MEASUREMENT OF ENZYMATIC ACTIVITY OF MEIOBENTHIC ORGANISMS: METHODOLOGY AND ECOLOGICAL APPLICATION (1)

by

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Résumé

Pour leur alimentation, les organismes méiobenthiques dépendent directement du stock de matière organique particulaire (POM) dans le sédiment. Abstraction faite de quelques rares espèces, on n'a guère réalisé jusqu'à ce jour d'études sur la digestion et le taux de dégradation chez des Annélides, des Turbellariés et chez d'autres taxons voisins. Pour la présente étude, les mesures d'activité protéolytique et hydrolytique (α-amylase, β-D-glucosidase) ont été choisies comme indices principaux pour estimer le taux de dégradation de la matière organique dans la méiofaune. Basés sur des expériences antérieures sur l'influence des procédés d'homogénéisation, de température et de salinité sur l'activité enzymatique, les résultats ont été assez nets en ce qui concerne la stabilité et l'activité des enzymes, pourvu que l'estimation de ces dernières se produise dans des conditions adéquates. Des expériences de dégradation de la matière organique ont été effectuées dans des systèmes sédimentaires marins pour évaluer l'importance de la méio- et de la microfaune dans la dégradation de la matière organique. Par rapport à l'activité de la β-D-glucosidase, ces expériences ont mis en évidence la contribution des organismes méiobenthiques pour le tiers de l'activité au taux de la dégradation. Dans un programme du Kieler Bucht ("Hausgarten", juin 1980), l'activité digestive des Oligochètes (*Lumbricillus lineatus*, *Grania postclitellochaeta*, *Peloscolex benedeni*) et des Némertiens (*Cephalothrix* sp.) a été étudiée pendant une journée. Les résultats obtenus ont mis en évidence que la dégradation des glucides (α-amylase-activité) par les Oligochètes et par *Cephalothrix* sp. suit un rythme journalier pendant lequel le maximum de l'activité a été observé le matin et vers midi.

Introduction

The aim of the investigation presented was to follow short-term activities of meiobenthic organisms from natural sediments. Enzymatic measurements promised to be adequately sensitive to achieve direct results.

Very few ecological studies have been carried out on the enzymatic decomposition of biological polymers by marine meiobenthic

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organisms (Nielsen, 1966; Faubel and Meyer-Reil, 1981), especially compared with macrofaunal groups such as mollusks or arthropods (Kristensen, 1972). The most common polymers are protein and carbohydrates which undergo degradation by diverse decomposers in the benthic environment. To a certain extent, meiofauna organisms are involved in these degradation on particulate organic matter as a food source (Fenchel, 1970; Tenore, 1977; Gerlach, 1978; Riemann and Schrage, 1978; Meyer-Reil and Faubel, 1980).

Various methods have been used in the past to assess biological activity in aquatic environments and soils (references *in* Verstraete *et al.*, 1976; Wieser and Zech, 1976). Corpe and Winters (1972) and Kim and ZoBell (1972) demonstrated that α-amylase, β-D-glucosidase and proteolytic enzymes are secretion products of marine bacteria. The authors suggested that the degradation and mineralization of particulate organic matter result from the activities of these enzymes secreted from living cells as well as liberated during the lysis of cells, originating from micro-organisms either in the bottom sediments or throughout the water column or in the intestines of animals.

Generally, it is known that in lower metazoa the mode of digestion is an intra- and/or extracellular process. This takes place in the digestive tract in which larger food particles have to be introduced prior to being subjected to hydrolysis. Qualitative tests in different benthic animals revealed that the secretion of α-amylase and proteolytic enzymes is located in the digestive system (Nielsen, 1966; Michel, 1977; Marsden, 1963), known from higher metazoa. The synthesis of β-D-glucosidase by intestinal walls has been pointed out, for example, in *Mesenchytraeus glandulosus* (Nielsen, 1962) and other wrack invertebrates (Nielsen, 1966). Very little information exists about enzymes of Turbellaria (Jennings, 1974).

Based on the knowledge of determining enzymatic decomposition of organic matter by micro-organisms, pioneered by microbiologists (Corpe and Winters, 1972; Kim and ZoBell, 1972 : Little *et al.*, 1979; Meyer-Reil, 1981), the authors intended to adopt the methods to meiobenthic organisms. Up to recently, this is the first approach to describe activities of natural meiobenthic individuals on the basis of feeding dependent enzymatic activity. Therefore, the main purpose of this ecophysiological approach is to standardize reliable methods of determining proteolytic and hydrolytic activity of meiobenthic organisms which allows to acquire information on short-term variations or rythms and to evaluate the contribution of meiobenthic animals to the decomposition of particulate organic matter in natural sediments.

Area of investigation

The organisms used in these studies were collected from the midlittoral sandy beach near "Falkenstein" in the brackish water Kiel Fjord and from a station in the investigation area called "Hausgarten", located at a depth of 10m in the brackish water Kiel Bight (Baltic Sea), during periods in June and August 1980. The

collection site at the beach near "Falkenstein" was the "Otoplanen-zone", a habitat where the beach sediments were subjected to brief periods of exposure to brackish water and air. Influenced by wave action, there is a high contact of Oligochaeta and Otoplanidae (Turbellaria) with accumulated marine plant debris from bottom runoff. In this region the dominant meiofauna components are Oligochaeta (*Lumbricillus lineatus* (O.F. Müller)), the rich supply of decaying algae making this habitat favourable for this species (Giere and Hausschildt, 1979). Other important meiobenthic species are the Turbellaria Otoplanidae: *Bothriomolus balticus* Meixner, 1938; *Itaspiella helgolandica helgolandica* Sopott, 1972; *Otoplanella schulzi* (Ax, 1951).

The meiofauna found in the "Hausgarten" area represents a wide spectrum of species. Nematodes were by far the most important group, making up from 59 to 82 percent of the total. Harpacticoids were next in abundance. Other meiofaunal taxa were less numerous, but they were found in all cores every time (Scheibel, 1976).

Material and methods

Sediment sampling.

