tracto digestivo y las tasas de respiración, sugiriendo que el incremento metabólico fue debido a la alimentación y a la actividad natatoria.

Palabras clave: zooplankton, metabolismo, alimentación.

Respiration in aquatic organisms gives a base line of consumption in the marine environment. Their measurement is of paramount importance in order to understand the flow of energy and matter in the ocean. Excluding temperature and size, the respiration rates are mostly affected by the level of feeding and animal activity, and the so-called specific dynamic action as the effect of the energy cost

of protein synthesis (Jobling, 1983; Kiørboe *et al.*, 1985; Brown and Cameron, 1991). Thus, the respiration rates of zooplankton must be influenced by conditions of quantity and quality of food in the environment, as was stated long ago by Marshall and Orr (1955).

Respiration is frequently measured in aquatic organisms but in most cases food is omitted during the experiments. The effect of food on the respiratory rate has not yet been studied very extensively

*Received February 27, 2002. Accepted December 12, 2002.

because of technical reasons related to the balance (closed bottle) method. Omori and Ikeda (1984) suggested that measurements of respiratory rates by the classical bottle method can be taken as somewhere between routine and active metabolism and this value depends on the previous feeding history of the animals. However, short-term variability of physiological rates remains unknown and this could cause underestimation of respiration. Classical measurements are normally performed in filtered seawater where suppression of feeding takes place and a decline in oxygen demand presumably occurs as organisms enter in starvation (Skjoldal *et al.*, 1984). Organisms can only reach high rates of respiration for short periods which should not be representative of the physiological condition. In such a procedure, the range of animal activity cannot be determined accurately because the rate measured is an average (or integrated) value of respiration during the experiment. These methodological problems restrain the assessment of metabolic rates in relation to ambient food (and therefore physical processes) in biological oceanography.

Thus, to study the factors affecting respiration rates in marine zooplankton it is necessary to know the scope of animal response in order to obtain a true picture of their metabolic budgets. The scope of activity can be assessed by the use of a flow-through system (FTS), which allows one to monitor physiological rates continuously, as well as to change the food environment of animals. Using this methodology, Kiørboe *et al.* (1985) observed that respiration rate was related to food concentration in the copepod *Acartia clausi*. Lampert (1986) and Thor (2000) were also able to measure the increase in metabolic rate at increasing food levels in an FTS. The method shows the scope of respiratory activity of animals, which in the cited studies ranged from two to more than four times the standard respiration rate.

Mackas and Burns (1986) were able to synchronise the feeding response of starved organisms of the copepod *Calanus pacificus* to a sudden increase in food. The copepods responded to food by increasing the ingestion rates as observed from the increase in gut fluorescence. They observed that gut fullness and swimming activity at the time of sampling were strongly correlated. We wondered whether metabolic rates of zooplankton respond to such increases in feeding. The aim of this work was therefore to study the variability of the metabolic response in zooplankton induced by the increase in ingestion due to different pulses of food. Short-term respiration rates

of the cladoceran *Daphnia magna* were measured, looking for the response of metabolic activity to the ingested food (measured by the gut fluorescence method) using a flow-through system. Organisms were forced into extreme situations of low and high levels of food in order to reflect the metabolic range that animals can experience in nature.

Daphnia magna was cultured in the laboratory and allowed to grow in saturating food concentrations in large containers. Food consisted of the freshwater phytoplankton *Selenastrum capricornutum* (Chlorophyta). In order to study the effect of food on the respiration rates we built a flow-through system (FTS) which consisted of two chambers (120 ml), one filled with organisms (10-15 individuals) acting as the experiment and the other acting as a control (Fig. 1). A peristaltic pump maintained a constant flow (36 and 60 ml·h⁻¹) from a well-aerated reservoir, which could be filled with filtered water and to which the food could be added at the selected time intervals.

