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THE RELATION BETWEEN THE CONCENTRATION OF SOME MAIN ELEMENTS AND THE STAGES OF MATURATION OF OVARIES IN COD (*GADUS MORRHUA*)

By

K. JULSHAMN and O. R. BRÆKKAN
Institute of Vitamin Research
Directorate of Fisheries, Bergen

ABSTRACT

The contents of the major elements sodium, potassium, calcium and magnesium, and the minor elements iron, manganese, zinc, copper, cobalt, lead, cadmium and mercury have been determined in relation to the reproductive cycle of ovaries in cod (*Gadus morrhua*). In addition were determined the cobalt-containing vitamin $B_{12}$by microbiological assay of some of the samples. All ovaries were collected from cod caught off the Western coast of Norway, and all analyses were based on freeze-dried samples. The results are reported on the basis of wet weight and dry matter, and the relations to the reproductive cycle are given graphically. The contents of calcium, potassium, magnesium and zinc, showed different but significant decreases during the reproductive cycle, whereas sodium, manganese and copper showed significant increases. Calculation of cobalt derived from vitamin B_{12} compared with total cobalt showed a high degree of correlation, indicating that cobalt in the ovaries is present as part of vitamin B_{12}. The levels of lead, cadmium and mercury were well below the limits given as provisional tolerable weekly intakes proposed by FAO/WHO.

INTRODUCTION

With improved methods for the determination of minerals and in particular trace elements, there has been an increased interest in their occurrence in marine organisms and their physiological significance. The present study reports on the relation between major and minor elements in ovaries of cod (*Gadus morrhua*) and the stage of the reproductive cycle. In a previous communication (JULSHAMN & BRAEKKAN, 1975) we reported on the relation between cobalt in ovaries from salmon (*Salmo salar*) and the stages of maturation. No information is available on the content of metals during the development of ovaries in fish. However, OGINO & YASUDA (1962) have investigated several inorganic constituents in eggs from rainbow trout (*Salmo gairdneri*) from before fertilization to the larval stage.

Vitamin B_{12} determinations were included in the present study, to find if the unique relation between cobalt from vitamin B_{12} and total cobalt found in ovaries from salmon (*Salmo salar*) could be observed in cod (JULSHAMN & BRAEKKAN, 1975).

METHODS

COLLECTION AND TREATMENT OF THE SAMPLES

Cod roes were collected during the first three months of 1974, from fish caught off the Western coast of Norway and brought alive to the fish market in Bergen. The fish were weighed, gutted and the ovaries taken out, brought to the laboratory and weighed. The state of maturation was classified according to the method described by SIVERTSEN (1935, 1939). The size of the ovaries as expressed by percentage of the body-weight was used as the criterion for the determination of maturation. The stages noted with Roman numerals I—VIII refers to groupings <0.5, 0.5—2, 2—4, 4—6, 6—8, 8—10, 10—13 and > 13 %. When possible, the ovaries from each individual fish were analyzed, with the exception of juvenile ovaries, where five to ten ovaries were pooled. Each ovary or sample was homogenized, an at least 50 g material was taken out for freeze-drying. The freeze-dried samples were homogenized in a blendor and stored in tightly closed jars until analysis.

All glasswares were washed carefully in Thernards mixture (60 % destilled water, 30 % conc. hydrochloric acid, 10 % hydrogen peroxide (30 %)), followed by rinsing with diluted hydrochloric acid, very careful rinsing with deionized water and drying at 90 °C.All reagents were analytical grade.

ANALYTICAL PROCEDURE

Destruction of organic matter in the freeze-dried samples was based on wet-digestion, utilizing a combination of heat and pressure. A mixture of 4 ml $HNO_3/HCLO_4$ (1:1) was added to the bottles, and the samples predigested over night. Four replicates from each samples and blanks were then placed in a pressure boiler and heated at 110°C for 2 hours. After cooling and addition of water, the solutions were warmed to expel excess volatile aci. The solutions were transferred to 25 ml volumetric flasks and made up to volume with redistilled water.

The analyses of sodium and potassium were carried out in emmision mode on a Perkin-Elmer 403 Atomic Absorption Spectro-photometer. Depression effect of disturbing ions was avoided by diluting aliquots of the samples with 1000 μg per ml of an other alkali metal. Calsium and magnesium were measured in absorption mode and in a matrix of 1 % lanthanum oxide solution to minimize interfering components. With the exception of cobalt and mercury the trace elements were determined by the principle of standard addition. The measurements were performed in an organic matrix with methylisobutylketone (MIBK), where the trace elements were chelated as dithiocarbamate (NaDDC). The method has been described in detail by JULSHAMN and BRAEKKAN (1974 a).