The sampling for meiofauna was carried out by pushing a short (0-15 cm) plastic core liner (4.9 cm inner diameter) into the sediment. For abundance analysis, the sediment in the core was immediately extruded and cut into segments at 2 cm levels. In the laboratory the samples were fixed with 8 percent formalin and sorted later on. Other samples were sorted within a day, while the animals remained alive. Separating the fauna from the sediment was performed by the shaking supernatant technique of Wieser (1960). Thorough shaking removed nearly all of the meiofauna organisms from the sediment. Weighing for biomass calculations was performed with a Mettler microbalance (range 10^{-3} to 10^{-7} g) and carbon was analyzed with a CHN-analyzer (Hewlett Packard 185 B). The organisms were washed well with filtered seawater (0.2µm), then pipetted onto a prepared GF/C Whatman filter and dried at 60° C. Mean grain size and organic matter content were analyzed as described by Meyer-Reil *et al.* (1978).

Enzyme preparations and assay techniques.

The enzymes (α-amylase, β-D-glucosidase, proteolytic enzymes) investigated were prepared from freshly sampled animals or homogenized sediment samples and essayed spectrophotometrically (PM 2K, Zeiss). These preparations were carried out a short time before assaying and the crude enzyme mixtures were always stored at 4°C. According to Curl and Sandberg (1961), at 25°C enzymatic activity diminishes by about 50 percent over a period of 6h and the activity is lost after 20h. The crude enzyme solutions were prepared by homogenization technique such as grinding with a glass rod, sonication (3 min, 50 watt; Sonifier B 12, Branson Sonic Power) or by Ultra-Turrax.

Animals separated from the sediment were washed well in 0.2 μm filter sterilized seawater and a distinct number depending on body length, was homogenized in 1.5 ml sterilized seawater (pH= 8.2). For determination of enzymatic activity of total fresh sediment samples (10 cm³), 10 ml sterilized seawater was added and the mixture sonified. The general assay procedure outlined below was followed in the enzymatic activity determinations of α-amylase, β-D-glucosidase, and proteolytic enzymes. For the determination of the activity of α-amylase and proteolytic enzymes, the reaction mixture contained as substrate amylopectin azure (AA) (Kim and ZoBell, 1972) and hide poder azure (HPA) (Little *et al.*, 1979), respectively. The assay mixture of β-D-glucosidase contained p-nitrophenyl-β-D-glucosid dissolved in $NaHCO_3$ buffer, pH 9, (Morrison *et al.*, 1977) as the substrate. Variables, as indicated in the text and figures, were homogenization techniques, temperature, and salinity. All experiments were performed in triplicate test tubes, accompanied by controls and blanks for each assay. Enzyme assays were carried out in glass centrifuge tubes containing: 1.5 ml sterilized seawater, 0.1 ml homogenized sample (crude enzymes) and the respective substrate. Incubation experiments using homogenized sediment samples were carried out in Erlenmeyer flasks (100 ml). These were charged with 10 cm³ sediment sample, sterilized seawater and the respective substrate. Measuring β-D-glucosidase activity the Erlenmeyer flasks were charged with only 10 cm³ sediment and p-nitrophenyl-β-D-glucosid. Before incubation, controls were heated at 100°C for 30 min. Blanks were charged with substrate only. The tubes were stoppered and, according to the test, placed in an incubator while gently shaking for 4 incubation times. As the end of each incubation time, the content of the tubes was centrifuged (5000 rpm for 15 min at 4°C). The supernatant was decanted and assayed spectrophotometrically at 595 nm for dye released from AA and HPA and at 410 nm for p-nitrophenol. Control values were substracted and activity rates were determined from the slope of the activity curves calculated by linear regression. The proteolytic rates were determined from the slope of the proteolysis curve of absorbances between 0.020 and 0.400. The straight lines were fitted to the data for each time course by the least squares method. It was always above 0.950 thus justifying the 95 percent confidence level.

RESULTS AND DISCUSSION

Efficiency of homogenization techniques

Different homogenization techniques were used for the preparation of crude enzyme extracts. As illustrated in Fig. 1A-C, the highest activity of α-amylase in *Lumbricillus lineatus* was obtained after grinding the tissue of whole organisms with a glass rod as well as by applying homogenization by Ultra-Turrax (Fig. 1B), and

sonication (Fig. 1C). Initially, after sonication more activity could be detected compared to treatment with Ultra-Turrax. However, after 2h of incubation, the readings of the samples homogenized by Ultra-Turrax are somewhat higher.

In order to estimate the activity of α-amylase after homogenization of the tissue which remains bound to particles, an expe-

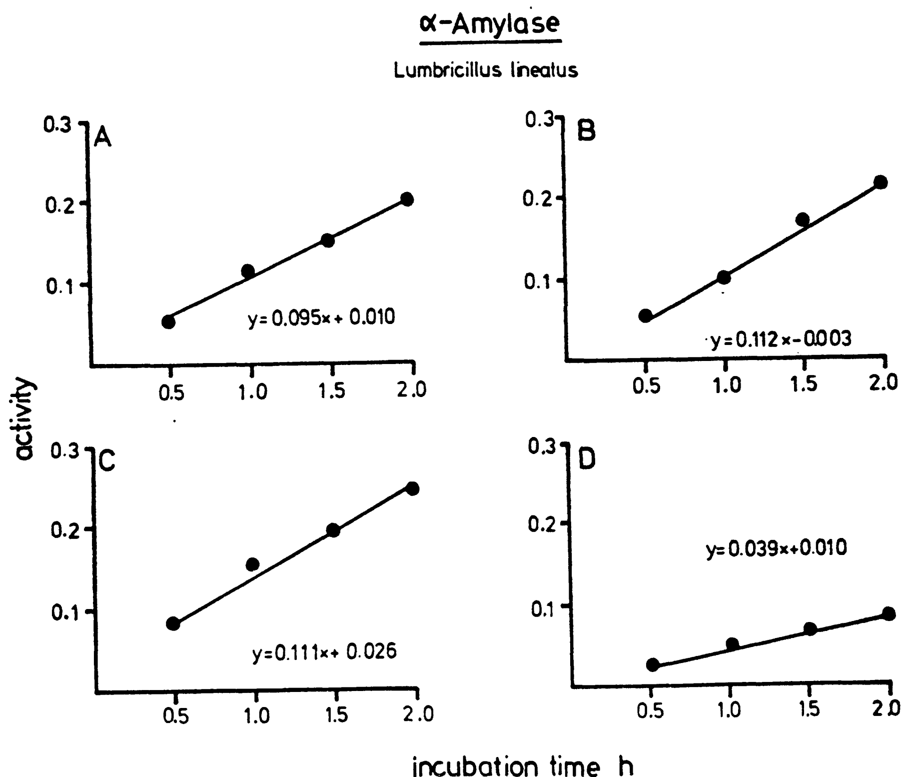

FIG. 1
Lumbricillus lineatus.

