FISKERIDIREKTORATETS SKRIFTER

SERIE ERNÆRING

VOL, 1, NO. 5

JULSHAMN, K.

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

I–VII

DIRECTORATE OF FISHERIES
BERGEN - NORWAY
1981

Fisk. Dir. Skr., Ser. Ernæring, Vol. 1: No. 5, 1981, 155–159.

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

By

KÅRE JULSHAMN

Institute of Vitamin Research
Directorate of Fisheries, Bergen.

PREFACE

The present study was carried out at the Institute of Vitamin Research, Directorate of Fisheries, between 1973 and 1979.

I wish to express my gratitude to the Head of the institute, prof. Olaf R. Brækkan for inspiring and interesting discussions. His help and criticism during the work with the presentation of the data has been much appreciated. My sincere thanks go to the Editor of Fisk. Dir. Skr., Ser. Ernæring, assoc. prof. Georg Lambertsen for having accepted the manuscripts for publication and his critical revision of the manuscripts is gratefully acknowledged.

Mr. P. A. Solheim, previous director of the Fishery Museum in Bergen is thanked for his valuable information and advice concerning the oyster farms on the Western coast of Norway. A study of the present type is basically dependent on good samples. The author is indebted to Mr. Magnus K. Røttingen for supplying and assessing ages of oysters from Innerøy used in papers I, II, IV, V and VI. Further to Mr. Per Dag F. Iversen for having provided samples from Svanøy which were used in papers I and II, and to Mr. Holger Ladegård for samples from Vågstranda used in papers I and II, and especially his help in age assessment of oysters used in paper III. Mr. Helge Thomsen made possible the transplantation study (paper VI) and the study on the extension of elements from industrial pollution in Sørfjorden (paper VII) with his diving and sampling under difficult conditions.

I am also indebted to Mr. Tore Neppelberg, Institute of Marine Research, Directorate of Fisheries, for helpful discussion of the statistical treatment and for helping with statistical analysis which were carried out on a Finnigan 6000 Series computer system for GC-MS at this institute. Further I should like to thank Mrs. Jorun Haugsnes and Mrs. Judit

Eriksen for their skilful technical assistance. Thanks are also due to Miss Margunn Ringøy and Mrs. Vigdis Rasmussen for their patience in typing and retyping of the manuscripts.

I am also indebted to Dr. Leif Rein Njaa for reading and commenting on the manuscripts. During the long time this study took place, also other collegues at the institute have from time to others given help and advice, for which I am grateful.

At the early stage of the study the late Dr. Gulbrand Lunde at the Central Institute for Industrial Research gave valuable help and advice based on his many years experience in trace elements analyses.

GENERAL INTRODUCTION

The relative pollution levels of aquatic environments by trace elements may be elucidated by analyses of water, sediments and/or indigenous biota. The more recent literature shows that element analyses of marine organisms is a method of choice for the evaluation of a marine recipient with regard to element burdens. The choice of indicator organisms for this purpose is critical as it must fulfil a number of requirements, e.g. as listed up by HAUG et al. (1974). Further, detailed knowledge is needed on environmental and biological factors determining the dynamics of elements in the tissues of potential indicator organisms, among these the effects of season, sexual maturation, size and age. The understanding of environmental and biological factors and the application of reliable and accurate analytical methods are of fundamental importance in pollution studies based on molluscs as indicator organisms.

The present studies started by establishing analytical procedures. At the start these were based on sample destruction by dry ashing and wet digestion in conjunction with organic solvent extraction. Cobalt was analysed in a study on vitamin B_{12} and determined by dry ashing followed by APDC/MIBK* as the chelating and extraction system (JULSHAMN and BRÆKKAN, 1974). A method was established for the simultaneous analysis of Mn, Fe, Cu, Zn, Cd and Pb using wet digestion and ion chelation by Na DDC (sodium diethyl dithiocarbamate), extracted into MIBK (JULSHAMN and BRÆKKAN, 1975). Investigations on marine organisms revealed some correlation between mercury and selenium and therefore a reliable method was worked out of simultaneous digestion of mercury and selenium (EGAAS and JULSHAMN, 1978). All procedures based on flame atomic absorption are reviewed elsewhere

*APDC: ammonium pyrrolidine dithiocarbamate
MIBK: methyl isobutyl ketone

(JULSHAMN et al., 1978). During the present studies the analytical methods for cadmium and lead were improved by a change from the extraction procedure to flameless atomic absorption, a method which allowed for the inclusion of aluminium, chromium and nickel in the program of analysis (JULSHAMN, 1977; JULSHAMN et al., 1978). Either procedure was checked by a reference sample purchased from U S. National Bureau of Standards (NBS) and by four intercalibration studies conducted by The International Council for the Exploration of the Sea (ICES) during the years 1976 to 1980 (TOPPING, 1979; 1980).

The study of biological effects of element levels were based on molluscs and algae obtained in oyster farms on the western coast of Norway. The oysters farms are all shielded from the open ocean and well removed from metal polluting industries. The work has been in progress during the years 1973–1979 and the problem of biological variations demanded repeated sampling to ensure realistic values. The samples used in paper I and II were collected monthly from October 1973 to November 1974 from three oyster farms, Innerøy, Svanøy and Vågstranda, whereas the samples used in paper III and IV were collected from the oyster farms at Vågstranda and Innerøy in October 1976 and September 1977, respectively. The molluscs used in paper V were collected at Innerøy in 1978. The accumulation study (paper VI) was based on molluscs taken from Innerøy in May 1976 and the depletion study was finished in August 1978. Finally, the samples used in paper VII were collected in October from Sørfjorden, a branch of the Hardangerfjord.

Paper I of this series discusses relative levels among species and geographical variations between the three oyster polls The zinc and copper concentrations in *Ostrea* were exceptionally high compared to values found in *Ascophyllum* and *Mytilus*. Differences in element concentrations were seen between the three polls of low element loads, but these differences were not consistent for the three species investigated, and did not reflect the water levels.

Paper II discusses the seasonal variations in the element concentrations during the year. Only minor variations in the tissue element contents was found through the year, except for manganese with minimum values in May and June and with a maximum peak in August. On the other hand there was a general decrease in the tissue element concentrations during maturation, followed by an increase after the spawning.

Paper III deals with element relations for size and age. The results show that the element concentrations depend on size as well as on age of the mussels. Consequently these parameters should be stated in studies on element contents of mussels, and should be included in calculations based on such studies.

Paper IV reports on the distribution of 17 elements in tissues of the species *Ostrea*, *Mytilus* and *Modiolus*. Ten of thirteen minor elements accumulated in the digestive system of *Modiolus*, and this tissue held 61 to 98% of the total element concent in the mussel. Particularly high concentrations were found for some elements, e.g. Mn: 2.2 g/kg, Zn: 19 g/kg and Pb: 100 mg/kg. In *Mytilus* 9 of 13 minor elements accumulated in the digestive system, whereas three elements accumulated in the gills and the mantle. In *Ostrea* only four elements accumulated in the digestive system, whereas four other elements accumulated in the gills and mantle. The other elements were equally distributed over the tissues. Higher concentrations of zinc, copper and cadmium in the heart may be noted.

Paper V describes the distribution of zinc, cadmium and copper obtained by analytical subcellular fractionation and Sephadex chromatography of the particle-free supernatants from two tissues in *Ostrea*, *Mytilus* and *Modiolus*. Molluscs from non-polluted seawater contain specific low molecular proteins which bind cadmium and copper, but not zinc.

Paper VI concerns transfer of *Ostrea* and *Mytilus* between two environments having widely different levels of cadmium and lead. The uptake of cadmium in *Ostrea* started after 60 days and a steady state was not reached during the 480 days period in Hardangerfjorden After transfer back to the original site, the depletion of cadmium was slow, and more than 70% of the maximum concentrations were still present after 360 days. *Mytilus* had a rapid uptake of cadmium and a steady state was reached after 60–90 days. After return to the original site, there was a rapid depletion of cadmium. The uptake of lead in *Ostrea* was not reached after two months. All tissues increased to the same extent. After transfer back to Innerøy, the tissue levels were halved in the gills within 2 months. Also *Mytilus* had a rapid uptake of lead. After return to Innerøy the depletion of lead was rapid in all tissues except the gills.

Paper VII studies the consequences of metal dilution out along Sørfjorden on *Mytilus* and *Ascophyllum*. Most element concentrations in the water decreased rapidly within the first 15 km. The cadmium values were linearly correlated to distance along the fjord. Shoots of *Ascophyllum* were useful indicators of the level of copper, zinc, cadmium and lead, as the contents were highly correlated to the distance. *Mytilus* was a useful indicator organisms for lead and mercury. The contents of copper, zinc and cadmium were relatively constant. The contents of lead and zinc in the *Mytilus* samples were among the highest recorded in field surveys.

REFERENCES

EGAAS, E. and JULSHAMN, K., 1978. At. Absorption Newslett. *17*, 135–138.

HAUG, A., MELSON, S. and OMANG, S., 1974. Environm. Pollut. *7*, 179–192.

JULSHAMN, K. and BRÆKKAN, O. R., 1974. At. Absorption Newslett. *13*, 139–141.

JULSHAMN, K. and BRÆKKAN, O. R., 1975. At. Absorption Newslett. *13*, 49–52.

JULSHAMN, K., 1977. At. Absorption Newslett. *16*, 149–150.

JULSHAMN, K., HAUGSNES, J., and UTNE, F., 1978. Fisk. Dir. Skr., Ser. Ernæring *1*, 117–135.

JULSHAMN, K., ANDERSEN, K.-J., WILLASSEN, Y. and BRÆKKAN, O. R., 1978. Anal Biochem. *88*, 552–559.

TOPPING, G., 1979. Ices Trace Metal Intercomparison Exercise 1978/79. DAFS Marine Laboratory, Aberdeen, Scotland.

TOPPING, G., 1980. Report on the 6th Ices Trace Metal Intercomparison Exercise for Cadmium and Lead in Biological Tissue.

Fisk. Dir. Skr., Ser. Ernæring, Vol. 1: No. 5, 1981, 161–182

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

I. Geographical variations in the contents of 10 elements in oyster (*Ostrea edulis*), common mussel (*Mytilus edulis*) and brown seaweed (*Ascophyllum nodosum*) from three oyster farms.

By

KÅRE JULSHAMN

Institute of Vitamin Research
Directorate of Fisheries, Bergen.

ABSTRACT

This study is the first of a series taken up to gain background knowledge on the use of mussels and seaweed as indicator organisms for element loads in sea water. Samples of water, cultured oysters (*Ostrea edulis*), common mussel (*Mytilus edulis*) and brown seaweed (*Ascophyllum nodosum*) were collected monthly from three oyster farms, resp. at Innerøy, Svanøy and Vågstranda on the western coast of Norway. The oyster polls are described, and salinities and temperatures through the year are given. Four major elements, sodium, potassium, magnesium and calcium, and 6 minor elements, manganese, iron, copper, zinc, cadmium and lead were measured by atomic absorption spectrophotometry in tissues and whole soft parts of the mussels and in 1–2-year-old shoots of seaweed. Details of the analytical procedure are given, and the accuracy of the methodology is discussed based on standard reference material. This paper gives average values for the element concentrations of the samples within the year and discusses relative levels and geographical variations between the three oyster polls, whereas the seasonal variations through the year are discussed in paper II of this series. The water analyses showed salinities near 29% for the three polls, and low minor element levels near the detection limit of the method for manganese, iron, copper, cadmium and lead. Only zinc values could be given with satisfactory accuracy. The values varied little between the polls, with the exception of manganese (0.4–0.8 microg/l), highest at Innerøy. Analyses of *Ascophyllum* shoots of 4-year classes showed that the contents of manganese and zinc increased with age, whereas the copper contents decreased with age. From the tables of results, some observations may be noted: All major element levels were higher in *Ascophyllum* than in the two molluscs. Higher levels of sodium and magnesium in *Mytilus* than in *Ostrea* may possibly be compensated by the high levels of zinc in *Ostrea*. Differences in major element concentrations were seen between the sites, but were not consistent.

The zinc and copper concentrations in *Ostrea* were exceptionally high with values of 2.8 to 4.9 g/kg zinc as compared to 80–300 mg/kg in *Ascophyllum* and *Mytilus*, and further 30–80 mg/kg copper compared to 5–10 mg/kg in the two other species. Other species

differences were higher iron (140–160 mg/kg) and cadmium (2–4 mg/kg) concentrations in *Ostrea* than in *Mytilus* and *Ascophyllum* (resp. 50–120 and 1–2 mg/kg), and a higher manganese level in *Ascophyllum* (12–25 mg/kg) compared to 4–13 mg/kg in the two molluscs. Variations between sites were seen for all minor elements analyzed, and the general trend was that the highest levels were found at Innerøy, whereas the values at Svanøy and Vågstranda were comparable. Particularly for *Ostrea* and *Ascophyllum* all minor element concentrations except those for iron were highest at Innerøy. The values for *Mytilus* were less consistent. All values given are based on dry weight.

In conclusion, these results showed that differences in element concentrations were seen between the three polls of low element loads, but these differences were not consistent for the three species investigated, and did not reflect the water levels.

INTRODUCTION

Studies on tissue element concentrations in shellfish have progressed along two major lines. Ranges of element concentrations in commercial species have been recorded to identify potentially hazardous levels from a nutritional standpoint, and element concentrations within a single species have been compared from different waters as a basis for environmental monitoring programmes. Detailed knowledge is needed on environmental and biological factors which determine the dynamics of elements in the tissues of shellfish, such as the effects of season, sexual maturation, size and age, further the impact of human activities on the processes involved. This paper is the first of a series of papers directed towards this goal.

Any abundance of trace elements in the aquatic environment can be studies by the analysis of elements in water, sediments or species of the indigenous biota (indicator organisms). To a large extent, indicator organisms are now supplanting water or sediments in element analyses as a means of giving a time-integrated moving average of the biological availability of the elements.

This paper compares 10 element concentrations in the water and in cultured oyster (*Ostrea edulis*), native common mussel (*Mytilus edulis*) and brown seaweed (*Ascophyllum nodosum*) from three oyster farms on the western coast of Norway.

METHODS

Hydrographical descriptions of the oyster polls

Innerøy

The Innerøy poll is situated about 30 km south of Bergen at the island of Innerøy (Fig. 1). The island is situated on the northern side of Bjørnafjorden, and the distance from the open ocean is about 30 km. The poll was described in details by KORRINGA (1976).

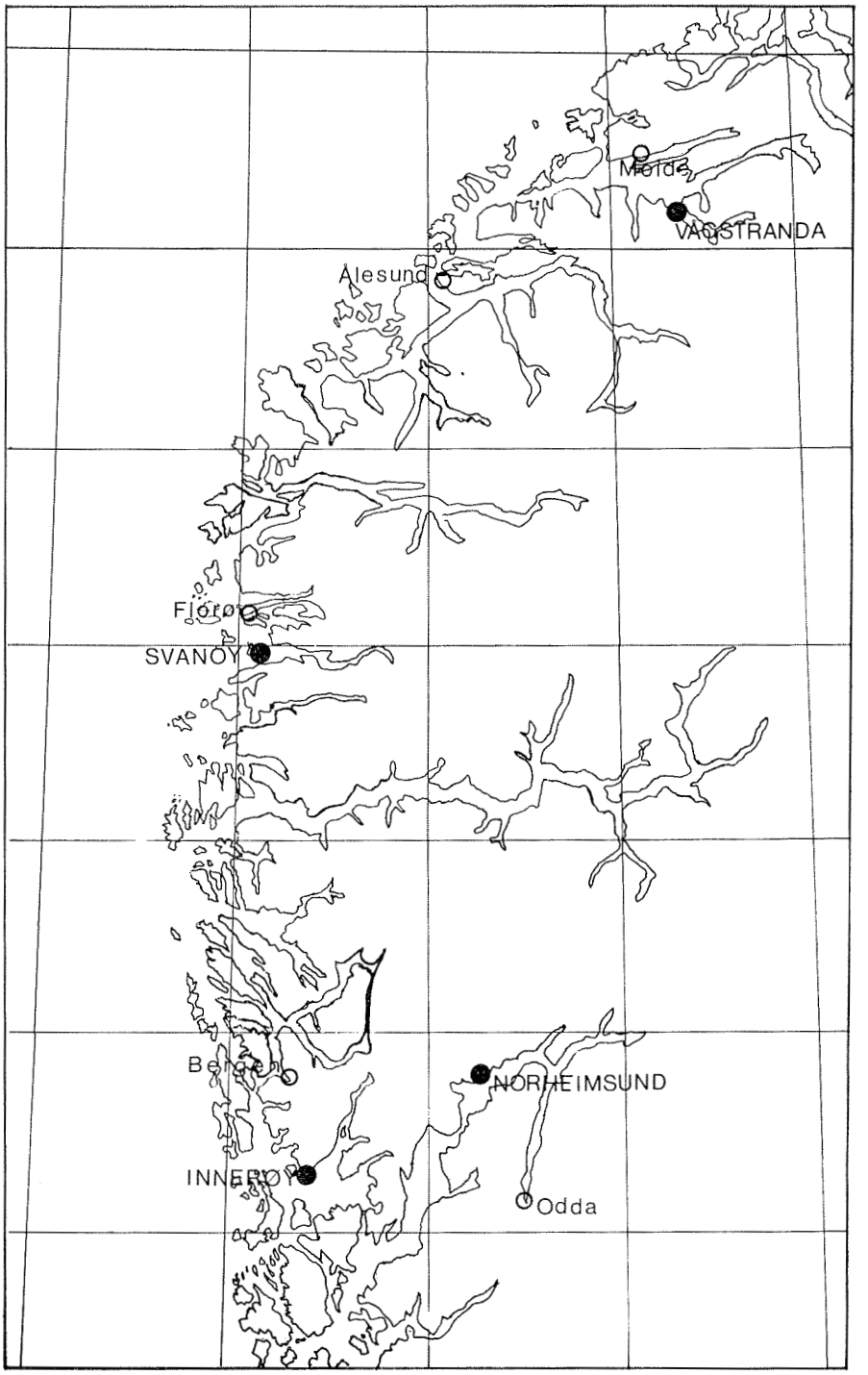


Fig. 1. Map of western Norway showing the position of the oyster farms at Innerøy, Svanøy and Vågstranda.

The oyster poll communicates with the open fjord through a narrow and shallow channel. A dam can close the channel completely and separates the inner (Indre poll) from the outer poll (Ytre poll), with its rather shallow inlet to the open fjord. The inner poll has a total area of about 80 000 m^2. It is sheltered on all sides by wooded slopes. The poll further connects with a shallow pool through a second dam. In the winter season this dam is closed so that the pool gradually fills with fresh water. Early in May the dam between the inner and outer polls is closed, to cut off the tidal exchange, and the dam is opened between the inner poll and the fresh water pool, thus, the inner poll receives an upper layer of fresh water. This results in a rise in the temperature of the salt water layer, necessary for the spawning.

Svanøy

Svanøy is an island 15 km south of the city of Florø (see Fig. 1). The island is about 10 km from the ocean. No spawning occurs in this water. The adult oysters are placed in wire netting baskets laid out in a shallow and narrow sound between Svanøy and a small island, Langøy. The depth here is 3 m at neaptide and the baskets are suspended 2.5 m below the surface with the aid of ropes stretched between the two islands.

Vågstranda

The Vågstranda poll is situated about 20 km south east of the city of Molde. The poll is connected on the north side with Romsdalsfjord and is about 50 km from the ocean. The poll communicates by a narrow and shallow channel with the open fjord. The poll cannot be closed, and the movement of the tide tends in part to equalize temperatures in the poll and the fjord. Nevertheless, the temperature is sufficiently high to give spatfall in August and September. It is sheltered on all sides, except to north where is the inlet to the fjord.

Sampling

Samples of oysters (*Ostrea edulis*), common mussel (*Mytilus edulis*), brown seaweed (*Ascphyllum nodosum*) and water were obtained at monthly intervals from the three oyster polls from November 1974 to October 1975. Salinity and temperature were measured at the sampling stations throughout the experiment.

Samples of 15 oysters from each poll were taken at nearly the same position throughout the year, to secure comparable conditions. The oysters were transported alive to the laboratory and sorted. The shells were opened, washed in distilled deionized water, dried in air and weighed.

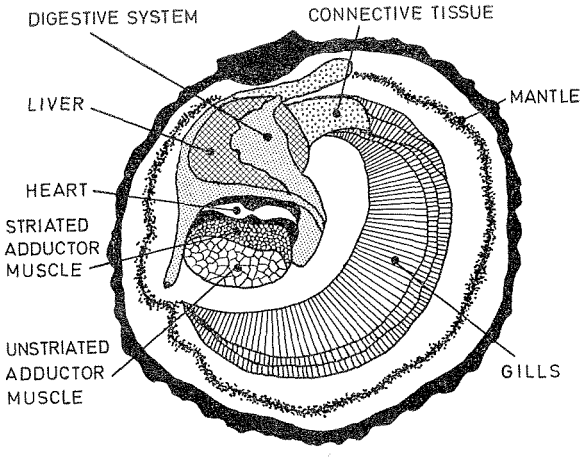


Fig. 2. Organs and tissues in oyster (*Ostrea edulis*).

Ten oysters weighing between 7 and 12 grams were dissected into muscle, mantle and gills and digestive system including connective tissue (Fig. 2). The tissue samples were pooled, weighed, freeze-dried until constant weight, homogenized in a mortar and stored in closed jars until analysis.

From each oyster poll 20–30 mussels with a size range from 45 mm to 55 mm (2–3 years old) were collected as near as possible to the oysters to minimize differences in nutritional supply between the species. The shells were transported alive to the laboratory and sorted. The soft parts were removed from the shells, washed in distilled deionized water and dried in the air. Ten mussels from Innerøy and Vågstranda were opened and dissected into muscle, foot, gonades, mantle, gills and digestive system (Fig. 3) (JULSHAMN, IV, 1981). In addition, 5–10 whole mussels were pooled monthly from each site.

The seaweed samples were washed by shaking twice in distilled deionised water (BRYAN and HUMMERSTONE, 1973) and at least 20 shoots of less than two years of age were pooled for analysis from each site. The values in Table 5 were based on a sampling of seaweed shoots of four age groups taken at Espegrend marine biological station. About 1 liter of water was taken at the level where the organisms were collected, transferred to a polyethylene flask and conserved with nitric acid.

Analysis

All elements were determined by atomic absorption spectrophotometry, using Perkin-Elmer models 370 and 403 equipped with Deuterium Background Corrector. Single element Intensitron hollow cathode lamps

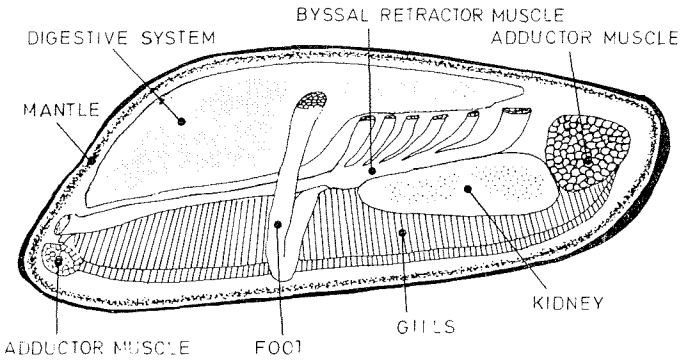


Fig. 3. Organs and tissues in sommon mussel (*Mytilus edulis*).

were used for calcium, magnesium and zinc. Sodium and potassium were measured in the emission mode and all other elements by flame absorption. Air-acetylene flame gas was used for all elements studied. Cadmium and lead were introduced to the flame from a sampling boat and all the other elements were sucked into the flame as solutions. The instrumental settings were according to the instrument instructions.

Water

250 ml of the water samples was irradiated in UV-light (Paus, 1973) prior to chelation with ammonium-pyrrolidine-dithiocarbamate and extracted into methyl-isobutyl-ketone (Brooks et al., 1967). The procedure of standard addition was used. The samples were aspirated into a fuel-lean air-acetylene flame, except in the determination of cadmium and lead where a sampling boat technique was used, under the working conditions described by Julshamn and Brækkan (1975). All complexes were stable for several hours except for manganese, and all samples were therefore analyzed for manganese immediately after extraction.

Wet digestion

To three replicates of 0.25 g of freeze-dried sample and blank were added 4 ml of nitric and perchloric acid (1:1 v/v, Merck p.a., conc.) in 10 ml capped vials (Sovirel). Appropriate standards of Mn, Fe, Cn, Zn, Cd and Pb were added to two of the three replicates (Julshamn and Brækkan, 1975). The samples were preashed over night followed by heating in a pressure cooker at 110°C for 2 hours. After cooling, redistilled water was added, the solutions were warmed to expel nitrious gases and were finally transferred to 25 ml volumetric flasks with redistilled water.

Pretreatment, ion-matrices

An addition of 10 mg per ml of lanthanum was used in the determination of calcium and magnesium. A 5% La-solution was made by dissolving 5.864 g lanthanum-III-oxide in 25 ml concentrated hydrochloric acid and diluting this to 100 ml with redistilled water. Samples as well as standard were prepared with the same concentrations of lanthanum. The final solutions, diluted for atomic absorption measurements, were always tested with the method of standard addition to secure the same standard response ratio in the sample as in the pure standard.

Pretreatment, chelation

Cadmium and lead were chelated using sodium diethyl dithiocarbamate (NaDDS) (5% in redistilled water) and extracting the complex into methyl isobutylketone (MIBK) (Merck p.a.) (JULSHAMN and BRÆKKAN, 1975). The MIBK-solutions were made up to 10 ml with water saturated with MIBK.

Standards

Stock solutions of most elements, 1 mg per ml, were obtained from Merck. Stock solutions of sodium and potassium were made from the corresponding chlorides (Merck, p.a.). The sample solutions were checked for physical and chemical interferences by standard addition.

Detection limit

Generally, the detection limit of the analyses is based on the variation of the blanks and the variation in the signal of the analyt. This may be given by the expression $\sqrt{S_s^2+S_b^2}$ where S_s is the standard deviation of a signal at a level corresponding to the limit of detection and S_b is the standard deviation of the blank. Detection limits for four elements in biological material and for six elements in seawater are given in Table 1.

Table 1a.
Detection limits of four elements in a biological sample. (Based on 0.25 g freeze-dried material.)

Element	mg/kg
Mn	1.0
Cu	1.2
Cd	0.04
Pb	0.2

Table 1b.
Detection limits of six elements in sea water. (Based on metal complexes between seawater and MIBK.)

Element	microg/l
Mn	0.2
Fe	0.3
Cu	0.2
Zn	0.1
Cd*	0.04
Pb*	0.2

*Sampling boat technique applied.

Accuracy

The methodology was checked for accuracy by measuring the recovery from the tissue samples of an added element. The recovery varied between 96% and 103% for all elements analyzed. The accuracy of results, however, can be confirmed only through agreement with certified levels in standard reference materials. The standard reference material NBS bovine liver (No. 1577) was available and was analyzed. Eight of the ten elements analyzed were certified, whereas the levels of magnesium and calcium were suggested. The analysis of the standard sample followed the methods above on the material received, whereas the moisture content was measured on a separate sample of the reference material and element levels re-calculated on this basis.

Accuracies were further tested by analyzing a sample of fish flour prepared by the Marine Laboratory, Aberdeen, and circulated for a collaborative analysis test on behalf of the International Council for the Exploration of the Sea (ICES, 1977).

RESULTS AND DISCUSSION

Analytical tests

The values found for the NBS bovine liver reference sample were in good agreement for most elements (Table 2). Somewhat high values were found for manganese, cadmium and lead. However, many element levels in the reference material do not reflect those normally present in oyster tissues and therefore it is impossible to encompass the range estimated for these trace elements. From Table 3 may be noted that the ICES collaborative values for cadmium and lead fell within such a wide range that the committee decided to repeat this part of the test.

Table 2. Analysis of NBS bovine liver No. 1577 (mg/kg). Values are given ± standard deviation.

Element	Found[1]	Certified
Na	2200±100	2430±130
K	8900±200	9700±600
Mg	620±15	605[2]
Ca	110±8	123[2]
Mn	13±1	10.3±1.0
Fe	250±10	270±20
Cu	190±10	193±10
Zn	130±2	130±10
Cd	0.33±0.04	0.27±0.04
Pb	0.41±0.30	0.34±0.08

[1] Standard addition method with 3 standard levels. 0.25 mg material was used as was recommended.
[2] Guideline only, not certified.

Table 3. Analysis of ICES fish flour sample issued 1977 (mg/kg).

Element	Found[1]	ICES[2]
Cu	4.2±0.1	3.67±0.50
Zn	36±1	36.6±2.5
Cd	0.04±0.05	0.20±0.552
Pb	0.81±0.20	1.2±1.5

[1] Standard addition method with 3 standard levels.
[2] The averages were calculated on values from 16–18 participating laboratories.

Water analyses

The element levels in the water samples are summarized in Table 4. No element gave significant differences between sites, but the analytical value of Mn, Fe, Cu, Cd and Pb were not sufficiently reliable for the low levels in uncontaminated water as seen by comparing the values of detection limits with the values from the water samples. Only zinc could be determined with sufficient accuracy in the coastal water. Manganese and iron levels found in the present study were lower than those obtained by STURGEON et al. (1979). The problem of distinguishing between dissolved and particulate bound elements may be pertinent in discussing different element values from water analyses. Published zinc values from coastal waters are generally below 10 microg/l (STURGEON et al., 1979; YEATS et al., 1978), but CAMPBELL and OTTAWAY (1977) in a study of

Table 4. Minor element concentrations (microg/l as averages of six bimonthly samples) and salinity in water samples taken from three oyster polls at the western coast of Norway. Standard deviation in italics.

Site	Innerøy		Svanøy		Vågstranda	
Salinity	28.8	*1.5*	28.9	*1.7*	29.5	*1.5*
Mn*	0.84	*0.40*	0.60	*0.35*	0.40	*0.30*
Fe*	1.0	*0.82*	1.1	*0.32*	0.95	*0.60*
Cu*	1.1	*0.50*	1.0	*0.25*	0.90	*0.42*
Zn	14	*4.0*	12	*5.2*	12	*4.7*
Cd*	0.07	*0.05*	0.06	*0.03*	0.06	*0.04*
Pb*	0.30	*0.20*	0.22	*0.16*	0.23	*0.12*

*Values less than the detection limits are included in the calculation as one half of the detection limit (See Table 1).

coastal seawater from England, found values in good agreement with the present ones. However, their cadmium values were higher.

Since the values in Table 4 were determined, analytical procedures for low element levels have been improved by introducing the graphite furnace, but the procedure still needs a solvent extraction step using a chelating agent, followed by a transfer back into HNO_3, (BRULAND et al., 1979). One difficulty lies in reducing the sea salt background by selective volatilization. Nevertheless, CAMPBELL and OTTAWAY (1977) analyzed zinc and cadmium in coastal waters by direct injection into an HGA-72 graphite furnace. They atomized both metals at 1490°C and could detect as little as 40 ng/l Cd and 1.7 microg/l Zn using the method of standard addition. Several recent instrumental improvements have increased the analytical performance for the determination of trace elements in uncontaminated seawater, and the procedure of simultaneous background correction is used to cope with the high salinity of the seawater in direct determination (STURGEON et al., 1979). An increased charring temperature may be allowed in the newer furnaces without risk of volatilization of the analyt. A further improvement lies in introducing the Zeeman background correction which will tolerate larger samples. Even with improved analytical procedures and instrumentations for the determination of trace elements in uncontaminated seawater, the problem of correct sampling will remain a serious one (JULSHAMN, VII, 1981).

Major elements

The salinity changes through the year are given in paper II of this series (JULSHAMN, II, 1981). The lowest salinity was observed at Innerøy (Table 4). This was not reflected in the samples of algae and molluscs, as

Table 5. Major and minor element concentrations (based on dry weight) in *Ascophyllum nodosum* shoots of 4 year classes.

Year class		1	2	3	4
Na	g/kg	38	35	36	32
K	,,	23	28	28	22
Mg	,,	8.3	8.5	8.9	8.5
Ca	,,	12	12	13	12
Mn	mg/kg	11	17	20	23
Fe	,,	58	28	38	60
Cu	,,	6.7	5.2	3.9	3.9
Zn	,,	110	260	310	420
Cd	,,	0.71	0.53	0.64	0.67
Pb	,,	1.4	1.1	0.98	1.5

the samples from Innerøy had the highest values of sodium in all three organisms (Tables 6–8). The highest sodium concentrations were found in *A. nodosum*, ranging from 26 to 30 g/kg between sites. Higher differences were obtained in the molluscs, particularly in *O. edulis* with 10 g/kg at Vågstranda and 16 g/kg at Innerøy. The major differences were seen in the digestive tissues.

High potassium levels were found in *A. nodosum* with 25 g/kg at Innerøy, corresponding to the values for sodium. The potassium levels in *M. edulis* were only half the sodium levels and with no differences between sites. Differences were found in digestive tissue from *O. edulis* with values ranging from 8 to 11 g/kg from Vågstranda to Innerøy. As discussed in paper VII of this series (JULSHAMN, VII, 1981), no significant effect was seen in the potassium content of *M. edulis* and *A. nodosum* of a wide range of salinity, ranging from 0.75% to 3.0%.

Table 6. Major and minor element concentrations (based on dry weight) in *Ascophyllum nodosum* taken from three oyster polls at the western coast of Norway. Averages of ten monthly samples from Vågstranda and twelve monthly samples from Innerøy and Svanøy. Standard deviation in italics.

Site		Innerøy		Svanøy		Vågstranda	
Na	g/kg	30	*2.2*	26	*5.0*	28	*3.0*
K	,,	25	*3.0*	16	*4.1*	23	*2.1*
Mg	,,	7.2	*0.32*	7.4	*0.3*	6.8	*0.30*
Ca	,,	11	*1.1*	11	*2.2*	10	*2.3*
Mn	mg/kg	25	*4.0*	12	*3.0*	16	*5.0*
Fe	,,	120	*20*	50	*10*	110	*25*
Cu	,,	9.5	*1.1*	6.0	*1.1*	4.5	*0.80*
Zn	,,	320	*50*	100	*20*	120	*40*
Cd	,,	1.8	*0.31*	0.80	*0.40*	1.1	*0.42*
Pb	,,	1.9	*0.50*	1.0	*0.39*	1.4	*0.32*

Table 7. Major element and protein concentrations in tissues (g/kg dry weight) of *Ostrea edulis* from three oyster polls at the western coast of Norway. Averages of twelve monthly samples. Standard deviation in italics.

Site	Tissue	Average tissue dry weight (g)		g dry weight per kg tissue		Protein g/kg	s.d.	Na g/kg	s.d.	K g/kg	s.d.	Mg g/kg	s.d.	Ca g/kg	s.d.
Innerøy	Muscle	0.23	*0.02*	209	*13*	731	*33*	14	*3.6*	11	*1.2*	2.2	*0.30*	2.7	*0.61*
	Mantle+gills	0.45	*0.07*	135	*16*	564	*20*	27	*5.4*	12	*1.2*	4.2	*0.64*	9.4	*3.7*
	Digestive system	1.05	*0.13*	249	*17*	365	*25*	11	*1.8*	11	*1.2*	1.5	*0.28*	1.5	*0.38*
	Calc. whole	1.73	*0.20*	202	*21*	466	*24*	16	*3.1*	11	*0.90*	2.3	*0.33*	3.8	*1.1*
Svanøy	Muscle	0.19	*0.03*	191	*15*	711	*21*	14	*2.6*	10	*2.1*	1.4	*0.52*	2.8	*0.83*
	Mantle+gills	0.34	*0.04*	137	*22*	550	*24*	27	*4.4*	10	*1.9*	3.1	*0.69*	9.9	*5.1*
	Digestive system	1.00	*0.12*	244	*26*	353	*34*	11	*3.0*	8.3	*2.6*	1.1	*0.40*	1.9	*0.79*
	Calc. whole	1.57	*0.16*	207	*21*	433	*20*	14	*2.8*	8.7	*2.2*	1.6	*0.42*	3.5	*1.2*
Vågstranda	Muscle	0.30	*0.05*	208	*15*	670	*43*	13	*3.2*	9.8	*3.0*	2.0	*0.41*	3.4	*1.3*
	Mantle+gills	0.48	*0.09*	144	*22*	537	*46*	19	*5.7*	13	*3.9*	3.5	*0.84*	9.2	*4.5*
	Digestive system	1.57	*0.29*	275	*22*	305	*32*	6.5	*2.0*	8.0	*1.6*	1.3	*0.45*	1.6	*0.69*
	Calc. whole	2.35	*0.39*	224	*18*	384	*41*	9.8	*2.6*	9.2	*2.0*	1.8	*0.46*	3.4	*1.3*

Table 8. Major element and protein concentrations in tissues and whole soft parts (g/kg dry weight) of *Mytilus edulis* from three oyster polls at the western coast of Norway. Averages of twelve monthly samples. Standard deviation in italics.

Site	Tissue	Average tissue dry weight (g)	g dry weight per kg tissue	Protein g/kg	Na g/kg	s.d.	K g/kg	s.d.	Mg g/kg	s.d.	Ca g/kg	s.d.
Innerøy	Muscle	0.15	212	708	15	*4.9*	10	*1.5*	2.9	*0.80*	1.2	*0.31*
	Foot	0.05	208	672	16	*4.8*	11	*1.5*	3.0	*0.92*	1.0	*0.25*
	Gonades	0.21	152	440	23	*11*	12	*2.7*	2.0	*0.50*	0.75	*0.14*
	Mantle	0.12	128	520	30	*10*	11	*0.66*	4.9	*1.5*	3.0	*0.26*
	Gills	0.14	112	515	45	*9.3*	10	*1.7*	6.9	*2.0*	4.7	*1.2*
	Digestive system	0.14	224	475	15	*5.1*	9.7	*3.0*	2.0	*0.55*	1.2	*0.30*
	Whole	0.92	138		28	*4.5*	11	*2.9*	3.2	*1.1*	2.4	*0.52*
Svanøy	Whole	1.05	182		23	*5.5*	11	*2.1*	2.7	*0.82*	3.3	*0.77*
Vågstranda	Muscle	0.20	231		14	*1.8*	11	*2.2*	3.4	*1.5*	2.1	*0.41*
	Foot	0.05	235		15	*2.9*	10	*2.2*	3.5	*1.6*	2.3	*0.63*
	Gonades	0.30	248		14	*6.1*	8.7	*1.8*	3.5	*1.7*	1.9	*0.88*
	Mantle	0.16	175		24	*5.0*	11	*2.0*	5.5	*2.5*	4.1	*1.4*
	Gills	0.15	152		28	*4.2*	11	*1.4*	8.3	*3.8*	4.5	*1.9*
	Digestive system	0.27	242		11	*1.8*	10	*2.7*	2.8	*1.4*	2.7	*0.60*
	Whole	1.35	188		19	*3.5*	11	*1.6*	4.2	*0.80*	3.4	*0.87*

The magnesium contents were again highest in *A. nodosum*, with values around 7 g/kg. The concentrations of magnesium in *M. edulis* were nearly double those in *O. edulis*. The generally higher concentrations of major elements in *M. edulis* than in *O. edulis* may well be compensated by a substantially higher zinc concentration in *O. edulis* than in *M. edulis*. The differences observed between the sites for magnesium in the two mollucs were equally distributed between the tissues. There were noteworthy differences in the levels of potassium and magnesium in the three organisms compared with the ambient waters. The concentration factors from water to organisms were ten times higher for potassium than for magnesium, and the magnesium concentrations seemed to be more related to the salinity than those of potassium.