For the determination of cobalt, portions of 10 g freeze-dried samples were preashed with infrared heat and ashed at 480 °C.The residue was dissolved in 0.1 N hydrochloric acid, chelated with ammonium pyrrolidine dithiocarbamate, extracted with MIBK and cobalt measured by atomic absorption as described by JULSHAMN & BRAEKKAN (1974 b).

For the determination of mercury, 1 g freeze-dried samples were digested with H_2SO_4/HNO_3 and V_2O_5. Hg(II)-salts were reduced to metallic mercury by the addition of $SnCl_2$, the mercury vapour blown through a gas cuvette and measured at a wavelength of 253.7 nm (MUNNS, 1972).

Vitamin B_{12} was determined microbiologically with *Lactobacillus leichmannii* as testorganism according to a slight modification of the method described earlier (THOMPSON et al. 1950). The extraction was carried out by autoclaving 0.5 g freeze-dried sample with 50 ml sodium acetate buffer of pH 4.5 + 5 ml 1 % KCN-solution for 15 min. at 15 lbs. pressure (120°C).

RESULTS AND DISCUSSION

In Table 1 are reported total dry matter based on freeze drying of the wet samples, further the concentration range and mean (± s.d.) based on wet weight, as well as the mean based on dry weight for the major elements sodium, potassium, calcium and magnesium for all eight maturation stages. In Table 2 are recorded the corresponding values for iron, manganese, zinc, copper and cobalt, and in Table 3 those for lead, cadmium and mercury.

In Fig. 1 are plotted percentages dry matter and in Fig. 2 and 4—7 the concentration of the different elements per g wet ovary against a logarithmic function of the stage of ovarian development. For each element are futher plotted two regression curves. One was calculated on

Table 1. The contents of the major elements sodium, potassium, calcium and magnesium in ovaries from cod (*Gadus morrhua*) in relation to the stages of the reproductive cycle.

		I	II	III	IV	V	VI	VII	VIII
No. of samples		3	7	11	14	11	9	5	11
Dry matter g/100g±s.d.		17.7±0.8	19.5±5.1	24.5±3.12	27.9±3.11	30.1±1.14	29.6±1.79	29.6±1.58	27.9±3.80
Na	Min.—max.	0.78—1.01	0.79—1.25	0.90—1.21	0.94—1.52	1.15—1.86	1.27—1.58	1.43—1.63	1.14—2.30
mg/g	M_w±s.d.	0.87±0.09	1.02±0.15	1.08±0.09	1.25±0.17	1.44±0.19	1.44±0.11	1.57±0.07	1.46±0.26
	M_d	5.23	5.25	4.40	4.48	4.78	4.86	5.22	6.67
K	Min.—max.	4.10—4.29	2.83—3.90	2.78—3.24	2.11—3.48	2.06—2.88	2.07—2.74	1.86—2.00	1.60—2.29
mg/g	M_w±s.d.	4.20±0.08	3.30±0.32	3.00±0.17	2.73±0.32	2.56±0.25	2.33±0.22	1.93±0.06	1.90±0.19
	M_d	25.16	16.96	12.24	9.81	8.46	7.85	6.52	6.78
Ca	Min.—max.	131—210	66—180	70—231	60—181	61—130	58—142	78—121	70—121
μg/g	M_w±s.d.	150±22	127±61	114±50	108±32	103±19	99±26	93±17	88±16
	M_d	899	653	465	388	342	344	315	315
Mg	Min.—max.	227—320	82—280	90—251	71—220	83—160	62—180	78—150	55—170
μg/g	M_w±s.d.	267±39	197±67	180±47	134±47	116±27	107±35	104±27	103±36
	M_d	1600	1013	730	479	386	360	353	367