Effect of different homogenization techniques on the activity development of α-amylase. Animal tissues were homogenized by sonication (A), Ultra-Turrax (B) and grinding with a glass rod (C). Removal of the particles in the homogenized tissue by filtration through 0.2μm cellulose ester membranes resulted in a drastic decrease in the activity of α-amylase (D). Activity (ΔE/58,2μg dwt/h) is expressed as changes in absorbance at 595nm (dwt = dry weight).

riment with untreated and with filtered homogenized samples (0.2 μm cellulose ester membranes) was carried out. Since in the filtered sample (Fig. 1D) the activity decreases approximately by one half, it must be concluded that most of the α-amylase activity remains bound to particles.

For instance, Verstraete *et al.* (1976) demonstrate in an identical experiment with saccharase that these enzymes are either intracellulary bound or are complexed to the suspended solids in the water.

Activity of individual enzymes

Proteolytic enzymes, α-amylase, and β-D-glucosidase activities (ΔE/μg dwt/h) (dwt=dry weight) were assayed using crude homogenates derived from *Lumbricillus lineatus* (Oligochaeta) and Proseriata (Turbellaria). By plotting incubation time versus activity, typical curves (straight lines) were obtained, as shown in Fig. 1-2. The convenient incubation temperature in these experiments was found to be 25°C for proteolytic enzymes and β-D-glucosidase and

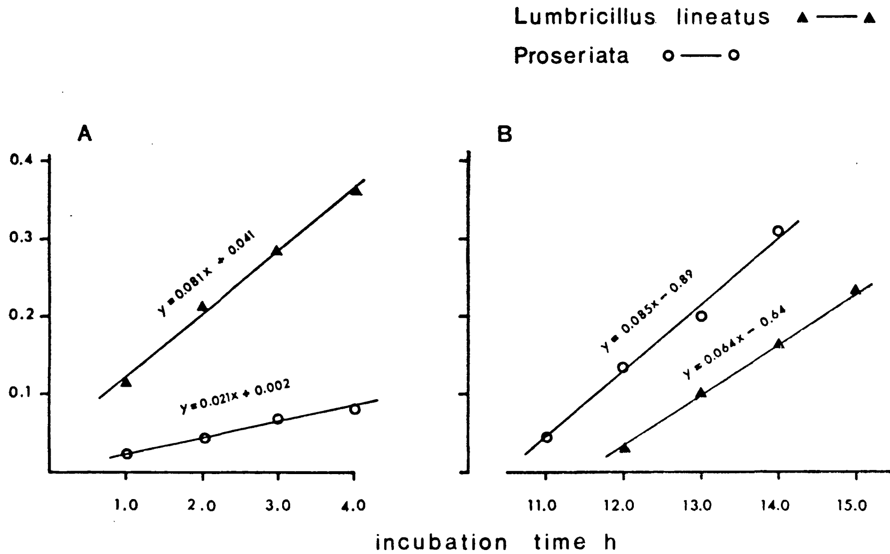

FIG. 2
Lumbricillus lineatus and Proseriata.

Time course of the activity of β-D-glucosidase (A) and proteolytic enzymes (B) in homogenized tissue from the oligochaete *L. lineatus* and Proseriata. Activity (ΔE/ 0,1mg dwt/h) is expressed as changes in absorbance at 410nm (β-D-glucosidase) and 595nm (proteolytic enzymes), respectively, (dwt = dry weight).

37°C for α-amylase. The results attained by assaying β-D-glucosidase activity (ΔE/100 μg dwt/h) of Proseriata were less pronounced compared with the activity (ΔE/100 μg dwt/h) of *L. lineatus* from digestive cells of animal's intestine (Fig. 2). The comparison of proteolytic activity (ΔE/100 μg dwt/h) of Proseriata and *L. lineatus* (Fig. 2), however, proves that the activity of Proseriata is somewhat higher than that of *L. lineatus*. Indeed, apart from some studies on β-D-glucosidase of marine macrofauna organisms (Yokoe and Yasumasu, 1964; Okada *et al.*, 1966; Kristensen, 1972; Lewis, 1980) very little information exists on this enzyme of meiobenthic organisms (see Nielsen, 1962; 1966). As mentioned above, some animals possess cellulases, but in many cases the degradation and mineralization of these polymers is believed to result from the activities of micro-organisms (ZoBell, 1968).

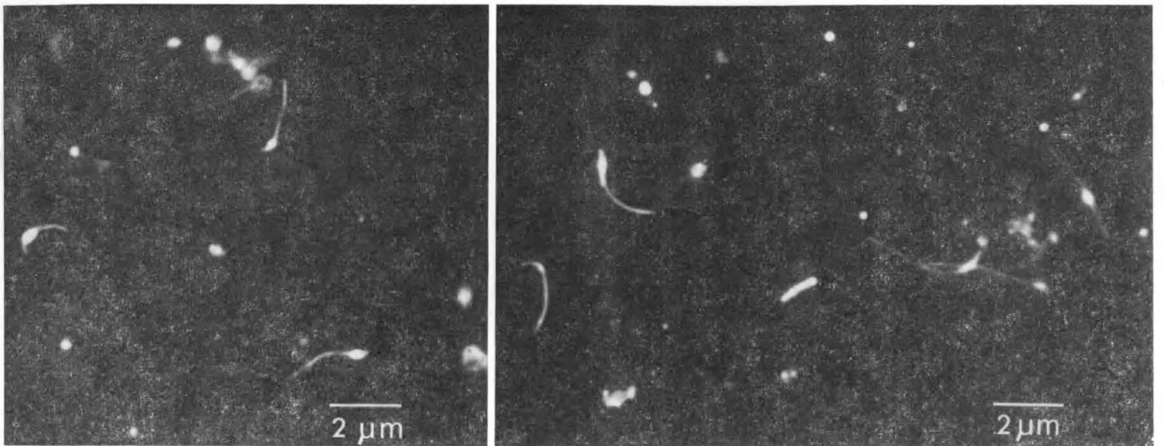

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PLANCHE I

Epifluorescence photograph of micro-organisms inhabiting *Lumbricillus lineatus* (Oligochaeta). Homogenized animals (cf. Material and methods) were diluted and filtered onto Nuclepore polycarbonate membranes prior to staining with acridine orange. Bar represents 2µm.