The levels of calcium in *A. nodosum* did not vary between the three sites. Substantially lower calcium concentrations were found in the molluscs than in *Ascophyllum*. The calcium contents in all tissues except gills were lower in *M. edulis* from Innerøy than from Vågstranda, and this was the only difference for calcium observed between the three polls. The calcium levels were not affected by the salinity as suggested by the results from *A. nodosum* and *M. edulis* from brackish water (JULSHAMN, VII, 1981).

Manganese, iron

Water levels of manganese were rather low with values below 1 microg/g (Table 4). The manganese contents in *Ascophyllum* increased in shoots from 1 to 4 years of age (Table 5). As algae do not respond to elements associated with organic or inorganic particulate matter, this may indicate that the response of *Ascophyllum* to the ambient dissolved concentration of manganese in water may be high, probably due to a very long biological half life of the element such as described for Zn^{65} in *Laminaria digitata* (BRYAN, 1969). The manganese contents in *A. nodosum* were lower at Svanøy and Vågstranda than at Innerøy (Table 6), possibly corresponding to the lower water levels. MORRIS and BALE (1975) found a poor agreement between the manganese content in water and in algae. The manganese concentrations in the two molluscs were significantly lower than in *A. nodosum* (Tables 9 and 10). *M. edulis* had the lowest contents, varying between 4.1 mg/kg at Vågstranda and 6.9 mg/kg at Innerøy, consistent with other results from uncontaminated waters (CROWLEY and MURPHY, 1976; MARTIN, 1979). Similar differences between sites but higher levels were obtained from *O. edulis*. The lower contents in molluscs compared to algae were somewhat surprising, since manganese exists mainly in particulate form in seawater as reported from

Table 9. Minor element concentrations in tissues (mg/kg dry weight) of *Ostrea edulis* from three oyster polls at the western coast of Norway. Averages of twelve monthly samples. Standard deviation in italics.

Site	Tissue	Mn mg/kg	Mn s.d.	Fe mg/kg	Fe s.d.	Cu mg/kg	Cu s.d.	Zn mg/kg	Zn s.d.	Cd mg/kg	Cd s.d.	Pb mg/kg	Pb s.d.
Innerøy	Muscle	3.2	*1.2*	60	*24*	23	*6.1*	1600	*380*	3.8	*1.1*	2.0	*1.1*
	Mantle+gills	31	*11*	220	*107*	135	*31*	8300	*2100*	5.2	*1.4*	2.0	*0.64*
	Digestive system	6.8	*2.3*	160	*31*	71	*16*	4200	*1500*	4.0	*1.3*	1.7	*0.69*
	Calc. whole	13	*4.0*	140	*56*	80	*16*	4900	*1600*	4.2	*1.1*	1.9	*0.35*
Svanøy	Muscle	3.8	*1.7*	49	*22*	19	*4.8*	2200	*520*	2.0	*0.45*	1.2	*0.60*
	Mantle+gills	19	*5.0*	170	*26*	115	*31*	10200	*1900*	4.0	*0.90*	2.8	*1.27*
	Digestive system	6.9	*2.2*	150	*34*	43	*10*	3800	*930*	2.4	*0.52*	1.1	*0.97*
	Calc. whole	8.9	*2.3*	140	*27*	55	*13*	4900	*937*	2.6	*0.52*	1.5	*0.76*
Vågstranda	Muscle	2.6	*0.81*	80	*40*	12	*5.3*	1200	*270*	1.6	*0.45*	0.82	*0.36*
	Mantle+gills	20	*4.2*	180	*36*	73	*39*	6200	*780*	2.6	*0.69*	1.3	*0.82*
	Digestive system	5.7	*1.6*	160	*72*	26	*13*	2100	*640*	1.6	*0.55*	0.49	*0.36*
	Calc. whole	8.2	*1.9*	160	*65*	34	*15*	2800	*550*	1.8	*0.36*	0.68	*0.38*

Table 10. Minor element concentrations in tissues and whole soft parts (mg/kg dry weight) of *Mytilus edulis* from three oyster polls at the western coast of Norway. Averages of twelve monthly samples. Standard deviation in italics.

Site	Tissue	Mn		Fe		Cu		Zn		Cd		Pb	
		mg/kg	s.d.	mg/kg	s.d.	mg/kg	s.d.	mg/kg	s.d.	mg/kg	s.d.	mg/kg	s.d.
Innerøy	Muscle	4.2	*1.8*	68	*15*	3.5	*1.1*	100	*33*	1.6	*1.0*	4.8	*1.8*
	Foot	5.8	*0.50*	45	*14*	3.9	*1.6*	115	*25*	1.8	*0.40*	4.6	*1.4*
	Gonades	4.3	*1.0*	72	*19*	3.0	*1.2*	55	*10*	0.95	*0.30*	2.5	*1.2*
	Mantle	5.6	*0.39*	80	*25*	4.2	*1.6*	60	*20*	2.3	*0.91*	4.2	*1.0*
	Gills	9.0	*1.4*	155	*40*	11	*2.5*	190	*31*	4.0	*1.2*	16	*4.2*
	Digestive system	9.1	*1.0*	345	*100*	14	*2.0*	200	*28*	3.8	*1.0*	7.8	*2.2*
	Whole	6.9	*1.2*	98	*21*	6.0	*1.2*	135	*23*	2.0	*0.45*	4.9	*1.2*
Svanøy	Whole	4.5	*1.5*	57	*15*	4.6	*0.74*	102	*12*	1.1	*0.20*	2.9	*0.85*
Vågstranda	Muscle	3.6	*0.87*	57	*17*	3.2	*1.8*	82	*25*	1.3	*0.65*	0.65	*0.40*
	Foot	4.3	*2.0*	63	*16*	4.5	*1.4*	84	*29*	1.1	*0.45*	1.1	*0.50*
	Gonades	3.8	*1.4*	64	*19*	5.5	*2.0*	32	*16*	0.93	*0.52*	0.75	*0.38*
	Mantle	4.9	*1.8*	77	*30*	7.0	*3.6*	48	*26*	1.9	*0.75*	1.3	*0.52*
	Gills	6.5	*2.6*	116	*32*	11	*3.8*	150	*68*	2.5	*1.1*	1.8	*0.78*
	Digestive system	7.1	*1.5*	389	*250*	11	*1.9*	91	*25*	2.4	*0.65*	1.5	*0.48*
	Whole	4.1	*1.0*	101	*35*	7.0	*2.6*	84	*20*	1.6	*0.40*	1.2	*0.40*

British waters (PRESTON et al., 1972) High concentrations of manganese in *O. edulis* may be related to the shell formation as FRAZIER (1975) found high levels of manganese in shells. High manganese levels were found in *M. edulis* from brackish waters (JULSHAMN, VII, 1981), probably corresponding to the findings by PHILLIPS (1976) that decreasing salinities increased the net uptake of cadmium and copper in *M. edulis*.

Iron exists almost totally in particulate forms in seawater. PRESTON et al. (1972) found average values of 93 to 99% of particulate iron in waters off the British Isles and HEAD (1971) reported 96% from Southampton. The concentrations of iron in shoots of *A. nodosum* varied, but did not increase with age (Table 5), as opposed to data from polluted areas for *Fucus vesiculosus* (BRYAN and HUMMERSTONE, 1973) and for *A. nodosum* (HAUG et al., 1974; JULSHAMN, VII, 1981). The iron levels in *A. nodosum* from the three sites (Table 6) varied between 50 mg/kg at Svanøy and 120 mg/kg at Innerøy, consistent with other analyses from uncontaminated waters; e.g. LUNDE (1970). The present values probably represent the natural range of iron in *A. nodosum*, and similar concentrations were reported from *Fucus vesiculosus* (PHILLIPS, 1979). *O. edulis* had higher iron contents than *M. edulis*, and the two mussels distributed iron differently between the tissues (JULSHAMN, IV, 1981). The iron is transported from gills and viscera to other tissues by amoebocytes in the haemolymph, the major proportion being deposited in the byssal threads (GEORGE et al., 1976). The metabolic pathways of iron in *O. edulis* may be different from that in *M. edulis*, and more directed through the gills. The iron values in *M. edulis* were lower than corresponding values from Scandinavia (PHILLIPS, 1978) and from California (MARTIN, 1979), but agreed well with results from Dorset, U.K. (BOYDEN, 1975). The iron contents in *O. edulis* were consistent with results from colorimetric analyses as early as 1941 by GAARDER and ALVSAKER (1941), who found 25 mg/kg fresh sample. It may be noticed that *A. nodosum* had higher iron contents than *M. edulis* both at Innerøy and Vågstranda, notwithstanding the mainly particulate form of iron in the water. LANDE (1973) found ten times higher iron values in *M. edulis* than in *A. nodosum* from contaminated effluents near mining industries, whereas there were no appreciable differences between the present values and the iron contents in *M. edulis* and *A. nodosum* from Sørfjorden with its low salinity and metal discharges from metallurgic factories (JULSHAMN, VII, 1981).

Copper

No differences were observed between the three sites in the copper contents of the water (Table 4). Copper contents of approximately

1 microg/l were in the range reported for unpolluted coastal waters (STURGEON, 1979). A slight decrease was found for the copper content in *A. nodosum* with increasing age of the shoots (Table 5), whereas the opposite trend was found in *A. nodosum* from polluted waters (HAUG et al., 1974; JULSHAMN, VII, 1981). Both *A. nodosum* and *M. edulis* had copper contents of less than 10 mg/kg, in good agreement with results from Reine in Lofoten (HAUG et al., 1974). Values reported from Spain and Portugal were from 6–4 mg/kg (STENNER and NICKLESS, 1975), and a similar range was reported from Scottish waters (TOPPING, 1973), whereas higher values were reported from California (MARTIN, 1979). The copper values found in *M. edulis* in the three coastal oyster farms corresponded to values found in the same species taken from the mouth of Sørfjorden (JULSHAMN, VII, 1981). The copper contents in tissues of *O. edulis* were significantly higher than found for the other species, and differences between sites were seen. The results were nevertheless among the lowest recorded for *O. edulis*. Values reported from uncontaminated areas in U.K. varied between 23 and 67 mg/kg wet weight (COOMBS, 1972; GEORGE et al., 1978). It is well documented that oysters can accumulate copper and can tolerate high concentrations (AYLING, 1974; IKUTA, 1967; RATKOWSKY et al., 1974; D'SILVA and QASIM, 1979). This effect was noted early (BOYCE and HERDMAN 1897); and given the colloquial name "green sickness" because of a green pigmentation in the oyster tissue. High copper concentrations are found in certain estuaries where mining wastes are washed out. Concentrations of copper as high as 450 mg/kg wet weight have been reported in oysters from Tasmania, Australia (MACKAY et al., 1975). Oysters placed in seawater of high copper concentrations presumably absorb more of the element than they can excrete, and are forced to bind the potentially toxic copper to an organic complex. The principal copper-binding protein in oysters occurs in granular haemolymph amoebocytes according to GEORGE et al. (1978). The different concentration levels of copper in *O. edulis* and *M. edulis* is well documented and also that *M. edulis* is capable of avoiding uptake of copper from ambient water with high copper levels (MANLEY and DAVENPORT, 1979). The present results suggest that *O. edulis* respond to small copper differences in the environment.

Zinc

The zinc contents in the water samples (Table 4) were rather high compared with some published values from uncontaminated areas. The zinc content increased dramatically with age in shoots of *A. nodosum* (Table 5). HAUG et al. (1974) demonstrated a similar increase in algae

collected in uncontaminated as well as contaminated waters. They found a similar increase for copper, which could be confirmed in the present work. *A. nodosum* had the highest zinc values at Innerøy and lowest at Svanøy (Table 6). The values from Innerøy were higher than those reported by HAUG et al. (1974). LUNDE (1970) found variations of zinc from 74 to 240 mg/kg dry weight from Reine in Lofoten. The ratio between the zinc levels in *A. nodosum* and in the water was $2.3.10^4$ at Innerøy and less than $1:10^4$ at Svanøy and Vågstranda. A ratio corresponding to that at Innerøy was found in Sørfjorden (JULSHAMN, VII, 1981). Smaller differences in zinc contents were found in the *M. edulis* samples than in those of *A. nodosum*, but the differences were significant between Innerøy and Vågstranda as well as between Innerøy and Svanøy. The fact that the digestive system of *M. edulis* at Innerøy had higher zinc contents than the mantle/gill tissue, whereas the mantle/gills had the highest concentrations at Vågstranda, may indicate a difference in the ratio between the dissolved and particulate zinc fractions in the ambient water. Several authors have reported that the uptake of metals by bivalves is higher from food than from water (PRESTON, 1971); PENTREATH, 1973; SCHULZ-BALDES, 1974; CUNNINGHAM and TRIPP, 1975). The zinc levels found in *M. edulis* were around the lower part of the range reported from other sites (PHILLIPS, 1976; MARTIN, 1979).

The zinc contents in *O. edulis* were nearly 40 times higher than in *M. edulis* (see JULSHAMN, IV, 1981, for further values). High zinc levels in oyster were found already in 1919 by HILTNER and WICHMANN, and substantial zinc differences between mussels and oysters were reported by BROOKS and RUMSEY (1965). The oysters had significantly lower zinc concentrations at Vågstranda than at Innerøy and Svanøy. The zinc values were in surprisingly good agreement with those reported by GAARDER and ALVSAKER (1941), who found 4.0 g/kg dry matter in whole *O. edulis* from "Ytre pollen" at Innerøy. This indicates that the zinc contents in the water has not increased during the 30 years span between the two investigations. The high zinc content has surprised several authors, and KORRINGA (1965) suggested that Norwegian flat oysters were contaminated compared to other species of oyster. Nevertheless, COOMBS (1972) and BOYDEN (1975) found natural high contents of zinc in the genus *O. edulis*, substantially higher than those reported for the Pacific oyster (*Crassostrea gigas*) and the American oyster (*Crassostrea virginica*) (PRINGLE et al., 1968). BOYDEN (1975) found a wide range of values in individuals from waters with high content and similar results were obtained in Paper VI of this series (JULSHAMN, VI, 1981). The zinc values given in tables 6 and 10 for *A. nodosum* and *M. edulis* may reflect normal zinc levels in water with a low zinc load. High contents of zinc in other

oysters e.g. in *C. gigas* are usually accompanied with high contents of copper (AYLING, 1973; MACKAY et al., 1975).

Cadmium, lead

The average cadmium contents in the water samples were below 0.1 microg/l and near the detection limit of the method. The values corresponded to the lower concentration range of values reported in the literature, which in coastal seawater ranged from 0.015 to 170 microg/l (CAMPBELL and OTTAWAY, 1977; STURGEON et al., 1979; SMITH and WINDOM, 1980). The cadmium levels in *A. nodosum* and *M. edulis* were low from all three sites, compared to the values reported in the literature (TOPPING, 1973; NIELSEN and NATHAN, 1975; PHILLIPS, 1976; MARTIN, 1979). The cadmium levels in *A. nodosum* and *M. edulis* were comparable with those found in corresponding samples from contaminated brackish waters (JULSHAMN, VII, 1981). The cadmium levels in species from contaminated water may be affected by the salinity, as PHILLIPS (1976) reported an increased uptake of cadmium at low salinities. Therefore concentration factors obtained from environments of different salinity may not be comparable. *O. edulis* also had low levels of cadmium compared to other reports on *Ostrea* (WATLING and WATLING, 1976; BOYDEN, 1977) and *Crassostrea commercialis* (RATKOWSKY et al., 1974, MACKAY et al., 1975). There were significant differences between sites. The cadmium and zinc contents in the different tissues were not proportional, and ratios between the zinc and cadmium concentrations of more than 1000 were among the highest reported from marine fauna.

Also lead contents in the water samples were near the detection limit for the method and the differences reflect the precision of the method. The reported concentrations of lead in sea water has changed over the past decades as refinements in analytical sensitivity and collection procedures have progressed. Furnace atomic absorption spectrophotometry is the most widely used method for determining trace metals in seawater and literature data range between 0.25 and 0.40 microg/l (STURGEON et al., 1979; BATLEY and MATOUSEK, 1977). The problems of determining lead contents in seawater are discussed in Paper VII in this series (JULSHAMN, VII, 1981). Analytical problems concerning lead in biological material of marine origin were discussed by TOPPING (1980), including results from an ICES intercalibration study. The lead concentrations found in the three species agreed with values reported from uncontaminated environments (TOPPING, 1973; HAUG et al., 1974; BOYDEN, 1977; PHILLIPS, 1978; MARTIN, 1979). The highest contents of lead were found in *M. edulis* tissues, corresponding with those reported

in Paper VI and VII in this series (JULSHAMN, VI and VII, 1981). The concentration factors between water and tissues were different from those obtained from waters with lower salinity and additional metal loads (JULSHAMN, VII, 1981). The availability of metals may depend upon chemical interactions in the water systems and further knowledge of such interactions are necessary for accurate assessments of the biological activity of elements in water.

REFERENCES

AYLING, G. J., 1974. Water Res. *8*, 729–738.

BATLEY, G. E. and MATOUSEK, J. P., 1977. Anal. Chem. *49*, 2031–2034.

BOYCE, M. B. and HERDMAN, W. A., 1897. Proc. R. Soc. *62*, 30–38.

BOYDEN, C. R., 1975. Mar. Pollut. Bull. *6*, 180–187.

BOYDEN, C. R., 1977. J. Mar. Biol. Ass. U.K. *57*, 675–714.

BROOKS, R. R. and RUMSEY, M. G., 1965. Limnol. Oceanogr. *10*, 521–527.

BROOKS, R.R., PRESLEY, B.J. and KAPLAN, I.R., 1967. Talanta *14*, 809–816.

BRULAND, K.W., FRANKS, R.P., KNAUER, G.A. and MARTIN, J.H., 1979. Anal. Chim. Acta *105*, 233.

BRYAN, G.W., 1969. J. Mar. Biol. Ass. U.K. *49*, 225–243.

BRYAN, G. W. and HUMMERSTONE, L.G., 1973. J. Mar. Biol. Ass. U.K. *53*, 705–720.

CAMPBELL, W.C. and OTTAWAY, J.M., 1977. Analyst *102*, 495–502.

COOMBS, T.L., 1972. Mar. Biol. *12*, 170–178.

CROWLEY, M. and MURPHY, C., 1976. Fishery Leaflet No. 81, 1–10.

CUNNINGHAM, P.A. and TRIPP, M.R., 1975. Mar. Biol. *31*, 311–320.

D'SILVA, C. and QASIM, S.Z., 1979. Mar. Biol. *52*, 343–346.

FRAZIER, J.M., 1975. Chesapeake Sci. *16*, 162–171.

GEORGE, S.G., PIRIE, B.J.S. and COOMBS, T.L., 1976. J. Exp. Mar. Biol. Ecol. *23*, 71–84.

GEORGE, S.G., PIRIE, B.J.S., CHEYNE, A.R., COOMBS, T.L. and GRANT, P.T., 1978. Mar. Biol. *45*, 145–156.

GAARDER, T. and ALVSAKER, E., 1941. Biologie and Chemie der Auster in den norwegischen Pollen. Bergens Mus. Aarb., Naturv. R., 1–236.

HAUG, A., MELSOM, S. and OMANG, S., 1974. Environm. Pollut. *7*, 179–192.

HEAD, P.G., 1971. J. Mar. Biol. Ass. U.K. *51*, 891–903.

HILTNER, R.S. and WICHMANN, H.J., 1919. J. Biol. Chem. *32*, 204–221.

IKUTA, K., 1967. Bull. Jap. Soc. Scient. Fish. *33*, 405–409.

ICES, Charlottenlund, DK-2920 Denmark, 1977. Cooperative Research Report No. 69.

JULSHAMN, K. and BRÆKKAN, O.R., 1975. At. Absorption Newslett. *14*, 49–52.

JULSHAMN, K., 1981. Studies on Major and Minor Elements in Molluscs in Western Norway, II, IV, VI–VII, Fisk. Dir. Skr., Ser. Ernæring, *1* No. 5.

KORRINGA, P., 1952. Recent advances in oyster biology. Quart. Rev. Biol. *27*, 266–308, 339–365.

KORRINGA, P., 1976. Farming the flat oysters of the genus *Ostrea*. Developments in aquaculture and fisheries science, volum 3. Elsevier.

LANDE, E., 1973. Environ. Pollut. *12*, 187–197.

LUNDE, G., 1970. J. Sci. Fd. Agric. *21*, 242–247.

MACKAY, N.J., KAZACOS, M.N., WILLIAMS, R.J. and LEEDOW, M.I., 1975. Mar. Pollut. Bull. *6*, 57–61.

MANLEY, A.R. and DAVENPORT, J., 1979. Bull. Environ. Contam. Toxicol. *22*, 739–744.
MARTIN, J.H., 1979. Bioaccumulation of heavy metals by littoral and pelagic marine organisms. Moss Landing Marine Laboratories, Moss Landing, California. EPA-600/3-79-038, 1–57.
MORRIS, A.W. and BALE, A.J., 1975. Estuar. Coastal Mar. Sci. *3*, 153–163.
NIELSEN, S.A. and NATHAN, A., 1975. N Z J. Mar. Freshwat. Res. *9*, 467–481.
PAUS, P.E., 1973. Z. Anal. Chem. *264*, 118–122.
PENTREATH, R.J., 1973. J. Mar. Biol. Ass. U.K. *53*, 127–143.
PHILLIPS, D.J.H., 1976. Mar. Biol. *38*, 59–69.
PHILLIPS, D.J.H., 1978. Mar. Biol. *46*, 147–156.
PHILLIPS, D.J.H., 1979. Environ. Pollut. *18*, 31–43.
PRESTON, A., JEFFERIES, D.F., DUTTON, J.W.R., HARVEY, B.R. and STEELE, A.K., 1972. Environ. Pollut. *3*, 69–82.
PRESTON, E.M., 1971. J. Exp. Mar. Biol. Ecol. *6*, 47–54.
PRINGLE, B.H., HISSONG, D.E., KATZ, E.L. and MULAWKA, S.T., 1968. J. Sanit. Engng. Div. Am. Soc. Civ. Engrs. *94*, 455–475.
RATKOWSKY, D.A., THROWER, S.J., EUSTACE, I.J. and OLLEY, J., 1974. J. Fish. Res. Bd. Can. *31*, 1165–1171.
SCHULZ-BALDES, M., 1974. Mar. Biol. *25*, 177–193.
SMITH, R.G. and WINDOM, H.L., 1980. Anal. Chim. Acta *113*, 39.
STENNER, R.D. and NICKLESS, G., 1975. Mar. Pollut. Bull. *6*, 89–92.
STURGEON, R.E., BERMAN, S.S., DESAULNIERS, A. and RUSSELL, D.S., 1979. Anal. Chem. *51*, 2364–2369.
TOPPING, G., 1973. Aquacult. *1*, 373–377.
TOPPING, G., 1980. Report on the 6th ICES trace metal intercomparison exercise for cadmium and lead in biological tissue. DAFS Marine Laboratory, Aberdeen, Scotland.
WATLING, H.R. and WATLING, R.J., 1976. Mar. Pollut. Bull. *7*, 45–48.
YEATS, P.A., BEWERS, J.M. and WALTON, A., 1978. Mar. Pollut. Bull. *9*, 264.

Fisk. Dir. Skr., Ser. Ernæring, Vol. 1: No. 5, 1981, 183–197

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

II. Seasonal variations in the contents of 10 elements in oysters (*Ostrea edulis*) from three oyster farms.

By

KÅRE JULSHAMN

Institute of Vitamin Research

Directorate of Fisheries, Bergen.

ABSTRACT

Samples of 3-year-old immature oyster, *Ostrea edulis*, were collected monthly through one year from 3 oyster farms at the western coast of Norway. The oysters were dissected into muscle, gills/mantle and digestive system and the tissues analyzed by atomic absorption spectrophotometry for 4 major and 6 minor elements. Average results from these analyses and for tissue dry weight and protein contents from the three oyster polls for each month are presented in tables. Salinities and temperatures were measured through the year. Yearly averages for each oyster poll were presented and discussed in the preceeding paper in this series. Only minor variations in the tissue element contents were found during the year, and none were statistically significant, except for manganese with minimum values in May and June and with a maximum peak in August.

In the month of August, maturating oysters of two stages of maturation, together with spawned and immature oysters were dissected and analyzed as above. From the tables of results, the following may be mentioned: Sodium, magnesium and calcium showed high concentrations in the maturating gonades and decreased concentrations in other tissues with the exception of very high levels of calcium in mantle/gill tissues, up to 29 g/kg before spawning, 4 times the corresponding values in immature specimens. There was a general decrease in the tissue concentration of manganese, iron, copper and zinc during maturation, and increasing values after spawning. In the digestive system, iron and zinc concentrations increased after spawning to values substantically higher than in immature specimens and so did the zinc concentration in mantle/gill tissue. Only small variations were seen in the tissue concentrations of cadmium and lead during maturation and spawning.

INTRODUCTION

Several reports have suggested seasonal fluctuations in the chemical composition of oysters. Fluctuations in trace element composition were reported by GAARDER and ALVSAKER, (1941); GALTSOFF, (1942, 1964);

PRINGLE et al., (1968); ROOSENBURG, (1969); COOMBS, (1972); KOPFLER and MAYER, (1973); FRAZIER, (1975); CUNNINGHAM and TRIPP, (1975) and ZAROOGIAN et al., (1979). A fundamental question is whether the element variations during the year may be attributable to accidental changes in the molluscs themselves or to variations in the environment (KNAUER and MARTIN, 1973; MORRIS, 1974).

Element variations through the year may be discussed as four topics, i.e. those related to shell growth and Ca metabolism (GALTSOFF, 1964; COOMBS, 1972), those related to feeding (KORRINGA, 1952; PRESTON, 1971), those related to spawning (GALTSOFF, 1964) and lastly those related to varying availability of the chemical forms of the elements in the water (RUCKER and VALENTINE, 1961; ROMERIL, 1971; WINDOM and SMITH, 1972).

This paper records seasonal changes during one year in protein and in 4 major and 6 minor element contents in tissues of oyster (*Ostrea edulis*). Hatchery-reared oysters were used to minimize biological variability, and 3 tissue groups were analyzed to gain some information on metabolic processes of the oyster tissues. Three different populations, sampled from oyster farms at Innerøy, Svanøy and Vågsstranda in western Norway were analyzed to obtain a realistic picture of seasonal variations. Oysters in different stages of spawn development were sampled in August from one poll.

METHODS

Sampling

Samples of about 15 oysters were taken at approximately monthly intervals, from three oyster farms during the period November 1974 to October 1975. The positions of the sampling stations are shown in Figure 1 and a description of the polls is given in part I of this series (JULSHAMN, I, 1981). Each supplier was asked to procure fifteen individuals of adult *O. edulis* from the age group of three years. The samples were all of immature oysters taken at nearly the same position throughout the year, to secure comparable conditions. Salinity and temperature were measured at the sampling stations throughout the experiment. The oysters were transported alive to the laboratory and sorted. The shells were opened, washed in redistilled water, dried in air and weighed. Ten oysters weighing between 7 and 12 grams were dissected into 1) muscle, 2) mantle and gills and 3) digestive system including connective tissue (Fig. 2). Maturating oysters were taken in August from the Innerøy poll and sorted into two groups representing two stages of the development containing grey and black eggs, respectively. In addition spawned specimens were col-

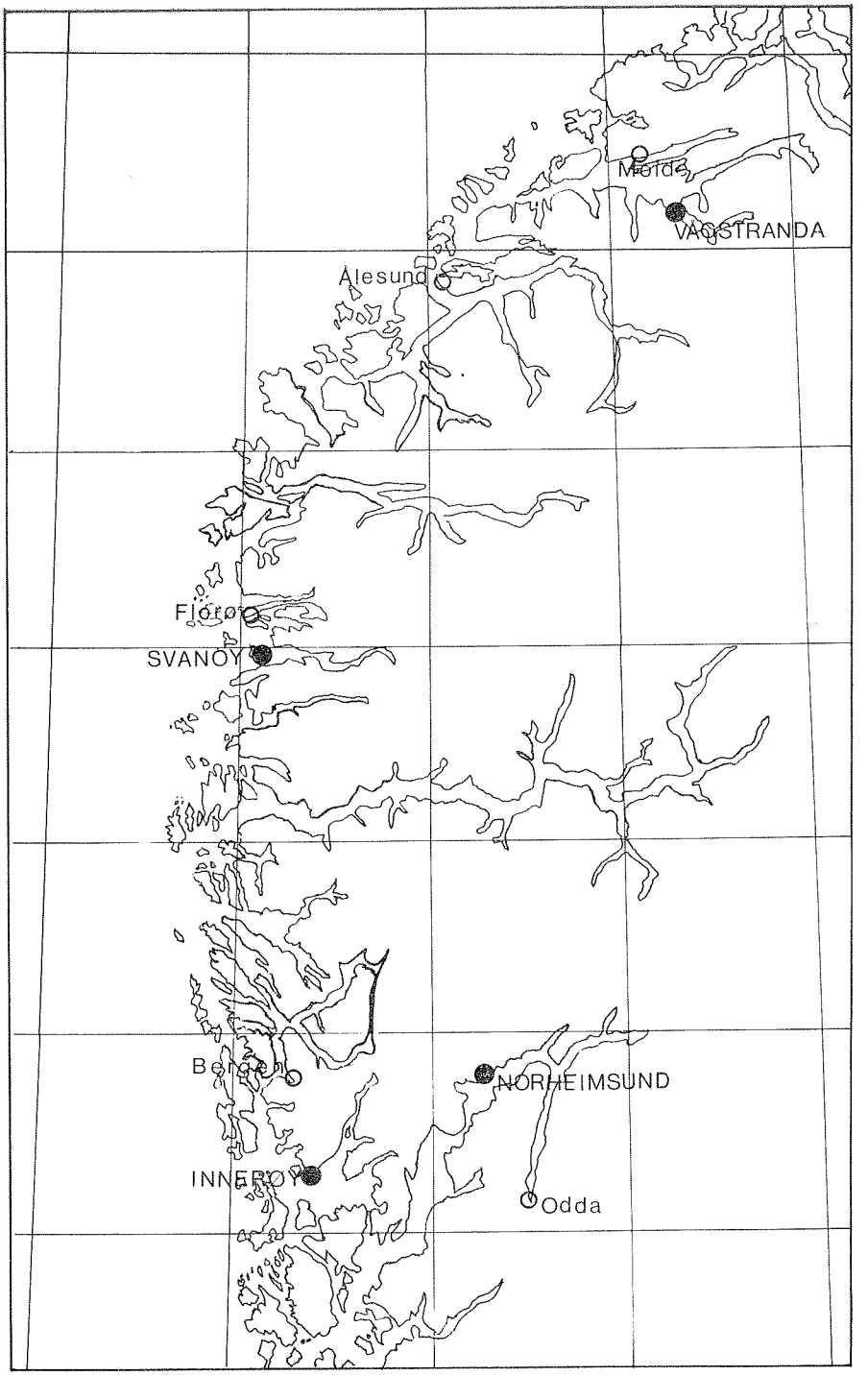


Fig. 1. Map of Western Norway showing the position of the oyster farms at Innerøy, Svanøy and Vågstranda.

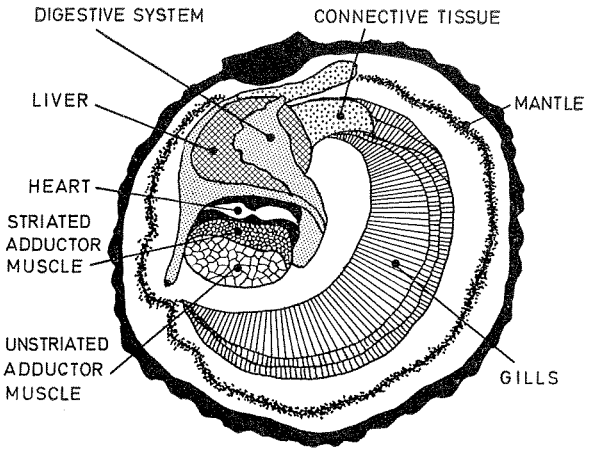


Fig. 2. Organs and tissues in oyster (*Ostrea edulis*).

lected and all were dissected as above. The tissue samples were pooled, homogenized, freezedried to constant weight and stored in capped jars until analysis.

Analysis

All samples were analysed for 10 elements by atomic absorption spectrophotometry after acid digestion. All elements were determined in air/acetylene flame. Cadmium and lead were determined in a boat and the other elements were sucked into the flame as solutions. Details of all procedures and instrumental settings are given in Paper I of this series (JULSHAMN, I, 1981).

Protein was determined by a micro-Kjeldahl technique as described by NJAA (1963).

RESULTS AND DISCUSSION

Averages of element concentrations through the year from each poll were given and discussed in the preceeding paper (JULSHAMN, I, 1981).

Dry matter and protein

Data on temperature and salinity are shown in Fig. 3. A decline of the temperature during the winter months was followed by increasing salinity. The temperature fell below 7°C between December and April. The salinity varied between 24 and 33‰ for the three sites through the year.

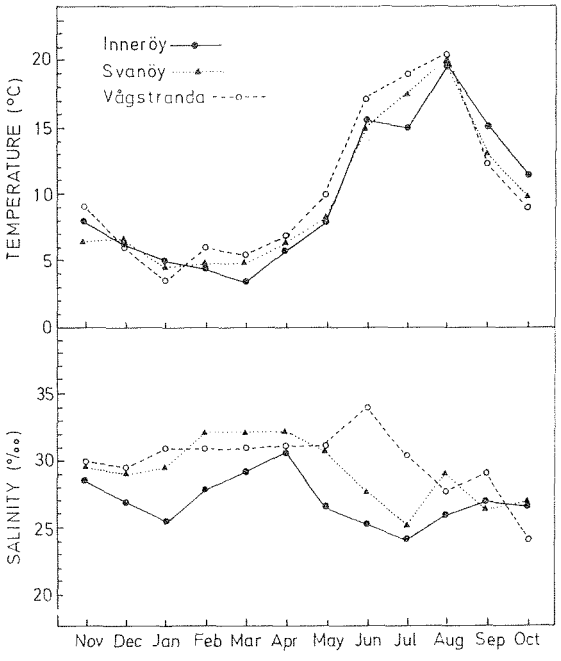


Fig. 3. Temperatures and salinities through one year at the three oyster polls.

Table 1 gives averages of tissue dry weights and protein contents from the ten oysters from each site sampled monthly through one year. The overall tissue weights changed very little indicating that the oysters were in similar conditions. The highest contents of dry matter were found in November to January, the lowest in April. GALTSOFF (1964) and FRAZIER (1975) found a rapid growth during autumn with storage of glycogen, followed by a decline in dry weight during the depletion of the glycogen stores in the winter. In disagreement with this, WALNE (1970) reported a maximum dry weight content between April and June. The highest protein contents were found in the muscle tissue, and the lowest in the digestive system. The digestive system accounted for more than 60% of the total dry matter, and the total protein content may vary with this tissue. The highest protein levels in the digestive system were found in January and June. Only minor protein variations were found in muscle and mantle/gills through the year.

Values from the oyster maturation stages are given in Table 2. There was a substantial loss of tissue weight during the maturation, particularly in the digestive system. The total weight of the digestive system in an oyster after spawning was 0.16 g compared to 1.22 g in an immature

Table 1. Contents of protein and major elements (g/kg dry weight) of *O. edulis* during one year, from Nov. 1973 to Oct. 1974. Values are further given as mg in the whole tissue.

Month	Tissues	Average tissue dry weight (g)	g dry weight per kg tissue	Protein		Na		K		Mg		Ca	
				g/kg	mg	g/kg	mg	g/kg	mg	g/kg	mg	g/kg	mg
Nov.	Muscle	0.25	224	693	173	17	4.3	13	3.1	1.9	0.49	3.5	0.85
	Mantle/gills	0.45	170	537	242	29	13	13	7.7	3.7	1.7	15	6.9
	Digestive system	1.3	286	293	381	10	13	10	14	1.7	2.4	1.8	2.5
	Sum/ave.	2.00	240	400	796	15	30	12	24	2.2	4.6	5.1	10
Dec.	Muscle	0.22	214	682	150	16	3.6	10	2.3	1.9	0.45	3.8	0.89
	Mantle/gills	0.42	165	507	213	30	13	10	4.3	4.4	1.9	13	5.7
	Digestive system	1.3	291	345	448	8.6	11	8.3	11	1.2	1.5	1.3	1.6
	Sum	1.94	241	420	812	14	28	9.0	17	2.0	3.8	4.3	8.2
Jan.	Muscle	0.24	205	648	156	15	3.5	12	2.8	1.9	0.45	3.0	0.68
	Mantle/gills	0.47	144	523	246	28	13	12	5.8	4.0	1.7	10	3.8
	Digestive system	1.3	281	447	581	8.5	10	8.6	11	1.0	1.5	1.3	1.5
	Sum	2.01	231	489	983	13	27	9.1	19	1.8	3.8	3.1	5.9
Feb.	Muscle	0.23	201	707	163	12	2.6	10	2.3	1.7	0.45	1.8	0.35
	Mantle/gills	0.44	132	522	230	23	9.6	11	4.6	3.5	1.6	8.6	4.3
	Digestive system	1.3	262	335	435	7.9	9.7	9.0	11	1.2	1.6	1.2	1.4
	Sum	1.97	209	420	828	12	22	9.5	18	1.8	3.8	3.3	6.1
Mar.	Muscle	0.23	195	720	165	12	2.8	9.3	2.2	1.9	0.46	3.6	0.92
	Mantle/gills	0.38	115	553	210	21	8.1	11	4.7	3.8	1.5	6.0	2.4
	Digestive system	1.2	249	342	410	11	13	8.6	11	1.3	1.6	2.0	2.5
	Sum	1.81	195	434	785	13	24	9.3	18	1.9	3.6	3.0	5.8
Apr.	Muscle	0.22	191	712	157	14	3.0	10	2.5	1.9	0.46	2.6	0.62
	Mantle/gills	0.39	119	545	213	24	9.3	12	4.8	3.2	1.3	7.3	3.0
	Digestive system	1.1	242	358	394	10	9.6	8.1	8.3	1.3	1.3	1.2	1.4
	Sum	1.71	189	447	764	14	22	9.2	16	1.8	3.1	3.0	5.0

May	Muscle	0.24	193	672	161	15	3.5	10	2.2	1.6	0.41	2.8	0.69
	Mantle/gills	0.44	133	553	243	26	12	10	4.7	3.5	1.6	6.8	3.0
	Digestive system	1.2	241	362	634	10	12	8.9	11	1.3	1.6	1.5	1.9
	Sum	1.88	204	446	838	15	27	9.3	18	1.9	3.7	2.9	5.6
Jun.	Muscle	0.24	195	703	169	13	3.2	8.7	2.0	1.6	0.39	2.7	0.67
	Mantle/gills	0.39	123	588	229	22	9.1	11	4.3	3.3	1.3	6.6	2.6
	Digestive system	1.2	247	415	498	8.1	10	8.2	10	1.3	1.6	1.3	1.7
	Sum	1.83	198	490	896	12	22	8.8	17	1.7	3.3	2.6	4.9
Jul.	Muscle	0.21	192	710	149	13	2.7	8.6	1.9	1.8	0.38	3.9	0.83
	Mantle/gills	0.36	146	553	199	20	7.4	10	3.8	3.1	1.1	12	6.2
	Digestive system	1.2	250	353	424	10	10	6.9	8.1	1.3	1.6	2.7	1.5
	Sum	1.77	211	436	772	12	20	7.9	14	1.7	3.0	4.9	10
Aug.	Muscle	0.25	214	737	184	11	2.8	10	2.4	2.0	0.51	2.6	0.67
	Mantle/gills	0.39	137	515	201	21	8.0	10	3.9	3.4	1.3	8.1	3.1
	Digestive system	1.1	243	333	366	9.5	9.7	8.7	9.9	1.5	1.7	2.3	2.5
	Sum	1.74	204	432	751	12	21	9.1	16	2.0	3.5	3.5	6.3
Sep.	Muscle	0.23	203	698	161	10	2.3	11	2.5	1.7	0.39	2.4	0.57
	Mantle/gills	0.56	198	575	322	18	7.3	13	5.2	2.9	1.2	8.5	3.4
	Digestive system	0.99	244	335	332	8.2	7.1	11	10	1.2	1.1	1.9	1.9
	Sum	1.78	201	458	815	12	17	12	18	1.7	2.7	3.6	5.8
Oct.	Muscle	0.28	197	672	188	12	3.2	11	3.0	1.9	0.52	3.4	1.0
	Mantle/gills	0.43	147	545	234	26	11	11	5.0	4.0	1.7	9.4	4.2
	Digestive system	1.2	246	325	390	9.5	11	8.6	10	1.4	1.6	1.6	1.8
	Sum	1.91	206	425	812	14	25	9.5	18	2.1	3.8	3.7	7.0

Table 2. Contents of major elements in tissues (g/kg dry weight) during maturation of oyster (*Ostrea edulis*) from Innerøy poll. Values are further given as mg in whole tissue.

