Netherlands Journal of Sea Research
14 (3/4) : 349–361 (1980)

TISSUE COMPOSITION AND REPRODUCTION OF MYTILUS EDULIS IN RELATION TO FOOD AVAILABILITY

by

H. PIETERS

Netherlands Institute for Fishery Investigations, Haringkade 1, 1976 CP IJmuiden, The Netherlands

J. H. KLUYTMANS, D. I. ZANDEE

Laboratory of Chemical Animal Physiology, State University of Utrecht, Padualaan 8, 3508 TB Utrecht, The Netherlands

and

G. C. CADÉE

Netherlands Institute for Sea Research, P.O. Box 59, 1790 AB Den Burg, Texel, The Netherlands

CONTENTS

I. INTRODUCTION

As part of an investigation into the influence of seasonal changes on the anaerobic metabolism of the sea mussel *Mytilus edulis* L., a description of the biochemical composition in relation to environmental factors and gametogenesis has already been published for mussels from the Dutch Wadden Sea (PIETERS *et al.*, 1979; ZURBURG *et al.*, 1979; KLUYTMANS *et al.*, 1980; ZANDEE, KLUYMTANS, ZURBURG & PIETERS, 1980).

Extensive studies of the circannual fluctuations in tissue composition have also been carried out for several other populations of bivalve molluscs (CHIPPERFIELD, 1953; GIESE, 1959; LUBET, 1959; LUBET & LEGALL, 1967; WILLIAMS, 1969; DE ZWAAN, 1971; SEED,

1975; DARE & EDWARDS, 1975; WALDOCK, 1979); some of these studies are concerned particularly with the interrelationships of gametogenic cycle and environmental factors, and some give detailed information on the variations of protein, glycogen and total lipid contents during the spawning period.

LUBET (1959) described the occurrence of several sheddings of eggs during the spring for a population of *Mytilus edulis* on the west coast of France. This phenomenon of repeated release of gametes could be detected owing to sudden strong decreases of protein and lipids, which were always followed by short-term increases.

The spawning process seems to be highly dependent on environmental circumstances, and the time and duration of the spawning season can be correlated particularly with latitude (GIESE, 1959; LUBET, 1959) shifting towards regions with higher latitude during the summer months (LUBET & LEGALL, 1967).

Food availability also exerts a significant influence upon the production of ripe gametes during the spawning process (BAYNE, 1976), whereas temperature has an indirect effect on the onset and duration of the gametogenic cycle (BAYNE, 1975).

The aim of this research was to obtain greater insight into the time course (in relation to biochemical changes) of the spawning process of mussels from the Dutch Wadden Sea. By weekly sampling from a culture bed we were able to establish a distinct pattern of changes in the biochemical composition, which could be correlated with the stages of the gametogenic cycle as described by CHIPPERFIELD (1953) and LUBET (1959).

Acknowledgements.—The authors are very grateful to Mr A. Kok, Mr J. Hegeman, and Misses P. R. Veenhof and A. de Bont for skillful technical assistance. They are greatly indebted to the late Mr F. J. de Koning. The authors also wish to express their thanks to the crews of the MS Eider and the former MS Griend of the Netherlands Institute for Sea Research.

II. MATERIAL AND METHODS

Mussels were collected weekly from a culture bed near the island of Texel in the Dutch Wadden Sea (Fig. 1), from the end of February till the beginning of July 1978. After being transported to the laboratory, the animals were cleaned, divided into groups according to shell length, and stored for 6 days in a tank of running sea water with a temperature almost identical to that of the sampling place. In this way the mussels did not experience any sudden temperature change which

might have caused stress. Mussels with a shell length between 6.5 and 7.0 cm were used for the determinations.