Generally, as suggested in some reports (ZoBell and Feltham, 1938; Jannasch, 1954; Odum, 1968; Corpe and Winters, 1972; Meyer-Reil and Faubel, 1980; and other authors cited in the last paper), a considerable amount of digestive enzymes could be derived from micro-organisms inhabiting the digestive tracts for taken up with nutrient supply. As illustrated in Plate I, from homogenized *Lumbricillus lineatus,* micro-organisms colonizing the digestive tract were analyzed by epifluorescence microscopy (Meyer-Reil *et al.,* 1978). From Plate I, the occurrence of a great number of filamentous bacteria, which are very seldom found in water or sediment samples of the corresponding station, becomes obvious.

Effect of pH, temperature and salinity

It has been well established (Okada *et al.,* 1968; Kim and ZoBell, 1972) that the pH value influences enzymatic activity rates. Enzymes are catalytically active over only a restricted pH range and usually have a quite pronounced optimum pH. This optimum is generally near pH 7. The activity of α-amylase on amylopectin azure is almost linear near its optimum pH 6.0 or in seawater (Kim and ZoBell, 1972).

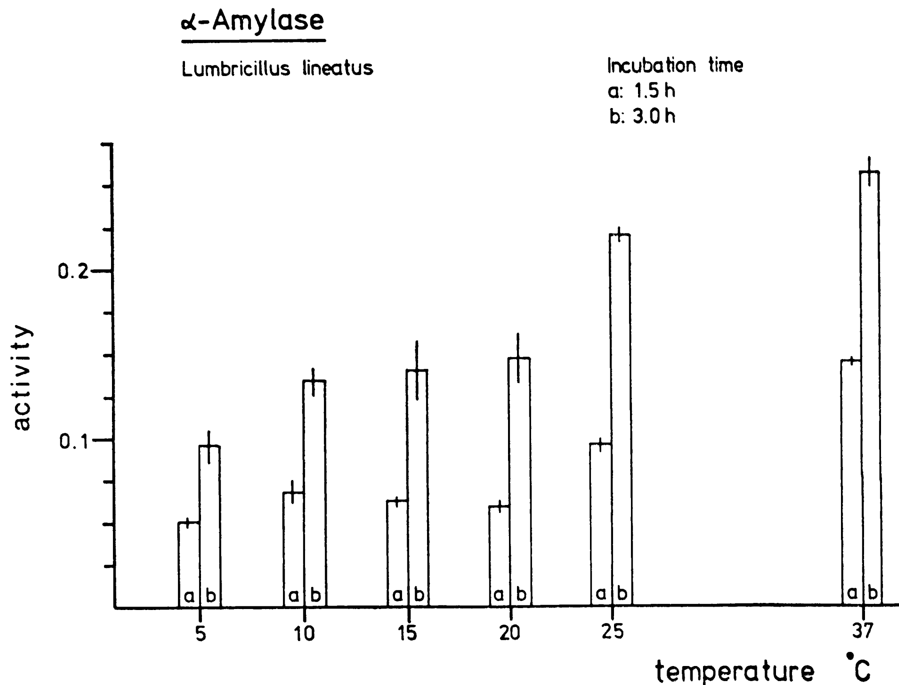

FIG. 3
Lumbricillus lineatus.

Effects of temperature on α-amylase activity in homogenized tissue from the oligochaete *L. lineatus*. Activity (ΔE/60,5μg dwt/h) is expressed as changes in absorbance at 595nm. Bars represent range of three measurements. (dwt = dry weight).

As illustrated in Figs. 3 and 4, increased activity of α-amylase was obtained with increasing temperature when incubated at temperatures of 5°, 10°, 15°, 20°, 25°, 37°C (pH 8.2). However, the pattern of the activity curves of α-amylase for *Lumbricillus lineatus* and Proseriata are quite different. The activity rates of crude α-amylase prepared from Proseriata as relatively low incubated at temperatures of 5° and 10°C, but relatively high activity rates of α-amylase

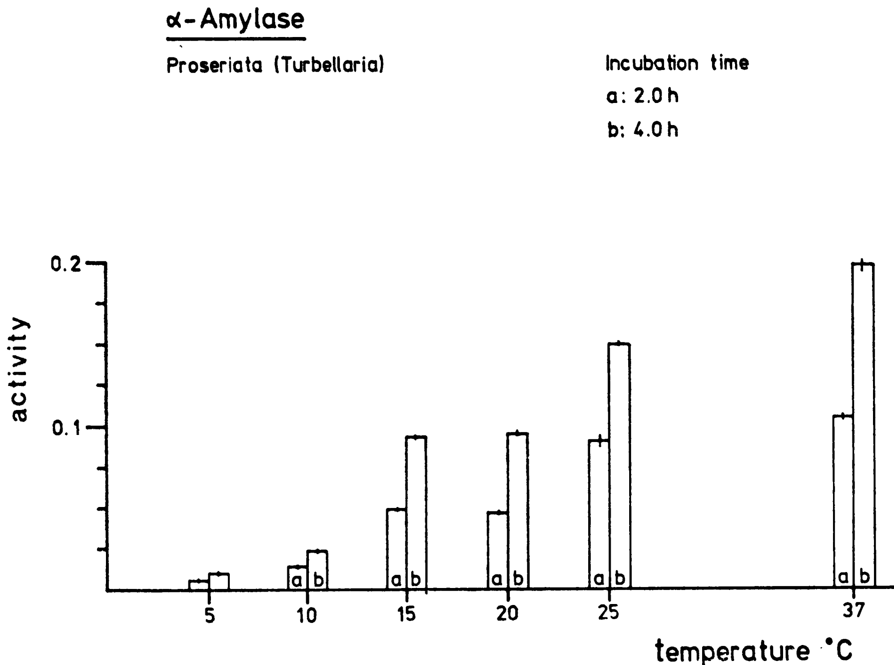

FIG. 4
Effects of temperature on α-amylase activity in homogenized tissue from Proseriata (Turbellaria). Activity (ΔE/45,2μg dwt/h) is expressed as changes in absorbance at 595nm (dwt = dry weight). Bars represent range of three measurements.

were obtained from *L. lineatus* even at 5°C (approximately 32 percent of the activity measured at 37°C). In both, a very strong increase in enzyme activity was observed when the temperature was raised from 20° to 25°C. At the transition from 10° to 15°C, only α-amylase from Proseriata shows a great increase, approximately the four-fold of the activity assayed at 10°C. Comparable results were obtained from enzyme activity measurements of β-D-glucosidase in sediment cores investigated by Meyer-Reil (1981).