	Tissue	No. ind.	Average tissue dry weight (g)	g dry weight per kg tissue	Na		K		Mg		Ca	
					g/kg	mg	g/kg	mg	g/kg	mg	g/kg	mg
1. stage of maturation	muscle	5	0.11	200	8.4	0.92	9.7	1.1	0.88	0.10	0.42	0.05
	mantle/gills	5	0.28	155	15	4.2	9.8	2.7	1.5	0.42	18	5.1
	digestive system	5	0.43	249	8.6	3.7	8.7	3.7	0.73	0.31	1.0	0.45
	gonades	5	0.32		49	16	7.7	2.5	4.9	1.6	21	6.7
2. stage of maturation	muscle	5	0.14	202	11	1.5	10	1.4	0.99	0.14	1.9	0.26
	mantle/gills	5	0.22	158	13	2.8	8.7	1.9	1.2	0.26	29	6.5
	digestive system	5	0.33	250	5.7	1.9	9.0	3.0	0.58	0.19	1.5	0.48
	gonades	5	0.26		49	13	6.7	1.7	4.6	1.2	13	3.4
After spawning	muscle	5	0.10	208	9.9	0.99	10	1.0	0.95	0.10	2.3	0.23
	mantle/gills	5	0.19	123	19	3.7	11	2.0	1.9	0.36	18	3.4
	digestive system	5	0.16	169	13	2.1	6.7	1.1	1.3	0.20	2.5	0.40
Immature	muscle	5	0.25	211	9.5	2.4	12	2.8	1.9	0.46	2.6	0.64
	mantle/gills	5	0.41	124	22	9.0	9.5	3.9	3.6	1.5	7.0	2.9
	digestive system	5	1.22	254	7.5	9.2	12	14	1.5	1.8	1.5	1.8

specimen. The dry weight content in the muscle tissue did not change during maturation and spawning, whereas in mantle and gills dry weight increased during the maturation and decreased after spawning. This is consistent with findings on *Crassostrea virginica* reported by GALTSOFF (1942, 1964). The protein contents were not measured in these samples.

Major elements (Tables 1 and 2)

The highest levels of sodium were found in mantle/gills and the lowest levels in the digestive tissue. The highest values in muscle and mantle/gills were recorded in November to January, whereas the samples from March to May showed the highest amounts in the digestive system, seemingly inconsistent with the variation in salinity. High sodium values were found in the gonades of the maturating oysters. The sodium content in muscle was little affected by maturation stages, whereas the contents in mantle/gills were low during the maturation. Only minor variations were found in the potassium concentrations through the year. The gonades of the maturating oysters had low potassium levels in contrast to the sodium levels. The loss of potassium was related to the loss of weight in muscle and mantle/gills, whereas the content in the digestive system had decreased in the oysters after spawning.

The highest magnesium levels were found in mantle/gills, more than 40% of this element lies in these tissues. Mantle/gills accounted for most of the element in the early winter, whereas the digestive system had the major part in the summer months. Seasonal changes were small in the muscle tissue. Highest magnesium levels were found in the gonades (4.9 g/kg) in maturing oysters, whereas the concentrations in muscle and mantle/gills were significantly lower than those in immature oysters. The loss was substantial in all tissues during maturation, decreasing more than the loss of weight. The magnesium content was regained in the digestive system after spawning. High calcium concentrations were found in the mantle/gills, and the highest values were found in the winter months, with a decrease during the shell growing months. This would be expected if the element components of the shell matrix are derived from the soft tissues and not absorbed directly from the external environment (FRAZIER, 1975). In the digestive system the highest levels were found in July and August. During the development of the gonades the calcium content increased from 7.0 g/kg to 29 g/kg, and declined to 18 g/kg in the spawned specimens. Only small changes were found in the muscle and digestive system.

Table 3. Contents of trace elements in tissues (mg/kg dry weight) of *O. edulis* during one year, from Nov. 1973 to Oct. 1974. Values are further given as microgram in whole tissue.

Month	Tissues	Mn		Fe		Cu		Zn		Cd		Pb	
		mg/kg	μg	mg/kg	μg	mg/kg	μg	mg/kg	μg	mg/kg	μg	mg/kg	μg
Nov.	Muscle	3.1	0.78	61	15.9	14	3.59	1400	346	2.3	0.56	1.4	0.32
	Mantle/gills	27	12.5	170	85.8	83	38.4	6800	3000	4.4	1.91	2.6	1.04
	Digestive system	6.2	7.97	160	210	37	45.6	2600	3250	2.6	3.09	1.4	1.73
	Sum	11	21.2	150	312	45	87.5	3400	6600	3.0	5.57	1.7	3.10
Dec.	Muscle	3.0	0.64	54	12.4	13	2.94	1600	349	2.0	0.44	1.0	0.23
	Mantle/gills	19	7.84	160	65.9	76	32.1	7200	3040	3.6	1.35	2.1	0.90
	Digestive system	5.5	6.96	120	156	27	34.1	2700	3420	1.6	1.97	0.41	0.53
	Sum	8.1	15.5	120	234	36	69.1	3500	6810	2.1	3.93	0.86	1.66
Jan.	Muscle	3.6	0.81	44	10.3	17	3.91	1500	332	2.5	0.38	1.8	0.44
	Mantle/gills	22	11.3	160	74.0	120	55.6	6600	3000	3.9	1.99	1.3	0.67
	Digestive system	5.9	6.99	110	137	49	60.5	2800	3260	2.0	2.26	0.78	0.94
	Sum	9.1	17.8	110	221	57	120.1	3400	6550	2.5	4.89	1.0	2.46
Feb.	Muscle	3.0	0.69	31	7.2	15	3.20	1600	348	2.2	0.51	1.5	0.35
	Mantle/gills	21	9.17	150	64.2	130	55.8	8400	3580	5.0	2.08	2.4	0.95
	Digestive system	6.7	8.20	110	134.0	56	62.6	3000	3420	2.4	2.71	1.7	1.89
	Sum	9.7	18.1	110	206	69	122	4000	7350	3.0	5.30	1.5	3.18
Mar.	Muscle	2.6	0.55	42	9.8	21	5.17	1600	362	1.4	0.29	1.8	0.40
	Mantle/gills	20	7.46	170	63.7	96	35.6	8300	2980	3.4	1.26	3.0	1.08
	Digestive system	6.0	6.92	120	146	55	69.7	3900	4640	2.1	2.49	1.2	1.58
	Sum	8.4	14.9	120	219	60	110	4700	8000	2.3	4.04	1.6	3.06
Apr.	Muscle	2.6	0.62	49	12.4	16	3.18	1800	379	2.4	0.52	1.2	0.33
	Mantle/gills	21	8.54	170	67.7	99	37.1	8700	3390	3.9	1.56	2.2	0.87
	Digestive system	6.0	5.73	130	126	53	41.4	3900	3240	2.7	2.24	0.64	0.60
	Sum	9.5	14.8	130	206	59	81.7	4800	7010	2.9	4.32	1.06	1.80

May	Muscle	2.1	0.49	57	13.9	14	3.31	1500	339	2.2	0.53	0.85	0.20
	Mantle/gills	17	7.93	190	85.7	120	55.2	9100	4110	3.6	1.68	1.9	0.83
	Digestive system	6.9	8.55	150	186	51	64.6	3800	4590	2.3	2.86	0.51	0.61
	Sum	8.8	17.0	150	286	62	123	4800	9030	2.6	5.07	0.85	1.65
Jun.	Muscle	2.1	0.53	59	15.0	16	3.71	1300	303	2.2	0.54	0.86	0.20
	Mantle/gills	15	6.26	170	66.7	98	38.5	9200	3490	3.5	1.38	1.1	0.45
	Digestive system	4.4	5.64	150	190	32	38.3	2600	3230	2.2	2.75	0.55	0.65
	Sum	6.5	12.4	140	274	44	80.5	3800	7030	2.5	4.67	0.69	1.30
Jul.	Muscle	3.9	0.85	64	13.5	17	3.8	1700	388	1.8	0.37	0.93	0.21
	Mantle/gills	23	8.50	150	55.3	99	35.7	7600	2730	2.6	0.94	1.9	0.68
	Digestive system	7.4	8.65	170	205	32	36.9	2800	3250	2.1	2.43	0.9	1.00
	Sum	10	18.0	150	274	44	76.4	3700	6380	2.2	3.75	1.1	1.89
Aug.	Muscle	4.8	1.16	77	20.2	21	4.91	2000	466	3.2	0.79	1.2	0.28
	Mantle/gills	31	12.0	260	104	100	39.0	8700	3630	3.6	1.36	2.0	0.74
	Digestive system	9.1	10.2	180	212	44	47.9	4200	4420	3.0	3.40	1.1	1.14
	Sum	13	23.3	190	336	55	91.8	4900	8180	3.2	5.55	1.3	2.16
Sep.	Muscle	4.0	0.92	100	24.1	24	5.19	2000	452	3.3	0.72	1.1	0.22
	Mantle/gills	29	11.5	270	108	120	48.7	8900	3410	3.9	1.56	1.8	0.71
	Digestive system	7.7	8.16	210	195	45	36.4	3400	3400	4.1	3.29	1.1	0.90
	Sum	13	20.6	210	327	62	90.3	4600	7260	3.9	5.58	1.3	1.87
Oct.	Muscle	3.4	1.02	130	43.6	13	3.70	2300	605	2.4	0.68	0.6	0.17
	Mantle/gills	23	10.3	190	87.7	89	35.0	9400	3900	3.1	1.31	1.2	0.49
	Digestive system	6.0	6.89	240	286	35	37.4	3200	3400	2.5	2.88	0.9	0.95
	Sum	9.7	18.2	210	417	45	76.0	4500	7880	2.6	4.87	0.9	1.61

Minor elements (Tables 3 and 4)

About 50% of the total manganese in oysters was found in mantle/gills. All tissues had a similar pattern of seasonal variations. The concentrations as well as total body contents had minimum values in May and June, increased substantially in July and reached a peak in August. This corresponds to data given for *Crassostrea virginica* (GALTSOFF, 1964; FRAZIER, 1975). The manganese content in the soft tissues is probably correlated with shell growth and with calcium metabolism. Maturation and spawning had a substantial influence on the manganese contents, with a decrease in the concentrations for all tissues, different from the calcium pattern. Half the contents was found in muscle, whereas the mantle/gills had only 8.3 mg/kg before spawning. In the digestive system the manganese fell to 3.2 and 3.5 mg/kg during the maturation and increased to 8.3 mg/kg after spawning, as compared to 10 mg/kg in immature individuals.

Only minor differences were found between the contents of iron in mantle/gills and digestive system, whereas the muscle had lower iron contents. The lowest levels in all tissues were found in February, and the highest values from August to October. This corresponded with the pattern for manganese contents which increased during the late summer. BRYAN (1974) found the highest iron values in the winter months in *Chlamys* and *Pecten*. The iron concentrations decreased in the tissues during maturation and the content in the digestive system increased substantially after spawning and reached 400 mg/kg as compared to 180 mg/kg in immature mussels.

The mantle/gills and digestive system in *O. edulis* accounted for similar amounts of the total copper content. The copper contents were low in November and December. FRAZIER (1975) found a peak in the copper content of *Crassostrea virginica* in early August followed by a decrease to winter levels, but this could not be seen in the present study. During maturation the copper content in mantle/gills decreased from 100 mg/kg to 36 mg/kg before spawning, and increased somewhat to 42 mg/kg after spawning. A similar pattern was obtained for the digestive system. A noteworthy low level of 5 mg/kg was found in the muscle of mature mussels, compared to 33 mg/kg in immature individuals.

The mantle/gills accounted for approximately 50% of the total zinc content. The zinc levels in muscle changed little from November to August, whereas the zinc content in mantle/gills increased from 6.8 g/kg in November to 9.2 g/kg in June, decreased in July and then increased again. A similar trend was found for the digestive system with an increase from November to May followed by a decrease in June and July and

Table 4. Contents of trace elements in tissues (mg/kg dry weight) during mautration of adult oyster (*Ostrea edulis*) from Innerøy poll. Values are further given as microgram in whole tissue.

	Tissue	Average tissue dry weight (g)	Mn		Fe		Cu		Zn		Cd		Pb	
			mg/kg	µg	mg/kg	µg	mg/kg	µg	mg/kg	µg	mg/kg	µg	mg/kg	µg
1. stage of maturation	muscle	0.11	2.8	0.31	46	5.1	4.7	0.52	2500	280	4.1	0.45	3.8	0.42
	mantle/gills	0.28	28	7.8	130	35	100	28	6700	1900	2.1	0.60	3.8	1.1
	digestive system	0.43	3.5	1.5	110	49	65	28	3900	1700	5.2	2.2	1.9	0.82
	gonades egg	0.32	14	4.5	189	60	8.6	2.8	760	240	1.4	0.45	1.4	0.45
2. stage of maturation	muscle	0.14	2.5	0.35	86	12	4.7	0.66	3300	460	2.7	0.38	1.9	0.27
	mantle/gills	0.22	8.3	1.8	200	44	36	7.9	6500	1400	3.2	0.71	2.8	0.62
	digestive system	0.33	3.2	1.1	150	49	19	6.2	4800	1600	5.7	1.9	3.3	1.1
	gonades	0.26	8.3	2.2	110	29	4.7	1.2	620	160	1.5	0.39	1.0	0.26
Spawned	muscle	0.10	2.8	0.58	63	6.3	5.5	0.55	2100	210	6.4	0.64	2.3	0.23
	mantle/gills	0.19	19	2.4	280	53	42	8.0	12000	2100	5.2	0.99	3.8	0.72
	digestive system	0.16	8.3	1.4	400	64	40	6.4	12000	2300	4.4	0.70	3.8	0.61
	muscle	0.25	5.0	1.2	100	25	33	8.1	4000	1000	5.2	1.3	1.4	0.35
Immature	mantle/gills	0.41	35	14	430	176	130	54	8600	3500	5.0	2.1	2.5	1.0
	digestive system	1.22	10	12.3	180	220	65	80	4000	4900	3.9	4.8	1.7	2.1

again an increase in August. Studying *Crassostrea virginica*, FRAZIER (1975) found a rapid increase in early summer followed by a dramatic loss of a large fraction of the body zinc during a one-month period in the middle of the shell growing season, August–September. A corresponding variation could not be seen in *O. edulis* in the present study. During maturation and spawning the zinc content in the oyster tissues showed a different pattern from the other elements studied. The loss in the tissue contents of zinc was related to the loss in weight during the gonadal development. An appreciable increase of zinc was found in mantle/gills and the digestive system after spawning.

The same contents of cadmium were found in muscle and digestive system, whereas mantle/gills had higher values. The seasonal pattern for cadmium in mantle/gills differed from those of copper and zinc, but with smaller variations than for muscle and the digestive system. High levels of cadmium were found throughout the winter months when little feeding occurred. BRYAN (1973) noticed the highest values in autumn and winter months. He observed that the cadmium concentrations in the tissues were generally highest when the phytoplancton productivity was low. The highest levels in muscle and digestive system were recorded in August and September. Small variations were seen in tissue concentrations during maturation and spawning.

The lead contents varied appreciably during the year, but scattered values suggest that the reproducibility of the analytical procedure was quite low at concentration levels around 1 mg/kg. There was a slow decrease in the lead contents in muscle and mantle/gills during maturation and spawning and a greater decrease in the digestive system.

REFERENCES

BRYAN, G.W., 1973. J. Mar. Biol. Ass. U.K. *53*, 145–166.

COOMBS, T.L., 1972. Mar. Biol. *12*, 170–178.

CUNNINGHAM, P.A. and TRIPP, M.R., 1975. Mar. Biol. *31*, 321–324.

FRAZIER, J.M., 1975. Chesapeake Sci. *16*, 162–171.

GAARDER, T. and ALVSAKER, E., 1941. Biologie und Chemie der Auster in den norwegischen Pollen. Bergens Mus. Aarb., Naturv. r., 1–236.

GALTSOFF, P.S., 1942. Physiol. Zool. *15*, 210–215.

GALTSOFF, P.S., 1964. The American oyster. *Crassostrea virginica* Gonclin. U.S. Fish Wildlife Serv., Fish. Bull. *64*, 1–480.

JULSHAMN, K., 1981. Studies on Major and Minor Elements in Molluscs in Western Norway. I, Fisk. Dir. Skr., Ser. Ernæring, 1, no. 5.

KNAUER, G.A. and MARTIN, J.H., 1973. Limnol. Oceanog. *18*, 597–604.

KOPFLER, F.C. and MAYER, J., 1973. Proc. National Shellfish. Ass. *63*, 27–34.

KORRINGA, P., 1952. Recent advances in oyster biology. Quart. Rev. Biol. *27*, 266–308, 339–365.

MORRIS, A.W., 1974. Mar. Poll. Bull. *5*, 54–59.
NJAA, L.R., 1963. Fisk. Dir. Skr., Ser. Tekn. Undersøk. *4*, No. 5.
PRESTON, E., 1971. J. Exp. Mar. Biol. Ecol. *6*, 47–54.
PRINGLE, B.H., HISSONG, D.E., KATZ, E.L. and MULAWKA, S.T., 1968. Proc.Am. Soc. Civil Eng., J. Sanitary Eng. Div. SA 3, 455–475.
ROMERIL, M.G., 1971. Mar. Biol. *9*, 347–354.
ROOSENBURG, W.H., 1969. Chesapeake Sci. *10*, 241–252.
RUCKER, J.B. and VALENTINE, J.W., 1961. Nature *190*, 1099–1100.
WALNE, P.R., 1970. London, Her Majesty's stationary office. Fishery Investig. II, *16*, No. 3. 1–35.
WINDOM, H.L. and SMITH, R.G., 1972. J. Fish. Res. Bd. Can. *29*, 450–452.
ZAROOGIAN, G.E., MORRISON, G. and HELTSHE, J.F., 1979. Mar. Biol. *52*, 189–196.

Fisk. Dir. Skr., Ser. Ernæring, Vol. 1: No. 5, 1981, s 199–214

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

III. Effects of size and age on the contents of 10 elements in oyster (*Ostrea edulis*), taken from unpolluted waters.

By

KÅRE JULSHAMN

Institute of Vitamin Research
Directorate of Fisheries, Bergen.

ABSTRACT

This paper reports on effects of size and age on ten element concentrations in oyster (*Ostrea edulis*). The samples comprised composite samples of oyster eggs and spat (year class 0, 1–3 months old) and composite and single specimen samples of oysters of year class 1 (13–15 months) and year class 2 (25–27 months). Average values for each group of samples are given on a dry weight basis, and results of a regression analysis are given as graphs and a table. Correlation matrices between elements were calculated for the samples of year classes 1 and 2.

Little effect of age and size were found for the contents of sodium, potassium and manganese. Increasing values from eggs through spats to constant levels in adult oysters were found for copper and cadmium. No effect of size could be seen for these elements. The concentrations of magnesium, calcium and iron all decreased with increasing size, with calcium giving the lowest regression coefficients. Calcium levels also decreased significantly from the high average value of 40 g/kg in eggs through all three year classes. The magnesium levels were relatively constant with age, whereas for iron was found an exceptional high concentration in the samples of oyster spat (average value near 2 g/kg dry weight), decreasing rapidly to less than a tenth of this level in the samples of year class 2. Zinc contents are very high in oysters, and for the present material were found average values increasing from 0.4 g/kg in eggs to 2.4 g/kg dry weight for year class 2. The zinc concentrations in all three year classes also increased with size as well as with age. The lead levels were low and near the detection limits of the methods, making it doubtful to draw conclusions as to effects of size and age. Most correlations between the elements were not significant. The best correlation in the samples of year classes 1 and 2 were found between the magnesium and calcium contents. Further correlations were found between the iron contents and those of sodium and manganese. It may be concluded that some element concentrations are dependent on size and age of oysters, and exact statements of size-ranges and age classes of mussels analyzed for element contents are therefore recommended.

INTRODUCTION

The two preceding papers in this series discussed the seasonal and geographical variations in element contents in oyster (JULSHAMN, I, II, 1981). Element contents are usually given on a weight-specific basis, e.g. as mg (microgram) per kg (g) wet or dry weight. Differences related to size and year class of the species may not appear in the results of an analysis. Consequently, it may be difficult to assess whether observed differences in element concentrations reflect the environmental background of the species analysed, or are due to variations in body size and age group.

Information on such variations can be obtained only indirectly from published tables of element contents. Among more comprehensive data available on element contents in oyster are those on zinc in *Ostrea edulis* (PRESTON, 1966) and *Crassostrea virginica* (HUGETT et al., 1973), cadmium in *C. gigas* (AYLING, 1974), cadmium, copper and zinc in C. *commercialis* (MACKAY et al., 1975), and iron, copper, zinc and cadmium in *C. gigas*, *C. margaritacea* and *O. edulis* (WATLING and WATLING, 1976). More recently a comprehensive work has been published by BOYDEN (1977) on the contents of manganese, iron, nickel, copper, zinc, cadmium and lead in *O. edulis* and *C. gigas* from unpolluted and polluted environments. SCHULZ-BALDES (1973) found that the highest element contents are often recorded from the smallest individuals. High standard deviations calculated on published data may be due to inherent biological variability more than to analytical errors. It is possible to minimize this variation by careful sampling with reference to condition, sex and to homogeneity of a genetic stock. This effect was demonstrated in paper *I* and *IV* in this series (JULSHAMN, I, IV, 1981).

This paper concerns the contents of 10 elements in oysters related to size and is based on analyses of single or pooled specimens from the age groups 0 to 3 years, with large weight variations. Further, oyster eggs were analyzed. The data were treated in a regression analysis.

METHODS

Sampling

All samples were obtained from an oyster farm at Vågstranda, Western Norway. The location was described in part I. (JULSHAMN, I, 1981). Oysters of a genetically homogeneous stock were taken on the same day in the smallest possible area of the basin to avoid any differences in the nutritional supply. The oysters were transported alive to the

laboratory and sorted into the age groups 0, 1 and 2 years, and within each age group according to size. Tissues were removed from the shells, washed briefly in redistilled water, dried in air and weighed. Small specimens were pooled to give samples of 0.5 g, and specimens of 0.5 g or more were analyzed individually. The sampling scheme is shown in Table 1. Eggs were collected in the same way from six oysters which had loose-floating eggs. All samples were freeze-dried, ground and stored in closed jars until analysis.

Analysis

All samples were analysed for ten elements by atomic absorption spectrophotometry after acid digestion. All elements except cadmium and lead were determined in an air/acetylene flame. Cadmium and lead were determined in a flameless graphite oven. The method of standard addition was used to correct for matrix effects for the determination of cadmium and lead. Details of the analytical procedures were given in part I (JULSHAMN, I, 1981).

Data treatment

The analytical values were calculated on the basis of total element content per oyster and taken through a computer programmed regression analysis relative to the dry weight of the corresponding oyster tissue. The overall best fit for all elements was given by the function $y=ax^b$ which relates the total element content y to the dry tissue weight x. This regression function gives a linear relationship in a double logarithmic plot where a is the real intercept value or the element content of a theoretical specimen of 1 g dry matter and b the regression coefficient or slope of the curve. A value for b near 1 thus corresponds to an element concentration independent of body weight, whereas values for b less than 1 show a decreasing element concentration with increasing body weight (dilution) and, correspondingly, values for $b>1$ means an increase in element concentration with increasing body weight (accumulation).

RESULTS AND DISGUSSION

A weight range of 1 to 10 was found for the oyster spat of year class 0, however, only five composite samples were analyzed (Table 1). The information obtained on size effects of element concentration was therefore more limited than from year class 1 comprising 32 samples with a

Table 1. Sampling scheme of oysters (*Ostrea edulis*), analysed for 10 elements.

Composite sample no.	Year class 0		Year class 1		Year class 2	
	1–3 months		13–15 months		25–27 months	
	Range* (mg)	Mean* (mg)	Range (mg)	Mean (mg)	Range (mg)	Mean (mg)
1	<50	25.3	<25	14.1		
2	50– 75	60.3	25– 40	37.7		
3	75–100	89.2	40– 50	42.4		
4	100–150	139	50– 75	59.2		
5	150–300	228	75–100	85.7		
6			100–175	164		
7			175–250	185		
8			250–350	331		
9			350–450	358		
Single specimen samples of more than 500 mg	—		23		23	
Total no. of samples	5		32		23	
Weight range of dry matter per specimen	4.5–48.5 mg		2.3–945 mg		0.531–3.518 g	

*based on wet weight.

weight range of 1 to 500. The 23 samples of year class 2 fell within a more narrow range of 0.5 to 3.5 g which also limited information on size effects.

The water content of the samples varied somewhat with preparative handling, and all samples were freeze-dried to reduce variability within the values of element concentrations. Most published data of element content in biological material are based on dry matter and consequently can be compared (see BOYDEN, 1977).

The average protein content based on dry weight decreased significantly ($P<0.05$) from year class 0 to 1, and further to year class 2 (Table 2 and fig. 1). There were also some effects of size on the protein content, all

Table 2. Protein contents and regression values relative to size in three year classes of oysters. (*Ostrea edulis*).

Year class	Regression coef. (b)	Correlation coef. (r)	Ave. cont. g/kg dry weight
0	1.09	0.99	504±21
1	0.96	0.99	464±26
2	0.94	0.98	430±46

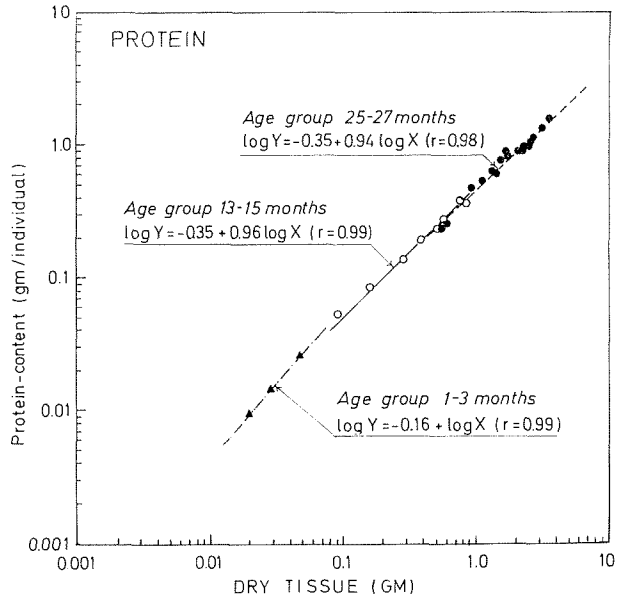


Fig. 1. Relations between the total protein contents and total dry weight per individual in *Ostrea edulis*.
Triangles: year class 0
Open circles: year class 1
Filled circles: year class 2

with high correlation coefficients. The protein contents increased with size in year class 0 and decreased with size in the year classes 1 and 2.

Average values for the contents of the 10 elements analyzed in oyster eggs and the oyster samples from each of the three year classes are com-

Table 3. Average element concentrations in oyster eggs and in samples of three year classes of oysters (*Ostrea edulis*). (Values in italics are ± standard deviation.)

Element		Eggs n=6		Year class 0 n=5		Year class 1 n=32		Year class 2 n=23	
Na	g/kg*	18.3	*6.0*	15.8	*1.0*	16.7	*5.1*	12.6	*2.6*
K	,,	7.3	*2.0*	13.1	*0.7*	12.8	*1.7*	11.8	*1.4*
Mg	,,	3.2	*0.6*	4.9	*2.2*	3.7	*1.5*	3.7	*2.3*
Ca	,,	40.0	*15.0*	26.8	*30.6*	11.6	*12.3*	4.9	*2.1*
Mn	mg/kg	10.0	*4.0*	17.7	*1.1*	13.8	*4.5*	16.6	*7.9*
Fe	,,	61	*20*	1990	*570*	270	*100*	190	*70*
Cu	,,	6.0	*2.0*	12.2	*1.5*	24.4	*8.7*	20.2	*7.4*
Zn	,,	360	*100*	660	*190*	1780	*750*	2390	*760*
Cd	,,	0.20	*0.10*	0.58	*0.10*	2.56	*0.90*	2.89	*0.68*
Pb	,,	0.40	*0.12*	1.68	*0.53*	1.16	*0.71*	1.18	*0.66*

*based on dry matter

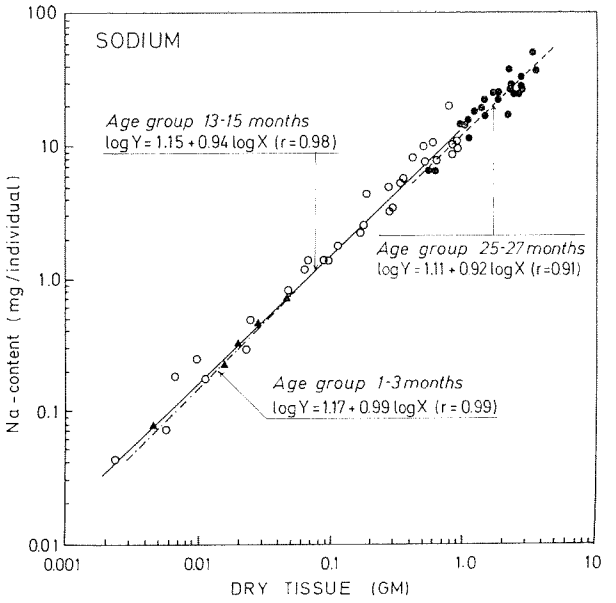
Table 4. Regression values calculated on the analytical results from the samples of oysters specified in Table 1. Total element content in sample is given by ax^b where x denotes dry weight of sample.

Element	Year class	a-value (content for 1 g dry weight)	b-value regression coef.	st. dev. of regression coef.	Correlation coefficient (r)
Na	0	14.8 g/kg	0.99	0.03	0.998
	1	14.2 ,,	0.94	0.03	0.985
	2	12.9 ,,	0.95	0.09	0.910
K	0	14.6 ,,	1.03	0.03	0.999
	1	12.8 ,,	1.00	0.01	0.997
	2	11.5 ,,	1.03	0.05	0.972
Mg	0	1.17 ,,	0.67	0.21	0.878
	1	2.48 ,,	0.84	0.03	0.983
	2	3.00 ,,	1.14	0.22	0.750
Ca	0	0.93 ,,	0.26	0.42	0.343
	1	3.13 ,,	0.56	0.05	0.889
	2	4.81 ,,	0.84	0.19	0.696
Mn	0	15.6 mg/kg	0.97	0.03	0.998
	1	11.1 ,,	0.91	0.03	0.987
	2	13.7 ,,	1.17	0.19	0.796
Fe	0	631 ,,	0.73	0.06	0.989
	1	179 ,,	0.83	0.02	0.988
	2	205 ,,	0.76	0.14	0.762
Cu	0	13.1 ,,	1.09	0.06	0.995
	1	19.4 ,,	0.91	0.03	0.981
	2	17.1 ,,	1.19	0.16	0.845
Zn	0	1290 ,,	1.19	0.15	0.978
	1	1900 ,,	1.07	0.07	0.981
	2	2010 ,,	1.25	0.10	0.960
Cd	0	0.40 ,,	0.91	0.10	0.981
	1	2.02 ,,	0.91	0.04	0.987
	2	2.67 ,,	1.11	0.10	0.924
Pb	0	0.67 ,,	0.78	0.15	0.948
	1	0.94 ,,	0.95	0.05	0.956
	2	1.06 ,,	0.87	0.29	0.543

piled in Table 3, and the corresponding results of the regression analyses in Table 4. The results will be discussed in pairs of two elements.

Sodium and potassium (Fig. 2 and 3)

Regression coefficients close to 1 with low standard deviations were found for Na and K contents in the three year classes, i.e. there were no



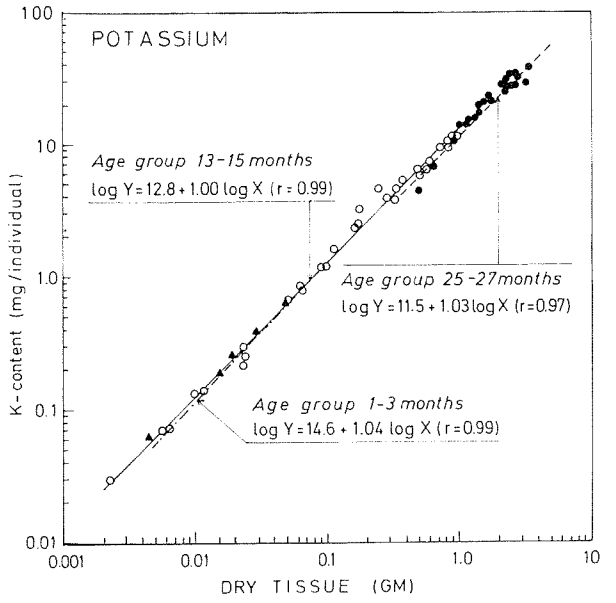


Fig. 2 and 3. Relations between the total sodium and potassium contents and total dry weight per individual in *Ostrea edulis*. Symbols description, see Fig. 1.

effect of size on element concentrations. The sodium to potassium ratio was 2.5:1 in oyster eggs, and fell to 1:1 in the two-year-old oysters. The differences between the Na and K levels were not significant within the samples of each year class.

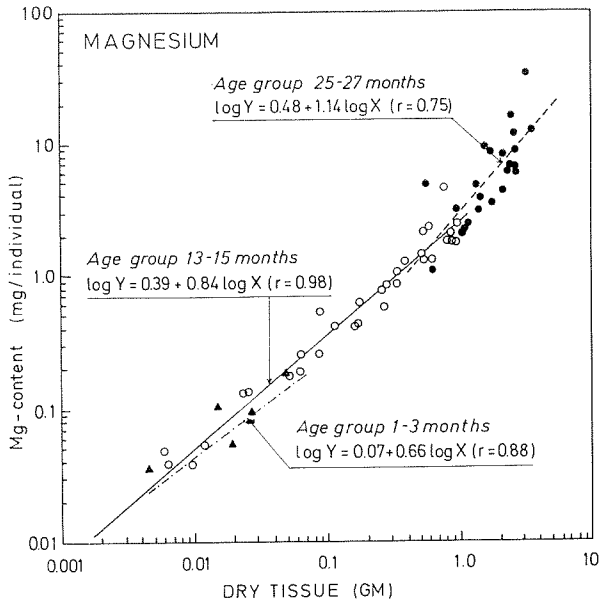
Magnesium and calcium (Fig. 4 and 5)

A relatively constant level of magnesium based on dry weight was found for oyster egg and all three year classes. Calcium, on the other hand, fell from an average level of 40 mg/g in eggs to 5 mg/g in the samples of the second year class i.e. from twelve times the content of Mg to a similar level. A pronounced effect of size upon the calcium concentration was also found, particularly noteworthy for the samples of year class 1, with a regression coefficient of 0.56 and a correlation coefficient of 0.89. The two other population groups gave scattered Ca-values with correspondingly low correlation coefficients. To a lesser extent the magnesium concentrations also decreased with increasing size, and year class 1 again gave the best correlation ($r=0.98$). For both elements the regression coefficients increased with the age of the oysters, for Mg of year class 2 even above 1. Correspondingly, the graphs show an upward trend. There was a significant positive correlation ($p>0.05$) between the Mg and Ca contents for the samples of year classes 1 and 2 (Table 5). An explanation for these size and age relations must presumably be sought in the development of the shell.

Manganese and iron (Fig. 6 and 7).

Relatively constant levels of manganese, between 10 and 20 mg/kg, were found in eggs and the samples of the three year classes. Further, the Mn levels were fairly independent of size, with regression coefficients between 0.91 and 1.17.

The iron levels gave a different picture. Noteworthy high concentrations, averaging 2 g/kg, were found in the samples of oyster spat (year class 0). This concentration contrasted sharply with an average value of 70 mg/kg in eggs, and values about 200 mg/kg in the samples of year classes 1 and 2. Without further investigations one can hardly put forward a theory as to the metabolic role of such a high level of iron in oyster spat, but some relation to the shell formations suggests itself. The regression analysis gave a clear relation of iron concentration to size. The concentrations decreased with increasing size of the oysters, with regression



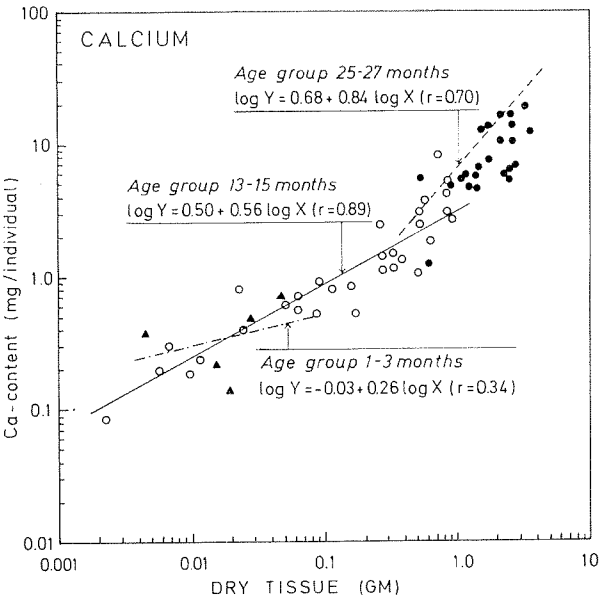
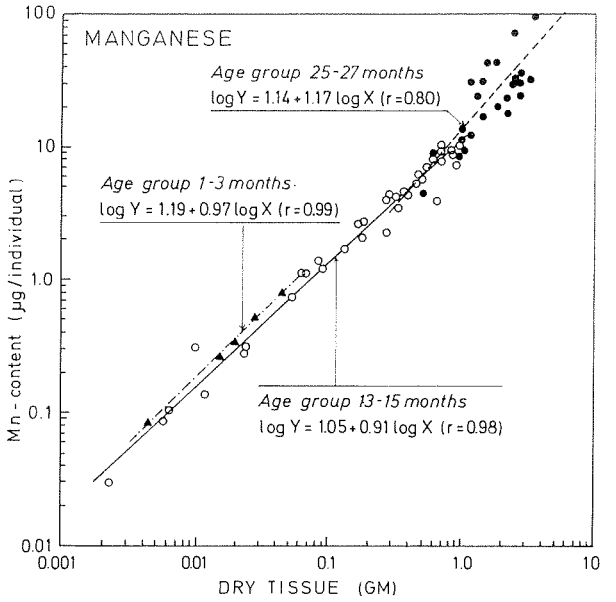


Fig. 4 and 5. Relations between the total magnesium and calcium contents and total dry weight per individual in *Ostrea edulis*. Symbols description, see Fig. 1.