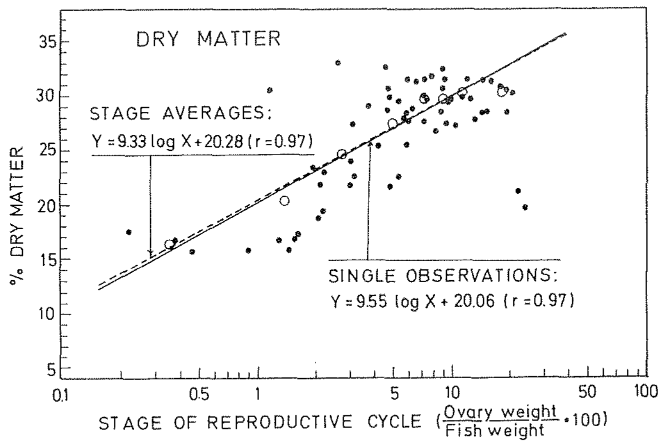

Fig. 1. Relation between percentage total dry matter and stage of reproductive cycle in cod (*Gadus morrhua*).

each single observation and the other on the stage averages given in Tables 1 and 2. The curves were fitted by a method of least squares to the logarithmic function, by the following equation:

$$Y = a_1 \cdot \log_{10} X + a_2$$

Figure 3 shows the relation between μmol sodium and μmol potassium during the maturation of the ovaries. The calculation of the least-square curve through the set of points were based on the simple linear regression equation. Otherwise, statistical treatment as t-test was performed on each element.

Total dry matter (Table 1 and Fig. 1) increased from 16.7 ± 0.80 at the first stage of the reproductive cycle (juvenile ovaries) and 19.5 ± 5.51 at stage II (early maturation) to values in the order of 28 to 30 at stages IV to VIII. A t-test showed no significant difference between stage I and II, but highly significant differences between stages I and III, and stages II and V ($0.001 < p < 0.01$). During the stages V to VIII the total dry matter remained rather constant. The general trend confirmed the findings reported by Braekkan & Boge (1959, 1962) in their studies on vitamins and the reproductive cycle of ovaries in cod. They pointed out that the increase in total dry matter during the first three stages of maturation mainly constitutes an increase in the protein content. Just before spawning a decrease in dry-matter could be observed. This decrease was not very marked, as the material did not comprise samples at imminent spawning stages.

In Fig. 1 may be noted that the curve based on single observations and the curve based on stage averages both gave the same correlation coefficient (r = 0.97).

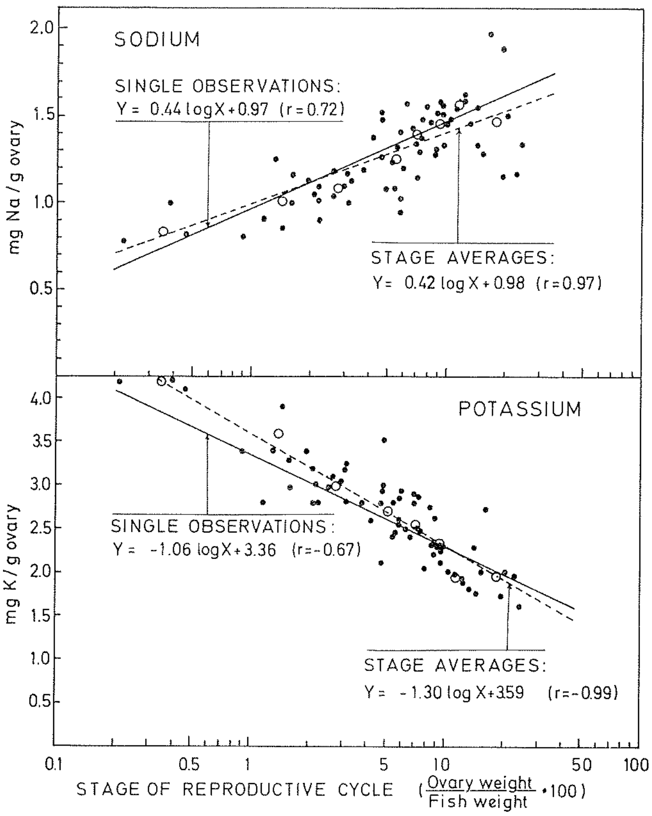

Fig. 2. Relations between mgNa/g and mg K/g wet weight and stage of reproductive cycle in cod (*Gadus morrhua*).