Within the salinity range studied (0, 8, 16, 24 and 32 permil S), different results were obtained for *Lumbricillus lineatus* and Proseriata (Fig. 5, 6). In the enzyme mixture of *L. lineatus*, the enzymatic activity ranges from 8 to 32 permil S with a peak around 16 permil S. In the extract from Proseriata, high enzymatic activity already arose at 16 permil S with a peak around 32 permil S. In both experiments, the activity of α-amylase in distilled water was completely lost. The peaks may reflect the optimal salinity range of

that habitat for these species. As reported (Giere, 1971; Sopott, 1972), both *L. lineatus* and the Proseriata (*Bothriomolus balticus, Itaspiella helgolandica* and *Otoplanella schulzi*) are also found in beaches of the North Sea with salinity values up to 32 permil S. Almost all species investigated show an eury-haline range in their enzymatic activity pattern displaying a peak proportional to the salinity of that area obviously being the proper habitat. The

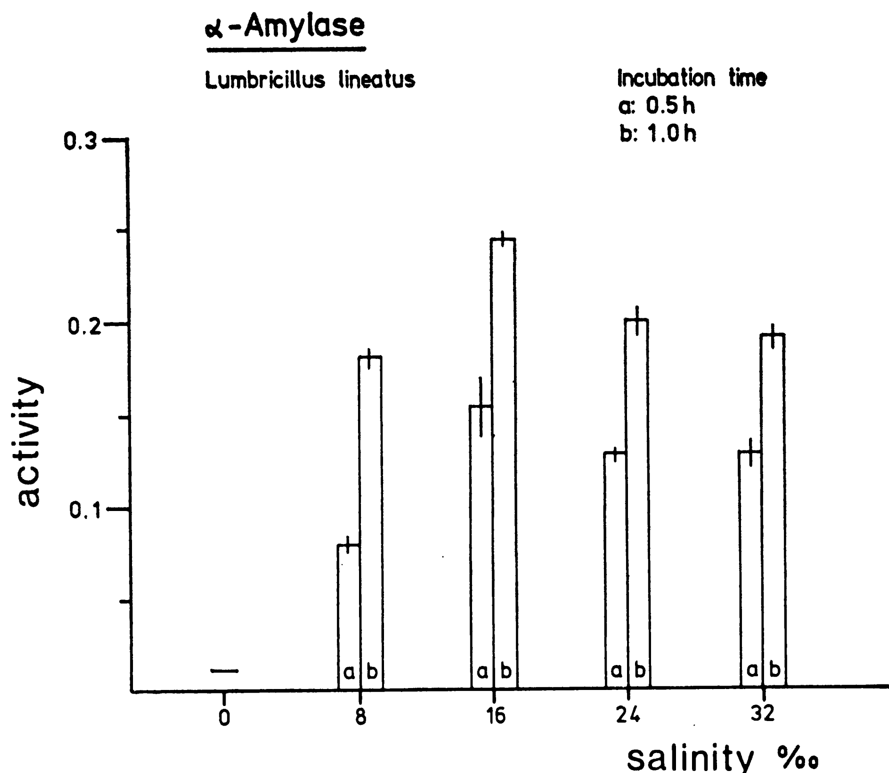

Fig. 5
Lumbricillus lineatus.

Effects of salinity on α-amylase activity in homogenized tissue from the oligochaete *L. lineatus*. Activity (ΔE/60,5μg dwt/h) is expressed as changes in absorbance at 595nm (dwt = dry weight). Bars represent range of three measurements.

dependence on salinity of amylase produced by moderately and extremely halophilic bacteria was reported by Onishi and Hidaka (1978). Accordingly, the activities of α-amylase were completely lost when assayed in distilled water and each micro-organism required a distinct range for maximal activity.

These preceding investigations of testing the influence of homogenization procedures, temperature and salinity of enzymatic activity are believed to be of importance prior to study enzymatic degradation and activity rates in a benthic system. The results obtained reveal that there is a fairly high reliability for enzymatic stabilities and activities, if the enzymatic assays are subjected to adequate

conditions. Based on this, experiments were performed to determine the contribution of meiofauna organisms to the decomposition of POM. Furthermore, it was investigated wether the enzymatic activity of meiofauna organisms follows diurnal cycles.

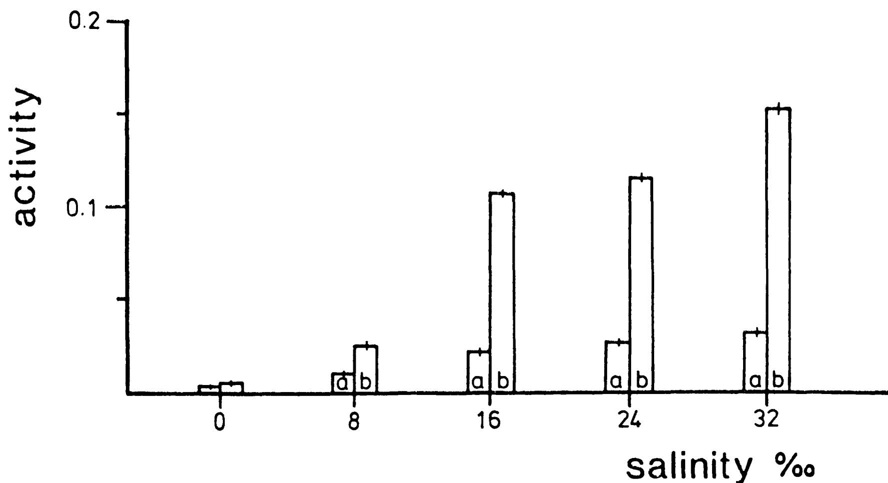

FIG. 6

Effects of salinity on α-amylase activity in homogenized tissue from Proseriata (Turbellaria). Activity (ΔE/45,2μg dwt/h) is expressed as changes in absorbance at 595nm (dwt = dry weight). Bars represent range of three measurements.