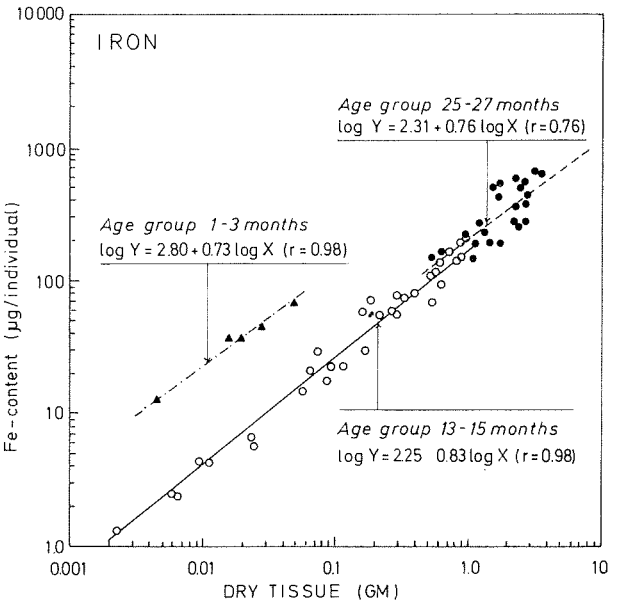


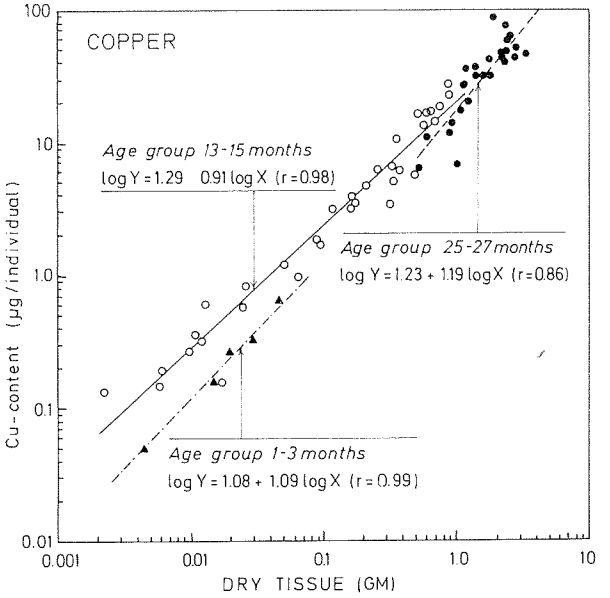
Fig. 6 and 7. Relations between the total manganese and iron contents and total dry weight per individual in *Ostrea edulis*. Symbols description, see Fig. 1.

coefficients between 0.73 and 0.83. The values from year classes 0 and 1 gave high correlation coefficients of 0.99. BOYDEN (1974, 1977) found similar effects in his investigations which did not include age effects.

Copper and zinc (Fig. 8 and 9)

The average copper concentrations increased from 6 mg/kg in oyster eggs through 12 mg/kg in oyster spats to values above 20 mg/kg in the samples of year class 1 and 2. These values are among the lowest reported for copper in oysters and are probably related to the low levels in the sea water in this oyster farm (see part I in this series, JULSHAMN, I, 1981). The increasing copper concentrations up to 1 year of age, followed by constant values, were parallel to those of cadmium (see below), and different from the manganese values which showed no increase, and from the zinc values, which increased through all four stages from eggs to the two-year-old samples. The regression coefficients were around 1.0 for the three year classes analysed, and the copper concentrations were therefore independent of size. This was also found by BOYDEN (1977) on samples of *Ostrea edulis*, but his values were 10 times higher than those reported here. MACKAY & al. (1975) found decreasing concentrations of copper with size and age for *Crassostrea commercialis* having a wet weight range of 2–14 g.

Zinc is a characteristic element for oysters, with concentrations more than 10 times higher than in *Mytilus edulis* of the same age and taken from the same site (JULSHAMN, I, 1981). Oysters have high levels of nitrogen-containing organic molecules of low molecular weights which presumably bind zinc ions, and the rate of uptake of zinc from the sea water is probably more dependent on the concentration of these complexing agents and less on the zinc concentration in the sea water. The high concentration of zinc found in oyster eggs of 0.36 mg per gram dry matter increased to 0.66 mg/g in oyster spat and further up to 2.4 mg/g in the two-year old oyster (Table 3). This increase of zinc concentration with age was parallel to an increase with size. Table 4 shows that zinc was the only element analysed with regression constants above 1 for all three year classes. These results were at variance with those of PRESTON (1966), who found a decrease in the zinc concentration with increasing size. His results were, however, based on specimens of a narrow weight range. MACKAY & al. (1975) also observed higher zinc values in small specimens of *C. commercialis*. BOYDEN (1977) found considerable variations in zinc concentration in oysters from a contaminated environment. Similar results are shown in part VI of this series (JULSHAMN, VI, 1981) based on transplantation studies of *O. edulis* and *M. edulis*.



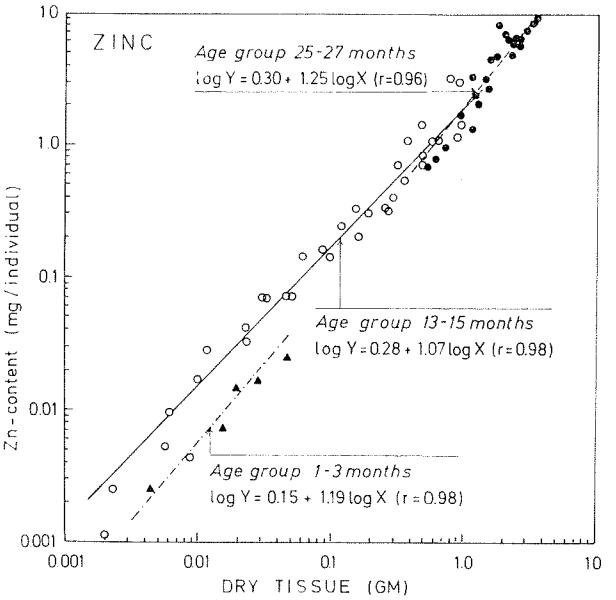


Fig. 8 and 9. Relations between the total copper and zinc contents and total dry weight per individual in *Ostrea edulis*. Symbols description, see Fig. 1.

Cadmium and lead (Fig. 10 and 11)

The cadmium contents increased from eggs through spat to a constant level in the samples of year classes one and two, corresponding to the copper contents, but at a lower level (2–3 microgram per gram Cd, and 20–30 microgram per gram Cu in adult oysters). The regression coefficients were not significantly different from 1.0, again as found for Cu. The cadmium concentrations were therefore independent of the size of oysters. Similar results were reported by BOYDEN (1977) and by NIELSEN (1975), the latter based on analyses of *Ostrea lutaria* of wet weight range 5–29 g.

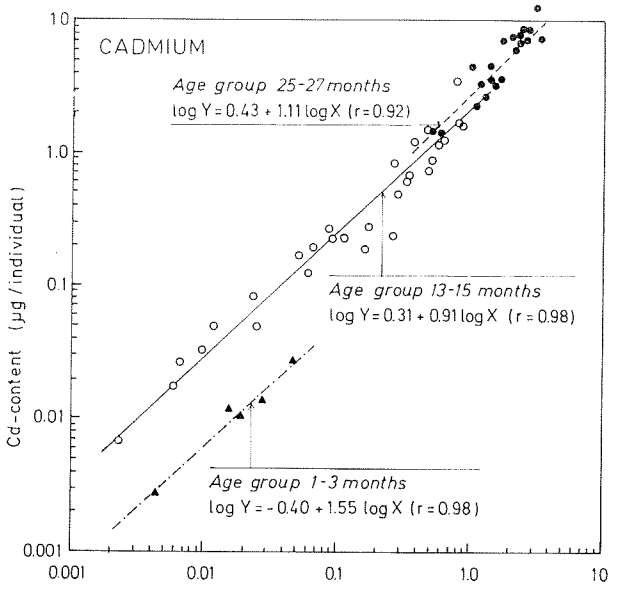
The values found for lead were low, and near the detection limit for the method of analysis. This was reflected in high standard deviations around the average values. Regression coefficients were below 1.0, but this effect may be associated with analytical difficulties at levels near the detection limit. This effect was demonstrated in a study on element contents in muscle biopsies by JULSHAMN and ANDERSEN (1979). The average values given in Table 3 for the three year classes were significantly different from the lead content in eggs, whereas the differences between the year classes were not significant.

Element correlations

Sufficient samples were analysed from year classes 1 and 2 for a calculation of correlation matrices between the elements. The results given in Table 5 gave one high positive correlation in both year classes, that of calcium and magnesium, as discussed above for these elements. The iron values were found to be positively correlated at the 95% level with sodium as well as with manganese in both year classes. Other correlations were not consistent in both year classes.

CONCLUSIONS

Calcium concentrations decreased tenfold from eggs to oysters of year classe 2, and iron contents were exceptionally high in spats and decreased tenfold to oysters of year class 2. Zinc concentrations increased substantially from 0.4 g/kg in eggs to 2.4 g/kg in oysters of year class 2. Cadmium concentrations increased from 0.2 mg/kg in eggs to 3 mg/kg in oysters of year class 2, whereas copper concentrations increased from 6 to 24 mg/kg from eggs to oysters of year class 1. Little or no effect of age were found for the concentrations of sodium, potassium, magnesium, manganese and lead.



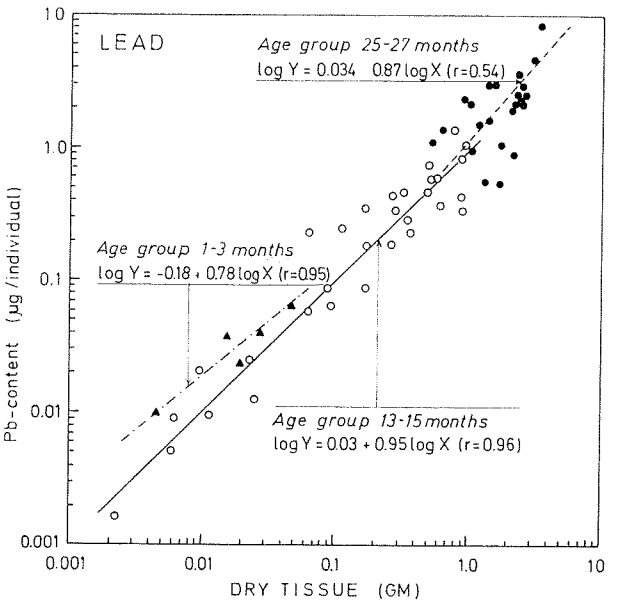


Fig. 10 and 11. Relations between the total cadmium and lead contents and total dry weight per individual in *Ostrea edulis*. Symbols description, see Fig. 1.

Table 5. Correlation matrices between element concentrations in the samples of year classes 1 and 2 of oyster (*Ostrea edulis*). For p=0.05, r=±0.35 for the 32 samples of year class 1 and ±0.41 for the 23 samples of year class 2.

Year class 1	Na	K	Mg	Ca	Mn	Fe	Cu	Zn	Cd	Pb
Na	1									
K	0.10	1								
Mg	*0.43*	—0.25	1							
Ca	0.32	—0.25	*0.74*	1						
Mn	*0.44*	0.01	0.23	0.23	1					
Fe	*0.42*	0.05	0.05	*0.65*	*0.49*	1				
Cu	0.12	—0.21	—0.21	*0.48*	0.02	0.30	1			
Zn	0.28	—0.13	—0.13	—0.19	0.03	—0.10	—0.08	1		
Cd	*0.46*	—0.20	*0.51*	*0.54*	0.20	*0.39*	0.23	*0.50*	1	
Pb	0.24	0.03	0.01	0.03	*0.47*	0.05	—0.18	0.04	—0.04	1

Year class 2	Na	K	Mg	Ca	Mn	Fe	Cu	Zn	Cd	Pb
Na	1									
K	0.05	1								
Mg	0.29	*—0.64*	1							
Ca	0.26	—0.35	*0.72*	1						
Mn	0.13	*0.49*	—0.11	—0.21	1					
Fe	*0.46*	0.06	0.36	0.29	*0.52*	1				
Cu	—0.36	0.22	—0.39	—0.36	—0.10	—0.33	1			
Zn	0.02	0.38	—0.21	—0.14	0.02	—0.40	0.01	1		
Cd	0.04	0.06	—0.18	—0.19	0.01	—0.13	0.17	0.11	1	
Pb	—0.10	*—0.55*	*0.44*	0.20	—0.36	0.19	—0.22	—0.25	*—0.41*	1

Regression coefficients below 1 for magnesium (year class 0 and 1) and for calcium and iron (all three year classes) showed that the concentrations of these three elements decreased with increasing size of the specimens. Regression coefficients above 1 were found in all three year classes for the concentrations of zinc, this element therefore increased in concentration with increasing size of the oyster. No clear effect of size on element concentrations were found for sodium, potassium, manganese, copper and cadmium. Low regression coefficients for lead concentrations may be due to values near the analytical detection limit.

One may conclude from this analytical study that element concentrations may depend on size and age of mussels, and that consequently these parameters should be stated in studies on element contents of mussels, and should be included in calculations based on such studies.

REFERENCES

AYLING, G.M., 1974. Water Res. *8*, 729–738.

BOYDEN, C.R., 1974. Nature *251*, 311–314.

BOYDEN, C.R., 1977. J. Mar. Biol. Ass. U.K. *57*, 675–714.

HUGGETT, R.J., BENDER, M.E. and SLONE, H.D., 1973. Water Res. *7*, 451–460.

JULSHAMN, K. and ANDERSEN, K.J., 1979. Anal. Biochem. *98*, 315–318.

JULSHAMN, K., 1981. Studies on Major and Minor Elements in Molluscs in Western Norway. I, II, IV. Fisk, Dir. Skr., Ser. Ernæring, 1, no. 5.

MACKAY, N.J., WILLIAMS, R.J., KACPRZAC, J.L., KAZVEOS, M.N., COLLINS, A.J. and AUTY, E.H., 1975. Aust. J. Mar. Freshw. Res. *26*, 31–46.

NIELSEN, S.A., 1975. Intern. J. Environ. Anal. Chem. *4*, 1–7.

PRESTON, A., 1966. In Radioecological Concentration Processes. Proceedings of the International Symposium, Stockholm, 1966 (ed. B. Åberg and F.P. Hungate) pp. 995–1004. Oxford. Pergamon Press.

SCHULZ-BALDES, M., 1973. Mar. Biol. *21*, 98, 102.

WATLING, H.R. and WATLING, R.J., 1976. Mar. Pollut. Bull. *7*, 45–48.

Fisk. Dir. Skr., Ser. Ernæring, Vol. 1: No. 5, 1981, s 215–234

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

IV. The distribution of 17 elements in different tissues of oyster (*Ostrea edulis*), common mussel (*Mytilus edulis*) and horse mussel (*Modiolus modiolus*) taken from unpolluted waters.

By

KÅRE JULSHAMN

Institute of Vitamin Research
Directorate of Fisheries, Bergen.

ABSTRACT

Samples of 3-year-old oysters, *Ostrea edulis*, and common mussels, *Mytilus edulis*, 45–55 mm, were collected from hanging baskets, and 12–18-year-old horse mussels, *Modiolus modiolus* were collected from the sandy floor from an oyster poll at Innerøy on the coast of Western Norway. The mussels were each dissected into 6 to 8 tissues which were analyzed by atomic absorption spectrophotometry for 4 major and 13 minor elements. Details of the analytical procedure are given and the results collected in 3 tables. Among the conclusions are the following observations: Ten of 13 minor elements accumulated in the digestive system of *M. modiolus*, and this tissue held 61 to 98% of the total element content in the mussel. The contents of manganese, iron, cobalt, zinc, cadmium, mercury and lead were exceptionally high in this tissue, e.g. Mn: 2.2 g/kg, Zn: 19 g/kg and Pb: 100 mg/kg. In *M. edulis* 9 of 13 minor elements accumulated in the digestive system, which held 34 to 61% of the total of these elements. Selenium, mercury and lead accumulated in the gills and mantle. Few values were exceptionally high, but concentrations of 60 mg/kg of Al in the digestive system and 22 mg/kg of Pb in the gills may be noted. In *O. edulis* only 4 elements: Co, Se, Ni and Pb accumulated in the digestive system, whereas 4 other elements: Mn, Cu, Zn and Al accumulated in the gills and mantle. Fe, As and Cd were equally distributed over the tissues in oysters. The high amounts of copper and zinc in oysters were concentrated in gills and mantle, and further in the heart which had the highest concentrations of the two elements: 280 mg/kg of Cu, and 23 g/kg of Zn. Chromium accumulated in the muscle tissue which held 30 to 40% of total Cr in all three mussels. The muscle tissue of *O. edulis* further accumulated mercury, with the concentration of 0.5 mg/kg. The high level of 21 mg/kg of cadmium in the heart of *O. edulis* may also be noted. All values given above are based on dry weight. Sodium and magnesium accumulated mainly in the gills and mantle of the three species. Calcium accumulated in the digestive system in *M. modiolus* representing 77% of the total Ca.

INTRODUCTION

The determination of whole body concentrations of elements in molluscs is an important aspect of environmental pollution control. Of equal importance, however, is the distribution and accumulation of these elements within the various tissues of the animal. Knowledge of the tissue distribution is necessary to identify specific tissues in different species that may be particularly affected by increasing environmental element concentrations. Much work has been done on mammals in this respect and the distribution patterns for many elements are known. The preceeding papers were concerned with factors influencing the element concentration of mussels from unpolluted waters such as season, size and site on the coast. This work deals with the distribution of sodium, potassium, magnesium, calcium, chromium, manganese, iron, cobalt, copper, zinc, selenium, aluminium, nickel, arsenic, cadmium, mercury and lead in tissues of oyster (*Ostrea edulis*), common mussel (*Mytilus edulis*) and horse mussel (*Modiolus modiolus*) taken from the Innerøy poll in Western Norway in September 1975. The water samples from this poll had very low levels of heavy metal ions (JULSHAMN, I, 1981) and the mussels grown there probably have an element distribution in the tissues not affected by additional metal loads.

METHODS

Sampling

Three species of bivalve molluscs were obtained from the Innerøy oyster poll, Western Norway (Fig. 1). The Innerøy poll is described in detail in Part I in this series (JULSHAMN, I, 1981). The oysters were grown in a hanging culture, suspended in baskets of wire netting in the inner poll.

Thirty specimens of adult *Ostrea edulis* were taken from a genetically homogenous stock of age group 3 years, and from the same basket in the poll to secure a similar food supply.

Thirty specimens of *Mytilus edulis* of 45 to 55 mm lengths were collected from the ropes and the baskets where the *O. edulis* had been taken to minimize differences in the nutritional supply between the two species. The larvae of *M. edulis* are carried by tidal currents to the oyster poll and the individuals would hardly belong to the same genetic stock. The samples of *O. edulis* and *M. edulis* were transferred to the outer poll which has a higher tide current to purge themselves.

Twenty specimens of *Modiolus modiolus*, partly hidden in the sandy floor, were collected from the bottom of the outer poll. The samples

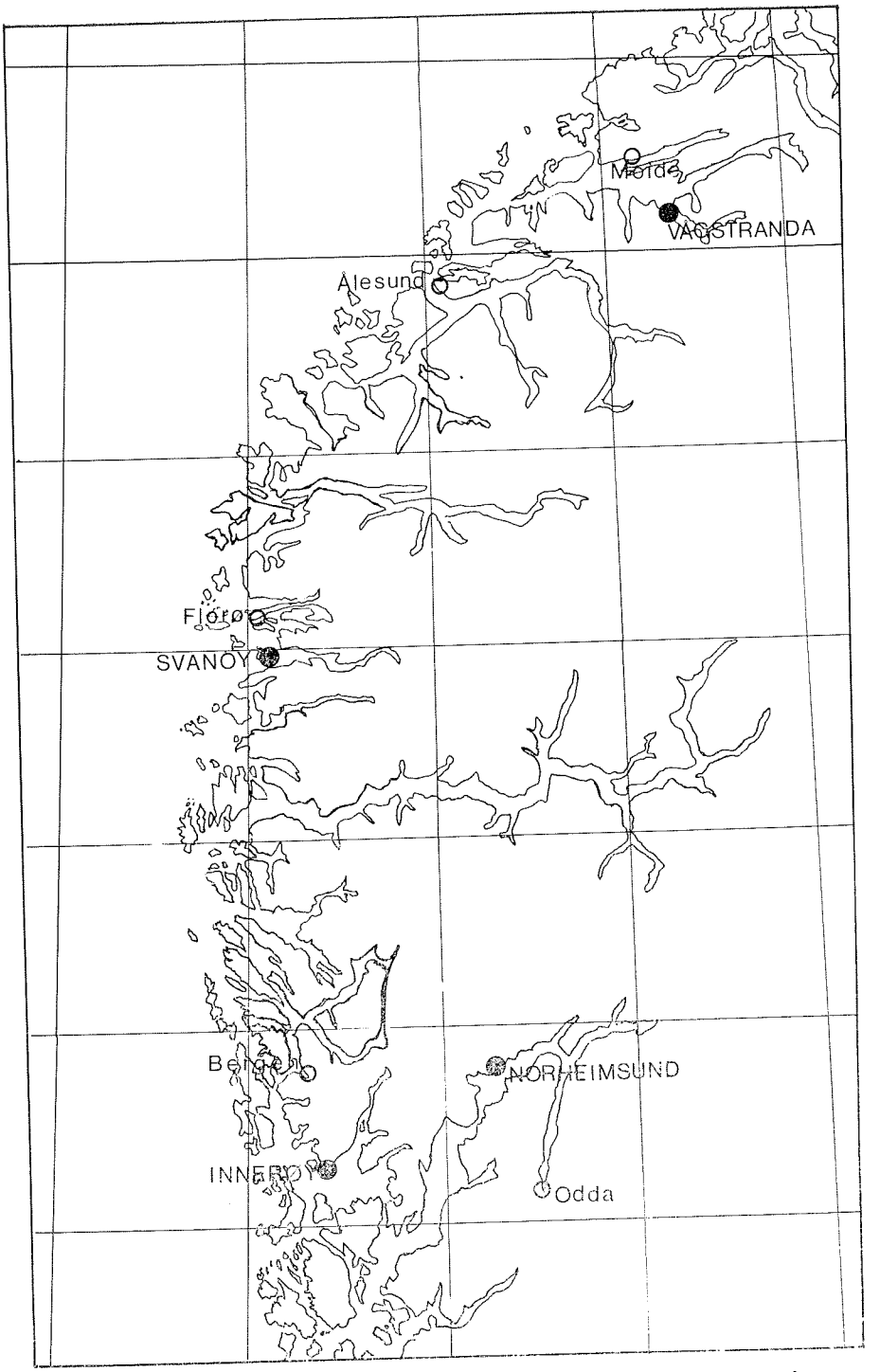


Fig. 1. Map of the western coast of Norway, showing the position of the oyster farm at Innerøy.

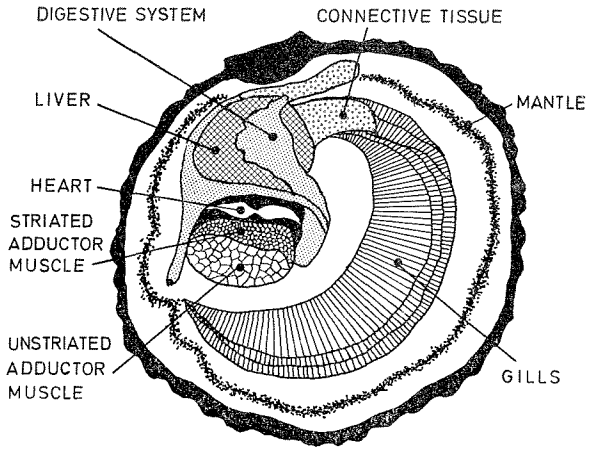


Fig. 2. Organs and tissues in oyster (*Ostrea edulis*).

ranged in age from twelve to eighteen years determined by counting the age-zones. All samples were transported alive to the laboratory and sorted.

Dissection

The shells were opened and the soft tissues dissected with the aid of stainless steel scissors. Only a few cuts were made to prevent contamination, and complete dissection of each organ could not be achieved. The digestive glands, stomach and intestine of oysters were pooled and named "digestive system" (Fig. 2). The white connective tissue consists mainly of labial palps and mouth. "Heart" consists of heart and pericardium. Eggs were collected in August from eight oysters which had loose-floating eggs. From *M. edulis* the soft tissue was dissected into foot (foot and foot retractor muscle), muscle (adductor muscle, byssal gland, byssal retractor muscle), mantle, gonades, gills and "digestive system" (consisting of heart, stomach, digestive gland, intestine and kidney) (Fig. 3). The shells of *M. modiolus* were shucked and the soft parts rinsed in distilled water, air dried and dissected into foot, white adductor muscle, dark (translucent) adductor muscle, mantle, gonades, gills and "digestive system" (heart, stomach, digestive gland, intestine and kidney) (Fig. 4). All tissue samples were freeze-dried, ground and stored in closed jars until analysis. The fluid was collected for the three species during the dissection procedures, particles sedimented by centrifugation and the supernatants were set aside for subsequent analysis.

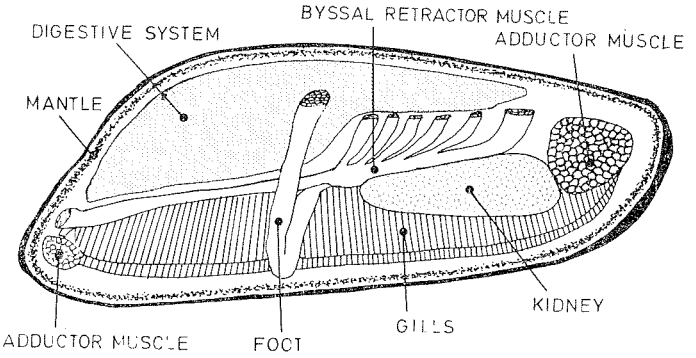


Fig. 3. Organs and tissues in common mussel (*Mytilus edulis*).

Analysis

All elements were determined by atomic absorption spectrophotometry (AAS). All elements except cobalt, arsenic, selenium and mercury were determined in an acid digest of nitric acid and perchloric acid. The procedure is described in Part I of this series (JULSHAMN, I, 1981). Aluminium, chromium, nickel, cadmium and lead were measured by flameless atomic absorption technique. The other elements were measured by flame atomic absorption.

For cobalt a dry ashing procedure was used. The white ash was dissolved in 0.1 N HCl, filtered and extracted into an organic system (JULSHAMN and BRAEKKAN, 1974). The element was measured in an air-acetylene flame. For arsenic the freeze-dried samples were soaked in a magnesium-solution used as ashing aid to improve the recovery of arsenic (UTHE et al., 1974). Samples for selenium and mercury determination

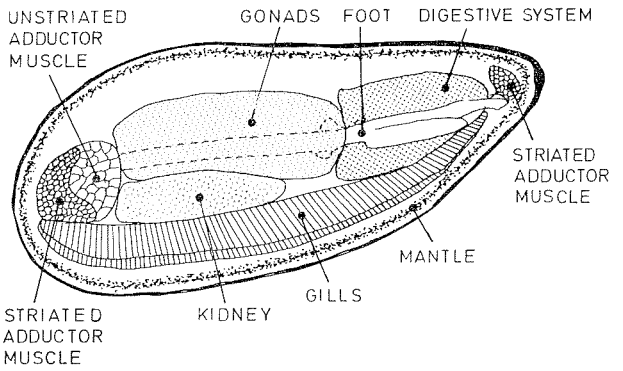


Fig. 4. Organs and tissues in horse mussel (*Modiolus modiolus*).

were digested simultanously by using nitric acid and sulphuric acid (1:1 v/v) containing 0.1% vanadium pentoxide (EGAAS and JULSHAMN, 1978).

Electrodeless discharge lamps (EDL) were used for arsenic, selenium and mercury. Mercury was measured by so-called "cold vapour" technique in a gas cuvette. A low-temperature argon-hydrogen flame was used for arsenic and selenium measurements. The solutions of arsenic and selenium were reduced by sodium boron hydride to give the corresponding hydrides which were vented into the flame. Prior to flameless analyses aliquotes of the nitric and perchloric acid digest were evaporated to dryness in platinum crucibles under an infrared lamp, and the residues were taken up in 1% nitric acid in acid-washed plastic tubes. This evaporation step must be introduced in methods for flameless atomic absorption determination (JULSHAMN, 1977), further, the flameless technique needs a standard addition procedure. The flameless measurements were carried out on a P–E Model 403 AAS, equipped with Deuterium Background Corrector, a Perkin-Elmer HGA-76 Graphite Furnace and a Model 056 Recorder. Conventional graphite tubes obtained from Perkin-Elmer were used. Single element Intensitron^R hollow cathode lamps were used for all elements investigated except aluminium for which a Scandinaviska Gen Tac AB hollow cathode lamp was used. All reagents were analytical grade. Stock solutions of all elements, 1000 mg/l, were obtained from Merck, Darmstadt. All test solutions were prepared immediately prior to use. Argon with a purity >99.9% was used to sheath the graphite tubes at a flow rate of 60 ml/min.

Ten microliter samples were introduced into the HGA-76 furnace with an Oxford Laboratories Sampler fitted with disposable plastic tips. Instrumental settings were in accordance with the manufacturers'

Table 1. Instrumental settings.

	Al	Cd	Cr	Ni	Pb
Wavelength, nm	309.3	228.8	357.9	232.0	283.3
Spectral Bandwidth, nm	0.7	0.7	0.7	0.2	0.7
Drying Temp. °C (Time, sec.)	105 (20)	105 (20)	105 (20)	105 (20)	105 (20)
Ramp Charring Temp., °C	450	350	450	450	450
Charring Temp., °C (Time, sec.)	1400 (20)	350 (20)	800 (20)	1000 (20)	450 (20)
Atomize Temp., °C (Time, sec.)	2650 (3)	1900 (3)	2650 (3)	2650 (3)	2080 (3)

recommendation with the exceptions given in Table 1. The charring and atomization temperatures and times were checked stepwise for each element investigated, and the optimal temperatures thus obtained are given in Table 1.

RESULTS AND DISCUSSION

Sodium and potassium

Na^+ together with Cl^- are the major electrolytes of the extracellular fluids and of the sea water environment. The lowest levels of sodium were found in the tissues of the digestive system, and the highest values in the tissues of mantle and gills (Table 2). These tissues regulate the uptake of salts and the body fluid osmolality. The concentration in the body fluids were lower than in the ambient water, with the exception of *M. modiolus* which had 15 g/l of sodium in the body fluid corresponding to the ambient water (JULSHAMN, I, 1981).

The concentrations of potassium varied less than those of sodium between the tissues. There was a close correspondance between the weight percentages of the body tissues and the contents of potassium as percentages of the total amounts. The highest difference was found in the tissues of *O. edulis* with 8 g/kg in the digestive system and 13 g/kg in the heart. The potassium level in the ambient sea water was 0.40 g/l (JULSHAMN, I, 1981), whereas the concentrations of potassium in the body fluids varied between 0.47 and 1.2 g/l.

The equivalent ratio between the sodium and potassium contents were highest in gills and mantle and lowest in the digestive system. The equivalent ratio in the digestive system of *O. edulis* was less than one.

Magnesium and calcium

The highest contents of magnesium were found in the gills for the three species studied (Table 2). There was a clear accumulation of Mg in the gills which represented 8–12% of the total tissue weight, but 20–30% of the total magnesium in the three species. High values were also recorded in the mantle of *M. edulis* and *M. modiolus* and in the heart in *O. edulis*.

The gills and mantle of *O. edulis* and *M. edulis* were high in calcium whereas in *M. modiolus* the digestive system accounted for 77% of the total calcium. For *O. edulis* and *M. edulis* the gills represented 37–38% of the total calcium, whereas the digestive system accounted for 17–18%. The highest Ca concentration recorded, 19 g/kg, was found in the gonades

Table 2. Tissue weights and contents of major elements in tissues and body fluid (g/l) of oyster (*Ostrea edulis*), common mussel (*Mytilus edulis*) and horse mussel (*Modiolus modiolus*).

Tissue	(g) dry weight per kg sample	Tissue dry weight g	%*	Na g/kg**	Na %***	K g/kg	K %	Mg g/kg	Mg %	Ca g/kg	Ca %
Oyster, total sample weight 13.2 g											
Striated adductor muscle	283	0.23	*7*	16	*9*	9.6	*8*	2.6	*11*	2.4	*5*
Unstriated adductor muscle	268	0.26	*8*	15	*10*	12	*11*	2.0	*9*	1.6	*4*
Gonades				27		7.1		4.2		19	
Mantle	181	0.18	*6*	27	*12*	11	*7*	2.5	*8*	8.9	*14*
Gills	142	0.34	*11*	31	*27*	11	*13*	4.8	*28*	13	*37*
Heart	695	0.03	*1*	26	*2*	13	*1*	5.2	*3*	6.0	*2*
Connective tissue	325	0.79	*25*	8.0	*16*	8.2	*22*	1.2	*17*	2.5	*17*
Digestive system	319	1.37	*43*	6.9	*24*	8.1	*38*	1.1	*25*	1.5	*18*
Body fluid				9.8		0.76		1.3		0.40	
Remainder 1.3 g											
Common mussel, total sample weight 7.6 g											
Adductor muscle	291	0.29	*22*	17	*19*	12	*21*	2.4	*19*	0.79	*12*
Foot	257	0.06	*4*	16	*4*	13	*5*	2.5	*4*	0.85	*3*
Gonades	274	0.38	*28*	10	*15*	11	*27*	1.5	*15*	0.59	*12*
Mantle	161	0.14	*10*	36	*20*	12	*11*	4.9	*19*	2.7	*20*
Gills	135	0.16	*12*	45	*28*	12	*13*	6.7	*29*	4.5	*38*
Digestive system	301	0.32	*24*	11	*14*	11	*23*	1.6	*14*	0.92	*16*
Body fluid						0.47		1.3		0.80	
Remainder 1.9 g											

Horse mussel, total sample weight 22.2 g												
Striated adductor muscle	233	0.35	*9*	19	*7*	9.3	*8*	2.7	*6*	1.2	*2*	
Unstriated adductor muscle	242	0.65	*17*	13	*9*	10	*17*	2.2	*9*	0.66	*2*	
Foot	228	0.64	*17*	18	*12*	11	*17*	2.7	*11*	1.0	*4*	
Gonades	161	0.30	*8*	35	*11*	11	*8*	4.5	*9*	2.1	*4*	
Mantle	134	0.35	*9*	42	*15*	10	*9*	6.1	*14*	2.6	*5*	
Gills	97	0.32	*8*	65	*22*	10	*8*	9.6	*20*	3.5	*6*	
Digestive system	228	1.26	*33*	19	*25*	9.8	*32*	3.6	*30*	11	*77*	
Body fluid				15		1.2		1.1		0.56		
Remainder 1.9 g												

* Tissues as percent of total body weight.

** Dry weight.

***The total contents of the tissues as percent of total element in the organism.

of *O. edulis*. In all tissues of *O. edulis* except muscle, the magnesium levels were lower than the calcium levels, whereas in the two other molluscs the opposite was the case, with the exception of the digestive system of *M. modiolus*. As for potassium higher values of magnesium and calcium were found in the body fluid than in the ambient water, and the magnesium values were 1.5 to 3 times higher than the calcium values.

Essential elements (Cr, Mn, Fe, Co, Cu, Zn, Se) Table 3

The muscle tissues accounted for 30 to 40% of the total chromium in the three species, an accumulation ratio of 1.7–2 over the tissue average. Low levels were found in the gonades and mantle. This may correspond with the finding that the chromium content in fish muscle increased significantly when the chromium level in the ambient water increased (JULSHAMN et al., 1978 c). SEGAR et al. (1971) found lower values for *M. modiolus* than those reported here. Chromium (III) is the nutritionally required form, and no efforts have been made to separate chromium of different oxidation states.

Substantially higher manganese contents were found in *M. modiolus* than in the other species. The exceptional high content of 2.2 g/kg was found in the digestive system, and this tissue accumulated 92.5% of the total manganese in *M. modiolus*. As early as 1910 BRADLEY reported high manganese contents in *M. modiolus*. SEGAR et al. (1971) also found high manganese levels in *M. modiolus* tissues, but did not find a particularly high concentration in the digestive system. JULSHAMN and ANDERSEN (1981 b) found that the manganese content of the digestive system was associated with the nuclei fraction of the cells. *O. edulis* accumulated Mn in the gills, which had 40 mg/kg, whereas the levels of manganese were generally low in *M. edulis*.

Relatively wide variations were found in the iron contents of the tissues studied. The highest values were recorded in the digestive system of *M. edulis* and *M. modiolus*, the only accumulating tissue of these species, representing 61 and 71% of the total iron respectively. None of the tissues of *O. edulis* accumulated iron, and the highest concentration was found in the heart. The high concentration levels in the muscle of *O. edulis* reported by BROOKS and RUMSEY (1965) could not be confirmed in the present study. According to GEORGE et al. (1976), the major routes of iron uptake in *M. edulis* are through the gills, gut and membrane-bound vesicles in kidney. About 30% of the iron in the gut is not adsorbed but voided as faeces, whereas the absorbed iron is transported to other tissues by the haemolymph, and deposited in the byssal threads. There is little

Table 3. Contents of essential elements in tissues and body fluid (mg/l) of oyster (*Ostrea edulis*), common mussel (*Mytilus edulis*) and horse mussel (*Modiolus modiolus*).

Tissues	Cr		Mn		Fe		Co		Cu		Zn		Se	
	mg/kg*	%**	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
Oyster														
Striated adductor muscle	1.7	*11*	4.3	*2*	65	*4*	0.09	*2*	27	*3*	3700	*4*	1.1	*2*
Unstriated adductor muscle	2.5	*19*	6.4	*5*	48	*4*	0.11	*3*	14	*2*	3100	*4*	1.7	*3*
Gonades	0.45		12		75		0.05		16		1100		1.8	
Mantle	0.72	*4*	36	*14*	150	*8*	0.19	*3*	180	*14*	13000	*12*	3.1	*2*
Gills	0.90	*9*	40	*30*	130	*13*	0.25	*10*	150	*23*	13000	*23*	5.3	*15*
Heart	5.3	*5*	9.8	*1*	360	*3*	0.88	*3*	280	*4*	23000	*4*		
Connective tissue	1.1	*25*	14	*25*	88	*20*	0.11	*10*	51	*18*	4200	*20*	1.3	*8*
Digestive system	0.72	*28*	8.2	*24*	140	*48*	0.44	*67*	62	*38*	4600	*33*	6.2	*69*
Body fluid	0.10		0.54		6		<0.01		2.4		98		n.d.	
Common mussel														
Adductor muscle	4.2	*38*	4.2	*15*	55	*10*	0.28	*11*	1.4	*5*	150	*24*	1.0	*12*
Foot	2.9	*5*	6.0	*5*	25	*1*	0.25	*3*	2.1	*1*	91	*3*	0.73	*2*
Gonades	1.9	*22*	4.0	*19*	50	*11*	0.10	*5*	6.2	*27*	47	*10*	0.83	*13*
Mantle	1.4	*6*	5.2	*9*	45	*4*	0.33	*7*	3.5	*2*	100	*8*	1.1	*6*
Gills	0.90	*4*	9.2	*18*	140	*13*	0.66	*15*	8.6	*15*	190	*16*	5.6	*38*
Digestive system	2.5	*25*	8.5	*34*	320	*61*	1.40	*61*	14	*50*	230	*39*	2.1	*29*
Body fluid	0.20		0.20		4		<0.01		0.80		6.6		<0.10	
Horse mussel														
Striated adductor muscle	3.5	*17*	270	*3.2*	43	*2*	<0.35		4.1	*2*	360	*0.5*	0.29	*1*
Unstriated adductor muscle	2.6	*24*	44	*1.0*	57	*3*	<0.20		2.9	*2*	330	*0.9*	0.51	*3*
Foot	1.5	*13*	51	*1.1*	74	*4*	0.25	*2*	4.2	*3*	450	*1.1*	0.69	*4*
Gonades	0.90	*4*	70	*0.7*	320	*8*	<0.15		2.6	*1*	510	*0.6*	1.1	*3*
Mantle	1.1	*5*	28	*0.3*	150	*5*	0.13	*1*	8.6	*3*	310	*0.4*	1.0	*4*
Gills	1.2	*5*	120	*1.3*	260	*7*	0.85	*8*	11	*4*	870	*1.1*	5.7	*19*
Digestive system	1.8	*32*	2200	*92.5*	660	*71*	9.5	*89*	62	*85*	19000	*95.4*	4.9	*65*
Body fluid	0.10		9.1		4		0.01		0.90		81		n.d.	