Respiration rates in the FTS were measured at 19°C and calculated by multiplying the difference in oxygen concentration between the control and the experimental bottles by the flow rate instead of measuring it at the inflow and outflow. Oxygen values were recorded every 20 seconds using a Strathkelvin Instruments (SI) pulse electrode. Experimental measurements were taken 24 hours after the organisms were introduced into the experimental chamber in order to allow the FTS to stabilise, but also to minimise stress effects and, in some experiments, to be sure that animals were in starvation. After this time, we replaced filtered water with 300 ml of saturating

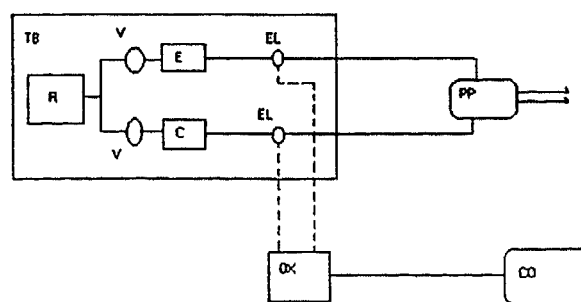


FIG. 1. – Schematic representation of the flow-through system used in our experiments. R is the reservoir containing the food source or well-aired water. Freshwater and food could be changed in this reservoir without allowing bubbles to enter into the system. TB stands for thermostatic bath, C and E are the control and experimental chambers, EL are the oxygen electrodes, V is a three-way valve which allows one to inject oxygen-free or saturated sea-water in order to calibrate the oxymeter, OX is the oxymeter, CO is the computer and PP the peristaltic pump. The solid lines indicate flow of water.

levels of *Selenastrum capricornutum* and measured their concentration (cells·ml⁻¹) at the out-flow in the control and experimental chambers. When the reservoir was empty, filtered water was replaced and the experiment continued. Water containing food was allowed to reach the temperature of incubation prior to the food treatment. The small differences in oxygen content due to water with and without food were prevented by the use of the control chamber.

Organisms used for gut fluorescence measurements were incubated in parallel to oxygen consumption measurements. A batch of 60-70 organisms were placed in the same conditions of temperature, flow and food as the others in the FTS. The volume of the chamber was 250 ml and, after introduction of phytoplankton, 3-4 animals were picked-up every hour for 20-24 hours. These individuals were immediately placed in an Eppendorf tube and frozen at -80°C. For the measurement of gut fluorescence, samples were homogenized in a teflon pestle at 0-4°C and an aliquot of the homogenate was placed in a test tube with 10 ml of 90% acetone and stored at -20°C (24 hours). Fluorescence of the samples was measured before and after acidification in a Turner Design fluorometer, previously calibrated with pure chlorophyll (Yentsch and Menzel, 1963). Pigments were calculated with the equations given by Strickland and Parsons (1972) slightly modified to

$$\text{Chlorophyll} = k \cdot (F_o - F_a) \cdot \text{individual}^{-1}$$

$$\text{Pheopigments} = k \cdot (R \cdot F_a - F_o) \cdot \text{individual}^{-1}$$

where k is the instrument calibration constant, F_o and F_a are the fluorescence readings before and after acidification and R is the acidification coefficient. Gut pigment concentration in this study refers to the addition of chlorophyll and pheopigments.

Experiments performed with *Daphnia magna* showed the effect of changes in food concentration (Fig 2). Respiration rates varied from 0.5 to 4 $\mu\text{O}_2 \cdot \text{ind}^{-1} \cdot \text{h}^{-1}$ depending on the presence or not of food. The increase in respiration rates lasted for about 20 hours after the introduction of food. The increase in respiration rates after the introduction of food was reproducible in our experiment (Fig. 2). Additional experiments were made at flushing times (chamber volume/flow rate) of 2 (Figs. 3a,b) and 3.3 hours (Fig. 3c). Total removing of food was observed after 4-5 times the flushing time. After the introduction of food, an increase in respiration rates