Sediment activity

Aliquots of sediment (10cm³; 1cm depth) collected from the sandy beach of Falkenstein were used for studying the activity of β-D-glucosidase originated through the activity of micro-organisms and of meiofauna individuals. The following components of the samples were incubated for assaying β-glucosidase activity: (a) sediment samples untreated, (b) sediment samples homogenized by sonication, and (c) the interstitial fauna of the sample extracted by shaking and decanting the supernatant. Category (c) was homogenized as well as (b). As the results illustrate in Fig. 7, in each category (a, b, c) activity of β-D-glucosidase was recorded but, as expected, the enzymatic activity of each assay exhibits different activity values. In (b), the curve represents the highest activity, and comparatively in (a) and (c) lower activities are recorded.

Adding up these activity values of the components (a) and (c), the activity values of curve (b) will be obtained. The share of the degradation rate of meiobenthic individuals according to β-D-glucosidase activity amounts to about a third of the total activity.

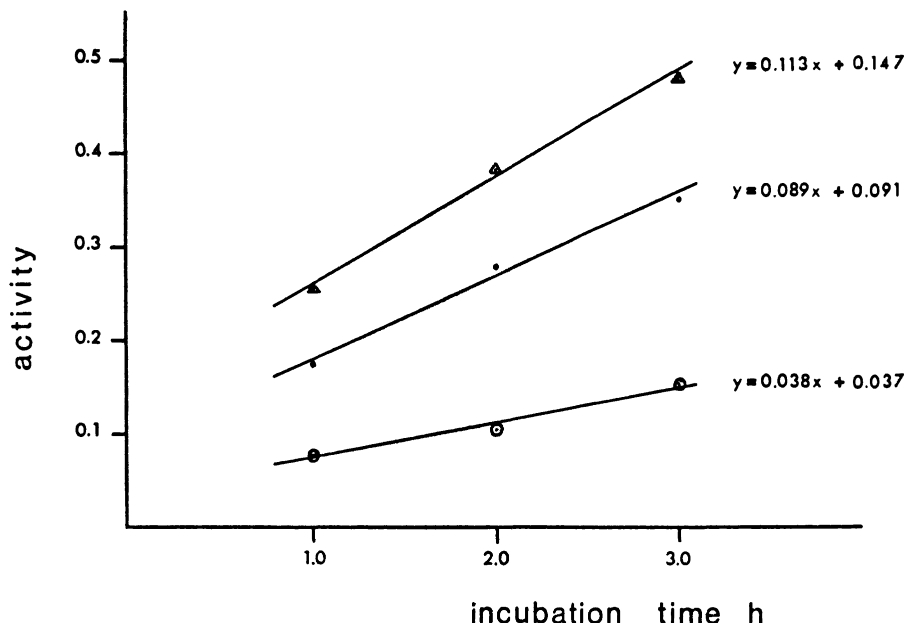

FIG. 7

Time course development of α-amylase activity in sediments and interstitial fauna. - ——— - sediment untreated, Δ ——— Δ sediment sonicated, o ——— o interstitial fauna sonicated. Activity (ΔE/10cm³ sed./h) is expressed as changes in absorbance at 595nm. Equations represent the corresponding regression lines.

Diel fluctuations

In June 1980, a field experiment was carried out in the "Hausgarten" area of the Kiel Bight to study diel variations of biological and chemical parameters. The sediment sampling area was marked by a grid of squares in order to obtain statistically valid samples. Salinity, temperature and wind remained relatively constant during the study period. Samples were withdrawn from the sediment at 4h intervals during a 36h cycle by divers. Oligochaetes (***Lumbricillus** lineatus, Grania postclitellochaeta, Peloscolex benedeni*) and Nemertini (*Cephalothrix* sp.) were separated by decantation while alive. For each assay, equal amounts in number and weight of both organisms groups (Fig. 8) were taken to prepare crude enzyme extracts of animal tissues. The measurements of α-amylase activity were performed after an incubation period of three hours. Plotting incubation time versus activity (Fig. 8) reveals that the decomposition of carbohydrates (α-amylase activity) in Oligochaeta and *Cephalothrix* sp. followed a diel cycle.

Comparable results of diel fluctuations could be demonstrated

for biomass and uptake of dissolved organic substances by bacteria, as well as for living biomass in the sediment cores (ATP) (G. Graf and L.-A. Meyer-Reil, unpublished data). These fluctuations may also depend on vertical movements of *Peloscolex benedeni* belonging to the Tubificidae (Oligochaeta).

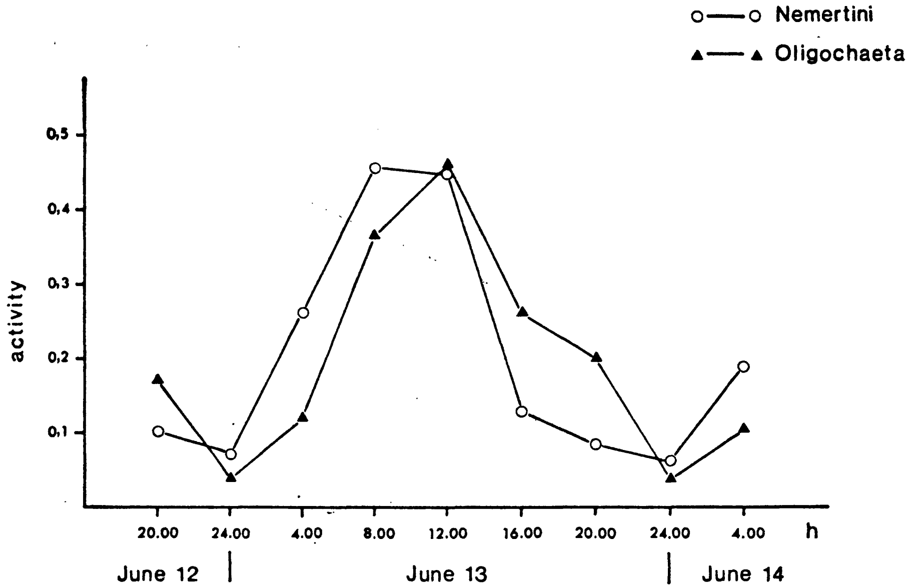

FIG. 8

Diel fluctuations of α-amylase (ΔE/26,5μg dwt/h) (dwt = dry weight) in homogenized tissue from Oligochaeta and Nemertini, respectively, at a 10m sandy sediment station in the Kiel Bight ("Hausgarten", Baltic Sea; FRG; June 12-14, 1980).