* Dry weight.

**The total contents of the tissue as percent of total element in the organism.

free iron within the cytoplasma (GEORGE et al., 1976; JULSHAMN and ANDERSEN, 1980 b).

The digestive system accumulated resp. 67, 61 and 98% of the total cobalt content in *O. edulis*, *M. edulis* and *M. modiolus*. Correspondingly, the highest concentrations of vitamin B_{12} were found in the digestive systems (JULSHAMN and BRAEKKAN, unpublished data). SEGAR et al. (1971) reported the highest cobalt levels in gills and mantle of *M. modiolus*, whereas PENTREATH (1973) reported the highest values in stomach, digestive gland and gonades of *M. edulis*. Marine invertebrates from unpolluted waters may accumulate many times the amount of cobalt found as part of vitamin B_{12} in the tissues (JULSHAMN and BRAEKKAN, unpublished data). The low contents of cobalt in the gonades were in agreement with data from ovaries of *Salmo salar* (JULSHAMN and BRAEKKAN, 1975) and *Gadus morrhua* (JULSHAMN and BRAEKKAN, 1976).

Much higher copper concentrations were found in the heart, mantle and gills of *O. edulis* than in any other tissue examined. The mantle and gills of *O. edulis*, representing 17% of the total tissues, accounted for 37% of the total copper. This is in accordance with published data (IKUTA, 1967; WOLFE, 1970; ROMERIL, 1971). The digestive system accounted for the major part of copper in *M. edulis* and *M. modiolus* with 50% and 85% of the total copper, respectively. A corresponding accumulation was not found in *M. modiolus* by SEGAR et al. (1971) or in *M. edulis* by DELHAYE and CORNET (1975). Tissues accumulating copper have been reported in other mussel species, e.g. the hepatopancreas of *Crangon vulgaris* by DJANGMAH (1970) and the kidney of *Chlamys* and the digestive gland of *Pecten* by BRYAN (1973). Different responses to copper of different mollusc species as reported by MANLEY and DAVENPORT (1979) may influence the copper distribution in species from low ambient water levels. The high levels of copper in gills and mantle of *O. edulis* was explained by a high accumulation of copper binding amoebocytes in these tissues (GEORGE et al., 1978). The copper contents in *O. edulis* were found equally distributed between the cytosol and particulate fraction of the cells in liver and the digestive system (JULSHAMN and ANDERSEN, V, 1981). The high contents of copper in the digestive system of *M. edulis* and *M. modiolus* may be concentrated together with iron and lead in membrane limited vesicles in epithelial cells, mainly in the kidney (GEORGE et al., 1976). Nevertheless, a major part of the copper in the digestive system of *M. edulis* and *M. modiolus* was found in the cell cytosol fraction by JULSHAMN and ANDERSEN, (V, 1981).

The zinc contents are very high in *O. edulis* tissues (JULSHAMN, I, 1981). The extreme value of 23 g/kg, only comparable to that of sodium, was found in the heart. The gills accounted for 23% of the total body zinc,

and had the highest accumulation of this element. These findings differ from those reported by COOMBS (1972), who found no organs concentrating zinc over the body average. On the other hand, the results agree well with the findings by GEORGE et al. (1978). There is a discrepancy between total tissue content of zinc and enzyme-bound zinc in *O. edulis* in that only 5% of the total zinc corresponds to the amount of the enzyme-bound zinc or to that found associated to a high molecular protein in the cell cytosol fraction (COOMBS, 1972; JULSHAMN and ANDERSEN, 1981 b). Similar relations were reported from *Crassostrea virginica* (WOLFE, 1970). Zinc and copper were similarly distributed between the tissues of *O. edulis*, even if the two elements play different roles in biochemical systems.

The tissues of *M. edulis* had much lower contents of zinc than those of *O. edulis*, and the element was evenly distributed through most of the tissues examined with some accumulation in the digestive system, and a low level in the gonades. This illustrates the species difference between the two mussels, living literally side by side. The digestive system of *M. modiolus* was particularly rich in zinc, with a concentration of 19 g/kg. More than 95% of the total zinc content in *M. modiolus* was deposited in this tissue. The major part of the zinc content in digestive system could be related to the particulate fraction in a cell fractionating study by JULSHAMN and ANDERSEN, (V, 1981). COOMBS and GEORGE (1978) suggested that zinc was associated with a lysosomal-vacuolar detoxification system in the digestive system of *M. edulis*.

The highest selenium concentrations were found in the gills with 5–6 mg/kg, and in the digestive system with 2–6 mg/kg in the three species. FOWLER and BENAYOUN (1976) have reported a similar distribution of selenium in the tissues of *Mytilus galloprovincialis* obtained from unpolluted water. Corresponding selenium levels were found in other samples of marine origin (LUNDE, 1970; JULSHAMN et al., 1978 a,b). Marine organisms appear to have higher contents of selenium than terrestrial animals (LUNDE, 1970), but few data are available on the distribution of selenium in the tissues of most marine organisms. Selenium has received much attention as an essential element, and also as an extremely toxic element comparable with mercury and cadmium. Further information, however, is required to assess selenium transport paths and accumulation mechanisms in different organisms. The lipid-soluble selenoorganic compounds described by LUNDE (1973 a) may be an important contribution in this field. Recently, increasing attention has been given to the relationship between selenium and mercury in marine organisms (GANTHER et al. (1972); MACKAY et al. (1975); KOEMAN et al. (1973); MARTIN et al. (1976); GLICKSTEIN (1978).

Table 4. Contents of some minor elements in tissues and body fluids (mg/l) of oyster (*Ostrea edulis*), common mussel (*Mytilus edulis*) and horse mussel (*Modiolus modiolus*).

Tissues	Al		Ni		As		Cd		Hg		Pb	
	mg/kg*	%**	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
Oyster												
Striated adductor muscle	12	*10*	5.8	*12*	3.1	*3*	4.5	*4*	0.48	*18*	0.85	*3*
Unstriated adductor muscle	15	*14*	1.9	*4*	6.9	*6*	7.5	*8*	0.17	*6*	0.72	*3*
Gonades	3.3		2.8		4.8		1.5		0.05		2.0	
Mantle	15	*10*	2.5	*5*	15	*10*	5.9	*4*	0.27	*8*	1.2	*3*
Gills	21	*27*	1.6	*4*	11	*13*	10	*13*	0.25	*15*	1.8	*9*
Heart	n.d.		n.d.		13	*1*	21	*2*	n.d.		<0.10	
Connective tissue	3.5	*10*	1.6	*12*	6.2	*17*	7.5	*22*	0.13	*16*	2.4	*28*
Digestive system	5.6	*29*	5.2	*63*	10	*50*	9.2	*48*	0.17	*37*	2.8	*55*
Body fluid	n.d.		n.d.		n.d.		0.25		n.d.		<0.20	
Common mussel												
Adductor mussel	27	*19*	2.0	*21*	2.1	*5*	0.90	*11*	0.04	*8*	3.2	*10*
Foot	33	*5*	1.4	*4*	6.3	*3*	2.7	*7*	0.05	*2*	4.0	*3*
Gonades	6.0	*6*	0.90	*11*	12	*20*	0.63	*10*	0.02	*6*	4.4	*18*
Mantle	26	*9*	2.0	*11*	6.8	*8*	1.2	*7*	0.18	*23*	3.6	*5*
Gills	34	*13*	2.8	*18*	15	*18*	3.3	*23*	0.16	*23*	22	*37*
Digestive system	60	*48*	3.2	*36*	20	*47*	3.0	*42*	0.17	*38*	8.2	*27*
Body fluid	n.d.		n.d.		n.d.		0.02		<0.01		n.d.	
Horse mussel												
Striated adductor muscle	7.9	*10*	1.5	*4*	6.5	*4*	0.76	*0.3*	0.27	*2*	2.5	*0.7*
Unstriated adductor muscle	7.4	*17*	1.9	*10*	7.1	*8*	1.1	*0.7*	0.33	*4*	5.2	*2.5*
Foot	11	*25*	1.2	*6*	8.0	*8*	1.6	*0.9*	0.52	*7*	4.7	*2.2*
Gonades	18	*20*	1.5	*4*	27	*13*	0.95	*0.3*	0.69	*4*	2.4	*0.5*
Mantle	9.0	*12*	3.2	*9*	36	*21*	1.4	*0.5*	1.3	*9*	0.5	*0.2*
Gills	4.5	*5*	2.1	*6*	19	*10*	7.1	*2.0*	0.39	*2*	6.1	*1.5*
Digestive system	2.3	*11*	6.0	*61*	17	*36*	82	*95*	2.8	*71*	100	*92.5*
Body fluid	0.8		<0.5		n.d.		0.40		0.04		1.3	

* Dry weight.

**The total contents of the tissues as percent of total element in the organism.

Non-essential elements (Al, Ni, As, Cd, Hg, Pb)

The aluminium concentrations in *O. edulis* varied between 3 and 21 mg/kg (Table 4). Similar levels were found in *M. modiolus* tissues, whereas the tissues of *M. edulis* had higher concentrations of aluminium ranging from 6 to 60 mg/kg. The highest tissue concentrations as well as accumulation of aluminium were found in the gills of *O. edulis,* the digestive system of *M. edulis* and in the foot and gonades of *M. modiolus.* Few data on aluminium in marine molluscs are available, and few comparisons could be made. The only new data available were reported for tissues of *M. modiolus* by SEGAR et al. (1971), who found values more than 10 times higher than those given in Table 4. The contents of aluminium in foods of plant origin depend upon the soil, and range from 0.2 to 50 mg/kg dry weight, whereas the contents of foods of animal origin are shown to be lower (UNDERWOOD, 1977). Until recently, aluminium has been regarded as a harmless element, but new experiences in clinical medicine have suggested a toxic effect of aluminium on human and animal tissues (BERLYNE et al., 1970, 1972 and CRAPPER et al., 1976).

The distribution pattern of nickel followed to some extent that of the essential elements, and the digestive system accumulated and accounted for the major part of the tissue element content. Further accumulation of nickel was found in the muscle tissue of *O. edulis* and the gills of *M. edulis.* The tissue levels may indicate low levels in the water according to FRIEDRICH and FILICE (1976), whose values from *M. edulis* were higher than those in Table 4, whereas the values reported by SEGAR et al. (1971) from *M. modiolus* corresponded well with the present results. Mussels as food had nickel contents in the same range according to GEERT et al. (1978). In most of their samples the nickel content was less than 0.5 mg/kg fresh weight.

The highest levels of arsenic were found in mantle and gonades of *M. modiolus,* in mantle and heart of *O. edulis* and in the digestive system, gills and gonades of *M. edulis.* The lowest values were found in muscle and foot tissues. Accumulation over the tissue averages were found only in the mantle of *M. modiolus* and the digestive system of *M. edulis.* The concentration levels reported in Table 4 for *O. edulis* and *M. edulis* fell within the main range (1.0–4.0 mg/kg fresh weight), whereas those for *M. modiolus* fell within the high range (>4 mg/kg) according to the ranges proposed for fish and fish products by JULSHAMN et al. (1978 a). From that listing, crustacean products and pelagic fish had arsenic levels in the high range, corresponding to available arsenic and feeding habits (VINOGRADOV, 1953; BOHN and MCELROY, 1976). Certain marine organisms probably synthesize arseno-organic compounds from inorganic arsenic, whereas no

evidence points to a corresponding ability in terrestrial organisms (VASKOVSKY et al., 1972; LUNDE, 1970, 1973 a,b; PENROSE, 1975). LUNDE (1973 b) found that the arsenic in marine organisms was present both as water-soluble and lipid-soluble arseno-organic compounds. Recently, EDMONDS and FRANCESCONI (1977) identified an organic form of arsenic in Australian rock lobster (*Panulinus longgipes cygnus*) as arsenobetaine. There seems to be a broad agreement that the arsenic in marine organisms is not found as inorganic ions (arsenite/arsenate), and that the organic forms of arsenic probably are less toxic than the inorganic ions. However, a discussion published in 1972 in Fishing News International concludes that there is confusion in this matter.

The tabulated values of cadmium showed no specific burden in organs of *O. edulis* nor in *M. edulis*, whereas the digestive system of *M. modiolus* had the high concentration of 82 mg/kg, which accounted for 95% of the total cadmium burden in the body. This agree well with data given by JULSHAMN and ANDERSEN (V, 1981). Correspondingly high values were not reported in the gut and digestive glands of *M. modiolus* by SEGAR et al. (1971). In *O. edulis* the highest content was found in the heart tissue with 21 mg/kg. BROOKS and RUMSEY (1967) found that the heart had the ability to accumulate cadmium. The body fluid cadmium level was particularly low in *M. edulis*. The values found in *O. edulis* were similar to those reported in paper I and II (JULSHAMN, I, II, 1981), whereas those in *M. edulis* were lower than those reported in paper III, (JULSHAMN, III, 1981). Cadmium levels in molluscs used as food, even from unpolluted waters, are among the highest known in foods. The concentration levels found in molluscs in the present work were a hundred times higher than those reported for fish and fish products (JULSHAMN et al., 1978 a,b). The wide variations in cadmium contents among species may be associated with different metal binding protein systems (CHOU et al., 1978; GEORGE et al., 1979; RIDLINGTON and FOWLER, 1979; GEORGE and PIRIE, 1979; MARSHALL and TALBOT, 1979; JULSHAMN and ANDERSEN, V, 1981; JULSHAMN, VI, 1981).

Low concentrations of mercury were found in the tissues of *O. edulis* and *M. edulis*, whereas substantially higher levels were obtained in tissues of *M. modiolus*, particularly in the digestive system and the mantle. The mercury contents found in the present study correspond well with those reported in fish and fish product from the North Sea. Low mercury levels were found in gonades, foot and muscle tissue with the exception of a surprisingly high values in the striated adductor muscle in *O. edulis* (0.48 mg/kg), accounting for 18% of the total body mercury. High mercury contents have been found in the muscle of pelagic fish (JULSHAMN et al., 1978 c), where the mercury is bound mainly as methyl mercury.

JERNELÖV and LANN (1971) found that little methyl mercury was excreted and that eventually it was stored in muscle and brain tissues of fish. It may be worth noting that while the gills of *O. edulis* and *M. edulis* showed some accumulation of mercury, the gills of the sanddwelling *M. modiolus* had a very low mercury content, and a high accumulation in the digestive system.

Most tissues of *O. edulis* had low lead concentrations, consistent with other results from these studies (JULSHAMN, I, VI, 1981). The connective tissue and digestive system accounted for the major part, 83%, of the total lead in *O. edulis*. In *M. edulis* the gills was the only accumulating organ, and held 37% of the total body content. This distribution of lead was comparable with that of selenium and calcium. MARSHALL and TALBOT (1978) reported an equiatomic ratio between lead and calcium in a lead loaded system. They found lead as crystalline extracellular deposits in the capillary wall, and assumed that Pb occurred with Ca as a mixed complex carbonate. They further suggested that Pb was transported rapidly through the cells and deposited extracellularly in an inert form. JULSHAMN and ANDERSEN (1980 a,b) found that lead was bound to the particulate fraction of the cells. A striking feature was a lead concentration of 100 mg/kg, accounting for 92.5% of the total body lead in the digestive system of *M. modiolus*. Correspondingly a high concentration of calcium was found in the digestive system of *M. modiolus*.

CONCLUSIONS

Knowledge of the accumulation of elements in the digestive system relative to the gills and mantle is of value in elucidating the uptake routes in molluscs. For the sake of convenience, the accumulation ratio of an element in a tissue may be defined as the ratio between the content of an element in a tissue as percentage of the total element content in the mollusc, and the weight percentage of this tissue of the total soft part of the mollusc. The following conclusions may then be drawn from the tables 2–4:

1. In the sand-dwelling horse mussel, *M. modiolus*, 10 of the 13 minor elements analysed accumulated in the digestive system with an average accumulation ratio of 2.5, and this tissue held 61 to 98% of the total element contents of these ten in the mollusc. Seven of the ten elements: manganese, iron, cobalt, zinc, cadmium, mercury and lead showed exceptionally high values in the digestive system of *M. modiolus*. Among these values were: 2.2 g/kg of manganese, 19 g/kg of zinc and 100 mg/kg of lead. Of the remaining three elements, chromium accumulated in the muscle (see below) aluminium in the gonades and in the foot, and arsenic in the gonades and the mantle.

2. In the common mussel, *M. edulis*, taken from the hanging baskets, 9 of the 13 minor elements analysed had an average accumulation ratio of 2 in the digestive system and the tissue held 34 to 61% of these elements. The three elements selenium, mercury and lead showed an accumulation ratio of 2 in the gills and mantle tissue, whereas chromium accumulated in the muscle. Two high values may be noted: 60 mg/kg of aluminium in the digestive system, and 22 mg/kg of lead in the gills.

3. In oysters, *O. edulis*, taken from the same basket as the *Mytilus*, only four minor elements accumulated in the digestive system with an average ratio of 1.5: cobalt, selenium, nickel and lead. Four elements accumulated in the gills/mantle tissue with an average ratio of 2.25: manganese, copper, zinc and aluminium. Iron, arsenic and cadmium were equally distributed over all tissues, whereas chromium accumulated in the muscle and the heart and mercury in the muscle tissue. Copper and zinc, of which *O. edulis* is known to hold high amounts, (JULSHAMN, I, 1981) were found accumulated in the gills and the mantle with a ratio of 3, and with concentrations of 150–180 mg/kg of copper and 13 g/kg of zinc. However, the highest concentrations of these elements were found in the heart, with 280 mg/kg of copper and 23 g/kg of zinc.

4. Chromium was the only element accumulating in the muscle tissue in all three molluscs, with an accumulation ratio of 1.7 to 2, and representing 30 to 40% of the total chromium content in the molluscs.

5. A particular mention may be given cadmium, mercury and lead, of interest in pollution studies. All three accumulated with high levels in the digestive system of *M. modiolus*, they accumulated in both the digestive system and the gills in *M. edulis*, where the level of lead in the gills was the only high value, and lastly, no particular accumulation was seen for the three elements in *O. edulis*, with the exception of the high level of 21 mg/kg of cadmium in the heart, and the accumulation ratio of 2.5 for mercury in the muscle tissue (0.5 mg/kg).

6. Of the four major elements analyzed, sodium and magnesium accumulated mainly in the gills and the mantle, so did calcium in *O. edulis* and *M. edulis*, whereas in *M. modiolus*, calcium accumulated in the digestive system. Potassium, as an intracellular element, showed an equal distribution over the tissues.

REFERENCES

BERLYNE, G.M., BEN ARI, J., PEST, D., WEINBERGER, J., STERN, M., GILMORE, G.R. and LEVINE, R., 1970. Lancet No. 1, 494–496.

BERLYNE, G.M., YAGIL, R., BEN ARI, J., WEINBERGER, J., KNOPF, E. and DANOSTICH, G.M., 1972. Lancet No. 2, 564–567.

BOHN, A. and MCELROY, R.O., 1976. J. Fish. Res. Board. Can. *33*, 2836.

Bradley, H.C., 1910. J. Biol. Chem. *8*, 237.
Bryan, G.W., 1973. J. Mar. Biol. Ass. U.K. *53*, 145–166.
Brooks, R.R. and Rumsey, M.G., 1965. Limnol. Oceanogr. *10*, 521–527.
Brooks, R.R. and Rumsey, M.G., 1967. Aust. J. Mar. Freshwat. Res. *15*, 53–61.
Chou, C.L., Uthe, J.F. and Zook, E.G., 1978. J. Fish. Res. Board. Can. *3*, 409–413.
Coombs, T.L., 1972. Mar. Biol. *12*, 170–178.
Coombs, T.L. and George, S.G., 1978. Proceedings of the 12th European Symposium on Marine Biology, Stirling, Scotland, 1977 (Ed. D.S. Mclusky and A.J. Berry), pp. 179–187. Oxford and New York: Pergamon Press.
Crapper, D.R., Krishnan, S.S. and Quittkat, S., 1976. Brain, *99*, 67–80.
Delhaye, W. and Cornet, D., 1975. Comp. Biochem. Physiol, *50A*, 511–518.
Djangmah, J.S., 1970. Comp. Biochem. Physiol. *32*, 709–731.
Edmonds, J.S. and Francesconi, K.A., 1977. Nature *265*, 436.
Egaas, E. and Julshamn, K., 1978. At. Absorption Newsl. *17*, 135–138.
Fishing News International, 1972, *11*, 73 and 88.
Fowler, S.W. and Benayoun, G., 1976. Bull. Environ. Contam. Toxicol. *16*, 339–346.
Friedrich, A.R. and Filice, F.P., 1976. Bull. Environ. Contam. Toxicol. *16*, 750–755.
Ganther, H.E., Gondie, C., Sunde, M.L., Kopechy, M.J., Wagner, P., Sang-Hwan, O.H. and Holkstra, W.G., 1972. Science *175*, 1122.
Geert, E., Bosch-Tibbesma, G. van den, Douma, F.F., 1978. Z. Lebensm. Unters. Forsch. *166*, 145–147.
George, S.G., Pirie, B.J.S. and Coombs, T.L., 1976. J. Exp. Mar. Biol. Ecol. *23*, 71–84.
George, S.G., Pirie, B.J.S., Cheyne, A.R., Coombs, T.L. and Grant, P.T., 1978. Mar. Biol. *45*, 147–156.
George, S.G. and Pirie, B.J.S., 1979. Biochim. Biophys. Acta, *580*, 234–244.
George, S.G., Carpene, E., Coombs, T.L., Overnell, J. and Youngson, H., 1979. Biochim. Biophys. Acta *580*, 225–233.
Glickstein, N., 1978. Mar. Biol. *49*, 113–117.
Ikuta, K., 1967. Bull. Jap. Soc. Scient. Fish. *33*, 405–409.
Jernelöv, H. and Lann, H., 1971. Oikos *22*, 408.
Julshamn, K. and Braekkan, O.R., 1974. At. Absorption Newslett. *12*, 139.
Julshamn, K. and Braekkan, O.R., 1975. Comp. Biochem. Physiol. *52B*, 381.
Julshamn, K. and Braekkan, O.R., 1976. Fisk. Dir. Skr., Ser. Ernæring *1*, 1–15.
Julshamn, K., 1977. At. Absorption Newslett. *16*, 149–150.
Julshamn, K., Haugsnes, J. and Utne, F., 1978a. Fisk. Dir. Skr., Ser. Ernæring, *1*, 117–135.
Julshamn, K., Haugsnes, J. and Eriksen, J., 1978b. Fisk. Dir. Skr., Ser. Ernæring, *1*, 137–144.
Julshamn, K., Haugsnes, J. og Secher, K., 1978c. Rapport Nr. 2/78 Fiskeridirektoratets Vitamininstitutt, Bergen.
Julshamn, K., 1981. Studies on Major and Minor Elements in Molluscs in Western Norway. I, II, III, VI, VII, Fisk. Dir. Skr., Ser. Ernæring, *1*, No. 5.
Julshamn, K. and Andersen, K.J., 1981a. Studies on Major and Minor Elements in Molluscs in Western Norway. V, Fisk. Dir. Skr., Ser. Ernæring, *1*, No. 5.
Julshamn, K. and Andersen, K.J., 1981b, to be published.
Koeman, J.J., Peeters, W.H.M., Koudstaal, C.H.M., Tjioe, P.S. and De Goeij, J.J.M., 1973. Nature *245*, 385.
Lunde, G., 1970. J. Sci. Fd. Agric. *21*, 242–247.
Lunde, G., 1973a. J. Sci. Fd. Agric. *24*, 413–419.
Lunde, G., 1973b. J. Sci. Fd. Agric. *24*, 1021–1027.

MACKAY, N.J., KAZACOS, M.N., WILLIAMS, R.J. and LEEDOW, M.I., 1975. Mar. Pollut. Bull. *6*, 57–61.

MANLEY, A.R. and DAVENPORT, J., 1979. Bull. Environ. Contam. Toxicol. *22*, 739–744.

MARSHALL, A.T. and TALBOT, V., 1979. Chem. Biol. Interactions. *27*, 111–123.

MARTIN, J.H., ELLIOTT, P.D., ANDERLINI, V.C., GIRVIN, D., JACOBS, S.A., RISEBROUGH, R.W., DELONG, R.L. and GILMARTIN, W.G., 1976. Mar. Biol. *35*, 91–104.

PENROSE, W.R., 1975. J. Fish. Res. Board. Can. *32*, 2385.

PENTREATH, R.J., 1973. J. Mar. Biol. Ass. U.K. *53*, 127–143.

RIDLINGTON, J.W. and FOWLER, B.A., 1979. Chem. Biol. Interactions. *25*, 127–138.

ROMERIL, M.G., 1971. Mar. Biol. *9*, 347–354.

SEGAR, D.A., COLLINS, J.D. and RILEY, J.P., 1971. J. Mar. Biol. Ass. U.K. *51*, 131–136.

UNDERWOOD, E.J., 1977. Trace Elements in Human and Animal Nutrition, e. ed., Academic Press, London.

UTHE, J.F., FREEMAN, H.C., JOHNSTONE, J.R. and MICHALIK, P., 1974. J. AOAC *57*, 1363–1365.

VASKOWSKY, V.E., KOROTCHENKO, O.D., KOSHELEVA, L.P. and LEVIN, V.S., 1972. Comp. Biochem. Physiol. *41B*, 777.

VINOGRADOV, A.P., 1953. "The Elementary Chemical Composition of Marine Organisms". Transl. from the Russian. Sears Found. Mar. Res., Mean II, 647 pp. Yale Univ.

WOLFE, D.A., 1970. J. Fish. Res. Bd. Can. *27*, 47–57.

Fisk. Dir. Skr., Ser. Ernæring, Vol. 1: No. 5, 1981, s 235–245

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

V. Protein binding of zinc, cadmium and copper in tissues of oyster (*Ostrea edulis*), common mussel (*Mytilus edulis*) and horse mussel (*Modiolus modiolus*), taken from unpolluted waters.

By

KÅRE JULSHAMN[1] and KNUT-JAN ANDERSEN[2]

ABSTRACT

Three species of molluscs: mature oyster, *Ostrea edulis*, common mussel, *Mytilus edulis* (45–55 mm length) and adult horse mussel, *Modiolus modiolus* were sampled from an oyster farm at Innerøy, Western Norway. Kidney and digestive system tissues were taken from *Mytilus* and *Modiolus*, liver and digestive system tissue from *Ostrea*. The homogenized tissues were ultra-centrifuged at 8 mill. g x min. Aliquots of the cytosols were chromatographed on Sephadex G-75 and fractions of Ve/Vo between 1 and 3 collected. Homogenates, pellets and the cytosol fractions were analyzed by atomic absorption spectrophotometry for zinc, cadmium and copper. The oyster liver homogenate cytosol was also chromatographed on Sephadex G-25, and the fractions analyzed as above. Figures give gel filtration profiles of the three elements, and tables give tissue contents, distribution percentages of the elements and recoveries from the Sephadex column.

60–90% of the tissue zinc content was found in the particulate fraction, and a further part was seen in the high molecular (>30000 daltons) cytosol fraction. This fraction represented 25% in the *Mytilus* tissues. Zinc was also present in the low molecular, non-protein fraction, especially in the oyster tissues where it amounted to 30–40% of the total zinc content. This fraction emerged from a Sephadex G-25 column near the taurine peak. A zinc-associated low molecular weight protein corresponding to the metallothioneins was not found in these experiments.

The tissue cadmium contents in *Ostrea* were distributed similarly to the zinc content. In the *Mytilus* and *Modiolus* tissue cytosols cadmium was found distributed over a wide range of molecular weights, among them in the range of 15000 to 25000 daltons corresponding to the literature described metallothioneins. 54% of the cadmium in *Modiolus* digestive oyster tissue was associated with a protein fraction of molecular weight around 15000 daltons.

The tissue copper contents were also found in several cytosol fractions in all three molluscs, with 30–60% in the particulate fraction, a further 6–35% in the high mole-

[1] Institute of Vitamin Research, Directorate of Fisheries, Bergen.

[2] Section for Clinical Research and Molecular Medicine, Medical Dept. A, University of Bergen.

cular cytosol fraction, up to 42% in the low molecular non-protein fraction and lastly associated with low molecular proteins in all tissues analyzed.

It is concluded that the molluscs from non-polluted seawater contain specific low-molecular proteins which bind cadmium and copper, but not zinc.

INTRODUCTION

The first isolation and characterization of a metallothionein was reported by MARGOSHES and VALLEE (1957). They found a noninduced, low molecular weight protein in equine kidney tissue, containing zinc and cadmium. This protein had the ususual features of a high content of cysteine (about 30%) while lacking histidine and aromatic amino acids (KÄGI and VALLEE, 1960). Proteins from many vertebrates have later been described acting as metallothioneins towards several metal ions including cadmium, copper, zinc, silver and mercury (WEBB, 1975; BREMNER and DAVIES, 1975; BREMNER and YOUNG, 1976). As will be discussed later, there has also been a recent interest in similar metal-binding proteins in marine invertebrates.

The present paper describes the distribution of zinc, cadmium and copper obtained by analytical subcellular fractionation and Sephadex chromatography of the particle-free supernatants from two tissues in common mussel, horse mussel and oyster from a non-polluted marine environment.

METHODS

Samples

Mature specimens of *Ostrea edulis* from a three-year-old population, and mature specimens of *Mytilus edulis* with a size range of 45 mm to 55 mm were obtained from the Innerøy oyster poll, Western Norway. This poll was described in paper I in this series (JULSHAMN, I, 1981). Specimens of *Modiolus modiolus* were also obtained from the outer poll. The molluscs were transported alive to the laboratory. The shells were opened and rinsed in distilled water. Kidney and the digestive system were collected from *Mytilus* and *Modiolus* and the liver and the digestive system were collected from *Ostrea*. The methods of dissection were described by JULSHAMN (IV, 1981).

Tissue fractionation

The pooled tissue samples were homogenized in 0.44 M sucrose and 50 mM Tris-HCl at pH 7.5, using a Potter-Elvehjem homogenizer (size

C; Thomas Co., Philadelphia, PA., USA). The pestle was rotated at 1000 rev/min and the homogenate adjusted to a final volume of 10 ml/g tissue. Particle-free supernatants (cytosols) were obtained by sedimentation at 8 106000 g x min in an MSE Superspeed 65 ultracentrifuge (angle rotor 43114–123; r_{min}=3.5 cm; r_{max}=9.3 cm). Aliquots of the homogenates, cytosols and pellets were analyzed for copper, zinc and cadmium.

Gel filtration experiments

The cytosol fractions were chromatographed at 4°C on a Sephadex G-75 column (Pharmacia) (2.5 x 100 cm) previously equilibrated with 10 mM Tris-HCl at pH 8.0. The amount of sample applied varied from 1 to 5 ml. Blue Dextran 2000, cytochrome C (13000 D), ribonuclease A (13600 D), myoglobin (17800 D) and chymotrypsinogen (25000 D) were used for the calibration of the column with respect to void volume (Vo) and effluent volumes (Ve). The ratios of Ve/Vo obtained were 2.0, 1.92, 1.74, 1.54 and 1.0 for the five standard products, respectively. The effluent was monitored at 207 nm and at 254 nm using the LBK 2089 Uvicord III with a LBK 6520 6-Channel DC recorder. The flow rate was 25 ml/h and fractions of 5 ml were collected and analyzed for copper, zinc and cadmium.

The oyster liver homogenate cyrosol fraction was also chromatographed on a Sephadex G-25 Column (Pharmacia). The column was equilibrated with 10 mM Tris-HCl at pH 8.0. The instrument settings, flow rate and collection of fractions were as described for the Sephadex G-75 column.

Element analyses

The contents of copper, zinc and cadmium in the different fractions were determined by atomic absorption spectrophotometry (AAS) after nitric-perchloric acid digestion. The acid was removed from the samples by evaporation and the residues were diluted in 5% (v/v) nitric acid. Copper and zinc were determined by flame atomic absorption, while cadmium was determined by flameless atomic absorption using the principle of standard addition to correct for matrix effects. Fractions obtained by gel filtration were aspirated directly into the flame for the determination of zinc. From the same fractions, aliquots of 20 μl were charred at 400°C for 40 sec. in the graphite tubes for direct determination of copper and cadmium. All details of the analytical procedures were described previously (JULSHAMN, I, IV, 1981).

RESULTS AND DISCUSSION

The tissue concentrations found in the homogenates showed small variations within each species, except that the zinc content in the liver of *O. edulis* was only half of the content in the digestive system (Table 2). The tissue concentrations of the elements analyzed agreed well with previous results (JULSHAMN, IV, 1981).

In a study on the subcellular distribution of metals based on the same mollusc tissues, the authors demonstrated by the use of marker-enzymes the problems of particle disruption as well as redistribution during the homogenization and the fractionation procedure (JULSHAMN and ANDERSEN, 1981). Obviously, some of the metal binding protein originally present in the nuclear or the mitrochondrial-lysosomal fractions of the cells may be released into the cytosol fraction by the treatment. The Potter-Elvehjem equipment seemed to give the best and most reproducible procedure available with the lowest possible organelle breakage in the homogenization step. Table 1 gives the quantities of each element

Table 1. Sephadex G-75 gel filtration of cytosols from tissues homogeneates of three mollusc species.

Tissue (L=Liver, K=Kidney, D=Digestive system)		Content in cytosol applied (microgram)	Recovery microgram	Recovery %
Zinc				
Ostrea	L	75	68.0	91
	D	132	102	77
Mytilus	K	14.2	12.2	86
	D	10.6	9.8	92
Modiolus	K	11.5	8.7	76
	D	84	74	88
Cadmium				
Ostrea	L	0.25	0.20	80
	D	0.26	0.21	81
Mytilus	K	0.48	0.31	65
	D	0.33	0.25	76
Modiolus	K	0.21	0.17	81
	D	2.90	2.36	81
Copper				
Ostrea	L	1.1	1.0	91
	D	1.7	1.2	71
Mytilus	K	0.54	0.63	117
	D	0.65	0.55	85
Modiolus	K	0.13	0.14	108
	D	1.8	1.5	83

Table 2. Sephadex G-75 gel filtration of tissue homogenates from three molluscs. Tissue concentrations and percentages in the fractions.

Tissue (L=Liver, K=Kidney, D=Digestive system)		Conc.[a] in tissue homogenate	Percentages in particulate and cytosol fractions				
			Particulate	>30000[b]	~25000	~15000	<10000
Zinc							
O. edulis	L	590	61	6			33
	D	1200	59	1			40
M. edulis	K	110	69	25			6
	D	99	70	26			4
M. modiolus	K	6200	89	4			7
	D	7200	91	4			5
Cadmium							
O. edulis	L	1.7	54	9			37
	D	1.3	42	5	4		49
M. edulis	K	2.0	28	40		4	28
	D	1.3	6	71	11	5	7
M. modiolus	K	23	28	37	11	14	10
	D	27	21	4		54	21
Copper							
O. edulis	L	8.5	58	6		15	21
	D	13	49	35	4	8	4
M. edulis	K	3.3	60	12	17	8	3
	D	3.2	55	20	5	4	16
M. modiolus	K	16	34	11		14	42
	D	20	29	8	6	45	12

[a] Microgram/gram wet weight.
[b] Molecular weight (daltons).

in the aliquots applied to the Sephadex G-75 column, as well as the recoveries of the elements as sums of the analyzed fractions from the column. The recoveries averaged 85% and varied between 65 and 117%, the copper values having the highest deviations from the averages. Fig. 1–3 show the profiles obtained by gel filtration of the cytosol fractions from the digestive system of the three species. Generally, three main peaks of elements and UV-absorbing material were seen. The first peak eluted as a distinct fraction in the void volume. The second composite peak appeared in a volume of 1.5 to 2.0 times the void volume, and the third eluted in a volume of 2.5 to 3.0 times the void volume.

Table 2 gives percentage values for the element contents in the pellet or the particulate fraction from the centrifugation, and further the percentages of the elements recovered in each fraction from the Sephadex gel filtration of the cytosols.

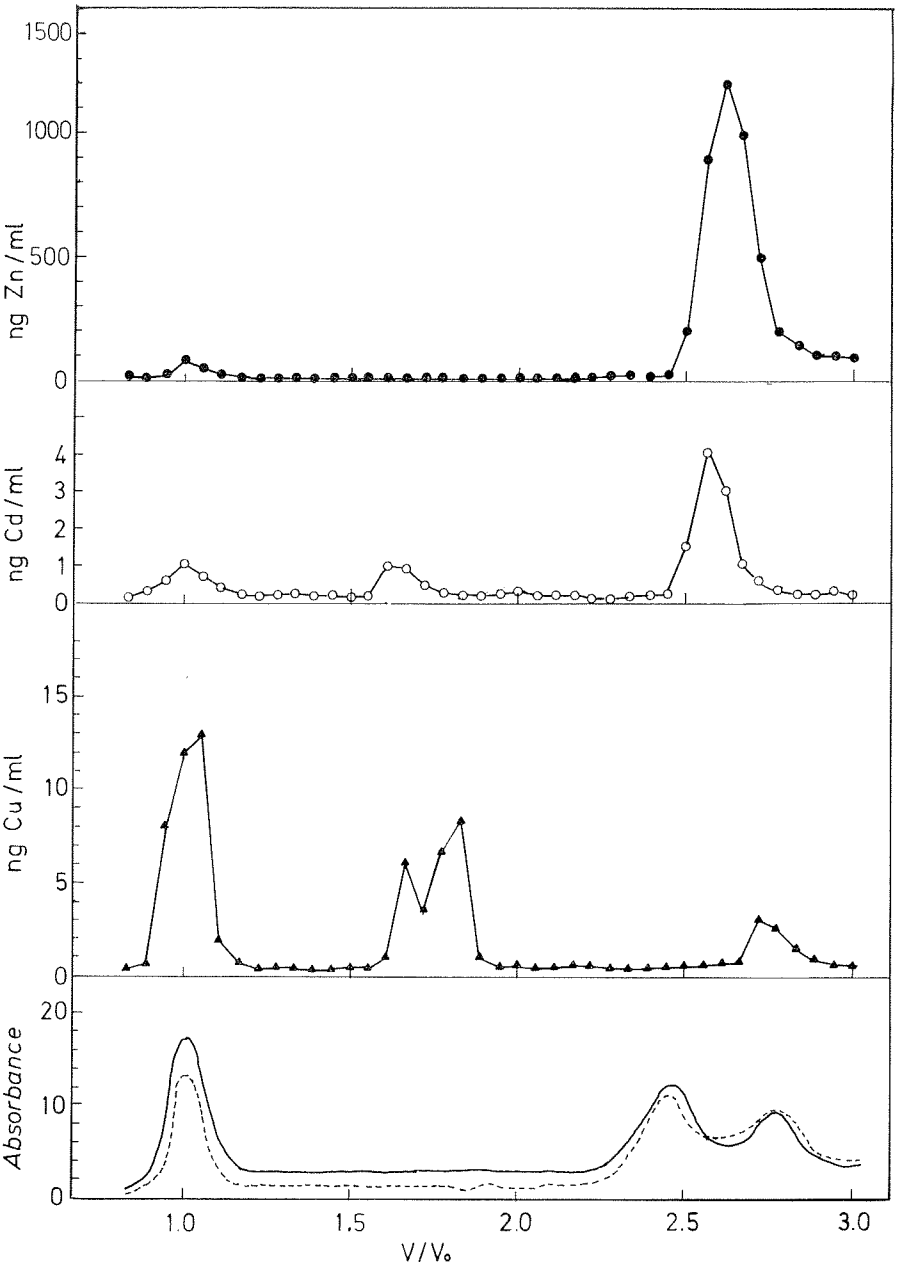


Fig. 1. Sephadex G-75 gel filtration profiles of zinc, cadmium and copper contents in digestive tissue cytosol fractions from *Ostrea edulis*.