Sodium values are given in Table 1 and Fig. 2. Based on wet weight sodium showed a slow increase from juvenile ovaries (stage I) to stage V from 0.87 $\pm$ 0.099 to 1.41 $\pm$ 0.192 mg/g. From stage V the content remained fairly constant. t-Test showed no significant difference between stage I and II ($0.05 < p < 0.1$). The difference between stage II and V was highly significant ($p < 0.001$), while the differences between later stages were not significant. The curve based on single observations (Fig. 2) showed a correlation coefficient $r = 0.72$, whereas calculations based on stage averages showed an improved correlation ($r = 0.97$). Calculations to a linear as well as an exponential function were tried, but did not give improved correlations.

Potassium. The levels of potassium (Table 1 and Fig. 2) showed an opposite effect of maturation, compared to sodium. This element decreased markedly through the maturation stages. The table showed a rapid and significant decrease from 4.2 $\pm$ 0.078 mg/g wet weight in juvenile to 3.31 $\pm$ 0.323 mg/g potassium in stage II, further to 1.90 $\pm$ 0.19 at the end of the maturation in stage VIII. t-Test on the analyzed values gave highly significant differences between stages I and II ($0.001 < p < 0.01$), stages II and V, and V and VIII. A logarithmic function gave the best relationship with a correlation of r = —0.67, and a least square treatment on the stage averages gave a very good correlation of r = —0.996. Fitting of observations to a linear and exponential function gave for the single observations correlations of —0.62 and —0.19, and stage averages —0.95 and —0.67, respectively.

In Fig. 3 are plotted the relation between μmol potassium and sodium per g wet weight in the ovaries. LEIVESTAD (1965) reported on the K/Na ratio in the muscle of different fish caught at different localities, and found the sum of the cations to be reasonably constant, distributed around 145 μmol/g. Similar values were reported in cod muscle by ELIASSEN et al. (1960) and in rat and frog sarcoplasm by HODGIN (1951). A least square treatment of the same relations in the ovaries from cod gave a regression coefficient of —0.50 with r = —0.56. Although there was an apparent inverse relation between the elements during the maturation, a constancy of the K + Na concentrations could not be observed. It should, however, be pointed out that the present values refer to the whole ovaries and do not represent the egg cells alone.

Calcium levels are given in Table I and Fig. 4. They showed a slight but significant decrease (on 1 % level) from 150 ± 22 μg/g in juvenile to 127 ± 61 μg/g fresh weight at stage II. The corresponding values as μg/g

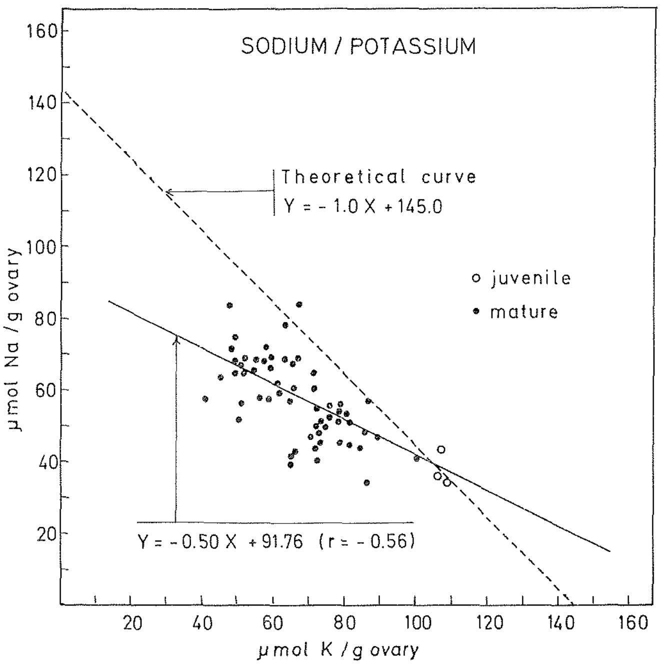

Fig. 3. Relation between μmol Na/g and μmol K/g ovary during the maturation.

