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Histopathological changes in the mussel *Mytilus edulis* L. at the outlet from a titanium dioxide plant in Northern Baltic

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A histopathological examination of a mussel sample (*Mytilus edulis* L.) from a polluted bottom in the Bothnian Sea, off the town of Pori, was made. The sampling station is situated near the outlet tube of a titanium dioxide plant. A control sample was collected from an unpolluted area from the Bothnian Sea. The mussels in the Pori sample had histopathological changes in their gonads and digestive diverticulas. Stomachs, intestines and kidneys showed an extensive physiological response to their environment. Mussels in the Pori sample were in the breeding stage, though the date of sampling (September) is outside the breeding period. The control mussels were not spawning. The spawning of the Pori mussels was abnormal. In the male follicles ripe sperm was cytolysed. The female follicles contained anomalous ova, granulocytes and cellular debris. The plasma membranes of the ova loosened from the vitelline membranes. In the digestive diverticula there was no striated border against the lumen of the tubules. The tubules were filled with exudate and the digestive cells had erosion of the cytoplasm. The stomach and intestine epitheliums contained macrophages and granulocytes. The kidney tubules of some mussels stained dark due to metal sequestration. There was excretion of macrophages in the kidney tubules. The changes in the tissues of the mussels are considered to be responses to an acid and heavy metal stress.

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1. Introduction

The greatest loader of the Bothnian Sea (the southern part of the Gulf of Bothnia) is a titanium dioxide plant near the town of Pori (Fig. 1). Main discharges are sulphuric acid, ferrosulphate, titanium dioxide and heavy metals aluminium, mangane, vanadine, zinc, chrome, nickel and cobolt. The discharge waters are conducted in outlet tubes 4.7 kilometers from the shoreline at a depth of 17 meters. pH of the discharge water is 1 and it contains 17 g/l of ferrous sulphate (Häkkilä 1983). In the open shore off Pori mixing of sea water is efficient and the area, where undiluted waste water is found, is limited (Häkkilä et al. 1978). A 3 km^2 bottom area adjacent to the waste outlet is covered with TiO_2-mud from the outlet tube. Ferric hydroxide flakes cover the bottom in an area of some 120 km^2. Since the density of the waste water (1.1 g/cm^3) is greater than that of the brackish water (1.005 g/cm^3), it keeps close to the bottom and clides on the bottom to a wider area. Spreading of the waste water depends on the weather conditions.

According to benthic fauna investigations very polluted bottom, with no or few bottom animals, is found in an area of 8 km^2 around the waste discharge. Polluted bottom, with very low densities, biomasses and number of species, is found in an area of 18 km^2. Just around the outlet tube there is a bottom area that is continuously exposed to the discharge waters. Around this there are areas that are occasionally exposed to poorly diluted waste waters (Häkkilä et al. 1978).

The Pori sea area is also affected by the polluted water from the river Kokemäenjoki. The pollution of the river is considered to be organic, increasing the densities and biomasses of the bottom animals. The river water spreads in the surface waters and dilutes relatively soon. Plain eutrophication in coastal sea water is restricted to a narrow belt. In winter the river water spreads under ice to a wider area. The pollution of the river water appears e.g. as low oxygen content, high heavy metal concentrations and high contents of bacteria and oxygen consuming organic matter (Häkkilä et al. 1978).

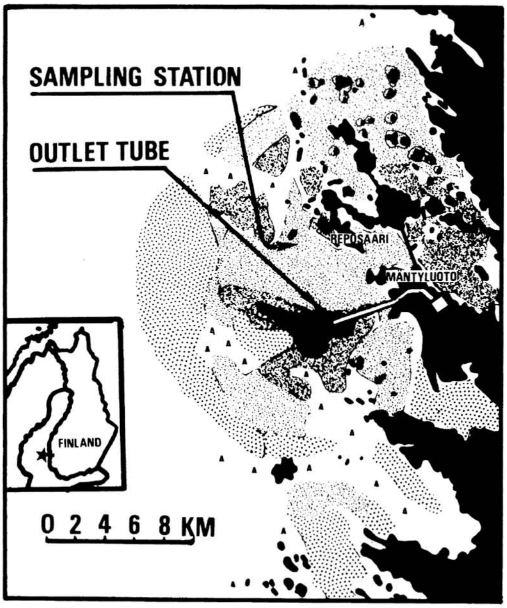

Fig. 1. A map over the sea area off the town of Pori. The darkest areas represent the most polluted parts, according to the benthic fauna investigations (from Häkkilä 1983).

Benthic fauna investigations at the study area are based on ecological on/off-monitoring. The physiological state or the histopathological status of the animals still living in the area has not been investigated. It has been claimed that the eye pathology observed in the Bothnian Sea herrings as well as the diminishing of trawl catches are due to industrial effluent from the titanium dioxide plant in Pori (Järvinen 1982). Histopathological and bacteriological examinations do not provide sufficient support for this hypothesis (Bylund 1983).

The International Mussel Watch is a programme that uses the common mussel (*Mytilus edulis* or *M. californianus*) for monitoring marine pollution. This programme is used for detecting heavy metals, oil, halogenated hydrogarbons and transuranic elements. Its methodology consists of measuring the bioaccumulation of the pollutants, conducting physiological tests in the laboratory and finally histopathological analysis (Goldberg 1980).

Histopathological examination is performed to evaluate the health of an organism as determined by the morphological changes in the cells and tissues. The most useful are specific stress indicators, changes in the tissues that are caused by certain, known pollutants. Amount of accumulation of a certain pollutant does not reveal its influence on the health of the organism. Stress indicators, detected in histopathological preparations, reveal minor disturbances in the organism. Besides straight effects of the pollutants to the tissues the defensive capacity of the mussels descends due to the stressing effect of the pollutants, after which they are more liable to parasitic and microbial infections. The use of the histopathological monitoring using the common mussel as a test organism is most reasonable when questions concerning local pollution are to be resolved, an advantage gained by the sessile nature of the organism.