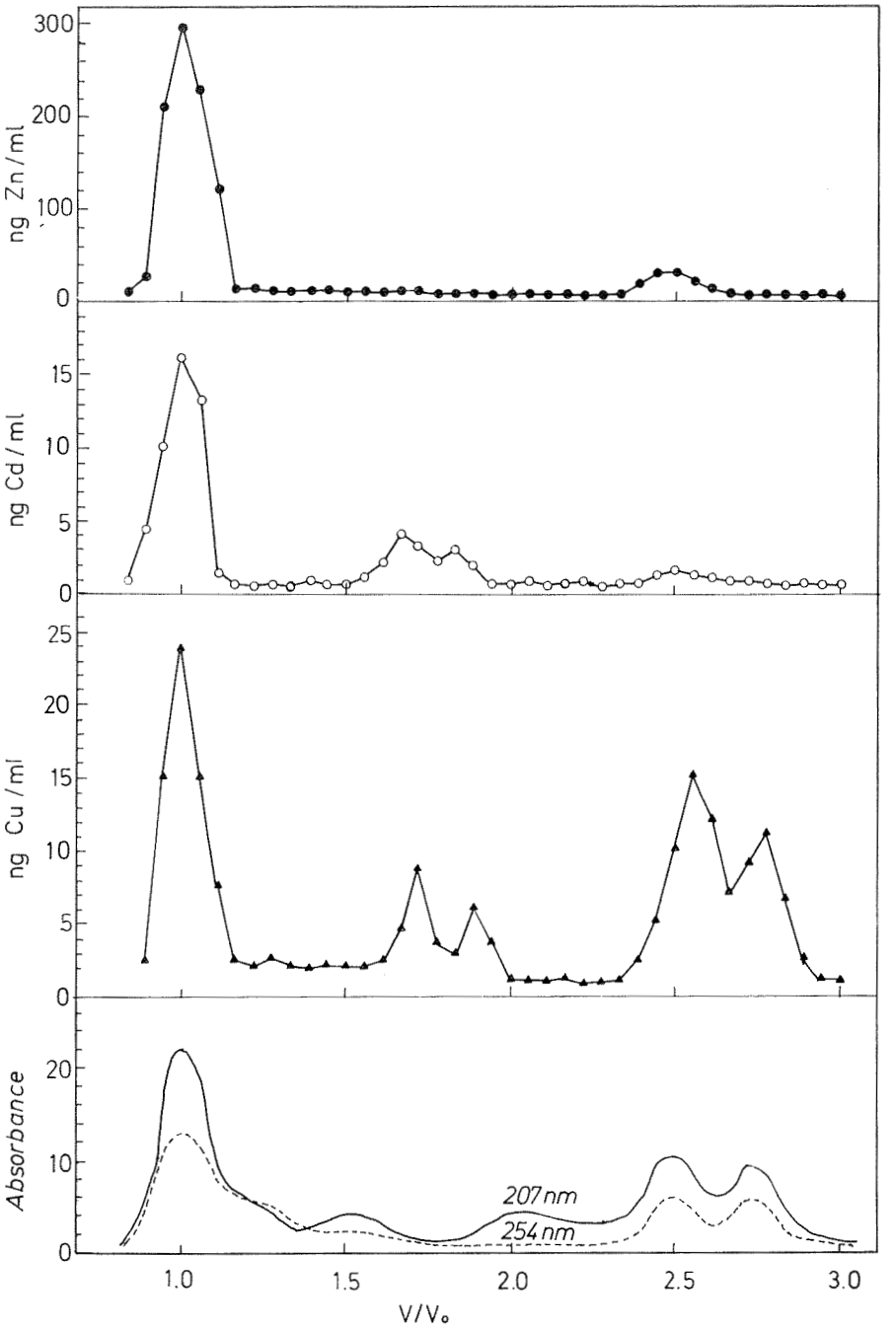


Fig. 2. Sephadex G-75 gel filtration profiles of zinc, cadmium and copper contents in digestive tissue cytosol fractions from *Mytilis edulis*.

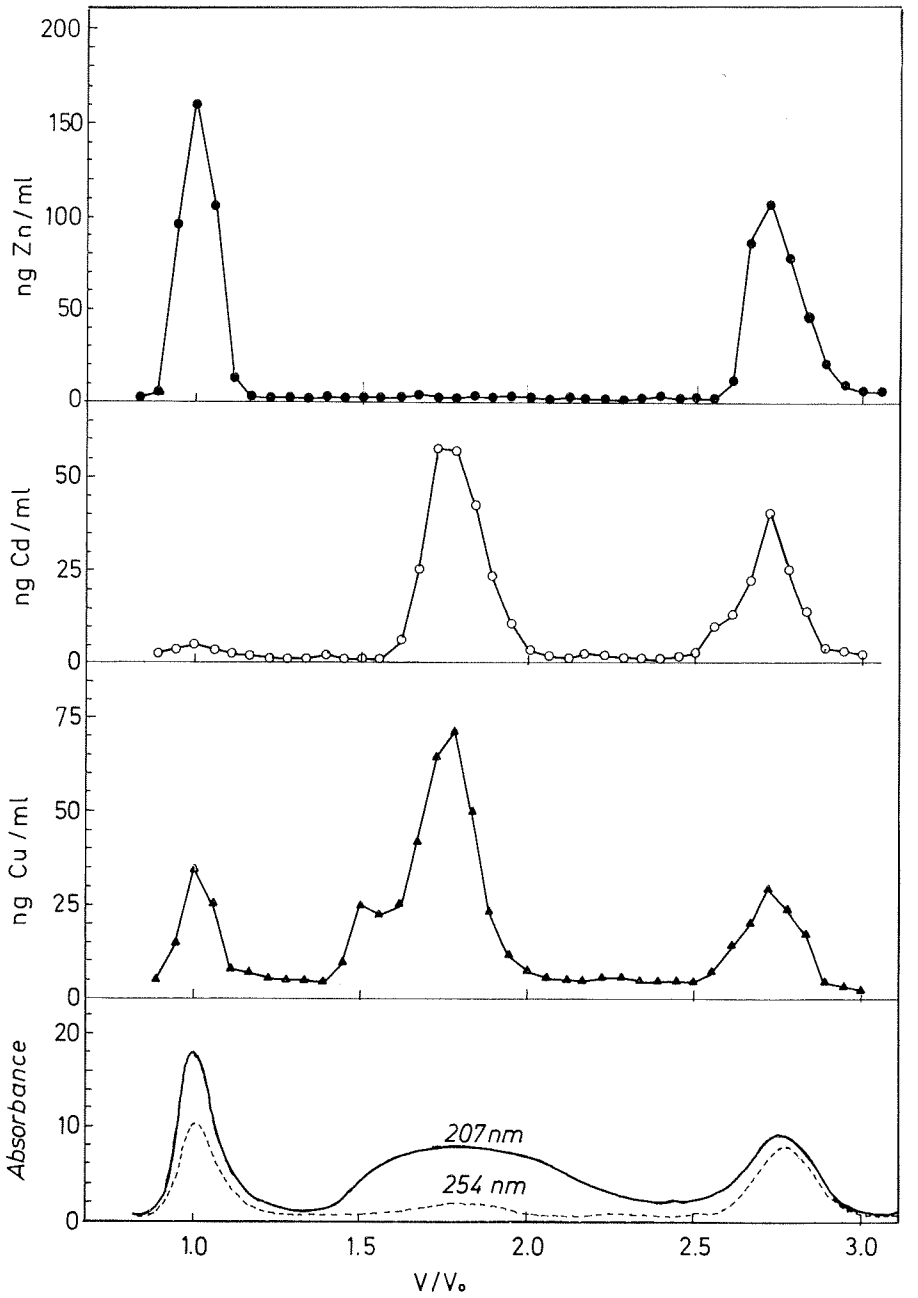


Fig. 3. Sephadex G-75 gel filtration profiles of zinc, cadmium and copper contents in digestive tissue cytosol fractions from *Modiolus modiolus*.

Zinc

The particulate fractions accounted for 60%, 70% and 90% of the zinc content in the tissues of *O. edulis*, *M. edulis* and *M. modiolus*, respectively, in agreement with the low cytosol extraction ratio previously reported for *Patella vulgata* and *Patella intermedia* (HOWARD and NICKLESS, 1977a) and for *O. edulis* (COOMBS, 1972; HOWARD and NICKLESS, 1977b). The cytosol zinc appeared in peak one and three after gel filtration (Fig. 1–3). In *O. edulis* 33 and 40% of the total zinc in the two tissues was found in the low molecular fraction of less than 10000 daltons. This fraction was investigated further by gel filtration of a sample of liver cytosol through a Sephadex G-25 column. These fractions were analysed for zinc, cadmium and taurine and were monitored at 207 and 254 nm (Table 3). A main peak at V/Vo=2.1 accounted for 72% of the zinc content and a further 16% of the applied zinc eluted in a tailing peak centered around V/Vo=2.25. The amino acid peak, consisting mainly of taurine, eluted somewhat displaced relative to the zinc peak. Taurine was present in amounts 20 times in excess of zinc. High amounts of free amino acids is known to be present in tissues of marine invertebrates. The present data agreed well with data reported by COOMBS (1974) who found that in oysters exposed to elevated element levels zinc was complexed to components with molecular weights less than 5000. The present experiments show that in the three molluscs studied, zinc was associated with at least three different compounds. The major part was firmly bound to cellular particles. A second part was found in the cytosol fraction bound to proteins of molecular weights over 30000 daltons. This was the major cytosol fraction in *M. edulis*. Finally, one part was associated with non-protein compounds of low molecular weights particularly prominent in *O. edulis*. A zinc-binding protein of low molecular weight could not be observed in these three molluscs taken in water of low pollution levels. A metalbinding protein has been reported to complex with zinc in goldfish injected with zinc (MARAFANTA, 1976).

Table 3. Gel filtration of *Ostrea edulis* liver homogenate cytosol on Sephadex G-25.

Substances applied, UV-absorbance	V/Vo of fractions 1.00	1.90	2.10	2.25
Cd, 0.13 microg	20	—	80	—
Zn, 37.5 microg	12	—	72	16
Taurin, 2.34 mg	38	62	—	—
Absorbance at 207 nm	+	+	—	—
Absorbance at 280 nm	+	+	—	—

Cadmium

Approximately ten times more cadmium were found in the tissues of *M. modiolus* than in *O. edulis* and *M. edulis*. The tissue distributions of cadmium in *M. edulis* and *M. modiolus* were different from those obtained for zinc, whereas the cadmium distribution in *O. edulis* tissues was similar to that for zinc. Cadmium was found mainly in the cytosol fractions, with the exception of the 54% bound to the particulate fraction in the liver of *O. edulis*. The major part of the cytosol cadmium content in *O. edulis*, representing 37% and 49% in the two tissues, was associated with the low molecular weight fraction. On a Sephadex G-25 column, the liver cytosol fraction gave one cadmium peak partly overlapping the major zinc peak and related to the non-protein low molecular compounds (Table 3). CHOU et al. (1978) found in polarographic studies that this cadmium fraction occurred non-complexed. Cadmium peaks corresponding to molecular weights around 7400, as described for exposed *O. edulis* by RIDLINGTON and FOWLER (1979) and CASTERLINE and YIP (1975) could not be ascertained. The distribution pattern for cadmium was similar in *M. edulis* and *M. modiolus*, in spite of the 10-fold difference in the tissue contents. A noteworthy exception was the high proportion of cadmium (71%) in the high molecular weight cytosol fraction from the digestive system of *M. edulis*, combined with the exceptional low content in the particulate fraction. In contrast to the zinc distribution cadmium was found in the low molecular protein fractions in these two molluscs. In *M. edulis* the major part of the Cd was associated with high molecular weight proteins (>30000 daltons), with a further double peak in the lower molecular weight range. Similar results were reported by NÖEL-LAMBOT (1976). The *M. modiolus* kidney tissue gave the same profile, whereas in the digestive system 54% of the total tissue cadmium was found in a fraction of Ve/Vo=1.75, related to proteins with molecular weights between 10000 and 20000 daltons. A cadmium complexing protein with a molecular weight above 20000 daltons has been suggested (GEORGE et al., 1979; HOWARD and NICKLESS, 1977a).

Copper

O. edulis and *M. modiolus* had the highest tissue concentrations of copper. From 30 to 60% of the total tissue copper contents were found in the particulate fractions, highest in *M. edulis*, lowest in *M. modiolus*.

The gel filtration profiles were more complex for copper than for the two other elements, with several peaks over a wide molecular weight range. In all tissue samples copper was found associated with high mole-

cular weight protein, and in the digestive system of *O. edulis* this was the major cytosol fraction, representing 35% of the total copper. All tissues also gave composite peaks of copper in the lower molecular weight protein range of 10000–25000 daltons. The proportions of these fractions varied widely, and the digestive system of *M. modiolus* had more than 50% of the total copper in this area. Finally, from 3–42% of the tissue copper contents eluted at Ve/Vo above 2.5, i.e. in the non-protein, low molecular weight range. The kidney tissue from *M. modiolus* had 42% of its total copper content in this fraction.

The identification of copper compounds related to low molecular weights (<10000 daltons), is complicated by the weak complexing strength and the ready exchangeability of this element. Previous reports also suggest that heavy metals such as Zn, Cd and Cu occur naturally in the unbound state in oyster (Casterline and Yip, 1975; Coombs, 1974; Chou et al., 1978).

One may conclude from this study that molluscs exposed to low levels of zinc, cadmium and copper in non-polluted sea water contain specific low-molecular proteins which bind cadmium and copper, but not zinc.

REFERENCES

Bremner, I. and Davies, N.T., 1975. J. Biochem. *149*, 733–738.

Bremner, I. and Young, B.W., 1976. J. Biochem. *157*, 517–520.

Casterline, J.L. and Yip. G., 1975. Arch. Environ. Contam. Toxicol. *3*, 319–339.

Chou, C.L., Uthe, J.F., and Zook, E.G., 1978. J. Fish. Res. Board Can. *35*, 409–413.

Coombs, T.L., 1972. Mar. Biol. *12*, 170–178.

Coombs, T.L., 1974. Mar. Biol. *28*, 1–10.

George, S.G., Carpene, E., Coombs, T.L., Overnell, J. and Youngson, A., 1979. Biochim. Biophys. Acta *580*, 225–233.

Howard, A.G. and Nickless, G., 1977 a. Chem-Biol. Interact. *16*, 107.

Howard, A.G. and Nickless, G., 1977 b. Chem-Biol. Interact. *17*, 257–263.

Julshamn, K., 1981. Studies on Major and Minor Elements in Molluscs in Western Norway. I–IV, Fisk. Dir. Skr., Ser. Ernæring, *1*, No. 5.

Julshamn, K. and Andersen, K.J., 1981. To be published.

Kägi, J.H.R. and Vallee, B.L., 1960. J. Biol. Chem. *235*, 3460–3465.

Marafanta, E., 1976. Experientia *32*, 149.

Margoshes, M. and Vallee, B.L., 1957. J. Am. Chem. Soc. *79*, 4813.

Nöel-Lambot, F., 1976. Experientia *32*, 324.

Ridlington, J.W. and Fowler, B.A., 1979. Chem.-Biol. Interact. *25*, 127–138.

Webb, M., 1975. Biochem. Soc. Trans. *3*, 632–634.

Fisk. Dir. Skr., Ser. Ernæring, Vol. 1: No. 5, 1981, s 247–265

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

VI. Accumulation and depletion of cadmium and lead and 5 further elements in tissues of oyster (*Ostrea edulis*), and common mussel (*Mytilus edulis*) by transfer between waters of highly different heavy metal loads.*

By

KÅRE JULSHAMN

Institute of Vitamin Research
Directorate of Fisheries, Bergen.

ABSTRACT

Adult oysters (*Ostrea edulis*) and adult common mussels (*Mytilus edulis*) from an oyster farm at Innerøy in West Norway were transferred to a site in Hardangerfjorden were the ambient water had concentrations of 0.5 microgram/l cadmium and 2.9 microgram/l lead as compared to 0.04 microgram/l Cd and 0.5 microgram/l Pb at Innerøy. Samples of oysters and mussels were removed at 12 intervals from 0 to 480 days. Thereafter the residual molluscs were transferred back to Innerøy and samples again removed at 4 intervals up to 360 days. The samples were dissected into 4–6 different tissues which were analyzed for cadmium and lead, and further for magnesium, calcium, iron, copper and zinc by atomic absorption spectrophotometry. The uptake of cadmium in oyster started after 60 days and a steady state was not reached during the 480 days period in Hardangerfjorden. There was little accumulation during the winter time with water temperatures below 7°C. The highest concentrations were reached in the gills, digestive system and mantle which had an overall uptake rate of 0.63 mg/kg/week. These concentrations were 3 to 6 times higher than the values at Innerøy as compared to a 13-fold increase in the water. After transfer back to the original site, the depletion of cadmium was slow, and more than 70% of the maximum concentrations were still present after 360 days. *Mytilus* had a rapid uptake of cadmium and the tissue concentrations reached a steady state after 60 to 90 days depending on the depth of the baskets. The levels increased 3–6 times as for oysters, and only the digestive system reached concentrations corresponding to those in the whole soft parts of indigenous mussels. After return to the original site, there was a rapid depletion of cadmium, particularly in the mantle, digestive system and gills and the concentrations were back to the original values within a year. The uptake of lead in oysters was very rapid and a constant level was reached after two months corresponding to 3 to 6 times the original values. All tissues increased

*A preliminary account of this work was presented at a symposium on ecotoxicology arranged by the Norwegian Research Councils at Sundvollen, Norway, 6–7. Nov. 1978.

to the same extent, pointing to a rapid transport of lead. After transfer back to Innerøy, the tissue levels were halved in the gills within 2 months. The depletion was somewhat slower in the other tissues, but all were back to the original values within a year.

Also *Mytilus* had a rapid uptake of lead, calculated to 7.8 mg/kg dry weight/week at 5 m depth, and 5.4 mg/kg/week at 10 m depth within the first two months. The highest levels of lead were found in the digestive system representing more than 40% of the total. After return to Innerøy the depletion of lead was rapid in all tissues except the gills, and 2 thirds of the lead contents were released within the first two months. All tissues were back to the original values within a year.

Values for the contents of magnesium, calcium, iron, copper and zinc in the tissue samples of oyster and common mussel are given in appendix tables.

INTRODUCTION

The relative pollution levels of aquatic environments by heavy metal elements can be elucidated by analyses of water, sediments and indigenous biota. Cadmium and lead are found partly in solution and partly in suspension adsorbed to organic or inorganic particulate matter in the water. Estuarine mixing of freshwater and saltwater may result in a decrease in the dissolved metal concentrations and a shift in the particulate fraction from mainly inorganic associated metal contents in freshwater to mainly organic associated (phytoplancton) in saltwater (PHILLIPS, 1977).

Water analyses have several disadvantages for the evaluation of pollution levels. One problem lies in the laborious preconcentration steps which involve possible contamination. The chemical form of the element in the water may in part make it unavailable to the biota, thereby overestimating the availability as based on the total element concentration. The main disadvantage of water analyses lies in the large variation in the element concentrations with changes in season, time of day, the extent of freshwater run-off, depth of sampling, the intermittent flow of industrial effluent and general hydrological factors such as tides and currents.

Analysis of sediments also may give insufficient information for the evaluation of pollution. The main problem here would be to estimate the amount of element available to the biota, and to correlate such data to the concentrations in water and in marine organisms (BRYAN and HUMMERSTONE, 1973; STENNER and NICKLESS, 1974, 1975; BOYDEN, 1975).

Sedentary organisms with wide distribution and comparative ease of collection should be favourable as indicators of pollution levels. The macroalgae reflect the dissolved concentrations of elements in the water, whereas mussels, being filter-feeders, take up trace elements from food and water, as well as from the ingestion of inorganic particulate material.

The five preceeding papers in this series treated factors influencing the

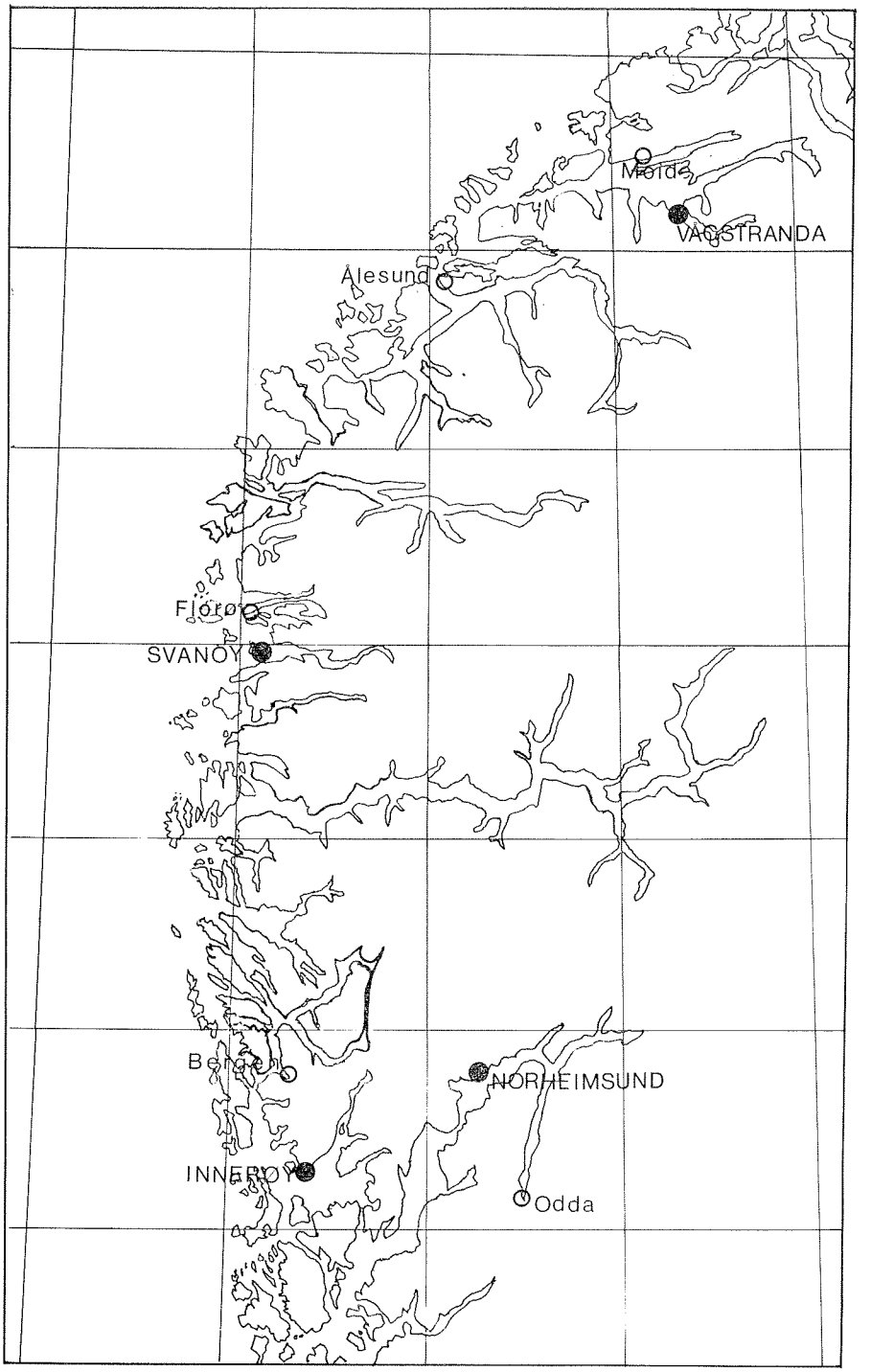


Fig. 1. Map over western Norway, showing the Innerøy oyster poll and the transfer site at Nordheimsund, Hardangerfjorden.

element concentration in mussels sampled from unpolluted waters. Relations based on season, growth sites, size, age, tissue distribution (JULSHAMN, I–IV, 1981) and metal-binding protein fractions in the tissues were discussed (JULSHAMN and ANDERSEN, V, 1981). This study concerns transfer of oysters (*Ostrea edulis*) and common mussels (*Mytilus edulis*) between two environments having widely different levels of cadmium and lead. Values for magnesium, calcium, iron, copper and zinc are presented in appendix tables.

METHODS

Sampling

Approximately 150 oysters, genetically similar, were collected from the Innerøy oyster farm on the first of May 1976. (Fig. 1). The oysters were taken from a three-year-old population, care being taken to obtain specimens of equal size (JULSHAMN, III, 1981). Further, 150 common mussels (*Mytilus edulis*) of a single year class, with a size range from 40 mm to 50 mm were collected at the same site. The mussels were immediately transferred to a site in Hardangerfjorden near Norheimsund and placed

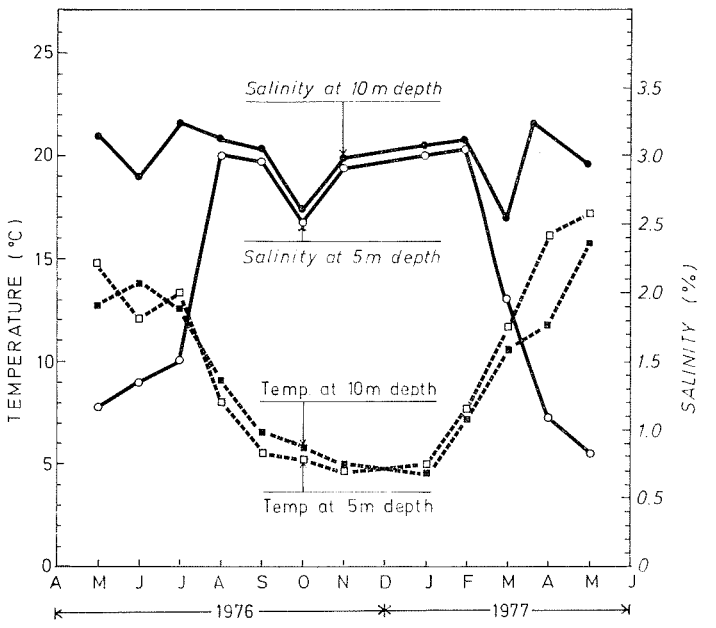


Fig. 2. Salinities and temperatures through the year at the transfer site, Nordheimsund, Hardangerfjorden.

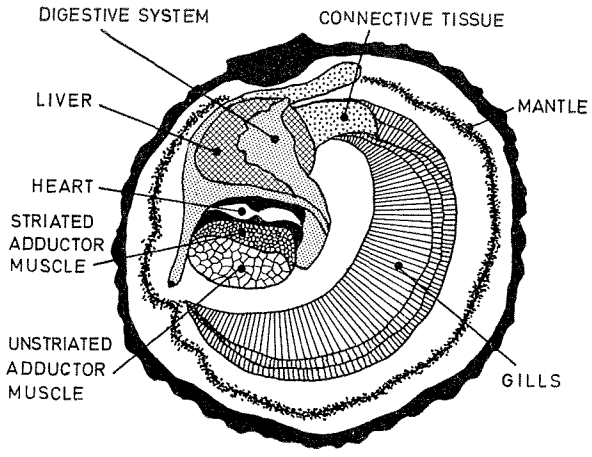


Fig. 3. Tissue distribution in oysters, *Ostrea edulis*.

in suspended wire netting baskets. The baskets were made to tolerate tough handling. One basket with common mussels was suspended 5 m below the surface with the aid of an anchored buoy. Two baskets with oysters and common mussels, respectively, were suspended ten meters below the surface. Samples were taken from the baskets after 0, 2, 4, 8, 16, 32, 64, 90, 150, 270, 390 and 480 days. Water samples were taken each time. Salinity and temperature were measured firstly at the sampling times and later at the 20th of each month throughout the experiment. The salinities at 5 m depth ranged from 0.8% to 3.0% at the Hardangerfjorden site (Fig. 2) and from 2.5% to 3.2% at Innerøy, the highest values measured during the winter season. Eight oysters were removed at each sampling time, opened, rinsed in distilled water, air-dried and the tissues removed and weighed. Five oysters weighing between 7 and 10 grams were dissected into mantle, gills, muscle and digestive system (Fig. 3). The pooled tissue samples were weighed, freeze-dried until constant weight, homogenized in a mortar and stored in closed jars until analysis. Three common mussels were taken from each basked at each sampling time, the tissues removed, pooled, freeze-dried and homogenized. Further five mussels were taken after 0, 150 and 480 days from the basket suspended ten meter below the surface. From these, the tissues were dissected into muscle, foot, gonades, mantle, gills and the digestive system and treated as for oysters (Fig. 4). Details of the methods of dissections of oysters and mussels are given in paper IV of the series (Julshamn, IV, 1981).

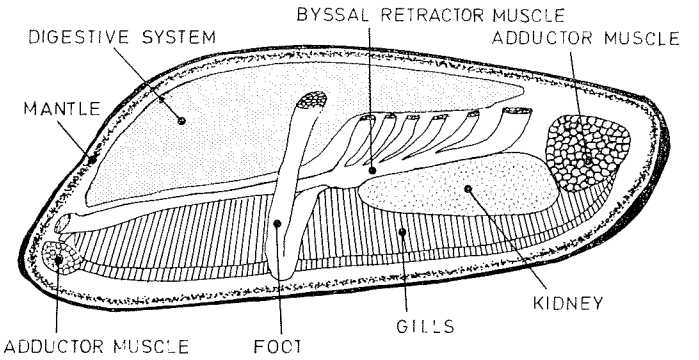


Fig. 4. Tissue distribution in common mussel, *Mytilus edulis*.

After 480 days the baskets with the remaining mussels were taken back to the original locality. Here, samples were taken after 60, 120, 240 and 360 days. The number of individuals and the sample preparation were as before.

Analysis

The freeze-dried samples were wet digested in "suprapur" concentrated nitric acid and perchloric acid (MERCK) in 10 ml test tubes. Nitrous gases were removed by cooking the digest, which thereafter was diluted to 25 ml. For cadmium and lead, two aliquots of each 2 ml were evaporated to dryness in platinum crucibles under an infrared lamp, and the residues were taken up in 2 ml 5% nitric acid in washed plastic tubes. The standard addition procedure was applied to 0.5 ml aliquots of the final solutions (JULSHAMN, 1977). These elements were measured with the flameless atomic absorption technique on a Perkin-Elmer 403 AAS equipped with a Deuterium Background Corrector and a Perkin-Elmer HGA-76 Graphite Furnace. Conventional graphite tubes obtained from Perkin-Elmer were used. Pure argon gas (>99%) was used to sheath the graphite tubes at a flow rate of 60 ml/min. Ten microliter samples were introduced into HGA-76 furnace with an Oxford Laboratories Sampler fitted with acid-washed disposable plastic tips. Further details of the analytical procedures, including analyses of the water samples, and the determination of sodium, calcium, iron, copper and zinc were given in part I of the series (JULSHAMN, I, 1981).

RESULTS AND DISCUSSION

Differences in cadmium and lead contents between the two stations

Most of the studies reported in the literature concern treatments of mussels in laboratory experiments with unrealistic high concentrations of metal ions added to the water. The concentrations of cadmium and lead were low in all water samples taken from the Innerøy poll, whereas the results from Hardangerfjorden gave evidence of a heavy load of cadmium and lead in the water (Table 1). The cadmium level increased from 0.04 microgram/l in the Innerøy poll to 0.5 microgram/l at the Hardangerfjord sampling station and the lead content increased from 0.5 to 2.9 microgram per liter. The variations in the water and problems concerning representative sampling of water are described and discussed in Parts I and VII of the present series (JULSHAMN, I and VII, 1981). The differences in the contents of cadmium and lead in indigenous *Ascophyllum nodosum* and *Mytilus edulis* at the two sampling stations corresponded to the water levels with increases of cadmium and lead in *A. nodosum* from 1.0 to 20 mg/kg and from 1.1 to 5.5 mg/kg, respectively, and increases of cadmium and lead in *M. edulis* from 1.2 to 25 mg/kg and from 4.4 to 70 mg/kg, respectively. For both organisms, the concentrations factors were higher for cadmium than for lead. The high concentration factor of 16 for lead in *M. edulis* compared to 5 and 6 respectively for *A. nodosum* and water is particularly noteworthy. This may indicate that cadmium and lead occur in different chemical forms in the water. *Ostrea edulis* is not

Table 1. Contents of cadmium and lead in sea water (microg/l) and in marine organisms (mg/kg dry weight) ±St. dev. from the Innerøy oyster poll and from Hardangerfjord.

	Innerøy		Hardangerfjord		Conc.* fact.
CADMIUM	N		N		
Sea water	6	0.04±0.02	5	0.5± 0.2	12
Ascophyllum	6	1.0 ±0.1	5	20 ± 2.5	20
Mytilus	12	1.2 ±0.4	5	25 ± 2.0	21
Ostrea	12	4.5 ±1.0	–	—	—
LEAD					
Sea water	6	0.5 ±0.3	5	2.9± 1.1	6
Ascophyllum	6	1.1 ±0.5	5	5.5± 3.0	5
Mytilus	12	4.4 ±0.7	5	70 ±10	16
Ostrea	12	1.5 ±0.4	–	—	—

*Concentration factor.

indigenous to Hardangerfjorden. Oysters reared in the Innerøy poll had substantially higher levels of cadmium than *M. edulis*, whereas the concentration of lead were lower than in *M. edulis*.

Cadmium – Ostrea

The uptake of cadmium in *Ostrea* started in July after a delay of 60 days in all tissues except muscle (Fig. 5). This is consistent with the report of FRAZIER (1976), who also found that the uptake took place rapidly in the summer and fall, but was delayed in the early spring. The cadmium uptake was somewhat delayed when the temperature rose above the minimum for metabolic turnover (>7°C). Probably, an uptake of cadmium must be preceeded by an increased protein synthesis, and this may be supressed in early spring with low water temperatures. No corresponding lag period could be observed for the cadmium uptake in *Mytilus edulis* or for the uptake of lead in the two mussel species.

The uptake levelled off in all tissues in October when the temperature fell below 7°C. The concentrations had then reached 19 mg/kg in mantle and gill and 18 mg/kg in the digestive system (Table 2). The initial rate of uptake varied from 1.2 to 0.75 microg/g per week in the four tissues. A low rate of uptake was seen during the winter months, probably corresponding to a low metabolic rate. According to ZAROOGIAN and CHEER (1976), the temperature seemed less critical for *Crassostrea virginica*. After 16 months the highest concentrations of cadmium were found in the gills, the digestive system and the mantle. The calculated overall rate of uptake was 0.63 microg/g per week for three tissues. The concentrations did not reach a steady state within the 480 days of the uptake period in any of the tissues. The cadmium levels in muscle were lower than in the other tissues studied at all sampling times, but the concentration factor was higher after 16 months. No experiments have been reported on cadmium uptakes from water of low element content in field experiments with uptake periods of 480 days. Experiments by BROOKS and RUMSBY (1965), EISLER et al. (1972) and ZAROOGIAN and CHEER (1976) did not last long enough to establish an equilibrium status. The maximum levels of cadmium reached after 480 days were 3 to 6.5 times the initial level (Table 2) which was well below the factor of 13 found in the water samples (Table 1). ZAROOGIAN and CHEER (1976) studied *Crassostrea* with an initial Cd level of 12 mg/kg and found an accumulation up to 105 mg/kg after 280 days in seawater containing 5 microgram/l of Cd, i.e. 10 times the water levels in the present experiments.

The return to the original poll took place after the spawning period was over for both species. Spawning has been implicated in metal deple-

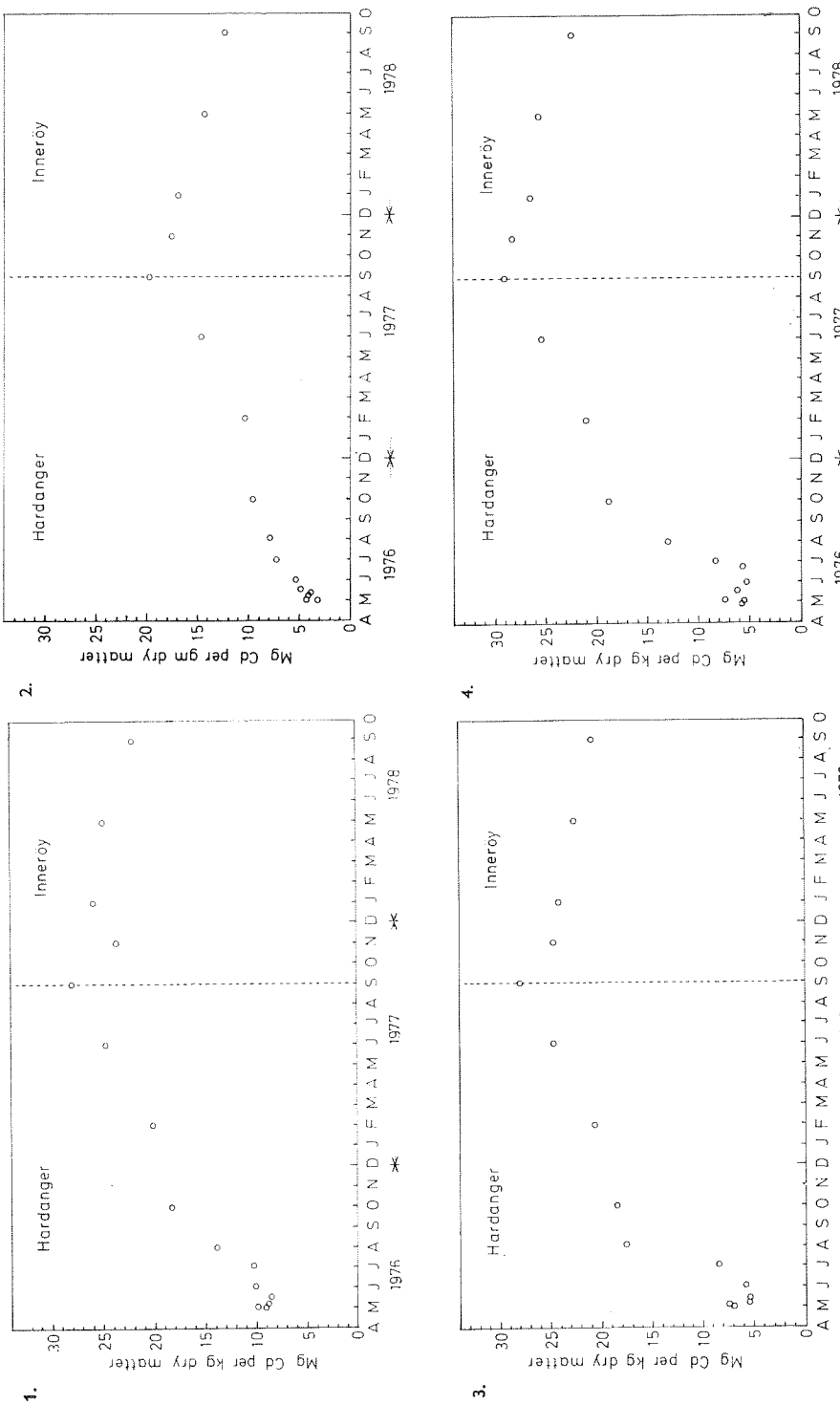


Fig. 5. Cadmium contents in four tissues of *Ostrea edulis* during the uptake period in Hardangerfjorden, and after the return to the Innerøy oyster poll. 1. Digestive system, 2. muscle, 3. mantle, 4. gills.