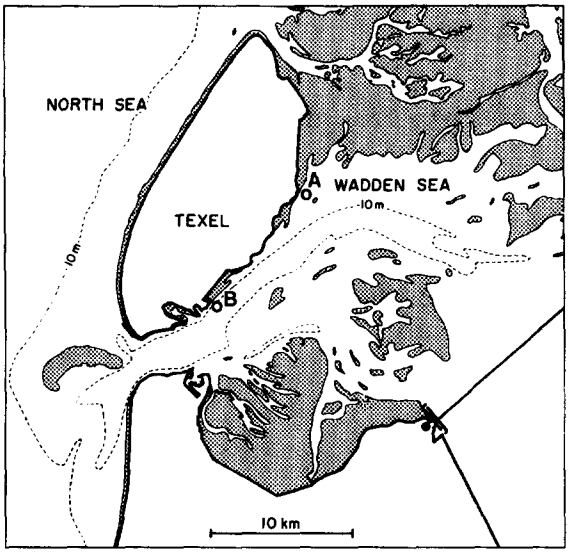

Fig. 1. Sampling locations in the western Dutch Wadden Sea: sublittoral mussel bed east of the island of Texel (A) and sea-water sampling location at Marsdiep tidal inlet (B).

Twenty-five mussels from each sample were removed from their shells, and the mantle tissue and the non-mantle tissues (whole animal minus the mantle tissue) were homogenized separately with 2 volumes of ice-cold bicarbonate buffer (0.02 M, pH 9.5) in a Sorvall Omni-mixer for 3 min at 20 000 rpm. Aliquots of the homogenates were taken in order to determine the dry weight and the biochemical constituents.

At two locations in the Wadden Sea, east of the island of Texel (Fig. 1), the surface temperature was measured and sea water samples were taken for determination of the chlorophyll concentration. In the sea water, collected from the Marsdiep tidal inlet, phytoplankton cell counts were made for analysis of species composition. Thereafter, ultrasonification of the sea water samples was carried out to disrupt the cell colonies present (CADÉE & HEGEMAN, 1974).

The content of functional chlorophyll a was determined using the method of LORENZEN (1967), and carried out at the laboratory of the Netherlands Institute for Sea Research.

The protein content of the tissues was determined according to the procedure of LOWRY *et al.* (1951), whereas the amount of glycogen was

measured spectrophotometrically as described by DE ZWAAN & ZANDEE (1972). The extraction of the total lipid content was carried out according to the method of FOLCH, LEES & SLOANE-STANLEY, 1957).

III. RESULTS

1. FLUCTUATIONS IN TISSUE DRY WEIGHT

During the breeding season the dry weight of mussels decreases until it reaches a minimum in May or June. Fig. 2a shows a detailed curve of the fluctuations in the dry weight of whole animals (mean value of 25 mussels), from the end of February through June 1978. Several rises in March, April and May were observed. After each rise there was a rapid reduction in the dry weight. The lowest values were determined during May.

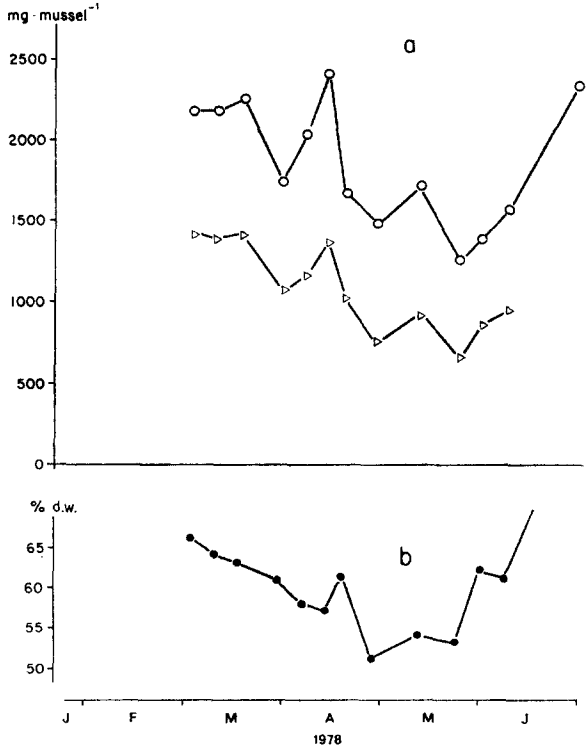

Fig. 2. a. Fluctuations in dry weight (○) and total amount of protein, lipid and glycogen (△) during the spawning season of 1978 (mg·animal^{-1} as the average of 25 mussels). b. Percentage of protein, lipid and glycogen as calculated from the total dry weight.