2. Material and methods

2.1. Sampling stations

A *Mytilus* sample was taken the 18th of September 1984 about 30 meters south-east from the shore of the reef Kaijakari by a drag (depth 5−6 meters), location 61°37′N 21°10′E from a stony bottom. The sampling station is located 6 km from the outlet tube of the titanium dioxide plant. The reef Kaijakari is situated at the border line, south of which low pH and high iron concentrations, indicating the field of effluence of the plant, can be detected from surface waters, but north of which the sea water does not differ from the unpolluted parts of the Bothnian Sea (Häkkilä 1981). The concentration of iron in near-bottom water near the sampling station can rise above 1 mg/l (Lehtonen, 1976). Under certain climatic conditions the discharge waters drift north toward Kaijakari also in the surface waters. The sampling place represents a belt that is occasionally exposed to the discharge waters (Häkkilä 1981). No bottom fauna analysis of this point is available since it represents a hard bottom. Soft bottoms around the sampling point represent semipolluted bottoms (Häkkilä et al. 1978). Measurings of iron concentrations or pH from the water phase just above the sampling station are not available.

According to bottom sediment studies the highest heavy metal concentrations in this area can be detected in the hollows west of Kaijakari (eg. Fe 10−15%, Ti 2%, Vn 160−490 mg/kg, Zn 250−350 mg/kg), these values being higher than just outside the outlet tube (Häkkilä 1980).

In the hollows a perfect destruction of the bottom fauna is possible, when poorly diluted discharge water enters there. Such a phenomenon has happened in 1978 west of Kaijakari and signs of a similar reaction are seen in the south-western hollows of Kaijakari. This incident was due to disturbances in the outlet tubes, after which waste water with pH 2 and iron concentration of 200−300 mg/l stopped in the hollow for a week (Häkkilä 1981).

The sampling station is in the field of influence of the river Kokemäenjoki. This heavily polluted river flows to the sea via two main routes. The other route, carring about 2/3 of the river water, flows through the sound of Reposaari and turns north between Kaijakari and Reposaari (Häkkilä et al. 1978). According to Häkkilä (1983) the sampling station in regard to the effects of the river water belongs to class IV, in which the effects of the river water rapidly decrease and appear as a slight eutrophication in the surface waters.

A control sample was taken during the same day from an unpolluted area from the Gulf of Bothnia. This sampling point is situated 70 kilometers south of the Pori sampling station near Pyhämaa (60°00'N 21°14'E). The sample was collected by a drag near scars north of the island Pitkäluoto. Bottom consisted of gravel and mud (depth 5–6 meters).

The Pori sample consisted of 59 specimens. They were externally normal. Shells were covered by *Balanus improvisus* and *Electra crustulenta*. 6 specimens were in poor condition, with slimy and flabby tissues. Mussels in the Pyhämaa sample (36 specimens) looked healthy.

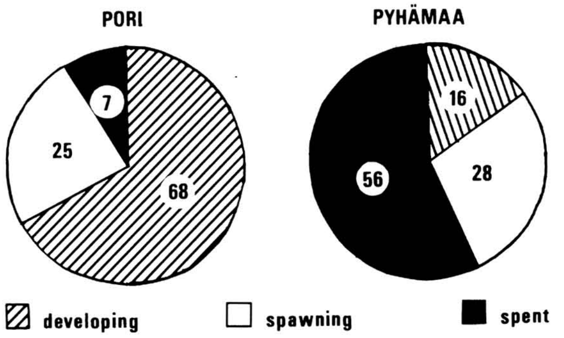

Fig. 2. The breeding stage of the mussels in the Pori and Pyhämaa samples in September. The proportions of developing, spawning and spent specimens expressed as percentages encircled.

2.2. Histological methods

The mussels were prepared 15 minutes after sampling and fixed in Helly's fixative (Barszcz & Yevich 1975). 8 μm thick sections were stained with Hematoxylin–Eosin. Since there were no mature gametes in the mussels in the sample from Pyhämaa, control photographs from the gonads (3A, 3B and 4A) were taken from a sample near Tvärminne Zoological Station, the southern coast of Finland, during reproductive period.

3. Results

Mussels collected from the polluted area off Pori were in poor condition and showed many pathological features. Histopathological findings were restricted to the gonads and the digestive diverticula. The stomach, the intestine and the kidney showed an extensive physiological response. In contrast the gills and muscles, as well as other tissues, were in good condition. There were no more signs of parasitic or microbial infections or granulocytomas in the Pori sample than in the control sample.

3.1. The gonads

The Pori sample was in the breeding stage. The developmental stage of the gonads was classified according to Seed (1969) and they were grouped as developing, spawning or spent. Results are summed in Fig. 2. During the breeding period genital canals ramify from the gonads to all tissues except foot, gills, muscles and the dorsolateral walls of the pericardium. Ciliated canals terminate in follicles, in which ova or sperm develop from germinal epithelium. Ovocytes are attached to the follicle epithelium and loosen when mature (Fig. 3 A). In an ovum under light microscope a vitelline membrane and a plasma membrane with microvilli are seen. Inside cytoplasm there is a large nucleus with chromosomes and a prominent nucleolus, sometimes secondary nucleoli (Fig. 3 B). In the male follicles spermatogonios lie immediately adjacent to the basal lamina. The cells differentiate progressively from the basal region of the follicle to the lumen. Next to lie spermatocytes and mature sperm nearest to the lumens of the follicles (Fig. 4 A).