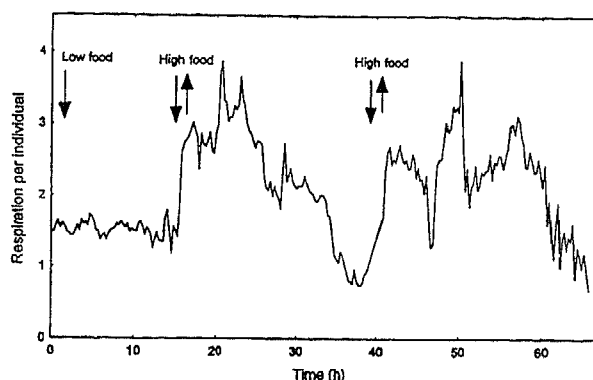


Fig. 2. — Respiration rates ($\mu\text{O}_2 \cdot \text{ind}^{-1} \cdot \text{h}^{-1}$) of *Daphnia magna* versus time using a flow-through system (experiment 1). The arrows indicate the start of a pulse of phytoplankton. See text for explanation.

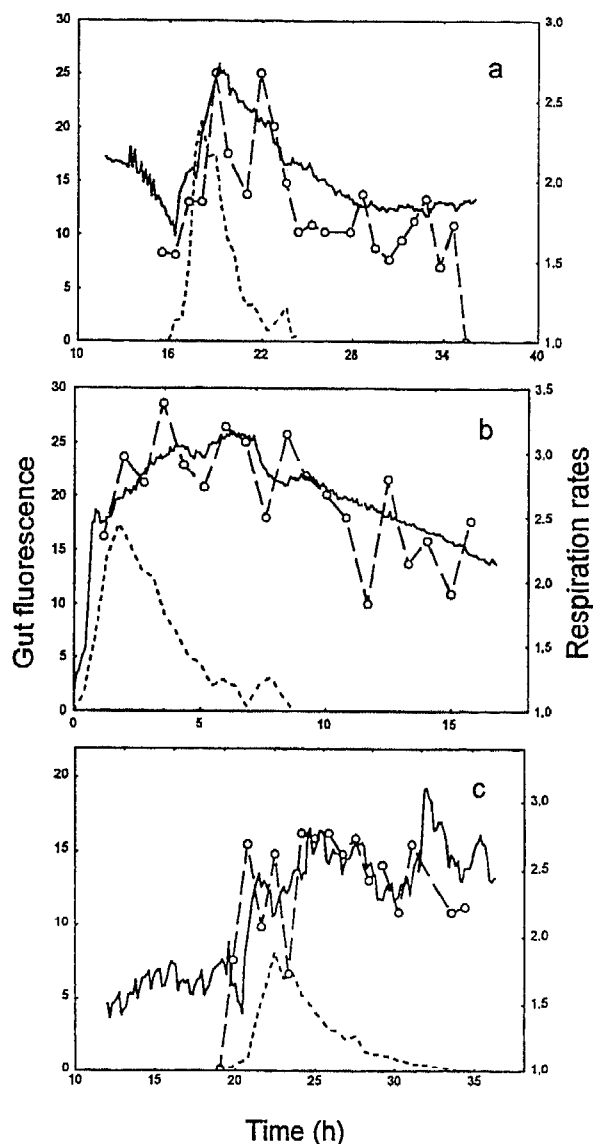


Fig. 3. — Respiration rates ($\mu\text{O}_2 \cdot \text{ind}^{-1} \cdot \text{h}^{-1}$) and gut fluorescence (ng pigments·ind⁻¹) of *Daphnia magna* versus time using a flow-through system at flushing times of 2 hours (a and b) and 3.3 hours (c). Cell density are given in relative units (right scale).