In the experiments carried out in the "Hausgarten" area the activity of α-amylase exhibited a peak in the morning and a continous decrease during afternoon and evening. The lowest amounts of relative activity were measured around midnight. The increase in α-amylase activity started again around 04.00h.

In all eucaryonts biological fluctuations or rhythms have been observed, but only recently have rhythms of enzyme activities also been detected (Harker, 1958; Halberg, 1960; Pittendrigh, 1960; Bünning, 1973; Rensing, 1973). Measurements of circadian fluctuations of enzyme activities have also been evaluated directly in crude extracts of homogenized animal tissues or indirectly through changes in metabolic activities (Glick *et al.*, 1961; van Pilsum and Halberg, 1964; Hardeland, 1969). The literature is very limited with respect to enzymatically dependent rhythms in invertebrates or even in meiobenthic organims. However, most recently diel fluctuations in microbiological and cheminal parameters could be demonstrated for bodies of water (Meyer-Reil *et al.*, 1979).

From the results presented the question arises wether these rhythms or fluctuations are of exogenous or endogenous nature. Generally, it is believed that circadian rhythms are endogenous

fluctuations, the properties of which are fixed genetically. However, this does not imply that endogenous rhythms are not directly influenced by environmental, i.e. exogenous factors such as temperature, lunarity, light intensity, atmospheric pressure, radiation and magnetic variation, since all these variables are subjected to periodicities of exactly 24h (Rensing, 1973).

Light has long been recognized as an initiating and controlling environmental factor of biological activity (Segal, 1970). Thus, the authors suggest that these diel cycles of feeding activity are strongly influenced by light intensity and coordinated with the cycle of day and night. *Cephalothrix* sp. is living on the sediment surface as well as other meiofauna and macrofauna individuals. However, the oligochaetes investigated occur in deeper sediment layers. As Fig. 8 illustrates, the enzymatic activity of *Cephalothrix* sp. starts increasing somewhat earlier than that of oligochaetes, which live in deeper sediments and may be activated by the activity of the surface community. Thus, light may be an important factor, indirectly regulating the activity of fauna in deeper sediment layers.

Summary

Meiobenthic organisms are greatly dependent on the availability of particulate organic matter (POM) in sediments. Except for very few species, however, natural enzymatic degradation rates of POM in annelids, turbellarians and related groups have been poorly investigated. Proteolytic and hydrolytic (α-amylase, β-D-glucosidase) activity measurements were the major indices examined in this study to determine trends in decomposing organic material by meiofauna organisms from natural sediment samples. Based on introductory investigations of testing the influences of homogenization procedures, temperature, and salinity on enzymatic activity, the results reveal a fairly high reliability for enzymatic stabilities and activities, if the enzymatic assays are subjected to adequate conditions. Experiments on degradation of organic matter in natural marine sediment systems were made to determine that part of decomposition which is originated by meiofauna and micro-organisms, respectively. The share of degradation rate of meiobenthic organisms according to β-D-glucosidase activity amounts to about a third of the total activity. In a field experiment in the Kiel Bight ("Hausgarten", June 1980), Oligochaeta (*Lumbricillus lineatus, Grania postclitellochaeta, Peloscolex benedeni*) and Nemertini (*Cephalothrix* sp.) were investigated for their activity of digestion over a diurnal cycle. The results reveal that the decomposition of carbohydrates (α-amylase activity) in Oligochaeta and *Cephalothrix* sp. follow a diurnal cycle showing maximum activity during morning and noon.

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REFERENCES

BÜNNING, E., 1973. — The physiological clock. Springer Verlag, New York, Heidelberg and Berlin, *pp.* 1-258.

CORPE, W.A. and WINTERS, H., 1972. — Hydrolytic enzymes of some periphytic marine bacteria. *Can. J. Microbiol.*, 18, *pp.* 1483-1490.

CURL, H. and SANDBERG, J., 1961. — The measurement of dehydrogenase activity in marine organisms. *J. Mar. Res.*, 19, *pp.* 123-138.

FAUBEL, A. and MEYER-REIL, L.-A., 1981. — Enzymatic decomposition of particulate organic matter by meiofauna. *Kieler Meeresforsch.*, Sonderh. 5, *pp.* 429-430.

FENCHEL, T., 1970. — Studies on the decomposition of organic detritus derived from the turtle grass *Thalassia testudinum*. *Limnol. Oceanogr.*, 15, *pp.* 14-20.

GERLACH, S.A., 1978. — Food-chain relationships in subtidal silty sand marine sediments and the role of meiofauna in stimulating bacterial productivity. *Oecologia* (Berl.), 33, *pp.* 55-69.

GIERE, O., 1971. — Beziehungen zwischen abiotischen Faktorensystem, Zonierung und Abundanz mariner Oligochaeten in einem Küstengebiet der Nordsee. *Thalassia Jugosl.*, 7, *pp.* 67-77.

GIERE, O. and HAUSSCHILD, D., 1979. — Experimental studies on the life cycle and production of the littoral oligochaete *Lumbricillus lineatus* and its response to oil pollution. *In:* Cyclic phenomena in marine plants and animals, E. Naylor and R.G. Hartnoll, ed., Pergamon Press, Oxford and New York, *pp.* 113-122.

GLICK, J.L., FERGUSON, R.B., GREENBERG, L.J. and HALBERG, F., 1961. — Circadian studies on succinic dehydrogenase, panthothenate, and biotin of rodent adrenal. *Am. J. Physiol.*, 200, *pp.* 811-814.

HALBERG, F., 1960. — Temporal coordination of physiologic function. Cold Spring Harbour Symp. quant. Biol., 25, *pp.* 289-310.

HARKER, J.E., 1958. — Diurnal rhythms in the animal kingdom. *Biol. Rev.*, 33, *pp.* 1-52.

JANNASCH, H.W., 1954. — Ökologische Untersuchungen der planktischen Bakterienflora im Golf von Neapel. *Naturwissenschaften*, 41, *pp.* 1-42.

JENNINGS, J.B., 1974. — Digestive physiology of the Turbellaria. *In:* Biology of the Turbellaria, N.W. Riser and M.P. Morse, ed., McGraw Hill Co., New York, *pp.* 173-197.