The spawning of the Pori mussels was abnormal. Female gonads were in a poorer condition than the male gonads. In the male gonads sperm developed but ripe sperm were cytolysed (Fig. 4 B). In some cases the male follicles were filled with granulocytes and no spermatogenic elements existed (Fig. 4 C). The female gonads were filled with anomalous ova, granulocytes and cellular debris (Fig. 3 C). The plasma membranes loosened from the vitelline membranes of the ova as the cytoplasm shrank (Fig. 3 D).

The gonads of the mussels from Pyhämaa represented normal gonads outside breeding season. There were no ripe gametes and the genital canals were replaced by connective tissue cells.

3.2. The digestive diverticula

The digestive diverticula of the mussels consists of blind tubules that ramify from the digestive tract. The walls of the ducts consist of a single, ciliated, columnar epithelium. The digestive tubules (Fig. 5 A) consist of digestive cells with vesicles and basophil cells with apical granules (Thompson et al. 1974, 1978). The tubules show a great variability according to the feeding rhythm, ranging from thick-

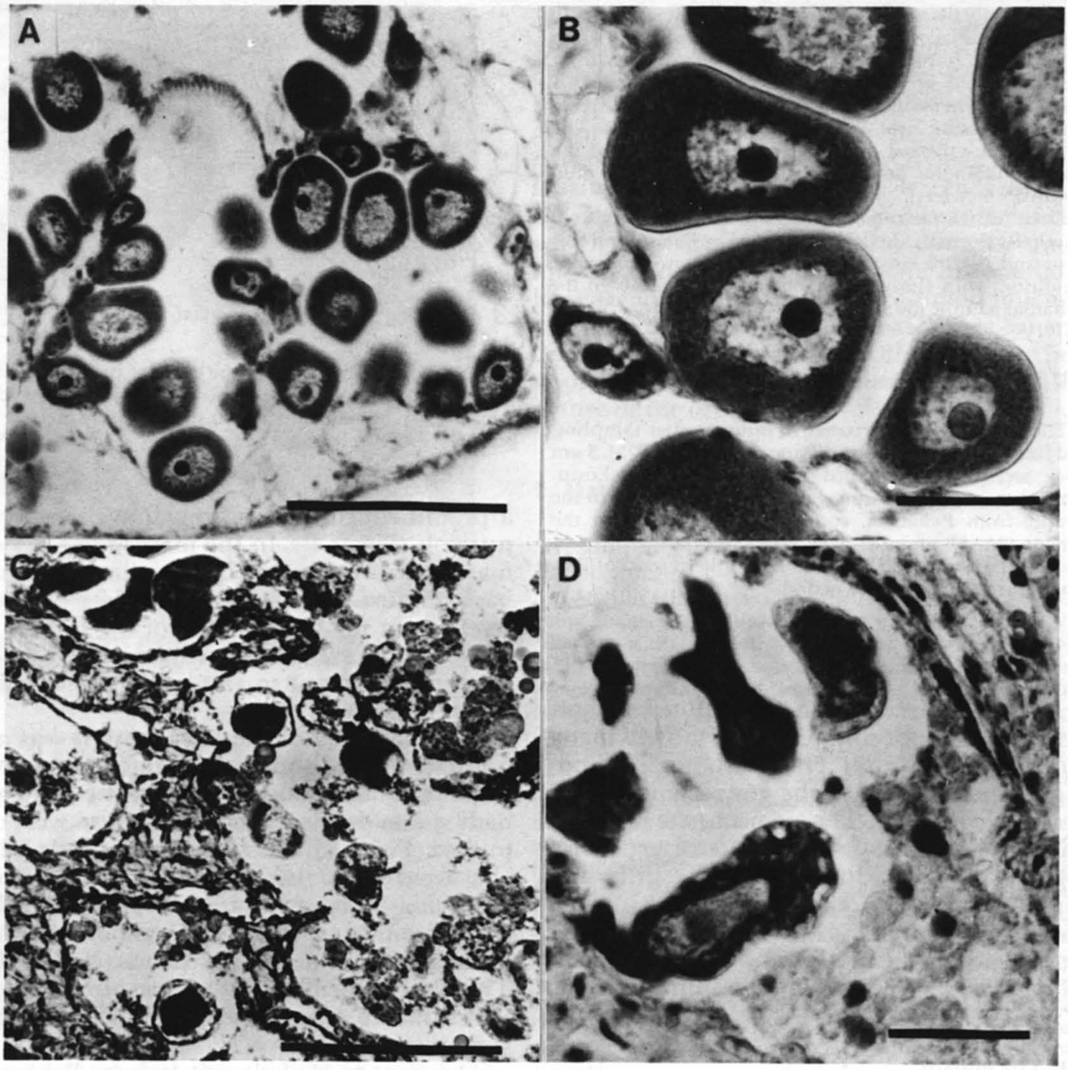

Fig. 3. Female gonads. — A. Control. Developing ovocytes in female follicles in the mantle of the mussel. Ovocytes are fastened to the follicle epithelium and loosen when mature. Scale bar 100 µm. — B. Control. Greater magnification of developing ovocytes in female follicles. The ovocytes are surrounded by vitelline membranes, under which lie the plasma membranes. In the ovocytes a large nucleus, chromosomes and a nucleoli are seen. Scale bar 25 µm. — C. Abnormal spawning in the mussels from the sample off Pori. The female follicles are filled with anomalous ova, granulocytes and cellular debris. Scale bar 100 µm. — D. Abnormal spawning in the mussels from the sample off Pori. Greater magnification of developing ovocytes in female follicles. The cytoplasm of the ova has shrunken and the plasma membranes loosen from the vitelline membranes. Scale bar 25 µm.

walled tubules with narrow lumen to wide thin-walled tubules (Langton 1975).