Comparing the curve with Fig. 3b which gives the functional chlorophyll a concentration at two locations in the Dutch Wadden Sea as a measure of the food availability, one observes a close similarity. Just before an increase in dry weight occurred, peak values were found for the chlorophyll content of the sea water. Therefore, an increase in food availability is directly reflected in tissue growth. On the other hand, the intense phytoplankton bloom in May (Figs 3b and 5) resulted in only a slight increase in the dry weight.

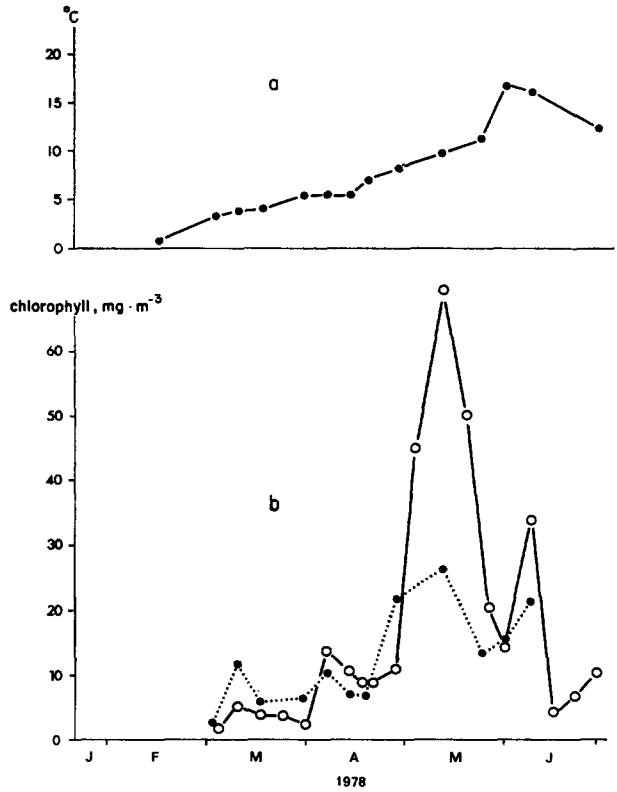

Fig. 3. a. Variation in sea water temperature near the sampling location for mussels A (°C). b. The concentrations of functional chlorophyll a at high tide for the sublittoral mussel bed near Texel A (○) and for surface water of the Marsdiep tidal inlet B (●).

The lower curve of Fig. 2a shows the amounts of protein, glycogen and lipids in mg per animal. The difference between these nutrient compounds and the total dry weight is due to dissolved organic compounds such as free amino acids, contributing 7 to 12% to the

total dry weight (unpublished results), sugars, betaine and many other organic substances of minor concentration and to the inorganic ion concentration.

Fig. 2b shows the amounts of protein, glycogen and lipids as percentage of the dry weight. The contribution of the nutrient compounds varies seasonally and reaches a minimum of nearly 50% in May after the main spawning.

Not until the beginning of June does the nutrient percentage rise again as result of a rapid increase in the glycogen content (Fig. 4).