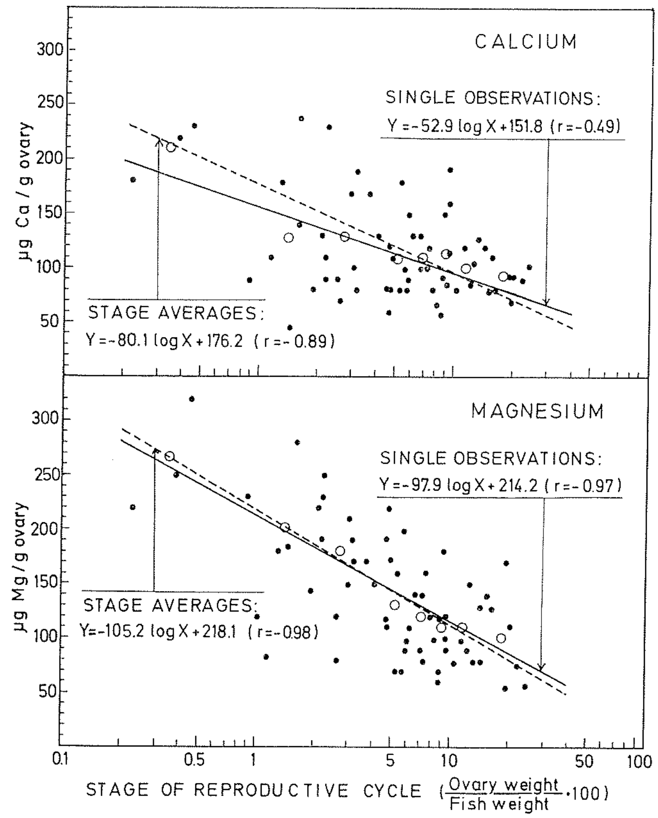

Fig. 4. Relations between μg Ca/g and μg Mg/g wet weight and stage of reproductive cycle in cod (*Gadus morrhua*).

dry matter were, however, of the same order. No significant differences were found between stages II and V nor between stages V and VIII. The observed values showed very high deviations within the stages as well as between different stages. The best correlation of the observations as fitted. to a logarithmic function based on single values as well as the stage averages gave rather low r-values, 0.45 and 0.89, respectively. Other functions were tried but with even lower correlation.

Magnesium levels were of the same order as those found for calcium. Magnesium decreased from 267 $\pm$ 39 μg/g in juvenile to 197 $\pm$ 67 μg/g at stage II, based on wet weight. The decrease continued to 116 $\pm$ 27 μg/g at stage V and to 103 $\pm$ 36 μg/g at the end of the maturation. A similar trend was observed on the basis of dry matter, from 1600 μg/g in juvenile to 367 μg/g at stage VIII (Table 1).

Juvenile ovaries (I) and ovaries in the first stage of maturation (II) showed no significant difference on 0.05 level in a t-test. The difference between stages II and V was highly significant ($0.005 < p < 0.01$) whereas no significant difference was found between stages V and VIII. The relation between magnesium in $\mu g/g$ fresh weight and the maturation stages of ovaries gave a very high correlation to a logarithmic function, r = 0.97 and 0.99, based on least square analysis on single observations and stage averages, respectively (Fig. 4).

Iron and manganese were plotted together as members of the iron family. The iron contents in ovaries of cod based on wet weight (Table 2 and Fig. 5) showed an increase from 11.6 ± 1.02 $\mu g/g$ at the juvenile stage to 15.4 ± 5.21 $\mu g/g$ at stage II, and further a slow decrease to 8.26 ± 1.52 $\mu g/g$ at stage VIII. t-Tests gave no significant difference on 0.05 level applied on stage I to II, but a significant difference between II and V ($0.01 < p < 0.05$) and a highly significant difference between stage V and VIII ($0.001 < p < 0.01$). Fig. 5 shows a semi-log plot of a least square curve with a correlation coefficient of —0.55 and —0.76 based on single values and stage averages, respectively. A better fitting could be made by linear regression with correlation coefficients of —0.57 and —0.84.