KIM, J. and ZOBELL, C.E., 1972. — Agarase, amylase, cellulase and chitinase activity at deep-sea pressures. *J. Oceanogr. Soc. Japan*, 28, *pp.* 131-137.

KRISTENSEN, J.H., 1972. — Carbohydrases of some marine invertebrates with notes on their food and on the natural occurrence of the carbohydrates studied. *Mar. Biol.* 14, *pp.* 130-142.

LEWIS, D.B., 1980. — Cellulase production in *Sabella penicillus* (Annelida: Polychaeta). *J. Mar. biol. Assoc. U.K.*, 60, *pp.* 1069-1070.

LITTLE, J.E., SJOGREN, R.E. and CARSON, G.R., 1979. — Measurement of proteolysis in natural waters. *Appl. environ. Microbiol.* 37, *pp.* 900-908.

MARSDEN, J.R., 1963. — Digestive enzymes of (the polychaete) *Hermodice caruncu-lata*. *Can. J. Zool.*, 41, *pp.* 159-164.

MEYER-REIL, L.-A., 1981. — Enzymatic decomposition of proteins and carbohydrates in marine sediments: methodology and field observations during spring. — *Kieler Meeresf. Sonderh.* 5, *pp.* 311-317.

MEYER-REIL, L.-A., BÖLTER, M., LIEBEZEIT, G. and SCHRAMM, W., 1979. — Short-term variations in microbiological and chemical parameters. *Mar. Ecol. Prog. Ser.* 1, *pp.* 1-6.

MEYER-REIL, L.-A., DAWSON, R., LIEBEZEIT, G. and TIEDGE, H., 1978. — Fluctuations and interactions of bacterial activity in sandy beach sediments and overlying waters. *Mar. Biol.*, 48, *pp.* 161-171.

MEYER-REIL, L.-A. and FAUBEL, A., 1980. — Uptake of organic matter by meiofauna organisms and interrelationships with bacteria. *Mar. Ecol. Prog. Ser.*, 3, *pp.* 251-256.

MICHEL, C., 1977. — Tissular localization of the digestive protease in sedentary polychaetous annelid *Sabellaria alveolata*. *Mar. Biol.*, 44, *pp.* 265-273.

MORRISON, S.J., KING, J.D., BOBBIE, R.J., BECHTOLD, R.E. and WHITE, D.C., 1977. — Evidence for microfloral succession on allochtonous plant litter in Apalachicola Bay, Florida, USA., *Mar. Biol.*, 41, *pp.* 229-240.

NIELSEN, C.O., 1962. — Carbohydrases of some wrack invertebrates. *Oikos*, 13, *pp.* 200-215.

NIELSEN, C.O., 1966. — Carbohydrases of some wrack invertebrates. *Natura Jutlandica*, 12, *pp.* 191-194.

ODUM, W.E., 1968. — The ecological significance of fine particle selection by striped mullet, *Mugil cephalus*. *Limnol. Oceanogr.*, 13, *pp.* 92-97.

OKADA, G., NISIZAWA, T. and NISIZAWA, K., 1966. — Cellulases of a marine mollusk *Dolabella* sp. *Biochem. J.*, 99, *pp.* 214-221.

ONISHI, H. and HIDAKA, O., 1978. — Purification and properties of amylase produced by a moderately halophilic *Acinetobacter* sp. *Can. J. Microbiol.*, 24, *pp.* 1017-1023.

PITTENDRIGH, C.S., 1960. — Circadian rhythms and the circadian organization of living systems. Cold Spring Harbour Symp. quant. Biol., 25, *pp.* 159-184.

RENSING, L., 1973. — Biologische Rhythmen und Regulation. Gustav Fischer Verlag, Stuttgart, *pp.* 1-265.

RIEMANN, F. and SCHRAGE, M., 1978. — The mucus-trap hypothesis on feeding of aquatic nematodes and implication for biodegradation and sediment texture. *Oecologia* (Berl.), 34, *pp.* 75-88.

RINDERKNECHT, H., GEOKAS, M.M., SILVERMAN, P. and HAVERBACK, B.J., 1968. — A new ultrasensitive method for the determination of proteolytic activity. *Clin. Chim. Acta*, 21, *pp.* 197-203.

SCHEIBEL, W., 1976. — Quantitative Untersuchungen am Meiobenthos eines Profils unterschiedlicher Sedimente in der westlichen Ostsee. *Helgoländer wiss. Meeresunters.*, 28, *pp.* 31-42.

SEGAL, E., 1970. — Environmental factors, 2.31 light. *In:* Marine ecology, O. Kinne, ed., Wiley-Interscience, London, New York, Sydney and Toronto, *pp.* 159-211.

SOPOTT, B., 1972. — Systematik und Ökologie von Proseriaten (Turbellaria) der deutschen Nordseeküste. *Mikrofauna Meeresboden*, 13, *pp.* 165-236.

TENORE, K.R., 1977. — Food chain pathways in detrital feeding benthic communities: a review, with new observations on sediment resuspension and detrital recycling. *In:* Ecology of marine benthos, B.C. Coull, ed., University of South Carolina Press, Columbia, *pp.* 37-53.

VAN PILSUM, J.F. and HALBERG, F., 1964. — Transaminase activity in the mouse kidney — an aspect of circadian enzyme activity. *Ann. N.Y. Acad. Sci*, 117, *pp.* 337-353.

VERSTRAETE, W., VOETS, J.P. and VAN LANCKER, P., 1976. — Evaluation of some enzymatic methods to measure the bioactivity of aquatic environments. *Hydrobiologia*, 49, *pp.* 257-266.

WIESER, W., 1960. — Benthic studies in Buzzard Bay. II. Meiofauna. *Limnol. Oceanogr.*, 5, *pp.* 121-137.

WIESER, W., and ZECH, M., 1976. — Dehydrogenases as tools in the study of marine sediments. *Mar. Biol.*, 36, *pp.* 113-122.

YOKOE, Y. and YASUMASU, J., 1964. — The distribution of cellulase in invertebrates. *Comp. Biochem. Physiol.*, 13, *pp.* 323-338.

ZOBELL, C.E., 1968. — Bacterial life in the deep sea. *Bull. Misaki mar. biol. Inst., Kyoto Univ.*, 12, *pp.* 77-96.

ZOBELL, C.E. and FELTHAM, C.B., 1938. — Bacteria as food for certain marine invertebrates. *J. Mar. Res.*, 4, *pp.* 312-327.