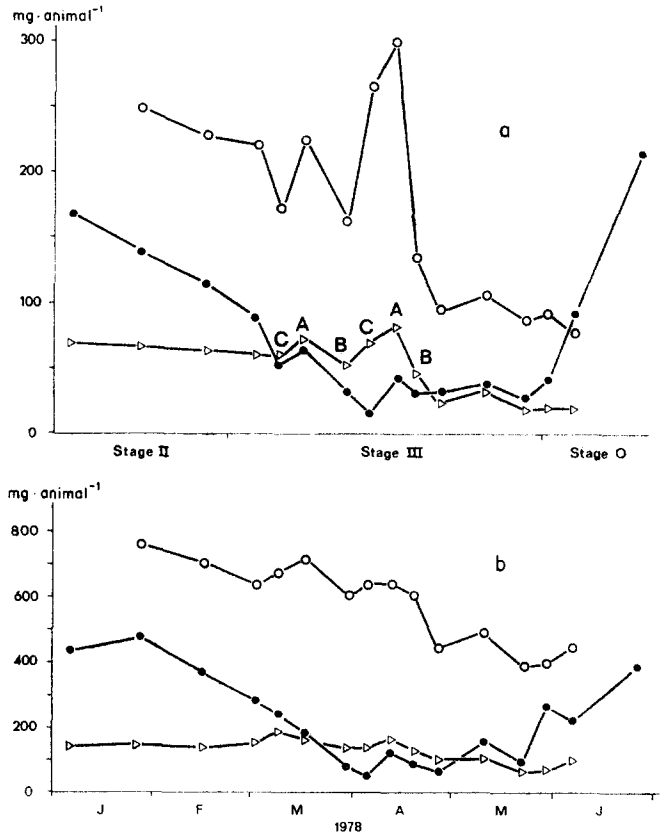

Fig. 4. Fluctuations in protein (○), glycogen (●) and total lipids (△) (mg dry weight per mussel) during 6 months of 1978 for mussels from a sublittoral mussel bed in relation to the stages of the gametogenic cycle with stage III subdivided into phases A, B and C. a. Mantle tissue. b. Non-mantle tissue.

2. BIOCHEMICAL COMPOSITION OF THE MANTLE AND NON-MANTLE TISSUES

The fluctuations in the protein, glycogen, and total lipid content are given in Fig. 4 for both the mantle and non-mantle tissues. Some sharp declines and increases in the protein and lipid content of the mantle tissue can be observed, as was already described for the total dry weight per mussel. Glycogen shows a similar tendency but to a lesser extent.

The rapid decreases in the protein and lipid content are probably the result of the release of sexual products into the water (LUBET, 1959, 1973; GABBOTT, 1975; PIETERS *et al.*, 1979; ZANDEE, HOLWERDA & DE ZWAAN, 1980; ZANDEE, KLUYTMANS, ZURBURG & PIETERS, 1980). Apparently, release of the gametes occurs more than once during the breeding season. A gradual decline in the energy reserves in discrete steps is observed in the non-mantle tissues as well. Apparently the main spawning in 1978 occurred in mid-April, when the largest reduction in the protein and lipid content was observed. Between early May and the beginning of June the amounts of all three nutrient compounds were maintained at minimum values, after which glycogen accumulated rapidly in the mantle again.

3. COMPOSITION OF THE EGGS

In April 1978 a group of mussels spawned spontaneously while being stored in a reservoir of running sea water in the laboratory. Part of the released eggs could be collected from the bottom of the reservoir and, after purification by means of gentle centrifugation and resuspension in buffer solution, the composition of the eggs was determined.

In Table I the biochemical composition of this sample of eggs is compared with the results of BAYNE *et al.* (1978). There is a striking conformity, the only difference being a somewhat higher protein content measured in our experiment.

TABLE I

Biochemical composition of the eggs of *Mytilus edulis* ($mg \cdot g^{-1}$ dry weight) compared with data obtained by BAYNE *et al.* (1978).