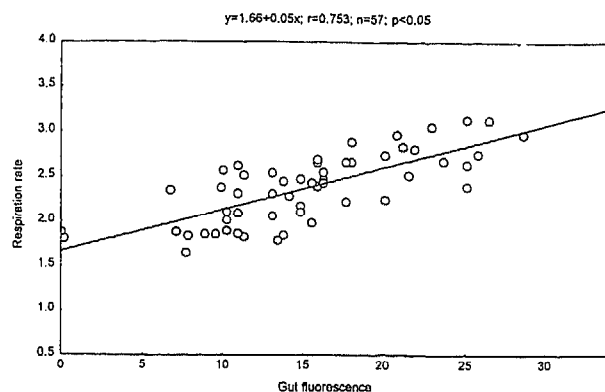


FIG. 4. – Relationship between gut fluorescence (ng pigments.ind⁻¹) and respiration rates (μlO₂.ind⁻¹.h⁻¹) in *Daphnia magna* in the experiments of Figure 3.

was always observed despite the previous metabolic level and was followed by a decrease in respiration rates after 10 to 15 hours, except in experiment 4 in which respiration rates remained at high levels for more than 14 hours. In these experiments, gut fluorescence was tightly coupled with the corresponding respiration rates (Fig. 3) and a significant relationship was observed between the index of feeding and the respiration rates obtained (Fig. 4).

Severe variability in respiration rates were observed in our experiments with the FTS. As the temperature in the system and the body size of the organisms did not change, respiration rates increased as a consequence of food availability. This effect agree with previous studies in which food level was the main cause of respiration rate variability (Conover and Lalli, 1974; Ikeda, 1976; Kiørboe *et al.*, 1985; Lampert, 1986; Thor, 2000). In our study, two pulses of food at saturating levels gave rise to two reproducible increases in respiration rates (Fig. 2). The average value of respiration was 2.07 μlO₂.ind⁻¹.h⁻¹ with maximum values of about 4 μlO₂.ind⁻¹.h⁻¹. Assuming that standard metabolic rates are in the range 18-22% of maximum rates (Kiørboe *et al.*, 1985; Macy *et al.*, 1999), our standard metabolic rate would be in the range 0.72-0.88 μlO₂.ind⁻¹.h⁻¹, which in fact is similar to the lower values obtained in the present work (see Fig. 2) and the range found in the literature of 0.70-0.92 μlO₂.ind⁻¹.h⁻¹ for *Daphnia magna* by Schindler (1968), Kersting and Van der Leeuw-Leegwater (1976), Goss and Bunting (1980) and Lampert (1986), among others. The similarity between our estimated standard respiration rates and the results of the bottle method is not surprising as no differences have been observed between food and non-

food treatments using this methodology (Christou and Moraitou-Apostolopoulou, 1995). The more than four-fold increase in respiration rates found in our work agree with the observations by Kiørboe *et al.* (1985) for the copepod *Acartia tonsa* and reflect the metabolic scope of zooplanktonic organisms. The observed increase in respiration rates suggests that feeding and the related activity (see Mackas and Burns, 1986) were responsible for the observed metabolic variability.

Our results suggest that the closed bottle method does not reflect the true metabolic rate of an animal during the diel cycle due to inherent variability in feeding (see Duval and Geen, 1976; Gyllenberg, 1981; Simard *et al.*, 1985; Durbin *et al.*, 1990; Atkinson *et al.*, 1992; Cervetto *et al.*, 1993). However, Lampert (1986) compared his FTS measurements in *Daphnia magna* with previous estimations of oxygen consumption rates using the closed bottle method and found no differences between the methods. The metabolic scope observed using the FTS was two-fold, and thus lower than that observed in the present work, which is comparable to that observed by other authors (Duval and Geen, 1976; Kiørboe *et al.*, 1985; Cervetto *et al.*, 1993; Thor, 2000). Our average value of respiration rates in experiment 1 (Fig. 2), in which the two pulses of food were given every 24 hours, was 2.07 μlO₂.ind⁻¹.h⁻¹. This value is more than twice the standard metabolism of those organisms. Therefore, metabolic rates in aquatic organisms should be estimated by taking into account their average feeding level in nature.