Digestive tubules of the Pori mussels were not regular: cells were unequal in size and there was no striated border against the lumens. The lumens of the tubules contained eosinophilic exudate and pinchings from the digestive cells (Fig. 5 B). The digestive cells had erosion of the cytoplasm and contained yellow-staining granules. The columnar epithelium of the ducts has become either a metaplastic cuboidal or squamous epithelium.

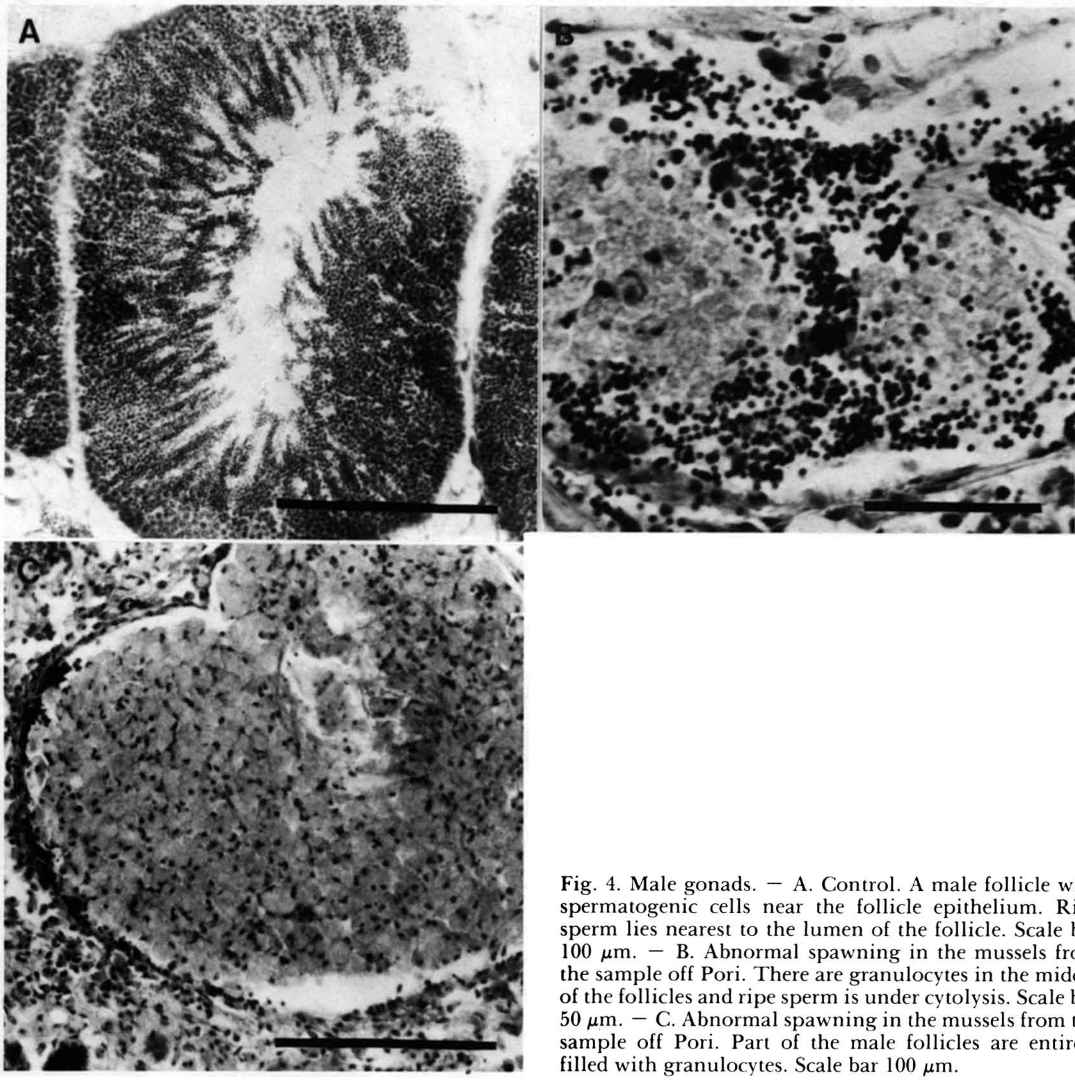

Fig. 4. Male gonads. — A. Control. A male follicle with spermatogenic cells near the follicle epithelium. Ripe sperm lies nearest to the lumen of the follicle. Scale bar 100 μm. — B. Abnormal spawning in the mussels from the sample off Pori. There are granulocytes in the middle of the follicles and ripe sperm is under cytolysis. Scale bar 50 μm. — C. Abnormal spawning in the mussels from the sample off Pori. Part of the male follicles are entirely filled with granulocytes. Scale bar 100 μm.

3.2. The stomach and the intestine

The epithelium of the intestine (Fig. 6 A and B) and stomach (Fig. 7 A and B) contained lot of macrophages and granulocytes. These cells formed large foci on the basal lamina of the epithelium. The lumens in the digestive tract were filled with foreign material.

3.3. The kidney

Most of the kidneys were normal, but showed an extensive physiological response to their environment. The normal epithelium of the kidney is columnar filled with light brown granules. Upon exposure these granules may become dark brown, gray or black, as in some cases in the Pori sample (Fig. 8). Extensive excretion of macrophages could be seen in the kidney epithelium.