Biochemical compounds	*Relative weight* ($mg \cdot g^{-1}$)	
	This study	BAYNE
Glycogen	18.8	20
Protein	526	400
Total lipids	208	210

IV. DISCUSSION

On the basis of the classification made by CHIPPERFIELD (1953), LUBET (1959) has given a modified scheme of stages of the gametogenic cycle. He divided the spawning stage (stage III) into 4 substages, *viz.*: IIIA the ripening stage in which the eggs ripen; IIIB the release of the gametes during which the protein and lipid levels decrease; IIIC the recovery stage during which lipids and proteins are built up again in the gonads; IIID the transitional stage prior to the resting period (stage O). In Fig. 4 these stages are marked with capitals at the corresponding changes in the biochemical composition of the mantle, observed in our experiments. There is a good agreement with the results of LUBET (1959). He too observed the occurrence of several massive egg sheddings within a few months. The release of gametes (stage IIIB) took place more than once during the breeding season in the Wadden Sea. After each release and the attendant decrease in the protein and lipid contents, the gonads entered upon a new recovery (stage IIIC), and via a ripening stage (not visualized by changes in protein or lipid) another release of gametes occurred.

The spawning probably occurred simultaneously in a great part of the population, as is demonstrated by discrete spawning observed in samples of 25 animals taken randomly from a culture bed area of more than one hectare. Possibly the mussels have an influence on each other (or are influenced by environmental factors) facilitating synchronization of the emissions. In areas situated close to the open sea, with a more intense water exchange, mutual influence is much less effective. This might explain why CHIPPERFIELD (1953) found no evidence of periodic spawning. Over a period of 4 years of investigation he observed only one discrete spawning period per year in any locality studied along the English coast. LUBET (1973) also described a difference in the spawning behaviour of mussels living in a sheltered estuary (Bay d'Arcachon) and mussels situated on the Atlantic coast at the same latitude in France. Only the former exhibited discrete emissions of gametes.

The fact that chlorophyll peaks (Fig. 3b) preceded the temporary increases in organic nutrients in the tissues (recovery stage IIIC, Fig. 4) before an emission of gametes, indicates a close relationship between food availability and the production of ripe gametes, as has already been observed by LUBET (1959). Also, according to BAYNE (1976) the extent of the recovery stage depends strongly on the nutritive condition of the mussels.

In coastal areas with minor differences between winter and summer sea water temperatures, stage III of the gametogenic cycle is extended.

The larger difference between winter and summer sea water temperatures in the Bay d'Arcachon resulted in a short stage III. Temperatures below 7° C and above 18° C inhibit the development of the gonads (CHIPPERFIELD, 1953; LUBET, 1959). In fact the same situation exists in relation to the spawning cycle in the Dutch Wadden Sea. Here the temperature varies from −1° C in winter to above 20° C in summer. This explains why there is a short spawning period from March till June, when temperatures are favourable.

The main release of sexual products occurred between April 15th and April 25th, during bright weather with few rainy days. The sea water temperature rose rapidly from 6° to 8° C during this period (Fig. 2). CHIPPERFIELD (1953) found that the spawning occurred during a temperature rise from 9° to 12° C. In addition to the differences in geographical location, these facts indicate a more indirect influence of temperature, as BAYNE (1975) already pointed out. In the so-called "day-degrees" hypothesis, BAYNE has given a more accurate description of the temperature influence by correlating the mean daily sea water temperature and the time, to give a number of "day-degrees". The beginning of spawning coincided closely with a certain number of day-degrees.

Table II summarizes the decreases in the dry weight and in the total biochemical components for the successive spawnings in March, April

TABLE II

Reduction in total dry weight and in nutrient compounds in *Mytilus edulis* (mg·mussel^{-1}) for three successive occasions during the spawning period of 1978.

Month in which reduction was measured	*Reduction in total weight (mg·mussel^{-1})*	*Reduction in protein, lipid and glycogen (mg·mussel^{-1})*		
		Mantle	*Non-mantle*	*Total tissue*
March	508	117	248	365
April	920	297	320	617
May	455	45	222	267

and May. The contribution of the amounts in mantle and non-mantle tissues to the total reduction in dry weight are very similar for the main spawning in April. In March, the mantle also contributes considerably to the total decrease, whereas in May, only a minor decline in protein, glycogen and lipids was observed in the mantle tissue. In spite of this, total dry weight did show a marked decrease in May, during which time the percentage of biochemical compounds remained unchanged at a low level (Fig. 2).