Table 2. Contents of cadmium and lead (mg/kg dry weight) in tissues of oyster (*Ostrea edulis*) after transfer to Hardangerfjorden (H), followed by return to original site, Innerøy (I).

Tissue	Site	Days	Cd	Conc. fact.	Pb	Conc. fact.
Muscle	H	0	3.1		1.1	
		150	9.6		5.5	
		270	11		6.9	
		480	20	6.5	7.0	6.5
	I	60	18		5.2	
		240	15		4.9	
		360	14	4.5	2.5	2
Mantle	H	0	6.8		2.8	
		150	19		11	
		270	21		10	
		480	28	4	8.4	3
	I	60	25		5.0	
		240	23		3.6	
		360	21	3	2.5	1
Gills	H	0	5.7		2.2	
		150	19		13	
		270	21		11	
		480	29	5	10	4.5
	I	60	27		5.0	
		240	26		4.4	
		360	22	4	2.2	1
Digestive system	H	0	9.0		1.5	
		150	18		8.5	
		270	20		6.8	
		480	28	3	9.1	6
	I	60	23		7.0	
		240	25		1.9	
		360	22	2.5	2.0	1.5

tion from oysters and common mussels (GALTSOFF, 1964; CUNNINGHAM and TRIPP, 1973, 1975a; FRAZIER, 1975, and JULSHAMN, II, 1981). The depletion rate was low for cadmium and this corresponds to a report by GREIG and WENZLOFF (1978). Their results suggest that *C.virginica* tissues retain much of their cadmium and copper when transferred to waters having lower levels of heavy metals. For other elements the rate of depletion may be governed by temperature, salinity, dosage, element concentration of the medium and time of exposure to the elements (SHUSTER and PRINGLE, 1969; CUNNINGHAM and TRIPP, 1975a). More

than 70% of the maximum levels of cadmium were still present in the tissues one year after transfer to the original site. A biological half life for cadmium can not be suggested from the data on oysters, as the depletion is clearly slower than that of other species and of other elements (PRINGLE et al., 1968; SCHULZ-BALDES, 1974; CUNNINGHAM and TRIPP, 1975b). Possibly, cadmium is partly bound to a low molecular weight protein in oyster such as the mammalian metallo-thioneins (JULSHAMN and ANDERSEN, V, 1981). This theory is not supported by CASTERLINE and YIP (1975) and CHOU et al. (1978).

Cadmium – Mytilus

The uptake started after a delay of less than 16 days. The rate of cadmium uptake from the 16th to the 32nd day was 2.2 microgram/g/week, i.e. 2 to 3 times the initial uptake rate in oysters. At 10 m depth the cadmium uptake levelled off already after 30 days when a body concentration of 8 mg/kg was reached. At 5 m depth a concentration of 15 mg/kg was reached after 90 days. A linear uptake was found only during the first few months. The concentrations at 5 m depth were significantly higher than those at 10 m depth. A slight decrease was found at both water depths during the winter (Fig. 6).

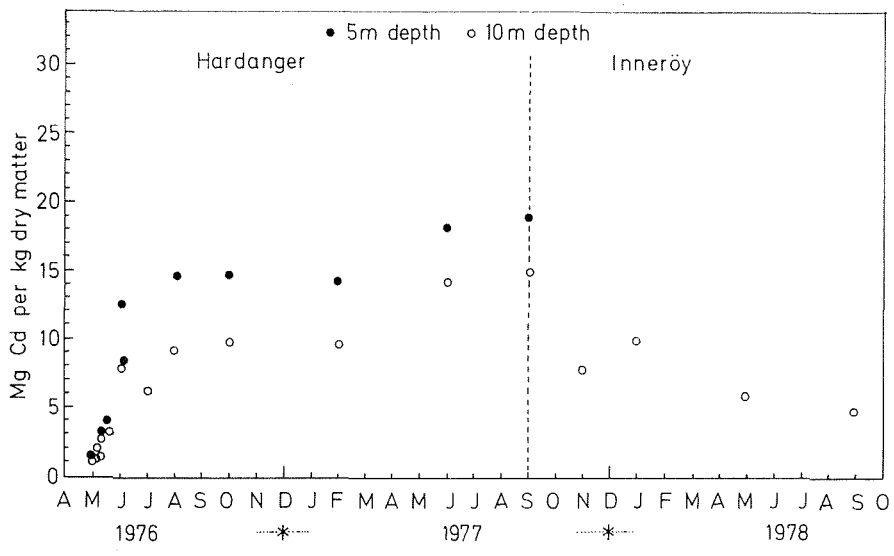


Fig. 6. Cadmium contents in the total soft tissues of *Mytilus edulis* during the uptake period in Hardangerfjorden, and after the return to the Innerøy oyster poll.

Table 3. Contents of cadmium and lead (mg/kg dry weight) in tissues of common mussel (*Mytilus edulis*) after transfer to Hardangerfjorden (H), followed by return to original site, Innerøy (I).

Tissue	Site	Days	Tissue % of total	Cd	Conc. fact.	% of total	Pb	Conc. fact.	% of total
Muscle	H	0	15	1.1		4.8	10		15
		150	33	2.3		5.1	30		30
		480	31	4.9	4.5	11	33	3.5	30
	I	60	22	3.0		9.3	12		21
		240	18	2.5		8.2	8.3		14
		360	15	1.8	1.5	6.3	9.5	1	15
Foot	H	0	4.7	2.5		3.5	5.1		2.6
		150	6.3	7.9		3.4	12		2.2
		480	7.0	6.5	2.5	3.1	17	3.5	3.6
	I	60	3.1	5.7		2.5	10		2.5
		240	5.0	1.6		2.8	8.6		4.0
		360	4.0	2.0	1	1.9	6.6	1	2.7
Gonades	H	0	23	1.2		8.6	4.2		9.4
		150	7.7	7.2		3.7	19		4.4
		480	22	9.8	8	16	18	4	9.9
	I	60	38	4.6		18	8.0		24
		240	27	3.3		12	6.8		17
		360	22	1.5	1	7.6	5.5	1.5	13
Mantle	H	0	13	2.4		10	4.0		5.7
		150	18	9.0		11	15		8.3
		480	12	15	6	12	15	4	5.1
	I	60	11	6.0		9.3	3.2		2.8
		240	13	4.9		8.0	3.5		4.1
		360	14	2.9	1	9.5	4.5	1	6.6
Gills	H	0	18	5.8		32	18		36
		150	9.4	17		11	38		11
		480	8.5	18	3	11	39	2	10
	I	60	9.3	10		13	28		21
		240	15	8.0		28	20		28
		360	24	7.5	1	42	16	1	41
Digestive system	H	0	26	4.9		38	11		31
		150	25	36		60	59		45
		480	20	29	6	46	68	6	41
	I	60	17	16		45	21		29
		240	23	11		42	15		32
		360	21	7.0	1.5	33	10	1	22

Table 3 shows the distribution of cadmium in tissues of *Mytilus*. The highest uptake was found in the digestive system which increased from 4.9 mg/kg to 36 mg/kg in 150 days, at which time it accounted for 60% of the total cadmium content. The uptake was lower in muscle, foot and gonades. The increased concentration of cadmium in the gills was counteracted with a relative weight decrease of the gills. Only the digestive system reached levels higher than the overall content in mussels indigenous to Hardangerfjorden. There was a rapid depletion of cadmium during the first 2–3 months after the mussels were transferred back to the original site. Between 40 and 50% of the cadmium was retained in the mantle, gonades and digestive system after 60 days. The cadmium depletion was slower in gills and muscle. These tissues lost 40–50% of the accumulated cadmium in 240 days, at which time the gills had regained their weight proportion. The overall rate of depletion was lower than the rate of uptake, but the levels of cadmium were back to the starting levels within a year.

Lead – Ostrea

There was a very rapid uptake of lead within the first two months in the digestive system, gills and mantle, after which an equilibrium level was reached and no further increase was observed (Fig. 7). The analytical values obtained varied much more than was the case for cadmium. All four tissues increased equally in their lead contents and consequently the transport from the sites of absorption to other tissues must have been rapid. Within the first summer, the lead content of the tissues increased 3 to 6 times corresponding with a concentration factor of 6 in the water (Tables 1 and 2). These results were consistent with those reported by ZAROOGIAN *et al.* (1979), who had a lead content in the control water of 0.12–0.16 µg/l and 1.0 and 3.3 µg/l in the experimental medium which gave concentration increases in *C. virginica* of 1.6, 6.6 and 11.4 mg/kg, respectively. Their experiments started in May and lasted for 20 weeks. The rate of uptake is probably influenced by the chemical binding of the element in the environment as discussed for lead in *C. virginica* (GEORGE and COOMBS, 1977) and mercury in *C. virginica* (CUNNINGHAM and TRIPP, 1975b).

All tissues had a high rate of depletion after transfer to the original site. The lead contents in the gills were halved in 60 days, whereas the other tissues were depleted at a somewhat slower rate. All tissue levels were nearly back to the original values within a year. These findings were consistent with the report by ZARGOOGIAN *et al.* (1979) who found that the *C. virginica* tissues were depleted rapidly until a certain residual

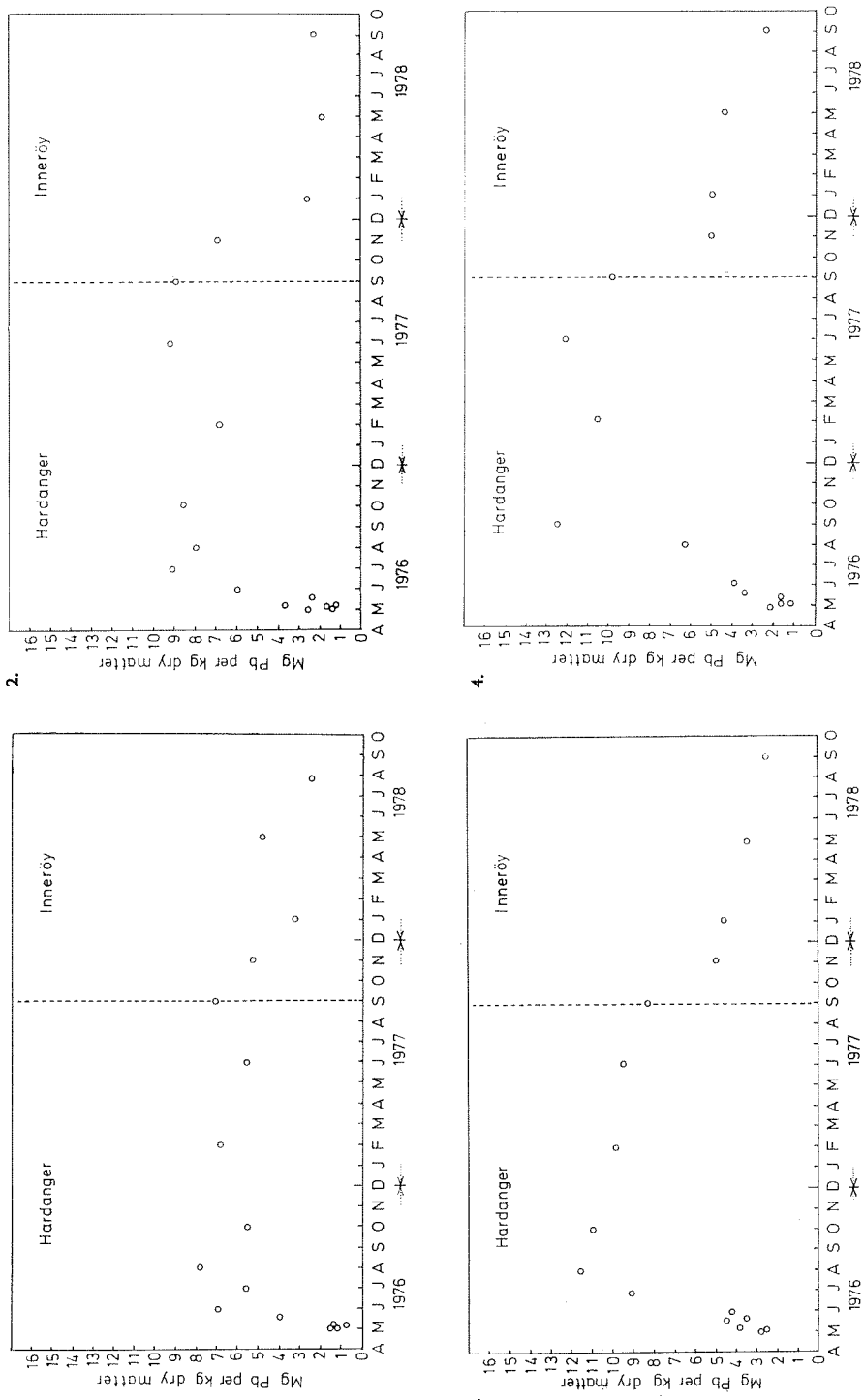


Fig. 7. Lead contents in four tissues of *Ostrea edulis* during the uptake period in Hardangerfjorden and after the return to the Innerøy oyster poll. 1. Muscle, 2. Digestive system, 3. Mantle, 4. Gills.

concentration was attained, after which the depletion proceeded more slowly. PRINGLE *et al.* (1968) concluded that lead was released at a constant rate.

Lead – Mytilus

A high rate of uptake was found at both depths for the first 3 months (Fig. 8). The uptake was calculated for the whole soft part to 7.8 microg/g/week at 5 m depth, and 5.4 microg/g/week at 10 m depth, based on the assumption of a linear uptake with time during the first 60 days of the experiment. At 10 m depth the lead concentration levelled off after 60 days and remained constant. Individual variations were highest at 5 m depth. The concentration factors found for *M. edulis* were similar to those for *O. edulis* (Table 2). The concentration factor at equilibrium, derived as the ratio between the levels in the mussels and in the water, was of the order reported by SCHULZ-BALDES (1974), calculated from laboratory treatments and extrapolated. According to her, the concentration factor can be given by a simple equation provided the organisms are in equilibrium with the ambient water; e.g. based on equal rates of uptake and loss:

$$[M] = 0.03\ [A]\infty$$

where $[A]\infty$ = lead concentration in the animal (mg/kg dry weight) at time $t = \infty$, i.e. equilibrium, and [M] = lead concentration in ambient

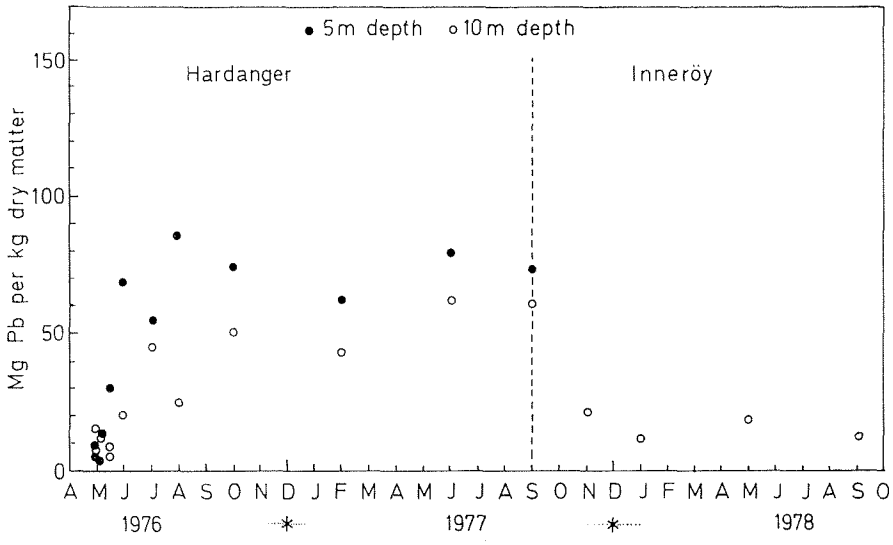


Fig. 8. Lead contents in the total soft tissues of *Mytilus edulis* during the uptake period in Hardangerfjorden, and after the return to the Innerøy oyster poll.

water. Used on the present results the equation should give a lead concentration of 2.1 microg/l in the ambient water for $[A]\infty = 70$ mg/kg.

Table 3 shows an increase in the weight of muscle tissue during the uptake period. Thus, the muscle accounted for an increasing part of the total lead content, from 15% at the start to 30% after 150 days. The highest levels of lead were found in the digestive system representing more than 40% of the total lead contents, and further in the muscle tissue, whereas the gills, decreasing in relative weight, accounted for a decreasing amount of lead during the uptake period.

The depletion rate was high in all tissues studied with the exception of the gills. In whole soft tissues 67% of the lead was released during the first 60 days. SCHULZ-BALDES (1974) reported a biological half-life of lead between 40 and 100 days, depending on the concentration in the ambient water. All tissue levels had returned to the original values within a year.

Other elements analyzed. Ostrea.

Appendix Table 1 shows the concentrations of magnesium, calcium, iron, copper and zinc in tissues of *O. edulis*. The magnesium levels decreased in Hardangerfjorden in all organs, with the highest decrease in the mantle, and returned to the original levels upon transfer to Innerøy. Calcium increased in muscle and mantle at the end of the stay in Hardangerfjorden, whereas a decrease was found in gills and in the digestive system. No trends could be seen for the iron contents. A copper uptake more than doubling the original values after 480 days was found in muscle and the digestive system, followed by a depletion upon transfer to Innerøy. The zinc values showed significant uptakes in all tissues analysed, and a slow depletion, with values well above the original levels a year after transfer back to Innerøy.

Other elements analysed. Mytilus.

As for *O. edulis*, the concentrations of magnesium decreased in all tissues during the stay in Hardangerfjorden, and increased again upon return to Innerøy. (App. Table 2). The calcium levels showed a similar trend for all tissues except for mantle. No trends could be found for the iron and copper contents. Significant increases in the zinc levels were observed, with maximum levels after transfer to Innerøy for several tissues. Only in muscle and foot did the zinc values return to the original levels after a year at Innerøy.

The variations in *Mytilus* tissue levels of these elements are further discussed in paper VII of the series (JULSHAMN, VII, 1981).

REFERENCES

BOYDEN, C.R., 1975. Mar. Pollut. Bull. *6*, 180–187.

BROOKS, R.R. and RUMSBY, M.G., 1965. Aust. J. Mar. Freshwat. Res. *15*, 53–61.

BRYAN, G.W. and HUMMERSTONE, 1973. J. Mar. Biol. Ass. U.K. *53*, 705–720.

CASTERLINE, J.L., and YIP, G., 1975. Arch. Environ. Contam. Toxicol. *3*, 319–339.

CHOU, C.L., UTHE, J.F. and ZOOK, E.G., 1978. J. Fish. Res. Board Can. *35*, 409–413.

CUNNINGHAM, P.A. and TRIPP, M.R., 1973. Mar. Biol. *20*, 14–19.

CUNNINGHAM, P.A. and TRIPP, M.R., 1975a. Mar. Biol. *31*, 311–319.

CUNNINGHAM, P.A. and TRIPP, M.R., 1975b. Mar. Biol. *31*, 321–334.

EISLER, R., ZAROOGIAN, G.E. and HENNEKEY, R.J., 1972. J. Fish. Res. Board Can. *29*, 1367–1369.

FRAZIER, J.M., 1975. Chesapeake Sci. *16*, 162–171.

FRAZIER, J.M., 1976. Chesapeake Sci. *17*, 188–197.

GALTSOFF, P.S., 1964. U.S. Fish Wildlife Serv. Fish. Bull. 64:iii+480p.

GEORGE, S.G. and COOMBS, T.L., 1977. Mar. Biol. *39*, 261–268.

GREIG, R.A. and WENZLOFF, D.R., 1978. Bull. Environm. Contam. Toxicol. *20*, 499–504.

JULSHAMN, K., 1977. At. Absorption Newsl. *16*, 149–150.

JULSHAMN, K., 1981, Studies on Major and Minor Elements in Molluscs in Western Norway. I–IV, VII. Fisk. Dir. Skr., Ser. Ernæring *1*, no. 5.

JULSHAMN, K. and ANDERSEN, K.J., 1981. Studies on Major and Minor Elements in Molluscs in Western Norway. V. Fisk. Dir. Skr. Ser., Ernæring, *1*, no. 5.

PHILLIPS, D.J.H., 1977. Environ. Pollut. *13*, 281–317.

PRINGLE, B.H., HISSONG, D.E., KATZ, E.L. and MULAWKA, S.T., 1968. Proc. Am. Soc. Civil Eng., J. Sanitary Eng. Div. SA3, 455–475.

SCHULZ-BALDES, M., 1974. Mar. Biol. *25*, 177–193.

SHUSTER, M. and PRINGLE, B.H., 1969. Proc. Natn. Shellfish Ass. *59*, 91–103.

STENNER, R.D. and NICKLESS, G., 1974. Water, Air Soil Pollut. *3*, 279–291.

STENNER, R.D. and NICKLESS, G., 1975. Mar. Pollut. Bull. *6*, 89–92.

ZAROOGIAN, G.E. and CHEER, S., 1976. Nature *261*, 408–410.

ZAROOGIAN, G.E., MORRISON, G. and HELTSHE, J.F., 1979. Mar. Biol. *52*, 189–196.

App. Table 1. Contents of magnesium and calcium (g/kg dry weight) and iron, copper and zinc (mg/kg dry weight) in tissues of *Ostrea edulis* after transfer to Hardangerfjorden (H), followed by return to original site, Innerøy (I).

Tissue	Site	Days	Mg	Ca	Fe	Cu	Zn
Muscle	H	0	3.0	2.5	60	28	3600
		150	1.9	2.7	78	26	4000
		480	1.9	5.3	160	65	5700
	I	60	2.9	1.4	67	53	6000
		240	2.9	2.9	100	58	5900
		360	3.5	2.2	88	40	4800
Mantle	H	0	7.0	8.9	190	180	9000
		150	4.8	9.2	440	190	11000
		480	2.2	16	230	200	17000
	I	60	3.9	14	300	230	19000
		240	8.9	3.8	380	280	18000
		360	7.8	8.0	270	220	15000
Gills	H	0	4.8	17	250	180	12000
		150	3.1	2.5	140	260	19000
		480	3.2	5.8	180	240	20000
	I	60	3.7	8.4	270	240	22000
		240	6.8	3.8	310	260	23000
		360	5.5	5.5	340	220	21000
Digestive system	H	0	2.5	1.5	120	70	4600
		150	1.3	0.72	95	90	6500
		480	1.3	0.98	140	150	13000
	I	60	1.7	0.90	130	110	11000
		240	3.3	0.92	160	120	11000
		360	2.9	1.1	150	95	7500

App. Table 2. Contents of magnesium and calcium (g/kg dry weight) and iron, copper and zinc (mg/kg dry weight) in tissues of *Mytilus edulis* after transfer to Hardangerfjorden (H), followed by return to original site, Innerøy (I).

Tissue	Site	Days	Mg	Ca	Fe	Cu	Zn
Muscle	H	0	3.9	1.0	41	5.6	76
		150	2.0	0.72	45	4.4	91
		480	1.5	0.51	40	4.2	150
	I	60	2.0	0.68	32	4.8	180
		240	3.1	1.3	50	5.7	89
		360	3.5	1.1	45	5.3	70
Foot	H	0	4.2	2.0	61	6.6	160
		150	2.3	0.99	53	12	320
		480	1.9	0.93	88	6.5	190
	I	60	2.2	1.1	43	7.5	180
		240	5.4	1.4	180	5.7	76
		360	3.9	1.6	58	6.0	120
Gonades	H	0	3.6	1.2	70	11	26
		150	1.9	1.0	100	12	100
		480	1.3	0.71	100	8.4	100
	I	60	2.0	0.96	93	11	380
		240	6.0	2.5	190	14	88
		360	5.2	1.3	90	12	55
Mantle	H	0	6.0	2.6	82	12	65
		150	4.2	1.8	78	12	110
		480	2.6	3.7	220	14	300
	I	60	4.6	3.0	68	11	100
		240	8.4	3.4	150	5.7	65
		360	7.5	2.2	110	6.9	84
Gills	H	0	10	6.2	150	14	120
		150	5.2	4.5	200	18	590
		490	3.9	2.5	180	17	380
	I	60	8.5	4.4	260	20	1240
		240	13	5.2	700	12	400
		360	12	4.9	170	13	210
Digestive system	H	0	3.3	1.4	210	14	190
		150	2.0	1.2	300	17	540
		480	1.8	1.3	480	12	440
	I	60	2.0	1.2	200	18	450
		240	5.0	2.0	380	13	760
		360	3.9	1.6	200	12	250

Fisk. Dir. Skr., Ser. Ernæring, Vol. 1: No. 5, 1981, s 267–287

STUDIES ON MAJOR AND MINOR ELEMENTS IN MOLLUSCS IN WESTERN NORWAY

VII. The contents of 12 elements, including copper, zinc, cadmium and lead, in common mussel (*Mytilus edulis*) and brown seaweed, (*Ascophyllum nodosum*) relative to the distance from the industrial sites in Sørfjorden, inner Hardangerfjord.

By

KÅRE JULSHAMN

Institute of Vitamin Research
Directorate of Fisheries, Bergen.

ABSTRACT

Samples of water, shoots of less than two years of age of brown seaweed (*Ascophyllum nodosum*) and adult common mussels (*Mytilus edulis*) were collected from 11 stations, 5 km apart, from the inner head of Sørfjorden, a branch of Hardangerfjorden, and out along the fjord. Industrial sites at the head of Sørfjorden discharge heavy metals to the water, among them copper, zinc, cadmium and lead. These four elements, and further iron, manganese, cobalt, mercury and the major elements sodium, potassium, magnesium and calcium were analyzed in the samples by atomic absorption spectrophotometry. The analytical values were treated in regression analyses. The results were discussed. Among the conclusions reached were the following points. 1. Most element concentrations in the water decreased rapidly within the first 15 km. The cadmium values were linearly correlated to distance along the fjord. 2. *Ascophyllum* shoots were useful indicators of the level of pollution from copper, zinc, cadmium and lead, as the contents were highly correlated to the distance. *Mytilis* was a useful indicator organism for lead and mercury, its contents of these two being highly correlated to distance, whereas the contents of copper, zinc and cadmium were relatively constant in *Mytilus*. 3. The contents of lead and zinc in the *Mytilus* samples were among the highest recorded in field surveys. The zinc contents from all sampling stations were 10 times higher than corresponding values from unpolluted water, pointing to a saturation of zinc under the prevailing conditions. The copper contents reached values only twice those from unpolluted waters, pointing to a barrier against further copper uptake in *Mytilus*. 4. Whereas the contents of the four major elements were generally positively correlated to distance and salinity in *Ascophyllum* as well as in *Mytilus*, the potassium contents in *Ascophyllum* was negatively correlated to distance along the fjord.

INTRODUCTION

The use of mussels as well as benthic algae as indicator species for metal contamination in estuarine or brackish waters is well established. GOLDBERG (1965) and PHILLIPS (1976 b, 1977 a, c, 1978) proposed the use of *M. edulis* as an indicator of metals in the aquatic ecosystem, whereas PRESTON et al. (1972), HAUG et al. (1974) and SEELIGER and EDWARDS (1977) reported that marine algae reflect the environment and magnify variations in the concentration of metals in seawater. Seaweeds may furthermore integrate metal concentrations in the water over relatively long intervals of time (PRESTON et al., 1972), giving a linear relationship between concentrations of metals in seawater and seaweeds (GUTKNECHT, 1965; BRYAN, 1969).

The four first papers in this series discussed natural factors influencing element contents in molluscs from unpolluted waters (JULSHAMN, I–IV, 1981). Paper VI concerned a study on the transfer of mussels between environments of different trace metal loads. This paper reports studies on the ability of indigenous common mussel (*Mytilus edulis*) and brown seaweed (*Ascophyllum nodosum*) to accumulate elements, particularly copper, zinc, cadmium and lead from a metal loaded environment. Comprehensive data are available from Sørfjorden (a branch of Hardangerfjorden on the west coast of Norway), including industrial effluents (*Miljøvernkomiteen*, 1974), hydrology (SVENSEN, 1973), water (SKEI et al., 1973, MELHUUS et al., 1978 b), sediment (SKEI et al., 1972, SKEI, 1975), algae (HAUG et al., 1974, MELHUUS et al., 1978 a, STENNER and NICKLESS, 1974) and common mussel (STENNER and NICKLESS, 1974).

The consequences on the organisms of metal dilution out along the fjord were studied, including regression analyses between the element levels in the water and the organisms. Twelve elements were analyzed in all samples, among them copper, cadmium, zinc and lead, as emission data were known on these from the factories at the fjord head.

METHODS

Sampling

Samples of water, brown seaweed (*Ascophyllum nodosum*) and common mussel (*Mytilus edulis*) were collected in September 1975 at eleven localities approximately 5 km apart from the inner head of Sørfjorden and out to Hardangerfjorden proper (Fig. 1). The samples of mussels and seaweed were collected half a meter below low tide to avoid effects of deposition of airborne particulates.

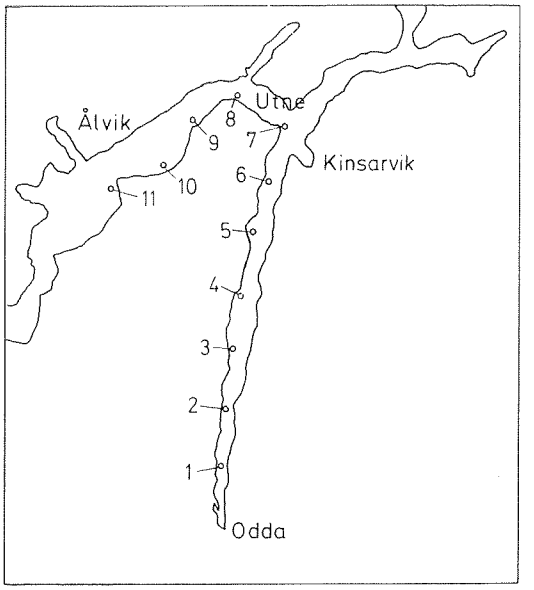


Fig. 1. Map of inner Hardangerfjorden/Sørfjorden, showing sampling stations at 5 to 55 km from inner fjord head.

At least 30 specimens of *Mytilus* of 30–40 mm length were sampled from each locality. Tissues were removed from the shells, washed briefly in distilled deionized water, dried in the air and pooled to give one sample from each locality. The seaweed samples were prepared as described in Paper I (JULSHAMN, I, 1981). At least 20 shoots of less than two years of age were pooled for analysis from each locality. About 2 liters of water were taken at the level where the organisms were collected, transferred to a polyethylene flask and conserved with nitric acid.

Analysis

Direct atomic absorption determination of trace elements in the complex matrix of sea water is not possible as the high salt content gives disturbing interferences. The water samples were irradiated in UV-light prior to chelation with ammonium-pyrrolidine-dithiocarbamate and extracted into methyl-isobutylketone (PAUS, 1973). The salinity was determined by a salinometer.

The pooled samples of *Mytilus* and *Ascophyllum* were freeze-dried to constant weight, ground and stored in capped jars until analysis. All elements were determined by atomic absorption spectrophotometry. Details of all methods, including digestion, pretreatments of the solutions

and instrumental modifications are given in Part I of this series (JULSHAMN, I, 1981). The results were run through a computerized regression analysis, fitted to a linear function ($y=a+bx$) or to the logarithmic function ($y=ax^b$).

RESULTS

Sørfjorden is a north–south trending extension of Hardangerfjorden, 1 to 2 km wide, 40 km long and 400 m deep, with several factories, among them a zinc factory at Odda at the head of the fjord (Fig. 1). Metal emissions to the fjord have varied, and in 1973 the output of Cu, Zu, Cd and Pb was estimated to 170, 3700, 38 and 1500 kg per day, respectively (*Miljøvernkomiteen*, 1974).

Water analyses

The concentrations of copper, zinc, cadmium and lead found in the water samples from the eleven stations are given in Table 1. There was a pronounced decrease in the water levels of all elements within the first 10 to 15 km from the industrial discharge. A three-fold dilution was observed between station 1 and 11 for zinc and lead, and a six-fold dilution for copper and cadmium, the latter value at station 10. Only the

Table 1. Heavy metal concentrations (microg/l) and salinity in water samples taken from Sørfjorden/inner Hardangerfjord.

Km from fjord head	Salinity ‰ (g/l)	Cu	Zn	Cd	Pb
5	3.1	14	140	1.8	4.2
10	3.1	8.6	70	1.6	2.1
15	5.6	5.2	72	1.2	2.8
20	3.5	3.2	70	1.8	1.6
25	4.5	2.0	50	1.2	1.0
30	7.0	4.9	52	1.2	1.3
35	5.6	5.3	40	0.40	1.4
40	7.0	8.1	40	0.60	1.3
45	7.2	5.5	42	0.80	1.0
50	7.5	6.0	50	0.30	1.0
55	8.3	2.4	48	0.10	1.3
Corr. coeff. lin.	0.91	—0.48	—0.72	—0.91	—0.75
Conc. factor at const. values		~3 (>15 km)	~3 (>25 km)	~6	~3 (>25 km)

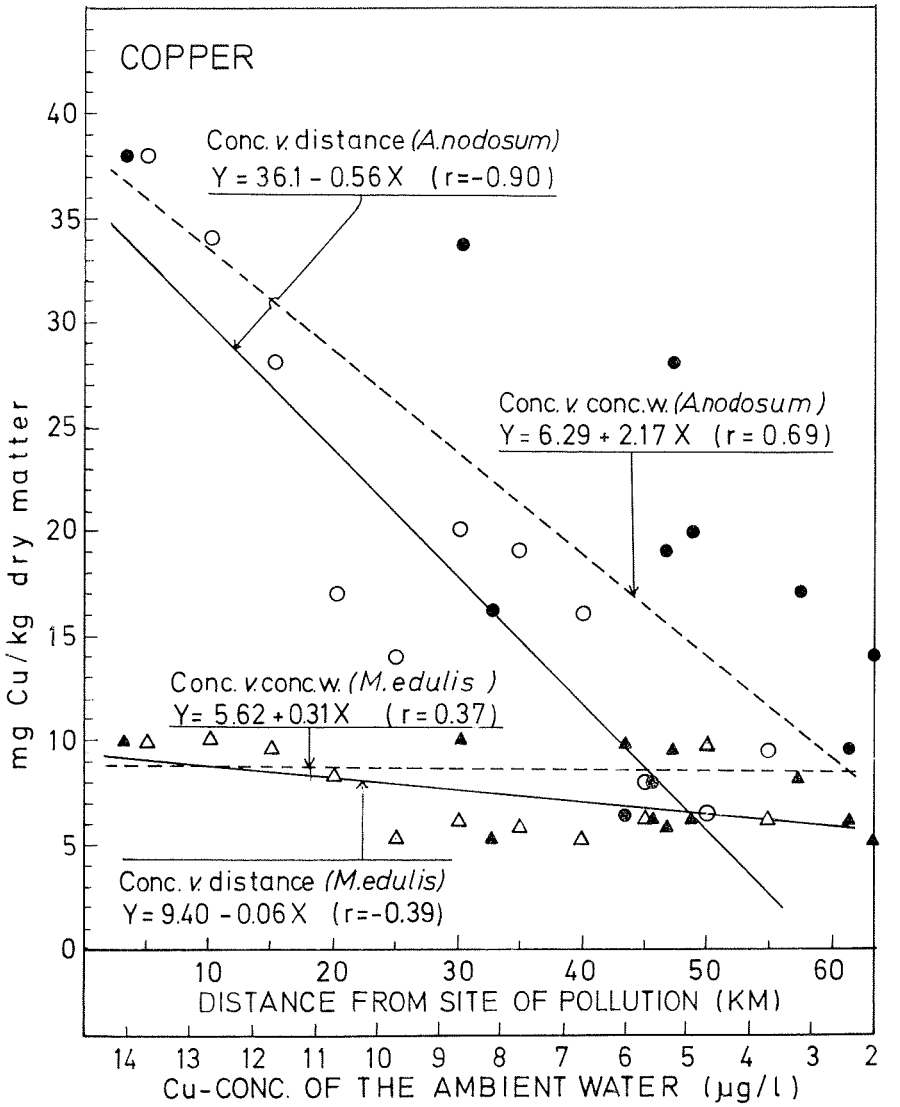


Fig. 2. Linear correlations for the copper contents in *Ascophyllum nodosum* and *Mytilus edulis* against distance from the fjord head and the water concentrations.

○ Concentrations in A. *nodosum* v. distance.
● Concentrations in *A. nodosum* v. concentration in water.
△ Concentrations in *M. edulis* v. distance.
▲ Concentrations in *M. edulis* v. concentration in water.

Table 2. Heavy metal concentrations (mg/kg dry weight) in samples of algae (*Ascophyllum nodosum*, A) and common mussel (*Mytilus edulis*, M) taken from Sørfjorden/inner Hardangerfjord.

Km from fjord head	Cu		Zn		Cd		Pb	
	A	*M*	A	*M*	A	*M*	A	*M*
5	38	*10*	3300	*710*	17	*35*	130	*530*
10	34	*10*	3100	*950*	27	*49*	38	*450*
15	28	*9.5*	2100	*1400*	16	*51*	13	*530*
20	17	*8.2*	2600	*1100*	17	*40*	7.5	*330*
25	14	*5.2*	1600	*1000*	7.8	*30*	4.2	*250*
30	20	*6.1*	1700	*1000*	7.6	*39*	2.5	*210*
35	19	*5.8*	1700	*950*	8.3	*33*	3.5	*260*
40	16	*5.2*	2600	*680*	8.2	*20*	2.9	*190*
45	7.9	*6.2*	1700	*380*	5.5	*22*	2.4	*130*
50	6.4	*9.8*	1300	*1000*	3.3	*22*	2.0	*230*
55	9.4	*6.1*	1700	*800*	5.5	*28*	2.7	*180*
Corr. coeff. lin.	—0.90	*—0.39*	—0.74	*—0.38*	—0.85	*—0.76*	—0.65	*—0.87*
Corr. coeff. log.*	—0.94	*—0.49*	—0.81	*—0.22*	—0.83	*—0.65*	—0.97	*—0.90*

*$y = ax^b$.

cadmium values were linearly correlated with the distance (r=—0.91), whereas the three other element levels were ca. constant from the 20 km station and out.

Copper

The copper content in *A. nodosum* was nearly four times higher than in *M. edulis* at the head of the fjord, but this difference diminished with increasing distance. The correlation between distance and copper concentration in *A. nodosum* was significant in a linear, as well as in a logarithmic function (Table 2, Fig. 2), whereas no corresponding correlation was found for the copper content in common mussel.