4. Discussion

At the date of sampling the mussels should not be in the breeding stage. The reproductive cycle of *Mytilus* in the northern Baltic has been investigated by Sunila (1981) and by Kautsky (1982). The most inactive period is

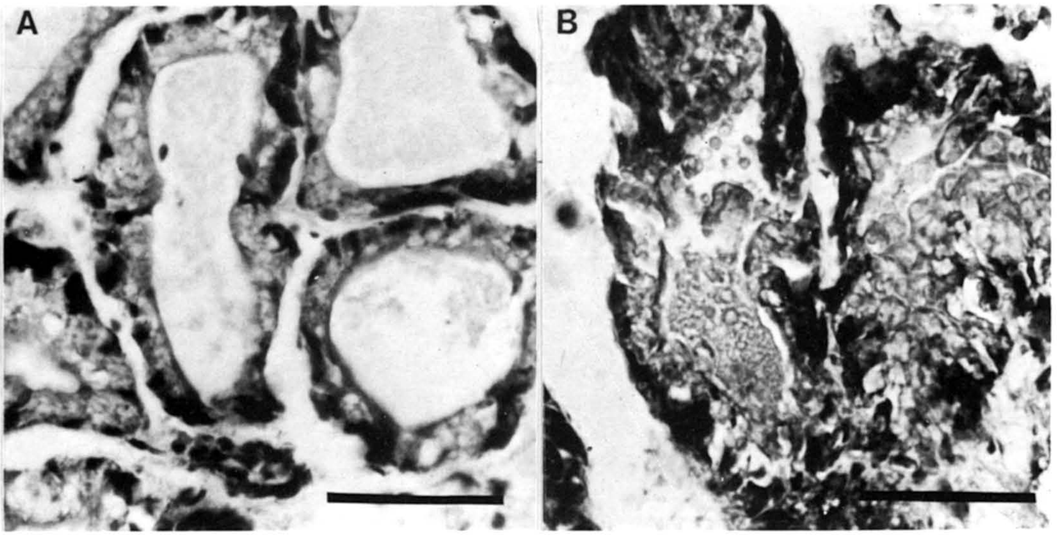

Fig. 5. Digestive diverticula. — A. Control. Pyhämaa. Digestive tubules are lined with a single, striated epithelium. Nuclei of the digestive cells are situated at the proximal ends and the apical ends are filled with vesicles. Scale bar 50 μm. — B. Abnormal digestive tubules in the mussels from the sample off Pori. There is erosion of the cytoplasm of the digestive cells. The lumens of the tubules contain exudate and pinchings from the digestive cells. Scale bar 50 μm.

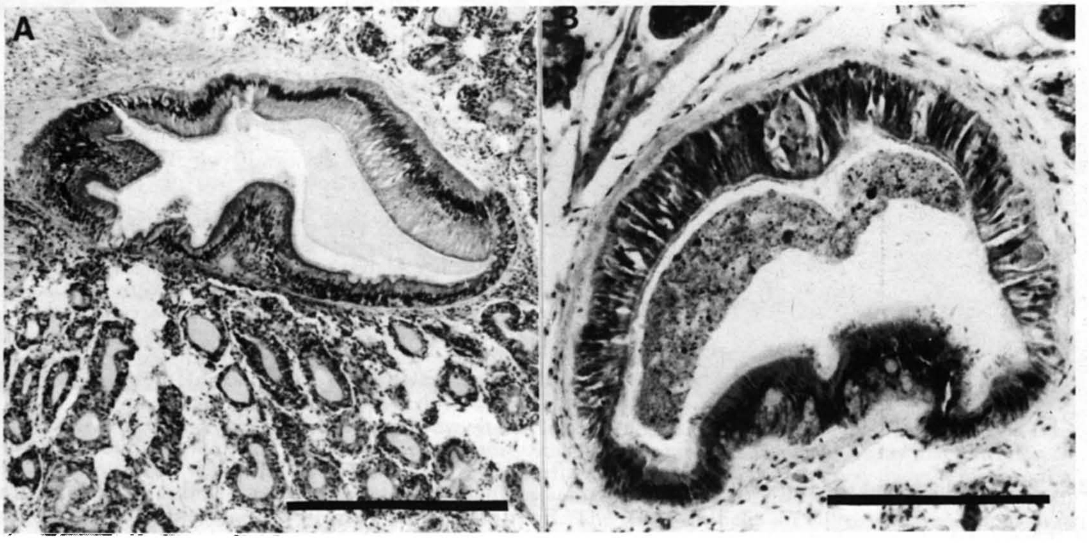

Fig. 6. Intestine. — A. Control. Pyhämaa. A cross section of the intestine. The walls are lined with a single, ciliated, columnar epithelium, which forms a typhosole in the lumen of the intestine. Scale bar 100 μm. — B. Intestine in the mussels from the sample off Pori. There were a lot of granulocytes and macrophages among the epithelial cells of the intestine wall. The hemocytes form large foci on the basal lamina of the epithelium. The intestine is filled with foreign material. Scale bar 100 μm.

from August, after spawning peak in the summer, to October, after which gametogenesis begins. First ripe gametes appear in the follicles in February (Sunila 1981). The Pori mussels were in the beginning of their spawning: developing stages dominated (Fig. 2). In the Pyhämaa sample the gonads were in the resting stage. After Bouxin (1956) mussels can be induced to spawn by a sudden phenomen, e.g. chemical stimulation.