Although plenty of food was available at the beginning of May

(Fig. 3), the increase in the biochemical components at that time is low in comparison with the increases in March and April (Fig. 4). Only the glycogen content of the non-mantle tissues increased during this period by about 100 mg on a dry weight basis. The subsequent decrease in the total amount of protein, lipid and glycogen is negligible for the mantle tissue but not for the non-mantle tissues (Table II). It is known that part of the ripe gametes can be stored in the visceral mass and mesosoma outside the mantle tissue.

Furthermore, glycogen reserves in the digestive gland can be used for the production and the ripening of eggs in the mantle tissue (vitellogenesis) as is pointed out by GABBOTT & BAYNE (1973), and THOMPSON, RATCLIFFE & BAYNE (1974). Although it cannot be concluded from our results that nutrient reserves were transferred from the digestive gland (non-mantle tissue) to the mantle tissue, and possibly used for the production of gametes in May, the observed decrease of biochemical compounds in the non-mantle tissues may be explained in this way.

An indication for a minor release of sexual products in May is found in Table I from which a protein to lipid ratio of 2.5 for the egg composition can be calculated. A similar calculation can be made for the successive sheddings using the amounts of protein and lipid decreases in the total mussel tissue. Consecutive ratios of 3.4, 3.3 and 2.1 were obtained for the declines in March, April and May. The ratio calculated for May, being smaller than that in the released egg cells, was even 60% lower than the ratio for the main spawning in April. This difference is not explained simply as the occurrence of a third production and subsequent release of gametes.

The observed delay in tissue growth in May may have been caused by environmental factors. Of the factors measured the quality of food showed the greatest change during this period, whereas the sea water temperature did not increase rapidly until the end of May (Fig. 3a).

An enormous bloom of *Phaeocystis pouchetii* occurred in May (Fig. 5). This organism forming glutinous colonies of thousands of cells, with a diameter of several mm, may give the sea a brownish colour, adheres to fishing nets and may also cause severe clogging of the gills of the herring, presumely even resulting in deviations of the migratory paths of the herring shoals (SAVAGE, 1930).

We suggest that the occurrence of the *Phaeocystis* bloom influenced the food collection by the mussels because the colonies adhere to their gills, and that a lowered food intake inhibited the recovery of the gonads and also the formation of new glycogen reserves. During periods of starvation, *Mytilus* utilizes organic nutrients from its tissues (BAYNE, 1973), which can even result in the resorption of ripe gametes

and recession of the gonads (BAYNE, 1975), with the subsequent reduction of the protein and lipid content. The *Phaeocystis* bloom in May 1978 very probably prevented in a greater or less degree a third spawning and retarded the build-up of new glycogen reserves. Although

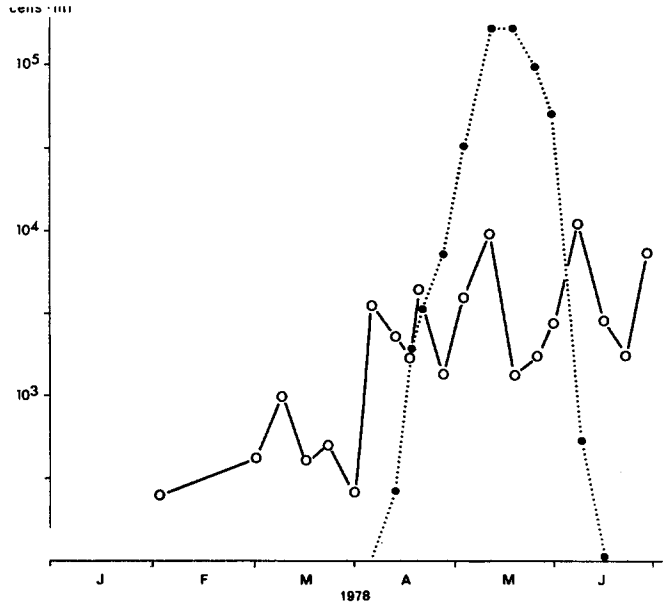

Fig. 5. The amounts of *Phaeocystis* algal cells (●) and of other algal cells (○) (cells per ml sea water) determined at high tide in the Marsdiep surface water.

the *Phaeocystis* bloom in May 1978 was more intense than observed in other years for which weekly phytoplankton counts are available (CADÉE & HEGEMAN, 1974, 1979; CADÉE, 1978), a *Phaeocystis* bloom was observed every year. This may indicate that the appearance of *Phaeocystis* in May influences the gamete production of mussels and retards new glycogen build-ups every year in the Dutch Wadden Sea.