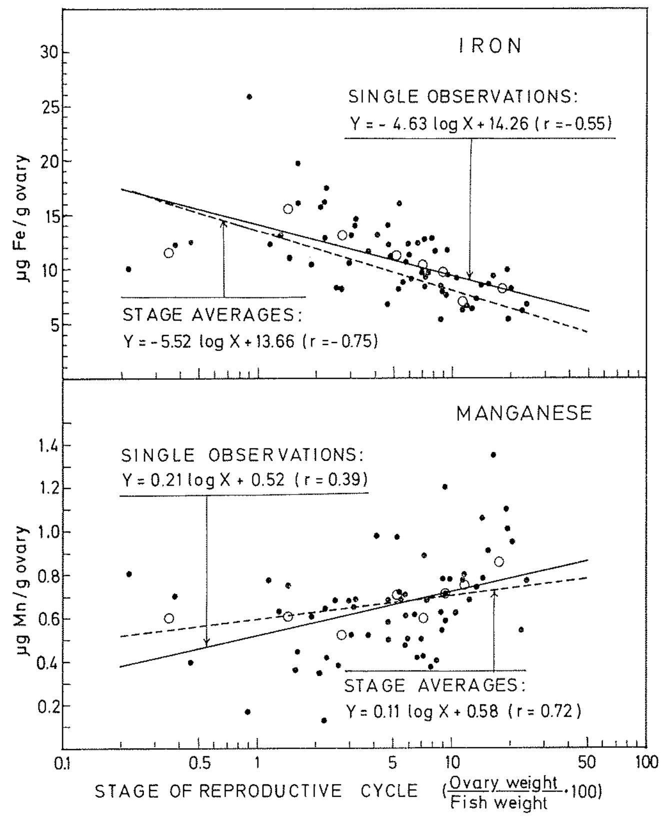

Fig. 5. Relations between μg Fe/g and μg Mn/g wet weight and stage of reproductive cycle in cod (*Gadus morrhua*).

Table 2. The distribution of the trace elements, iron, zinc, manganese, copper, cadmium, lead, cobalt during the maturation of the ovary in cod (*Gadus morrhua*).

		I	II	III	IV	V	VI	VII	VIII
No. of samples		3	7	11	14	11	9	5	11
Dry matter g/100g±s.d.		16.7±0.8	19.5±5.1	24.5±3.12	27.9±3.11	30.1±1.14	29.6±1.79	29.6±1.58	27.9±3.80
Fe	Min.—max.	10.2—12.5	10.5—25.9	8.2—17.5	6.8—16.1	8.4—12.8	5.4—11.8	6.4—9.2	5.3—11.6
μg/g	M_w±s.d.	11.6±1.0	15.4±5.2	13.0±2.94	11.3±2.28	10.05±1.76	9.24±2.10	7.36±1.20	8.26±2.25
	M_d	69.5	79.9	53.0	40.4	33.4	31.2	24.9	29.6
Mn	Min.—max.	0.25—0.75	0.17—0.77	0.13—0.68	0.48—0.98	0.38—0.89	0.40—1.20	0.68—0.80	0.54—1.10
μg/g	M_w±s.d.	0.45±0.15	0.53±0.20	0.51±0.17	0.67±0.15	0.67±0.19	0.70±0.22	0.72±0.15	0.88±0.18
	M_d	3.60	2.73	2.10	2.39	2.23	2.37	2.44	2.96
Zn	Min.—max.	117—119	50—107	56.6—92	42.3—78.0	29.0—56.3	39.6—76.0	37.8—53.0	33.4—63.8
μg/g	M_w±s.d.	118±0.9	78±20.7	69.2±10.1	54.8±11.5	47.5±9.3	53.1±11.1	47.3±5.7	47.4±8.9
	M_d	705	401	282.0	196.5	157.7	179.3	160	169.8
Cu	Min.—max.	0.80—0.91	0.52—1.27	0.85—1.48	1.10—2.07	1.29—1.60	0.61—1.71	0.88—1.89	0.99—2.30
μg/g	M_w±s.d.	0.84±0.05	0.96±0.23	1.15±0.16	1.65±0.23	1.43±0.20	1.29±0.34	1.31±0.26	1.75±0.31
	M_d	5.01	4.93	4.69	5.93	4.76	4.33	4.43	6.26
Co	Min.—max.	0.0212—0.0296	0.0145—0.020	0.010—0.0164	0.0099—0.014	0.0089—0.011	0.0078—0.0098	0.007—0.0087	0.002—0.0062
μg/g	M_w±s.d.	0.025±0.034	0.017±0.0019	0.013±0.0019	0.011±0.0013	0.010±0.0010	0.009±0.0012	0.007±0.0010	0.004±0.0013
	M_d	0.151	0.088	0.053	0.042	0.033	0.030	0.024	0.016