The deviating structure of mature ova in the Pori mussels reflect their response to the low

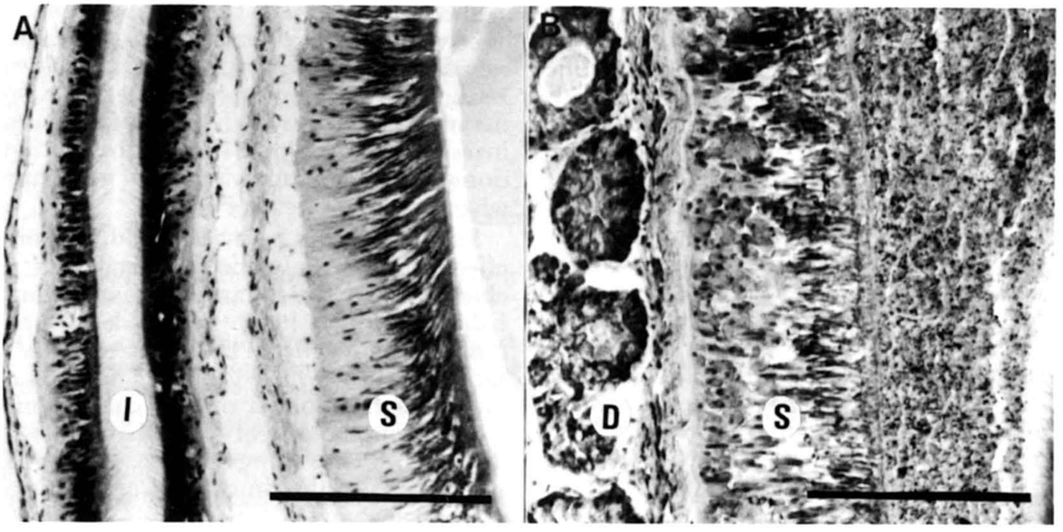

Fig. 7. Stomach. — A. Control. Pyhämaa. The epithelium of the stomach (S) is seen in the right and a longitudinal section of the intestine (I) in the left. Scale bar 100 μm. — B. Stomach in the mussels from the sample off Pori. It is filled with foreign material (in the right). There are granulocytes and macrophages among the epithelial cells (S). In the left tubules from the digestive diverticula are seen (D). Scale bar 100 μm.

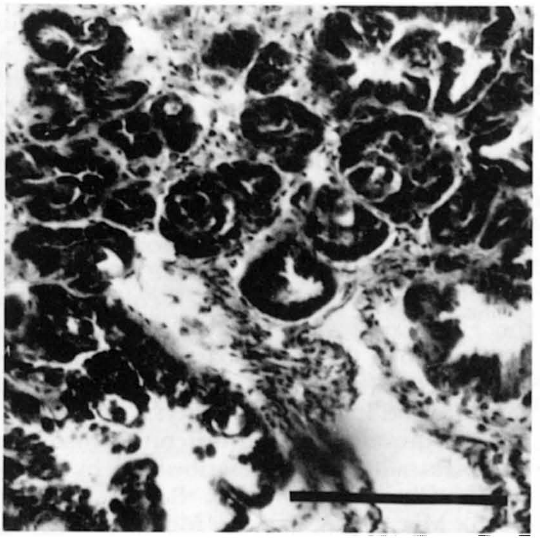

Fig. 8. Kidney. Kidney tubules in the mussels from the sample off Pori. The epithelium stains very dark because of the metals sequestered in the kidney cells. In the kidney tubules amebocyte excretion is seen. Scale bar 100 μm.

pH of their environment. The effects of hypertonic solutions on the ova of *Mytilus* have been studied by Humpreys (1962) by electron microscopy. Hypertonic sea water caused the main cytoplasmic mass of the egg to shrink away from the vitelline membrane, the latter exhibiting no osmotic properties.

At the end of the spawning season, some cytolysis in the male follicles is normal. Exposure to copper and cadmium in the laboratory induced migration of granulocytes to the male follicles and cytolysis of ripe sperm (Sunila 1984). In the Pori mussels, cytolysis in the follicles was more vigorous than in the aquaria tests in exposure concentrations 0.8 mg/l (24 h) of copper or 8.0 mg/l (24 h) of cadmium. Cytolysis during normal reproductive period occurs in residual gametes after the discharge of sperm to the seawater has occurred. In the Pori mussels all ripe sperm in still developing follicles was surrounded by granulocytes and cytolysed.

Histopathological changes in the digestive diverticula of the Pori mussels resemble those described by Calabrese et al. (1984). After a long-term exposure to copper the ducts were lined with a non-ciliated cuboidal or squamous epithelium, instead of a ciliated columnar epithelium. The tubules were dilatated and contained a pink-stained exudate in their lumens.

The kidneys of some of the Pori mussels were dark. Chemical and histopathological analysis have indicated that the excretory products of the kidneys are representative of the environment to which they are exposed. The kidneys can serve as biological monitors for these particular areas (Yevich 1980). In *Mytilus* the kidney mass consists of tubules. The epithelial cells of the tubules fill up with many fine granules. As the exposure increases, the cells become filled up with these granules. The cytoplasm appears to form a bud which is released as a vesicle into the lumen of the tubules from which it is excreted into the water through the urinary pore. Concretions of coalesced cytoplasmic bound granules and macrophages are found at times when the animals are stressed. These macrophages, containing metabolic byproducts and metals come to the kidney epithelium via renal sinuses or the surrounded tissues. George et al. (1982) have shown the particulates in the kidneys to be metals which are sequestered by phagosomes in the kidney cells.

Mussels in polluted areas often show heavy infestation with parasites (Yevich & Barzsz 1983). Granulocytomas also characterize mussels of polluted areas (Lowe & Moore 1979). There were no differences between the sampling stations in this respect.