The close correlation between respiration rates and gut fluorescence observed in the present work suggests a coupling between ingestion rates and metabolism and could help to correct for the effect of feeding in the assessment of metabolism in the field. Unfortunately, the gut fluorescence method suffers from the uncertainty of pigment destruction and the use of the close relationship found in the present work between respiration and gut fluorescence to predict the metabolic level of animals in the field is debatable. First, the rate at which pigments disappear in zooplankton guts is not constant and therefore is difficult to evaluate (see McLeroy-Etheridge and McManus, 1999). Secondly, zooplankton are omnivorous and the proportion of non-pigmented food in the gut varies from 10 to more than 80%, depending on the availability of phytoplankton and other non-pigmented protista (Dam *et al.*, 1995; Hernández-León *et al.*, 2002). The knowl-

edge of the daily integrated or average gut fluorescence that an organism can achieve in nature and its relationship with respiration rates suggests that it will provide a more realistic daily integrated or average estimate of metabolic requirements of zooplankton compared with the classical bottle method. This rate would still, however, be an underestimate of the true metabolic level because of pigment destruction and the amount of non-pigmented food in the gut.

Finally, further research on this relationship in e.g. different species of copepods of different size is required. The use of a flow-through system with a low chamber volume or higher flow rates in order to measure very short-term changes in respiration rates would increase its sensitivity and improve monitoring to follow the parallel changes in oxygen consumption and gut content of zooplankton. Nevertheless, the significant relationship obtained between both measurements suggests that feeding and the related swimming activity were responsible for the observed metabolic variability.

ACKNOWLEDGEMENTS

The authors wish to thank Dr. C. Almeida and P. Bécognée for their assistance in the measurements of gut fluorescence. This work was supported by projects Mesopelagic (MAR97-1036), Pelagic (1FD97-1084) and Coca (Ren 2000-1471-CO2-O2-MAR) from the CICYT (Spanish Ministry of Science and Technology) and the European Union (FEDER).