V. SUMMARY

Variations in the tissue composition of the sea mussel *Mytilus edulis* L. from culture beds in the western Dutch Wadden Sea were studied during the first half of 1978.

Protein, glycogen and lipids were determined in the mantle tissue and non-mantle tissues of *Mytilus*. The content was found to fluctuate with the repeated release of gametes during the spawning season. Gamete emissions were observed in March and April. In May, how-

ever, low levels of nutrient reserves in the tissues were prolonged, in spite of high algae concentrations in the sea water. These high concentrations were caused by an algal bloom of *Phaeocystis pouchetii*. The glutinous colonies probably prevented food intake and digestion by *Mytilus*, and resulted in a delay in gonad recovery.

Ultimately, at the beginning of June, glycogen content increased rapidly again, concomitant with a rapid rise in the sea water temperature.

VI. REFERENCES

BAYNE, B. L., 1973. Aspects of the metabolism of *Mytilus edulis* during starvation.—Neth. J. Sea Res. **7**: 399–410.

——, 1975. Reproduction in bivalve molluscs under environmental stress. In: F. J. VERNBERG. Physiological ecology of estuarine organisms. University of South Carolina Press, Columbia: 259–277.

——, 1976. Marine mussels, their ecology and physiology. IBP no. 10, Cambridge University Press.

BAYNE, B. L., D. L. HOLLAND, M. N. MOORE, D. W. LOWE & J. WIDDOWS, 1978. Further studies on the effects of stress in the adult on the eggs of *Mytilus edulis*.—J. mar. biol. Ass. U.K. **58**: 825–841.

CADÉE, G. C., 1978. On the origin of organic matter accumulating on tidal flats of Balgzand, Dutch Wadden Sea.—Hydrobiol. Bull. **12**: 145–150.

CADÉE, G. C. & J. HEGEMAN, 1974. Primary production of phytoplankton in the Dutch Wadden Sea.—Neth. J. Sea Res. **8**: 240–259.

——, 1979. Phytoplankton primary production, chlorophyll and composition in an inlet of the western Wadden Sea (Marsdiep).—Neth. J. Sea Res. **13**: 224–241.

CHIPPERFIELD, P. N. J., 1953. Observations on the breeding and settlement of *Mytilus edulis* (L.) in British waters.—J. mar. biol. Ass. U.K. **32**: 449–476.

DARE, P. J. & D. B. EDWARDS, 1975. Seasonal changes in flesh weight and biochemical composition of mussels (*Mytilus edulis* L.) in the Conwy estuary, North Wales.—J. exp. mar. Biol. Ecol. **18**: 89–97.

FOLCH, J., M. LEES & G. H. SLOANE-STANLEY, 1957. A simple method for the isolation and purification of total lipids from animal tissues.—J. biol. Chem. **226**: 497–509.

GABBOTT, P. A., 1975. Storage cycles in marine bivalve molluscs: A hypothesis concerning the relationship between glycogen metabolism and gametogenesis. In: H. BARNES. Proc. 9th Europ. mar. biol. Symp. Aberdeen University Press, Aberdeen: 191–211.

GABBOT, P. A. & B. L. BAYNE, 1973. Biochemical effects of temperature and nutritive stress on *Mytilus edulis* L.—J. mar. biol. Ass. U.K. **53**: 269–286.

GIESE, A. C., 1959. Comparative physiology. Annual reproductive cycles of marine invertebrates.—A. Rev. Physiol. **21**: 547–576.