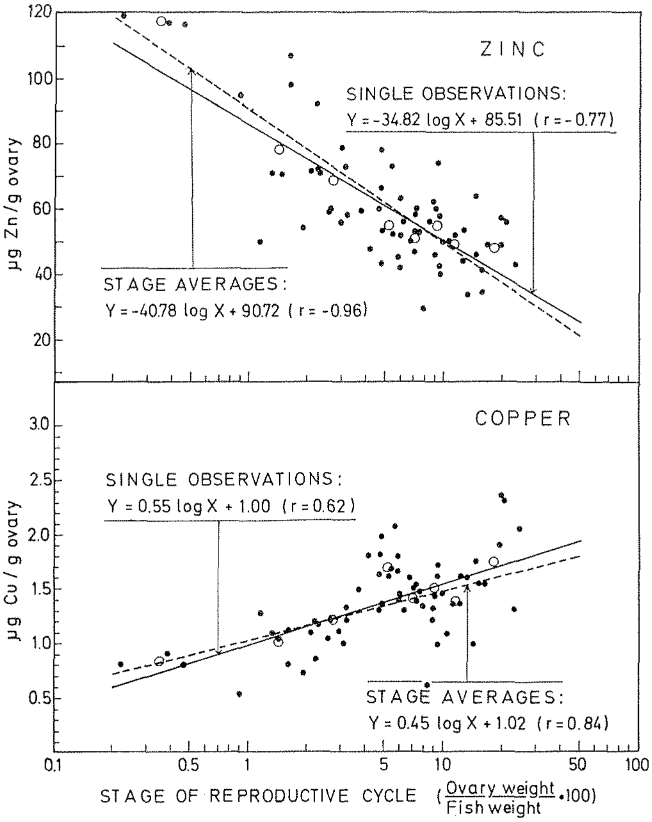

Fig. 6. Relations between µg Zn/g and µg Cu/g wet weight and stage of reproductive cycle in cod (*Gadus morrhua*).

Manganese levels showed an increase through the maturation of ovaries from 0.45 ± 0.15 at stage I to 0.88 ± 0.18 µg/g wet weight in stage VIII. t-Tests showed no significant differences between stages I and II, II and V, and V and VIII, but a significant difference between stages II and VIII on the 0.01 level. Calculated on the basis on dry matter, the values for manganese showed a decrease from the juvenile stage to stage V followed by a slow increase. The best relation between manganese and the stages of maturation was given by a linear regression curve based on stage averages with a correlation coefficient of 0.81.

Both manganese and iron levels were higher in roe compared to filet and soft roe of cod, and lower than that in liver (JULSHAMN & BRAEKKEN, 1975).

Zinc. The content of zinc in the ovaries of cod are given in Table 2, and related to the stages of the maturation in Fig. 6. This element showed a considerable decrease from stage I to II, and less markedly from II to V and remained fairly unchanged from V to VIII. The same trend was found from the values based on dry matter. t-Tests applied on the different stages showed highly significant differences between stages I and II, as well as II and V ($0.001<p<0.01$), but no significant difference between stages V and VIII, ($p>0.1$). From Fig. 6 may be noted the good correlation based on stage averages $r = 0.96$, whereas the regression curve based on single observations gave $r = 0.77$.

The levels of zinc were exeptionally high, 5 to 10 times higher than that of iron and further the zinc level in the ovary was higher than in any other organ of cod (JULSHAMN & BRAEKKAN, 1975). BERTRAND and VLADISCO, as early as 1922, reported that zinc plays a physiological role in fertilization; during spawning, zinc was transferred from the muscles to the sexual products of the males.

Copper levels are given in Table 2 and Fig. 6. The contents of copper seemed to increase during the maturation, and a t-test showed highly significant differences between stage II and V ($0.001<p<0.01$). The correlation was low based on single ($r = 0.62$) as well as stage averages ($r = 0.82$), nevertheless the logarithmic function gave the best fitting to a mathematical function. VINOGRADOV (1953) reported that the ratio Fe/Cu in the tissues of vertebrates remains constant, but this did not hold for ovaries of cod, the ratio decreased during maturation.

Cobalt is summarized in Table 2 and Fig. 7. The content of cobalt showed a decrease from 0.0252 ± 0.0034 at stage I to 0.0044 ± 0.0010 $\mu g/g$ wet weight in stage VIII. Between stages I and II, as well as between stages II and V, and between V and VIII, very highly significant differences ($p<0.001$) were found. This decrease was more marked than that observed in ovaries of salmon (*Salmo salar*)(JULSHAMN & BRAEKKAN, 1975). The least square calculation gave a correlation coefficient of $r = 0.93$ values and of $r = 0.99$ based on stage averages (Fig. 7).