According to Häkkilä (1980), highest concentrations of heavy metals (cadmium, copper, lead and mercury) in the study area in the clam *Macoma balthica* were found in the field of effluence of the river Kokemäenjoki. On the silty bottoms adjacent to the outlet tube, the metal contents of the clams were generally lower corresponding to those found in unpolluted areas of the Gulf of Bothnia. As the sampling station of this study is located on a hard bottom in the middle of the highest accumulations of heavy metals in the clams, the exposure to heavy metals can be considered to mainly originate from the river Kokemäenjoki. High concentrations of iron, titanium and vanadium in the study area characterize the waste water of the titanium dioxide plant (Häkkilä 1980). The effects of titanium or vanadium on the mussel *Mytilus edulis* have not been described. As yet no histopathological changes have been associated with the effects of iron in *Mytilus*. The iron concentrations in *Macoma balthica* in the Pori area are two times higher than in southern Bothnian Sea (Häkkilä 1982). Contents of heavy metals in benthic crustacea in the Bothnian Sea, off Pori, have been investigated by Sandler (1983). Exceptionally high heavy metal concentrations were not observed in the Pori coastal sea area. Kaitala (1981), in his studies of the estuaries of Finland found elevated copper concentrations in *Mytilus* off Pori. Concentrations of heavy metals in *Mytilus* in the estuaries of the Bothnian Sea were investigated by Häkkilä (1982). The concentrations of titanium and vanadium grew higher when getting nearer to Pori.

Laboratory tests have been conducted on the effects of sublethal concentrations of the discharge waters of the titanium dioxide plant on fishes (Lehtinen 1980). Brown deposits were observed in the gills of the exposed fishes. The x-ray analyses revealed that the deposits contained high amounts of iron and titanium. The histopathology of the gills was not studied (Lehtinen & Klingstedt 1983). The gills of the mussels in this study showed no histopathological changes.

The toxicity of the discharge waters of the titanium dioxide plant on crustacea (*Idotea balthica, Pontoporeia affinis* and *Gammarus* sp.) and a clam (*Macoma balthica*) has been studied by Häkkilä (1978). The toxicity of low pH and high contents of ferrous sulphate in brackish water have been studied by Bagge & Ilus (1975). Mussels (*Mytilus edulis*) tolerated discharge waters with these properties better than other tested animals. TL 50 (tolerance limit, 24 h) was 1000–1300 mg/l of ferrous sulphate and of pH 1.8.

The results of the histopathologic examination of the animals collected for the Mussel Watch Program in the USA are described by Yevich & Barzcz (1983). Mussels collected from the East Coast were in poorer health than mussels along the West Coast, especially mussels collected from areas of great population densities and industrial activity.

High prevalences of proliferative disorders in molluscs have been described e.g. by Farley (1969), Christensen (1974), Barry & Yevich (1975), Mix (1976), Lowe & Moore (1978), Yevich & Barzcz (1977) and Cosson-Mannevy et al. (1984), these cases mostly being associated with some chemical insult.

Histopathologic analysis when studying pollution problems in a brackish water area seems very promising. The Baltic Sea is more heavily loaded with organic and heavy metal wastes than the estuaries in true marine circumstances. The low salinity brings an additional stressing factor to the marine organisms. However, little is known about chronic histopathologic effects of pollutants in brackish water. More laboratory tests must be conducted and work done to applicate these research results to real environmental problems.

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References

Bagge, P. & Ilus, E. 1975: Ferrosulfaatin ja sitä sisältävien jätevesien akuutista myrkyllisyydestä murtovedessä. — Meri 3:46—64.

Barry, M. & Yevich, P. P. 1975: The ecological, chemical and histopathological evaluation of an oil spill site. III. Histopathological studies. — Mar. Pollut. Bull. 6:171—173.

Barszcz, C. A. & Yevich, P. P. 1975: The use of Helly's fixative for marine invertebrate histopathology. — Comp. Pathol. Bull. 7(3):4.

Bouxin, H. 1956: Observations sur le frai de Mytilus edulis var. galloprovincialis (Lmk.); Dates precises de frai et facteurs provoquant l'emission de produits genitaux. — Rapp. P.—v. Réun. Cons. Perm. Int. Explor. Mer 140:43—46.

Bylund, G. 1983: Porin edustan merialueen silakan terveydentila sekä silmävikaisten kalojen taudinkuva. (The health of Baltic herring and the symptons of eye disease in fish in Pori coastal waters). — Meri 12:203—212.

Calabrese, A., MacInnes, J. R., Nelson, D. A., Greig, R. A. & Yevich, P. P. 1984: Effects of long-term exposure to silver or copper on growth, bioaccumulation, and histopathology in the blue mussel, Mytilus edulis. — Mar. Envir. Res. 11(4):253—275.

Christensen, D. J., Farley, C. A. & Kern, F. G. 1974: Epizootic neoplasms in the clam Macoma balthica (L.) from Chesapeake Bay. — J. Natn. Cancer Inst. 52: 1739—1749.

Cosson-Mannevy, M. A., Wong, C. S. & Cretney, W. J. 1984: Putative neoplastic disorders in mussels (Mytilus edulis) from southern Vancouver Island waters, British Columbia. — J. Invert. Path. 44:151—160.

Farley, C. A. 1969: Sarcomatoid proliferative disease in a wild population of blue mussels (Mytilus edulis). — J. Natl. Cancer. 43:509—516.

George, S. G., Coombs, T. L. & Pirie, B. J. S. 1982: Characterization of metal-containing granules from the kidney of the common mussel, Mytilus edulis. — Biochem. Biophys. Acta 716:61—72.

Goldberg, E. D. 1980: The International Mussel Watch. Report of a Workshop Sponsored by the Environmental Studies Board. — Commission on Natural Resources. National Research Council. National Academy of Sciences. 248 pp. Washington D.C.