Zinc

The data for zinc showed a significant negative correlation between distance and concentration in *A. nodosum*, in a logaritmic as well as in a linear function (Table 2, Fig. 3). There was a positive correlation (r = 0.72) between the zinc concentrations in the water and in *A. nodosum*. The zinc content in *A. nodosum* was halved from station 1 to 11. The data for *M. edulis* were scattered around 900 mg/kg with no significant correla-

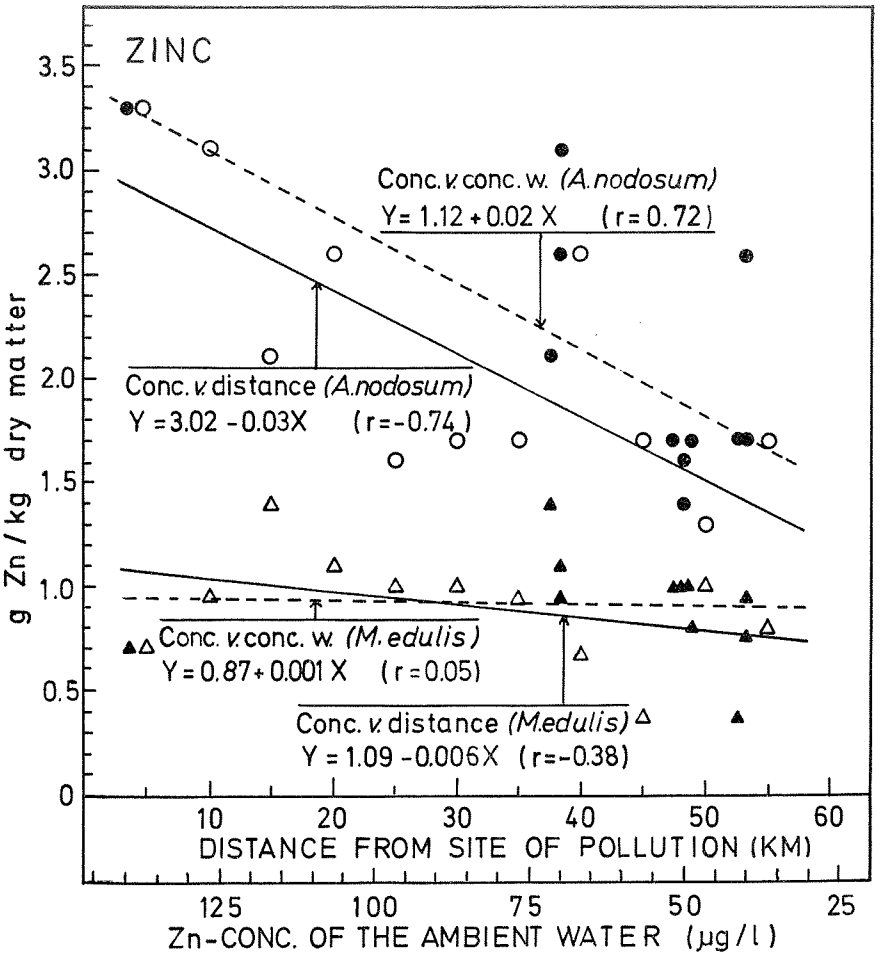


Fig. 3. Linear correlations for zinc, otherwise as for Fig. 2.

tions. Zinc levels were nearly five times higher in *A. nodosum* than in *M. edulis* at station 1, and this difference was reduced to 2:1 at station 11.

Cadmium

The cadmium concentrations in *A. nodosum* were significantly correlated to distance, as well as to the cadmium levels in the water samples (Table 2, Fig. 4). In *M. edulis* the cadmium levels were correlated to distance only. The cadmium content in *A. nodosum* fell to a third from the fjord head to mouth, whereas only a slight decrease was seen for the content in *M. edulis*. The cadmium levels in *M. edulis* were twice the values in *A. nodosum* at station 1, and the difference increased to 5:1 at station 11.

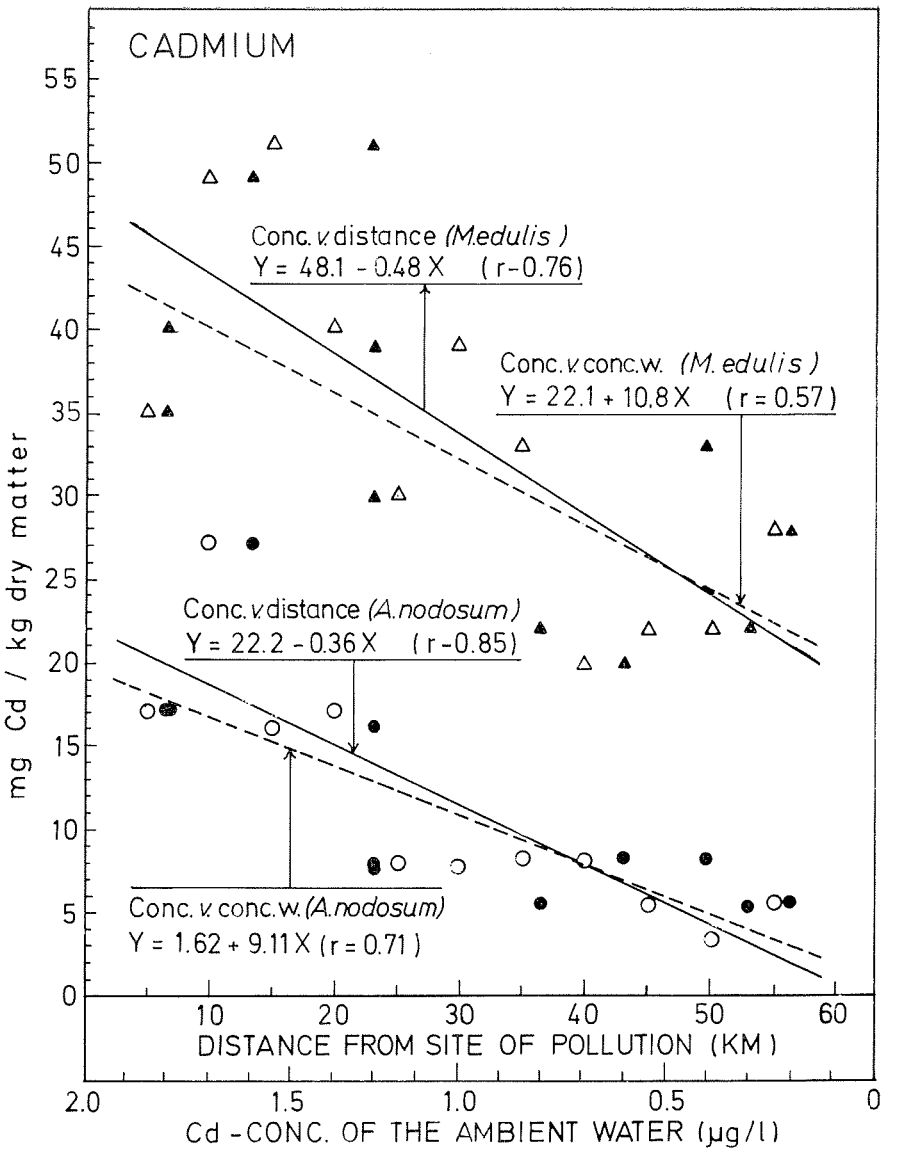


Fig. 4. Linear correlations for cadmium, otherwise as for Fig. 2.

Lead

The lead content in *A. nodosum* decreased substantially through the first 20 km, and the values were highly negatively correlated with distance in a logaritmic function (Table 2, Fig. 5). Significant correlations were also found between the lead concentrations in *M. edulis* and distance in a

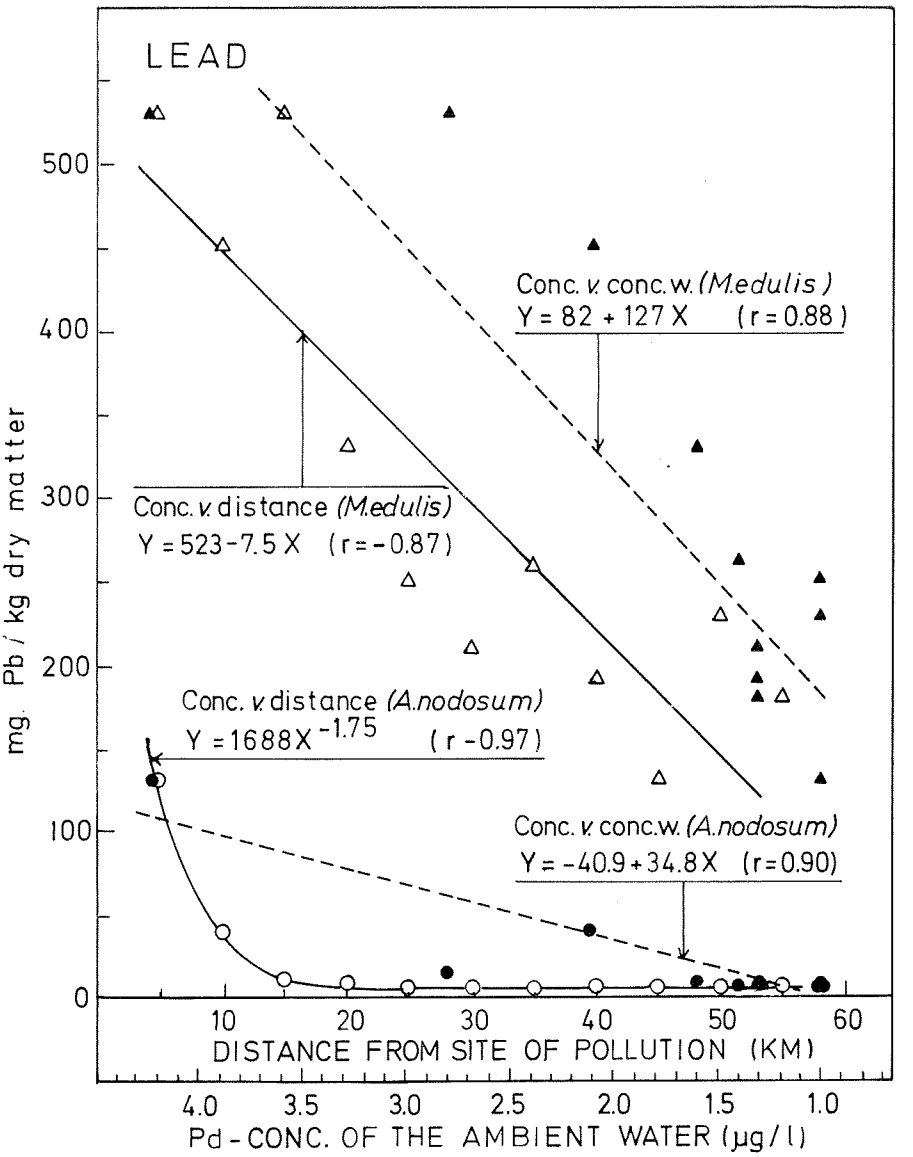


Fig. 5. Correlations for lead, otherwise as for Fig. 2.

linear and a logaritmic function. The lead contents in both organisms were further correlated with those in the water. The lead content in *M. edulis* decreased about three times from station 1 to 11, and were about four times higher in *M. edulis* than in *A. nodosum* at station 1, and this difference increased to 70:1 at station 11.

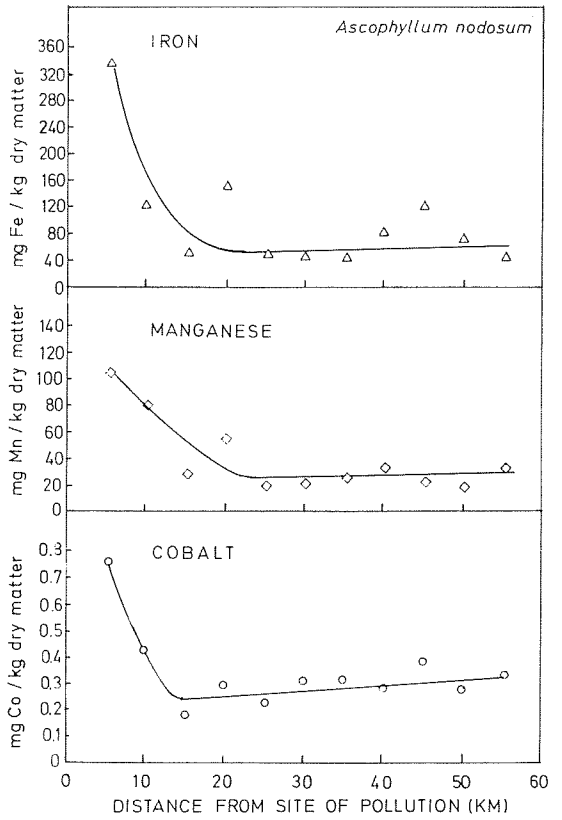


Fig. 6. Concentrations of iron, manganese and cobalt in *Ascophyllum nodosum*, plotted against distance from the head of Sørfjorden.

Manganese, iron, cobalt and mercury

The contents in *A. nodosum* of manganese, iron and cobalt fell substantially from station 1 to station 2, and all three concentrations were significantly negatively correlated to distance in a logaritmic function (Table 3, Fig. 6). No corresponding correlations were obtained in *M. edulis*. In *A. nodosum* the three element contents were further strongly correlated to each other, and also to the lead contents. No decrease with distance was found in the concentrations of mercury in *A. nodosum*, whereas the mercury levels in *M. edulis* were highly negatively correlated to the distance.

Sodium, potassium, magnesium and calcium

The sodium contents of both organisms studied increased along the fjord (Table 4). A highly positive correlation was found between the

Table 3. Heavy metal concentrations (mg/kg dry weight) in samples of algae (*Ascophyllum nodosum*, A) and common mussel (*Mytilus edulis*, M) taken from Sørfjorden/inner Hardangerfjord.

Km from	Mn		Fe		Co		Hg	
fjord head	A	*M*	A	*M*	A	*M*	A	*M*
5	120	*6.0*	330	*85*	0.75	*0.30*	0.64	*2.0*
10	80	*8.0*	130	*78*	0.40	*0.42*	0.84	*0.90*
15	25	*14*	48	*83*	0.16	*0.46*	1.1	*1.4*
20	53	*17*	150	*93*	0.31	*0.38*	0.40	*1.0*
25	19	*10*	43	*74*	0.21	*0.29*	0.10	*0.80*
30	19	*9.0*	42	*81*	0.32	*0.29*	0.32	*0.60*
35	22	*11*	40	*73*	0.32	*0.37*	0.98	*1.0*
40	33	*7.5*	96	*71*	0.28	*0.42*	0.64	*0.50*
45	26	*9.0*	120	*100*	0.36	*0.48*	0.86	*0.46*
50	19	*19*	68	*130*	0.28	*1.00*	0.64	*0.60*
55	27	*13*	36	*110*	0.32	*0.67*	0.60	—
Corr. coeff. lin.	—0.69	*0.35*	—0.56	*0.56*	—0.39	*0.63*	—0.04	*—0.81*
Corr. coeff. log.*	—0.86	*0.40*	—0.73	*0.40*	—0.61	*0.50*	—0.09	*—0.88*

*$y=ax^b$.

Table 4. Major element concentrations (g/kg dry weight) in samples of algae (*Ascophyllum nodosum*, A) and common mussel (*Mytilus edulis*, M) taken from Sørfjorden/inner Hardangerfjord.

Km from	Na		K		Mg		Ca	
fjord head	A	*M*	A	*M*	A	*M*	A	*M*
5	6.0	*2.0*	25	*2.7*	3.5	*0.34*	13	*1.8*
10	7.5	*2.0*	22	*3.8*	3.6	*0.39*	17	*1.2*
15	10	*2.8*	21	*4.6*	4.6	*0.51*	18	*3.3*
20	9.3	*2.3*	21	*4.2*	4.3	*0.45*	17	*1.8*
25	11	*2.2*	16	*4.6*	4.1	*0.50*	16	*2.0*
30	17	*3.3*	20	*5.3*	4.1	*0.50*	17	*1.7*
35	18	*4.2*	21	*5.7*	4.0	*0.65*	18	*1.6*
40	19	*3.6*	17	*6.5*	3.9	*0.45*	16	*2.8*
45	21	*4.6*	17	*8.8*	3.8	*0.58*	19	*4.5*
50	20	*7.4*	17	*9.1*	3.5	*0.85*	17	*5.2*
55	22	*6.0*	19	*8.4*	3.7	*0.63*	19	*2.6*
Corr. coeff. lin.	0.98	*0.88*	—0.72	*0.95*	—0.24	*0.78*	0.60	*0.60*
Corr. coeff. log.*	0.95	*0.82*	—0.63	*0.88*	—0.08	*0.69*	0.58	*0.62*

*$y=ax^b$.

sodium concentrations and the water salinity, as well as distance, particularly for the *Ascophyllum* values. A similar strong positive correlation was obtained between the potassium concentrations in *Mytilus* and salinity and distance, whereas the potassium concentrations in *A. nodosum*

decreased with distance. *M. edulis* showed the same pattern for magnesium, i.e. a significant correlation to distance and salinity, whereas the magnesium values in *A. nodosum* were constant through all sampling stations. The calcium contents in *M. edulis* were slightly correlated to the salinity, whereas those in *A. nodosum* gave no significant correlations. The four major element content were highly positively correlated to each other in *M. edulis* whereas this was not the case for *A. nodosum*.

DISCUSSION

Water analyses and metal loads

The concentration levels of copper, zinc and cadmium in the water samples were of the same order of magnitude as those reported from Sørfjorden by STENNER and NICKLESS (1974) and MELHUUS et al. (1978 a, b) and correspond well with values reported from other metal loaded areas (Table 5), with the exception of the concentrations of lead in the water samples which were at variance with other data from Sørfjorden. This may be due to influences of organic matter in the water samples and to the general problem of obtaining representative samples.

The wide variations in the concentrations of heavy metals in water samples at fixed sampling points have been clearly documented by MELHUUS et al (1978 b). Such variations may be caused, among other possibilities, by differences in season, time of day, the extent of freshwater

Table 5. Correlations between heavy metal concentrations in *Ascophyllum nodosum* (upper part) and in *Mytilus edulis* (lower part).

	Mn	Fe	Co	Cu	Zn	Cd
Fe	0.92					
Co	0.87	0.90				
Cu	0.78	—	—			
Zu	0.89	0.77	0.63	0.80		
Cd	0.70	—	—	0.84	0.83	
Pb	0.93	0.90	0.91	0.76	0.73	—
Fe	0.66					
Co	0.68	0.88				
Cu	—	—	—			
Zn	—	—	—	—		
Cd	—	—	—	—	0.69	
Pb	—	—	—	0.66	—	0.66

r for 9 DF and P=0.05=*0.60*
*N*on-significant correlations are not given.

run-off, depth of sampling, the intermittent flow of industrial effluent and hydrological factors such as tides and currents. The interacting effects of these variables may give wide variations in the concentrations of any element studied, and particularly in areas with brackish water. A time-intergrated water sampling equipment may be essential, thereby increasing the sample number at each site. Such improved equipment has been described recently by MELHUUS et al. (1978 b).

Uptake routes in A. nodosum and M. edulis

The metal contents reported here and those reported earlier from Sørfjorden show a substantial metal pollution towards the head of the fjord. The differences observed in the concentration relative to the distance profiles of *A. nodosum* and *M. edulis* may in part be due to different routes of metal uptake. The metal levels in seaweed are not subject to short term, erratic fluctuations of metal contents in the water, but integrate the dissolved metal concentrations in the water over relatively long intervals of time (PRESTON et al., 1972). It is widely accepted that this integration may be proportional to the ambient water mass for some dissolved metals (HAUG et al., 1974; SEELIGER and EDWARDS, 1977; MELHUUS et al., 1978 a). The cumulative effect of the uptake of several metals by algae has received little attention to date. If the uptake of metals in these organisms is a true ion exchange type of phenomenon, competition for binding sites may occur between metals and ion-displacement may take place (HAUG, 1961; HAUG and SMIDSRØD, 1967). This would cause an underestimation of the real metal burden when a complex metal matrix exist in the ambient water.

M. edulis is a filter feeder and accumulates metals by ingestion of phytoplankton and of inorganic particulates as well as by a direct uptake of dissolved metal ion.

Copper

The values found for copper in *A. nodosum* were substantially lower than those reported by HAUG et al. (1974) and MELHUUS et al. (1978 a). The discrepancy may in part be explained by differences in sampling. The samples used by MELHUUS et al. (1978 a) were based on shoots of a mixed age. The values given in Table 6 and results reported by HAUG et al. (1974), showed a significant increase in the copper content with increasing age of the shoots. Highly significant correlations were found between the copper levels in *A. nodosum* and in the water samples as well as the distance from the head of the fjord, whereas MELHUUS et al.

Table 6. Heavy metal concentrations in *Ascophyllum nodosum* shoots of 4 year classes from inner Hardangerfjord (mg/kg dry weight).

Year class	1	2	3	4
Mn	27	48	62	110
Fe	95	120	120	190
Cu	240	340	410	490
Zn	2300	3700	3900	4200
Cd	23	33	29	27
Pb	80	73	88	120

(1978 a) found no such correlation in samples of *A. nodosum* and *F. vesiculosus* from Sørfjorden. Possibly, a better correlation might be obtained if the samples of algae and water had been taken from the same place. MELHUUS et al. (1978 a) found approximately the same copper levels in *A. nodosum* and in *F. vesiculosus*, and the values agreed well with values from *F. vesiculosus* from Raritan Bay (SEELIGER and EDWARDS, 1977). The latter values were highly correlated with the copper levels in the sea water ($r = 0.95$). A higher proportion of the total copper in seawater was found in solution in Sørfjorden compared with the values from Raritan Bay (SKEI et al., 1973).

The contents of copper in *M. edulis* were substantially lower than in *A. nodosum* in the inner part of the fjord with high copper levels in the water. NICKLESS and STENNER (1974) found a similar trend. The copper content in *M. edulus* was little influenced by water levels of copper ranging from 2 to 14 microgram/l, and were not more than double those from unpolluted waters (JULSHAMN, I, 1981). PHILLIPS (1976 a) and DAVENPORT (1977) have proposed that *M. edulis* possesses a behavioural mechanism to avoid unnaturally high levels of copper. Similar low values were found in fish from copperexposed waters (JULSHAMN, unpublished data). PHILLIPS (1976 b) proposed a specific gill transport process for copper.

Zinc

The values found for zinc in *M. edulis* and *A. nodosum* are among the highest reported in the literature. The contents of zinc in *A. nodosum* correspond to previous results from Sørfjorden (Table 5). The sampling procedure may be less critical for zinc than for copper (Table 6). There was a better correlation between the zinc content in water and *A. nodosum* than found for copper. The ratio between the zinc levels in *A. nodosum* (Table 2) and in the water samples (Table 1) varied between $2.4{:}10^4$ and $6.5{:}10^4$. A similar ratio was obtained from unpolluted environments

(JULSHAMN, I, 1981). The present results agreed well with those reported on *A. nodosum* and *F. vesiculosus* by MELHUUS et al. (1978 a) and in *F. vesiculosus* by BRYAN and HUMMERSTONE (1973).

The zinc contents in *M. edulis* were ten times higher than in samples from unpolluted water (JULSHAMN, I, 1981). The maximum values were lower than those reported from Sørfjorden by STENNER and NICKLESS (1974). The discrepancy may be explained by size differences of the organisms analyzed and by the time of collection. The significant decrease of zinc content with the distance in the fjord water was not reflected in *M. edulis*, which had a relatively constant zinc content through all sampling stations. This suggests a saturation of zinc in *M. edulis* under the prevailing conditions. PHILLIPS (1977 b) described calculations of asymtotes of zinc uptake from similar concentrations as those reported in the present work. The zinc levels in *M. edulis* will probably be related to the water contents only in water with low zinc concentrations. This would cause an underestimation of zinc in *M. edulis* particularly when different metals coexist in the ambient water. The higher concentrations found in *A. nodosum*, may be due to a higher level of metal binding sites.

Cadmium

The discharge at the head of the fjord was 100 times higher for zinc than for cadmium in 1973. The cadmium levels found in *A. nodosum* agreed well with previous values (Table 7) and values from *F. vesiculosus* (PRESTON et al., 1972; MORRIS and BALE, 1975 and FOSTER, 1976). The concentrations were generally ten times higher than the values found in *A. nodosum* in unpolluted waters (JULSHAMN, I, 1981). The significant positive correlation between the cadmium levels in the water samples and in *A. nodosum* shows that this organism is a good indicator, not only of the total cadmium burden, but also for the dissolved proportion of the total Cd content. Zinc and cadmium contents in *A. nodosum* were significantly correlated, but the ratio between them increased from 150 to 330 from the inner to the outer part of the fjord (Table 5). This increase may follow the increasing salinity, and suggests that the dissolved part of the total metal content is higher for zinc than for cadmium (PHILLIPS, 1977 b).

The cadmium levels in *M. edulis* contrasted with those in *A. nodosum*, and only a weak correlation was seen between the zinc and cadmium values. Few data were available on cadmium in *M. edulis* from unpolluted environments. STENNER and NICKLESS (1974) reported a value of 140 mg/kg from Sørfjorden, sampled 15 km from the fjord head. Whereas there may have been an upper limit for the zinc content in *M. edulis*, a

Table 7. Reference values of heavy metal concentrations in water, *Ascophyllum* and *Mytilus*.

Authors	Locations	Cu	Zn	Cd	Pb	Mn	Fe	Hg
OPEN OCEANS								
(all values: microgram/l)								
Goldberg (1965)	Average seawater	3	10	0.11	0.03			
Preston et al. (1972)	N. Atlantic	0.05–0.80	1.4–7.0	0.01–0.41		0.03–0 09		
Chester and Stoner (1974)	Open ocean	0.1–3.9	0.3–3.0	0.02–0.17		0.07–0.37	0.5–4.1	
ESTUARINE AREAS								
(all values: microgram/l)								
Abdullah et al. (1972)	Bristol Channel, UK	2.1	10	1.1	1.2			
Butterworth et al. (1972)	Severn Estuary, UK	0.4–2.5	12–52	0.3–5.8	0.4–2.5			
Andersen et al. (1973)	Inner Oslofjord	8.4–10.4	41–73	0.7–0.9	1.0–2.8			
Boyden and Romeril (1974)	Bristol Channel UK	16.5–43	199–320	1.8–7.5	60–237	215–855	2410–900	
Stenner and Nickless (1974a)	Hardangerfjord	1–18	27–3560	0.01–85	2.9–12.7	0–62		
Boyden (1975)	Poole Harbour, UK	1–18	2–68	0.1–7.4		5–34	2–47	
Melhuus et al. (1978)	Hardangerfjord	1–23	8–900	0.5–9	1–92			
Present work	Hardangerfjord	2–14	40–140	0.1–1.8	1–4.2	0.4–8.0	1.6–4	
ASCOPHYLLUM								
(all values: mg/kg dry matter)								
Haug et al. (1974)	Trondheimsfjord	4–240	66–640	<0.7–1.0	<3–5			0.05–0.18
Haug et al. (1974)	Hardangerfjord	3–160	110–3700	0.7–16	<3–95			0.05–20.0
S enner and Nickless (1974a)	Hardangerfjord	4–44	160–1990	1.5–11.5	0.7–62			
Stenner and Nickless (1974a)	Skjerstadfjord	4–85	65–720	1.0–1.5	2–12			
Foster (1976)	Menai Straits, UK	6–18	82–236	1.8	2–6	10–35	54–120	
Melhuus et al. (1978a)	Hardangerfjord	11–125	1550–4000	6–16	4–105			
Present work	Hardangerfjord	6.4–38	1300–3300	3.3–27	2–130	19–120	36–340	0.1–1.1
MYTILUS								
(all values: mg/kg dry matter)								
Stenner and Nickless (1974a)	Hardangerfjord	3–22	170–2370	4.8–140	15–3100			
Stenner and Nickless (1974a)	Skjerstadfjord	15–130	105–280	1.9–4.7	2–6			
Present work	Hardangerfjord	5.2–10	710–1400	20–51	130–530	6–19	71–130	0.38–2.0

linear decrease correlated with distance was observed for cadmium. This difference between *A. nodosum* and *M. edulis* must reflect the different biological availability of cadmium, probably related to a decrease of the dissolved cadmium from the head to the mouth of the fjord. As discussed above, the decrease of soluble cadmium may be related to the increasing salinity.

Lead

The concentration of lead in the water samples as well as in *A. nodosum* from the inner part of the fjord were similar to earlier findings whereas the present data gave lower values than those previously reported from the outer part of Sørfjorden, (HAUG et al., 1974; STENNER and NICKLESS, 1974; MELHUUS et al., 1978 a). A similar trend was found for copper in *A. nodosum*, and there was a significant correlation besween these two elements (Table 5). There was a clear exponential correlation between the distance and the lead levels in *A. nodosum* ($r=-0.97$). Probably the values found for lead in the water samples were not quantitative because of a high content of particulate lead in the water. It is well documented that lead exists predominantly in particulate form in sea water (PRESTON et al., 1972; SEELIGER and EDWARDS, 1977). An investigation of the hydrology in Sørfjorden (SVENSEN, 1973) showed a circular current in the inner part of the fjord, whereas the average current elsewhere in the upper layer was towards the mouth of the fjord. Probably, more of the total lead pool is present as particulate lead towards the mouth of the fjord, an assumption that may be supported by the data given by SKEI et al. (1973). A logaritmic correlation between the distance and the lead contents in *A. nodosum* would therefore be reasonable. However, this is different from the results on *Bliclingia*, *Enteromorpha* and *Fucus* reported by SEELIGER and EDWARDS (1977) who found a close linear correlation between dissolved and particulate lead in algae. The present data for *A. nodosum* therefore suggest a different response to the total lead pool compared with the species studied by SEELIGER and EDWARDS (1977).

The concentrations of lead found in *M. edulis* were among the highest recorded in field surveys (PHILLIPS, 1978), probably reflecting higher lead levels in the water than those found by SCHULZ-BALDES (1974) and PHILLIPS (1978). It is therefore noteworthy that the data gave a significant correlation between distance and lead levels in *M. edulis*, as well as between the lead contents in the water and those in *M. edulis*. SCHULZ-BALDES (1974) found that *M. edulis* was an ideal indicator of the marine environment and reported a linear uptake of lead with time in *M. edulis* at levels from 5 to 5000 microg/l, further that the uptake via food and

water routes occurred at similar rates for the same exposure concentrations.

M. edulis may respond to the total lead pool, whereas *A. nodosum* responds only to the dissolved part of the lead.

Manganese, iron, cobalt and mercury.

Generally low concentrations were found for manganese, iron and cobalt, even in the inner part of the fjord. Already 10 km from the head of the fjord, the concentrations corresponded to those from uncontaminated water (LUNDE, 1970; FOSTER, 1976; JULSHAMN, I, 1980). Therefore the analytical values did not reflect a high level of pollution of these elements. In *A. nodosum*, the contents of these three elements were best correlated to the distance by a logaritmic function, and were also highly positively correlated to each other (Table 5). Probably, the elements exist predominantely in particulate form in seawater (PRESTON et al., 1972). SILLEN (1961) found that iron was bound in the particulate fraction as $Fe_2O_3 \cdot (H_2O)x$ and manganese was found as unchanged hydroxides such as $Mn(OH)_3$ or $Mn(OH)_4$. The chemistry of cobalt in seawater is poorly understood.

The levels found for the three element in *M. edulis* agreed well with data from uncontaminated areas (FOSTER, 1976). The data in Table 3 deviate clearly from those given by PHILLIP (1978) from Swedish waters. He found a significant correlation between lead and iron, whereas no correlation was obtained for manganese. Possibly, there is a very low biological availability for these elements in the water from Sørfjorden. The mercury content in *A. nodosum* decreased significantly from 1971 to 1974, as a result of new industrial processes involving a removal of mercury from the discharged water (HAUG et al., 1974). *A. nodosum* had relatively constant contents of mercury.

The mercury contents in *M. edulis* were double those in *A. nodosum* at the inner part of the fjord, but the difference levelled off further out. The levels at the mouth of the fjord were ten times higher than levels found in unpolluted areas (JULSHAMN, IV, 1981).

Sodium-potassium

There was a positive correlation between sodium and potassium in *M. edulis*, and a noteworthy negative correlation in *A. nodosum*. Generally, potassium dominated over sodium in both organisms studied. For *M. edulis* the ratio Na/K was between 0.5 and 1.0, whereas in *A. nodosum* the ratio increased from about 0.3 at station 1 to above 1.0 at station 11.

There was a discrepancy between these data for *M. edulis* compared with results given in Paper I in this series, which gave a predominance of sodium. This may be related to the difference in salinity. The ratio between sodium and potassium in *A. nodosum* may be more influenced by the salinity than in *M. edulis*. The concentration factor, defined by the ratio of the element in dried sample (mg/kg) and in the water sample (mg/l), was more than ten times higher for potassium than for sodium in the two organisms studied.

Magnesium – calcium

There was a predominance of calcium over magnesium in *M. edulis* with a ratio of about five to one. Both elements were significantly correlated to the salinity. The values for calcium agreed with values reported in *M. edulis* from seawater with high salinity (JULSHAMN, I, 1981), whereas the magnesium contents were influenced by the low salinity. The ratio between the magnesium content in *M. edulis* and salinity was about four for the present values as well as for the values reported in the Paper I of this series.

The calcium levels in *A. nodosum* were nearly constant, and remained higher than those reported from high salinity waters (JULSHAMN, I, 1981). The unexpectedly high calcium content may relate to a calcification process which occurs in a few species of algae (VINOGRADOV, 1953). Calcium predominated over magnesium in *A. nodosum*, and both concentrations were higher than in *M. edulis*.

CONCLUSIONS

1. Water analyses of the four heavy metals with known discharges to the fjord head showed a three-fold decrease in the concentrations of copper, zinc and lead through the first 15 to 25 km followed by constant values further out along the fjord. The *cadmium* concentrations decreased 6 times through the 11 sampling stations, and the values were highly linearly correlated with distance from fjord head.
2. Brown seaweed, *Ascophyllum nodosum* was found to be a useful indicator organism for the four polluting metals, *copper*, *zinc*, *cadmium* and *lead*, provided shoots of the same age were sampled. The contents in *Ascophyllum* based on dry weight, were highly correlated to distance from the fjord head in logaritmic function ($r = -0.81$ to -0.97) for all four elements and linearly for all except lead. The contents of copper, zinc and lead in *Ascophyllum* were further significantly cor-

related with the corresponding water concentrations. No correlation was seen for the mercury contents in *Ascophyllum*.

3. Common mussel, *Mytilus edulis* had relatively constant levels of copper, zinc and cadmium, and was therefore useless as an indicator organism for these element. The contents of *lead* and *mercury*, based on dry weight, were highly correlated with distance from the fjord head, linearly as well as logaritmically.
4. The lead contents in *Mytilus*, up to 530 mg/kg dry weight, were among the highest recorded in field surveys. The *Ascophyllum* values were also very high at the 5 km station, and decreased 10 times within the following 10 km.
5. In *Mytilus* the copper contents were little influenced by the water concentrations, and reached only double the values found from unpolluted waters, pointing to a mechanism to avoid high copper uptakes in *Mytilus*. The zinc contents in *Mytilus* were 10 times higher than in samples from unpolluted waters, and among the highest reported in the literature. The relatively constant values through all sampling stations pointed to a saturation of zinc in *Mytilus* under the prevailing conditions.
6. *Ascophyllum* enriched copper and zinc to a higher extent than *Mytilus*, whereas for cadmium and lead the levels in *Mytilus* were higher than in *Ascophyllum*.
7. The values for iron, manganese and cobalt in *Ascophyllum* were correlated logaritmically to the distance, pointing to increased levels in the inner part of the fjord, but already 10 km from the fjord head the values corresponded to those from uncontaminated waters. The contents in *Mytilus* of these three elements increased slightly out along the fjord.
8. The contents of sodium, potassium, magnesium and calcium were generally positively correlated to distance and salinity, with the exceptions of the potassium contents in *Ascophyllum* which gave a negative correlation to distance, and the magnesium contents in *Ascophyllum*, which were constant through all sampling stations.

REFERENCES

Abdullah, M.I., and Royle, L.G., 1974. J. Mar. Biol. Ass. U.K. *54*, 581–597.

Andersen, A.T., Dommasnes, A. and Hesthagen, I.H., 1973. Aquaculture *2*, 17–22.

Boyden, C.R., 1975. Mar. Pollut. Bull. *6*, 180–187.

Boyden, C.R. and Romeril, M.G., 1974. Mar. Pollut. Bull. *5*, 74–78.

Bryan, G.W., 1969. J. Mar. Biol. Ass. U.K. *49*, 225–243.

Bryan, G.W. and Hummerstone, L.G., 1973. J. Mar. Biol. Ass. U.K. *53*, 705–720.

Butterworth, J., Lester, P. and Nickless, G., 1972. Mar. Pollut. Bull. *3*, 72–74.

CHESTER, R. and STONER, J.H., 1974. Mar. Chem. *2*, 17–32.
DAVENPORT, J., 1977. J. Mar. Biol. Ass. U.K. *57*, 63.
FOSTER, P., 1976. Environm. Pollut. *10*, 45–53.
GOLDBERG, E.D., 1965. Chem. Oceanogr. *1*, 163–196.
GUTKNECHT, J., 1965. Limnol. Oceanogr. *10*, 58–66.
HAUG, A., 1961. Acta Chem. Scand. *15*, 1794–1795.
HAUG, A. and SMIDSRØD, O., 1967. Nature, Lond. *215*, 1167–1168.
HAUG, A., MELSON, S. and OMANG, S., 1974. Environm. Pollut. *7*, 179–192.
JULSHAMN, K., 1981. Studies on Major and Minor Elements in Molluscs in Western Norway, I–IV, VI, Fisk. Dir. Skr., Ser. Ernæring *1*, no. 5.
LUNDE, G., 1970. J. Sci. Fd. Agric. *21*, 416–418.
MELHUUS, A., SEIP, K.L., SEIP, H.M. and MYKLESTAD, S., 1978a. Environ. Pollut. *15*, 101–107.
MELHUUS, A., PAUS, P. and SEIP, H.M., 1978b. Concentrations of zinc, cadmium, copper and lead in Sørfjorden, Norway, SI rapport.
MORRIS, A.W. and BALE, A.J., 1975. Estuar. Coastal Mar. Sci. *3*, 153–163.
MILJØVERNKOMITEEN I ODDA, 1974, Final Report (in Norwegian), Odda, Norway.
PAUS, P.E., 1973. Z. Anal. Chem. *264*, 118–122.
PHILLIPS, D.J.H., 1976 a. Mar. Biol. *38*, 59–69.
PHILLIPS, D.J.H., 1976 b. Mar. Biol. *38*, 71–80.
PHILLIPS, D.J.H., 1977 a. Environm. Pollut. *13*, 281–317.
PHILLIPS, D.J.H., 1977 b. Mar. Biol. *41*, 79–88.
PHILLIPS, D.J.H., 1977 c. Mar. Biol. *43*, 283–291.
PHILLIPS, D.J.H., 1978. Mar. Biol. *46*, 147–156.
PRESTON, A., JEFFERIES, D.J., DUTTON, J.W.R., HARVEY, B.R. and STEELE, A.K., 1972. Environm. Pollut. *3*, 69–82.
SCHULZ-BALDES, M., 1974. Mar. Biol. *25*, 177–193.
SEELIGER, U. and EDWARDS, P., 1977. Mar. Pollut. Bull. *8*, 16–19.
SILL N, L.G., 1961. The physical chemistry of sea water. In: Oceanography, pp. 549–581. Ed. by M. Sears, Washington D.C. (Publs. Am. Ass. Advent Sci. No. 67).
SKEI, J.M., 1975. The Marine Chemistry of Sørfjorden, West Norway. Thesis presented for the degree of doctor of philosophy of the University of Edinburgh in the Faculty of Science.
SKEI, J.M., PRICE, N.B., and CALVERT, S.E., 1972. Water, Air and Soil Pollut. *1*, 452–461.
SKEI, J.M., PRICE, N.B. and CALVERT, S.E., 1973. Ambio *2*, 122–124.
STENNER, R.D. and NICKLESS, G., 1974. Water, Air and Soil Pollut. *3*, 279–291.
SVENSEN, H., 1973. Oceanographic investigations in Sørfjorden, 1972 (in Norwegian) Univ. of Bergen, Bergen, 1973.
VINOGRADOV, A.P., 1953. The Elemental Composition of Marine Organisms. New Haven, Sears Foundation.