REFERENCES

- Atkinson, A., P. Ward, R. Williams and S.A. Poulet. – 1992. Diel vertical migration and feeding of copepods at an oceanic site near south Georgia. *Mar. Biol.*, 113: 583-593.
- Brown, C.R. and J.N. Cameron. – 1991. The relationship between specific dynamic action (SDA) and protein synthesis rates in the channel catfish. *Physiol. Zool.*, 64: 298-309.
- Cervetto, G., R. Gaudy, M. Pagano, L. Saint-Jean, G. Verriopoulos, R. Arfi and M. Leveau. – 1993. Diel variations in *Acartia tonsa* feeding, respiration and egg production in a Mediterranean coastal lagoon. *J. Plankton Res.*, 15: 1207-1228.
- Conover, R.J. and C.M. Lalli. – 1974. Feeding and growth in *Clione limacina* (Phipps) a pteropod mollusc. II. Assimilation, metabolism and growth efficiency. *J. exp. mar. biol. Ecol.*, 16: 131-154.
- Christou, E.D. and M. Moraitou-Apostolopoulou. – 1995. Metabolism and feeding of mesozooplankton in the eastern Mediterranean (Hellenic coastal waters). *Mar. Ecol. Progr. Ser.*, 126: 39-48.
- Dam, H.G., X. Zhang, M. Butler and M.R. Roman. – 1995. Meso-zooplankton grazing and metabolism at the equator in the central Pacific: Implications for carbon and nitrogen fluxes. *Deep Sea Res. II*, 42: 735-756.
- Durbin, A.G., E.G. Durbin and E. Włodarczyk. – 1990. Diel feeding behaviour in the marine copepod *Acartia tonsa* in relation to food availability. *Mar. Ecol. Progr. Ser.*, 68: 23-45.
- Duval, W.S. and G.H. Geen. – 1976. Diel feeding and respiration rhythms in zooplankton. *Limnol. Oceanogr.*, 21: 823-829.
- Goss, L.B. and D.L. Bunting. – 1980. Temperature effects on zooplankton respiration. *Comp. Biochem. Physiol. A*, 66: 651-658.
- Gyllenberg, G. – 1981. *Eudiaptomus gracilis* (Copepoda, Calanoida): diel vertical migration in the field and diel oxygen consumption rhythm in the laboratory. *Ann. Zool. Fennici*, 18: 229-232.
- Hernández-León S., C. Almeida, A. Portillo-Hahnefeld, M. Gómez, J.M. Rodríguez and J. Aristegui. – 2002. Zooplankton biomass and indices of feeding and metabolism in relation to an upwelling filament off Northwest Africa. *J. Mar. Res.*, 80: 327-346.
- Ikeda, T. – 1976. The effect of laboratory conditions on the extrapolation of experimental measurements to the ecology of marine zooplankton. I. Effect of feeding condition on the respiration rate. *Bull. Plankton Soc. Japan*, 23: 51-60.
- Jobling, M. – 1983. Towards an explication of specific dynamic action (SDA). *J. Fish. Biol.*, 23: 549-555.
- Kersting, K. and Van der Leeuw-Leegwater, C. – 1976. Effect of food concentration on the respiration of *Daphnia magna*. *Hydrobiologia*, 49: 137-142.
- Kjørboe, T., F. Mohlenberg, K. Hamburger. – 1985. Bioenergetics of the planktonic copepod *Acartia tonsa*: relation between feeding, egg production and respiration, and composition of specific dynamic action. *Mar. Ecol. Progr. Ser.*, 26: 85-97.
- Lampert, W., 1986. Response of the respiratory rate of *Daphnia magna* to changing food conditions. *Oecologia*, 70: 495-501.
- Macy, W.K., A.G. Durbin and E.G. Durbin. – 1999. Metabolic rate in relation to temperature and swimming speed, and the cost of filter feeding in Atlantic menhaden, *Brevoortia tyrannus*. *Fish. Bull.*, 97: 282-293.
- McLeroy-Etheridge, S.L. and G.B. McManus. – 1999. Food type and concentration affect chlorophyll and carotenoid destruction during copepod feeding. *Limnol. Oceanogr.*, 44: 2005-2011.
- Mackas, D.L. and K.E. Burns. – 1986. Poststarvation feeding and swimming activity in *Calanus pacificus* and *Metridia pacifica*. *Limnol. Oceanogr.*, 31: 383-392.
- Marshall, S.M. and A.P. Orr. – 1955. The biology of a marine copepod, *Calanus finmarchicus* (Gunnerus). Oliver and Boyd, Edinburgh.
- Omori, M. and T. Ikeda. – 1984. *Methods in Marine Zooplankton Ecology*. John Wiley & Sons.
- Propp, M.V., M.R. Garber and V.I. Ryabusko. – 1982. Unstable processes in the metabolic rate measurements in flow-through systems. *Mar. Biol.*, 67: 47-49.
- Schindler, D.W. – 1968. Feeding, assimilation and respiration rates of *Daphnia magna* under various environmental conditions and their relation to production estimates. *J. Anim. Ecol.*, 37: 369-385.
- Simard, Y., G. Lacroix and L. Legendre. – 1985. In situ twilight grazing rhythm during diel vertical migrations of a scattering layer of *Calanus finmarchicus*. *Limnol. Oceanogr.*, 30: 598-606.
- Skjoldal, H.R., U. Båmstedt, J. Klinken and A. Laing. – 1984. Changes with time after capture in the metabolic activity of the carnivorous copepod *Euchaeta norvegica* Boeck. *J. exp. mar. biol. Ecol.*, 83: 195-210.
- Strickland, J.D.H. and T.R. Parsons. – 1972. A practical handbook of seawater analysis. *Fish. Res. Bd. Canada, Bulletin*, 167 p.
- Thor, P. – 2000. Relationship between specific dynamic action and protein deposition in calanoid copepods. *J. exp. mar. biol. Ecol.*, 245: 171-182.
- Yentsch, C.S. and D.W. Menzel. – 1963. A method for the determination of phytoplankton chlorophyll and phaeophytin by fluorescence. *Deep-Sea Res.*, 10: 221-231.

Scient. ed.: P. Jonsson