KLUYTMANS, J. H., D. I. ZANDEE, W. ZURBURG & H. PIETERS, 1980. The influence of seasonal changes on energy metabolism in *Mytilus edulis* (L.). III. Anaerobic energy metabolism.—Comp. Biochem. Physiol. **67B**: 307–315.

LORENZEN, G. J., 1967. Determination of chlorophyll and phaeopigments. Spectrophotometric equations.—Limnol. Oceanogr. **12** (2): 343–346.

LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR & R. J. RANDALL, 1951. Protein measurement with the Folin phenol reagent.—J. biol. Chem. **193**: 265–275.

LUBET, P., 1959. Recherches sur le cycle sexuel et l'émission des gamètes chez les Mytilidés et les Pectinidés.—Rev. Trav. Inst. (scient. techn.). Pêch. marit. **23** (4): 387–548.

——, 1973. Exposé synoptique des données biologiques sur la moule *Mytilus galloprovincialis* (L.). Synopsis F.A.O. sur les pêches No. 88 (SAST.-Moule 3, 16 (10) 028, 08 pag. var.) FAO, Rome.

LUBET, P. & P. LEGALL, 1967. Observations sur le cycle sexuel de *Mytilus edulis* L. à Luc sur Mer.—Bull. Soc. linn. Normandie **10**: 303–307.

PIETERS, H., J. H. KLUYTMANS, W. ZURBURG & D. I. ZANDEE, 1979. The influence of seasonal changes on energy metabolism in *Mytilus edulis* (L.). I. Growth rate and biochemical composition in relation to environmental parameters and spawning. In: E. NAYLOR & R. G. HARTNOLL. Cyclic phenomena in marine plants and animals. Pergamon Press, New York: 285–293.

SAVAGE, R. E., 1930. The influence of *Phaeocystis* on the migrations of the herring.—Fishery Invest., Lond. (2) **12** (2): 3–14.

SEED, R., 1975. Reproduction in *Mytilus* in European waters. Publ. d. Zool. di Napoli, Milan.

THOMPSON, R. J., N. A. RATCLIFFE & B. L. BAYNE, 1974. Effects of starvation on structure and function in the digestive gland of the mussel (*Mytilus edulis* L.).—J. mar. biol. Ass. U.K. **54**: 699–712.

WALDOCK, M. J., 1979. The fatty acid composition of the triacylgycerols of oysters, mussels and barnacles. University College of North Wales (Ph. D. thesis).

WILLIAMS, C. S., 1969. The effect of *Mytilicola intestinalis* on the biochemical composition of mussels.—J. mar. biol. Ass. U.K. **49**: 145–161.

ZANDEE, D. I., D. A. HOLWERDA & A. DE ZWAAN, 1980. Energy metabolism in bivalves and cephalopods. In: R. GILLES. Animals and environmental fitness. Pergamon Press, Oxford, New York: 185–206.

ZANDEE, D. I., J. H. KLUYTMANS, W. ZURBURG & H. PIETERS, 1980. Seasonal variations in biochemical composition of *Mytilus edulis* (L.) with reference to energy metabolism and gametogenesis.—Neth. J. Sea Res. **14** (2): 1–29.

ZURBURG, W., J. H. KLUYTMANS, H. PIETERS & D. I. ZANDEE, 1979. The influence of seasonal changes on energy metabolism in *Mytilus edulis* (L.). II. Organ specificity. In: E. NAYLOR & R. G. HARTNOLL. Cyclic phenomena in marine plants and animals. Pergamon Press, Oxford, New York: 293–300.

ZWAAN, A. DE, 1971. Het anaerobe koolhydraatmetabolisme in de zeemossel *Mytilus edulis* L. University of Utrecht: 1–83 (thesis).

ZWAAN, A. DE & D. I. ZANDEE, 1972. Body distribution and seasonal changes in the glycogen content of the common sea mussel *Mytilus edulis*.—Comp. Biochem. Physiol. **43A**: 53–58.