Vitamin B_{12} was determined in 2 to 3 samples at each stage of maturation by microbiological assay. The values found corresponded well with those reported by BRAEKKAN & BOGE (1962). Cobalt calculated on a molecular basis deriving from vitamin B_{12}, showed closely the same order of magnitude as well as the same relation during the different stages of maturation. When the 88 values reported by BRAEKKAN & BOGE were used as basis for calculation, the same close relation was found, with deviations of less than 10 %. Thus the same relation between total cobalt and cobalt derived from vitamin B_{12} could be observed in cod ovaries as reported for maturing ovaries in salmon (*Salmo salar*) by JULSHAMN & BRAEKKAN (1975).

In conclusion, the following pattern is seen: Sodium, copper and manganese concentrations increased during the maturation of the roe.

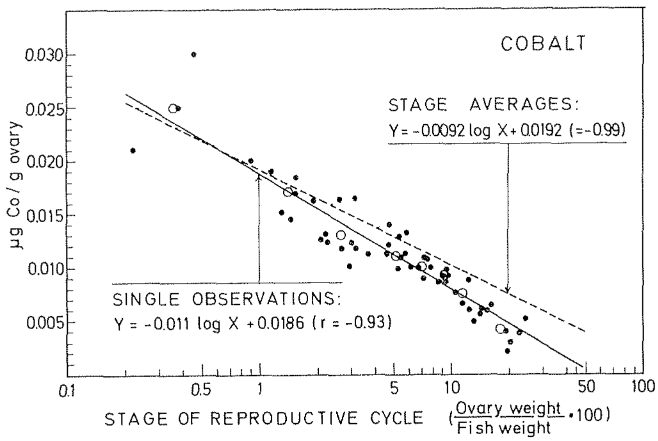

Fig. 7. Relation between μg Co/g and stage of reproductive cycle in cod (*Gadus morrhua*).

The levels of iron varied somewhat, but generally increased in the two first stages and then decreased. The intracellular elements calcium, potassium, magnesium as well as zinc decreased in concentration during the whole period of maturation, thereby giving decreasing levels relative to the protein contents of the roe. The best fitting of the curves to the observations were to logarithmic functions, when the stage averages were used. This indicated statistically that no systematic failure were introduced through collection and preparation of the samples.

The last three elements, lead, cadmium and mercury, are widely distributed in Nature, but considered to some extent contaminants arising from environmental pollution (Table 3).

Lead showed low, but somewhat fluctuating values based on wet weight. Calculated on dry matter a decrease may be observed.

Cadmium showed similar low values, but with a clearer decrease, and the values at stages VI to VIII were below the detection limit of the method.

Mercury was only analysed in samples at stage I and VIII, the values being 0.041 ± 0.005 in juvenile and 0.019 ± 0.009 μg/g wet weight in mature ovaries.

The values for lead, cadmium and mercury in cod roe in the present study were well below the limits given as provisional tolerable weekly intake proposed by the Joint FAO/WHO Expert Committee on Food Additives (1972).

Table 3. The distribution of the trace elements, lead, cadmium and mercury during the maturation of the ovary in cod (*Gadu morrhuas*).

		I	II	III	IV	V	VI	VII	VIII
No. of samples		3	7	11	14	11	9	5	11
Dry matter g/100g±s.d.		16.7±0.8	19.5±5.1	24.5±3.12	27.9±3.11	30.1±1.14	29.6±1.79	29.6±1.58	27.9±3.80
Pb	Min.—max.	0.08—0.1	0.10—0.64	tr.—0.98	tr.—0.48	tr.—0.30	tr.—0.73	tr.—0.35	tr.—0.30
μg/g	M_w±s.d.	0.10±0.01	0.25±0.165	0.27±0.24	0.15±0.20	0.10±0.12	0.17±0.24	0.10±0.10	0.15±0.10
	M_d	0.60	1.28	1.08	0.54	0.34	0.57	0.34	0.54
Cd	Min.—max.	0.01—0.05	tr.—0.03	tr.—0.04	tr.—0.03	tr.—0.01			
μg/g	M_w±s.d.	0.03±0.01	0.01±0.010	0.02±0.02	0.01±0.02	>0.01	N.D.	N.D.	N.D.
	M_d	0.11	0.05	0.096	0.05				
Hg	Min.—max.								
μg/g	M_w±s.d.	0.041±0.005							0.019±0.009
	M_d	0.25							0.068

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