Häkkilä, K. 1978: Titaanidioksiditehtaan jätevesien myrkyllisyydestä murtovedessä. (The toxicity of effluents of a titanium dioxide factory in brackish water). — Vesihallitus. Tiedotus 144:133—172. Helsinki.

—"— 1980: Pohjasedimenttien ja pohjaeläinten raskasmetalleista Porin edustan merialueella. (Heavy metals in sediments and bottom fauna in the sea area off Pori on the west coast of Finland). — Vesihallitus. Tiedotus 190:1—39. Helsinki.

—"— 1981: Vuorikemian titaanidioksiditehtaiden jätevedet Porin edustan merialueella. — Vesihallituksen Monistesarja 84:1—65. Helsinki.

—"— 1982: Selkämeren pohjaeliöstön raskasmetallitutkimukset. — Väliraportti 1-3. Turun vesipiirin vesitoimisto. 3 pp. Turku.

—"— 1983: Yhteenveto Porin edustalla ja Selkämerellä tehdyistä tutkimuksista, jotka saattavat valaista Selkämeren ekosysteemissä tapahtuneita muutoksia. (Previous investigations off the town of Pori and in Bothnian sea that may throw light on changes in the ecosystem of the Bothnian sea). — Meri 12:21—84.

Häkkilä, K., Hiltunen, P. & Mölsä, H. 1978: Vuorikemian titaanidioksiditehtaiden jätevesien vaikutuksista Porin edustan merialueen pohjaeläimistöön. (Effects of effluents of a titanium dioxide factory on the benthic macrofauna in a brackish water sea area off Pori). — Vesihallitus. Tiedotus 144:5—100. Helsinki.

Humpreys, W. J. 1962: Electron microscope studies on eggs of Mytilus edulis. — J. Ultrastr. Res. 7:467—487.

Järvinen, A. 1982: Hypoteesi silakan sideroottisesta silmäpatologiasta. — Master's Thesis. Dept of Limnology, Univ. of Helsinki. 52 pp.

Kaitala, S. 1981: Simpukoiden raskasmetallipitoisuuksista Suomen rannikkovesissä. — Meri 9:120—146.

Kautsky, N. 1982: Quantitative studies on gonad cycle, fecundity, reproductive output and recruitment in a Baltic Mytilus edulis population. — Mar. Biol. 68:143—160.

Langton, R. W. 1975: Synchrony in the digestive diverticula of Mytilus edulis L. — J. Mar. Biol. Ass. U.K. 55:221—229.

Lehtinen, K. J. 1980: Effects on fish exposed to effluent from a titanium dioxide industry and tested with rotatory-flow technique. — Ambio 9:31—33.

Lehtinen, K. J. & Klingstedt, G. 1983: X-ray microanalysis in the scanning electron microcope on fish gills affected by acidic heavy metal containing industrial effluents. — Aquatic Toxicol. 3:93—102.

Lehtonen, H. 1976: Tutkimus Kemira Oy:n Porin tehtaiden jätevesien kalataloudellisista vaikutuksista sekä kalataloudellinen tarkkailu- ja hoitosuunnitelma. — Riista- ja kalatalouden tutkimuslaitos, Kalantutkimusosasto. Tiedonantoja 6:1—292. Helsinki.

Lowe, D. M. & Moore, M. N. 1978: Cytology and quantitative cytochemistry of a proliferative atypical hemocytic condition in Mytilus edulis (Bivalvia, Mollusca). — J. Natl. Cancer Inst. 60:1455—1460.

—"— 1979: The cytology and occurrence of granulocytomas in mussels. — Mar. Pollut. Bull. 10:137—141.

Mix, M. 1976: A review of the cellular proliferative disorders of oysters (Ostrea lurida) from Yaquina Bay, Oregon. — Prog. Exp. Tumor Res.

20:275–282.
Sandler, H. 1983: Selkämeren pohjaeläimistön raskasmetallipitoisuudet. (Heavy metal concentrations of benthic invertebrates in the Bothnian sea). — Meri 12:84–93.
Seed, R. 1969: The ecology of Mytilus edulis L. on exposed rocky shores. — Oecologia 3:277–316.
Sunila, I. 1981: Reproduction of Mytilus edulis L. (Bivalvia) in a brackish water area, the Gulf of Finland. — Ann. Zool. Fennici 18:121–128.
— „ — 1984: Copper- and cadmium-induced histological changes in the mantle of Mytilus edulis L. (Bivalvia). — Limnologica 15:523–527.
Thompson, R. J., Ratcliffe, N. A. & Bayne, B. L. 1974: Effects of starvation on structure and function in the digestive gland of the mussel (Mytilus edulis L.). — J. Mar. Biol. Ass. U.K. 54:699–712.
Thompson, R. J., Bayne, C. J., Moore, M. N. & Carefoot, T. H. 1978: Haemolymph volume, changes in the biochemical composition of the blood, and cytological responses of the digestive cells in Mytilus californianus Conrad, induced by nutritional, thermal and exposure stress. — J. Comp. Physiol. 127:287–298.
Yevich, P. P. 1980: Comparative histophysiology of excretion in marine molluscs. — Thalassia Jugoslavica 16:399–403.
Yevich, P. P. & Barszcz, C. A. 1977: Neoplasia in soft-shell clams (Mya arenaria) collected from oil-impacted sites. — Ann. N.Y. Acad. Sci. 298:409–426.
— „ — 1983: Histopathology as a monitor for marine pollution. Results of the histopathologic examination of the animals collected for the U.S. 1976 Mussel Watch Program. — Rapp. P.-v. Réun. Cons. Perm. Int. Explor. Mer 182:96–102.

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