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THE HEALTH STATUS OF COMMERCIALLY EXPLOITED NATIVE FLAT OYSTERS (*Ostrea edulis*) IN NORWAY.

By

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ABSTRACT: The first systematic health survey of commercially exploited flat oysters (*Ostrea edulis*) has been carried out since 1989. The survey was performed on two important broodstocks in traditional oyster-production areas. No abnormal mortalities was registered, nor was any serious pathogen agent detected. Based on the results, some advice for monitoring and management of the stocks has been outlined.

RÉSUMÉ: La première campagne systématique de veille sanitaire d'huitres plates (*Ostrea edulis*) exploitées commercialement a été entreprise depuis 1989. Cette veille a eu lieu dans des zones de production ostréicole sur deux stocks importants de géniteurs. Aucune mortalité anormale n'a été constatée, de même qu'aucun agent pathogène n'a été détecté. Des consignes quant au contrôle et à la gestion des stocks ont été établies, basées sur ces résultats.

INTRODUCTION

The native oyster species in Norway is the Européan flat oyster *Ostrea edulis*. Farming of this species has long traditions in Norway (Helland-Hansen 1908, Gaarder and Bjerkan 1934), although the cultivation of any bivalve mollusc species in Norway has allways remained a minor industry. In recent years a few companies have also aimed at a commercial spat production of both indigenous species (*O. edulis*, the clam *Ruditapes decussatus* and scallops, *Pecten maximus*) and introduced species (Pacific oyster, *Crassostrea gigas*, and the manila clam *Ruditapes philippinarum*).

As no severe mortalities have been recorded in Norwegian stocks, the oysters were consideded free from diseases. However no systematic histological investigations have been performed. When the work with standardizing the zoosanitary control within the European common market was concretized (Anonymous 1991) the need for satisfactory monitoring of Norwegian stocks of commercially exploited bivalve molluscs was considered important.

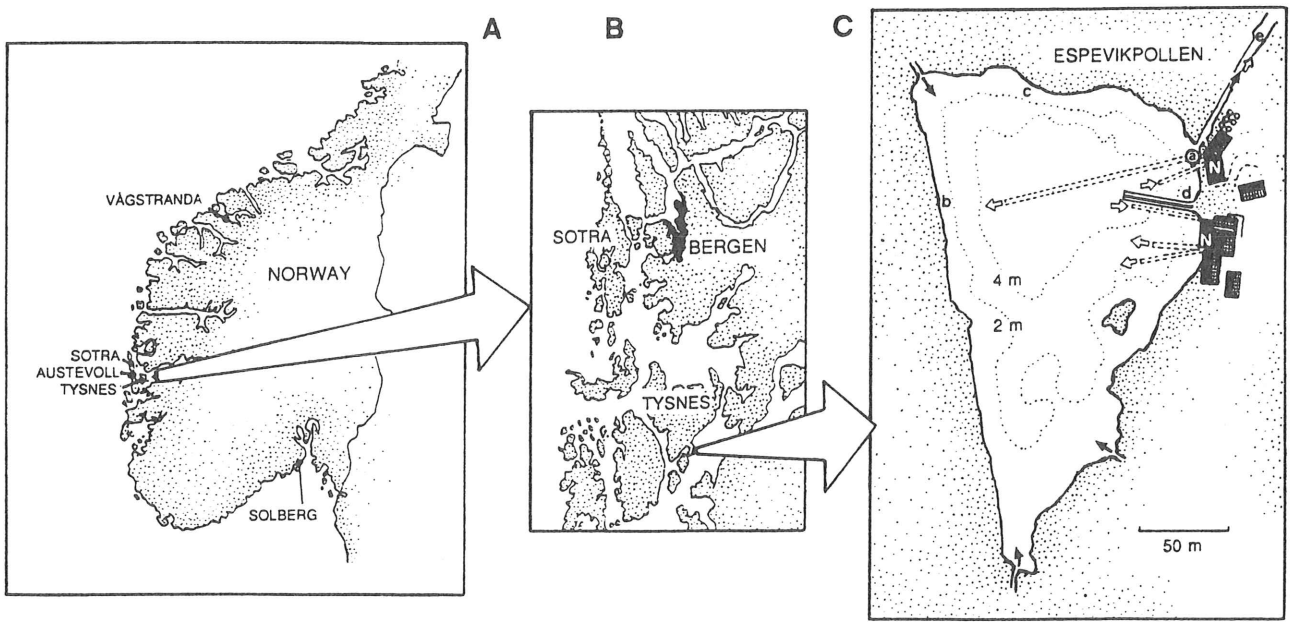

Figure 1: Map showing A: The southern part of Norway and some areas between which oysters have been moved. B: Detail from A, and C: The oyster poll and production fascilities at Espevik, Tysnes. Nurseries are marked "N". Black arrows show natural inlets of freshwater and the effluent (e). White arrows show directions of actively pumped water. "a" is mixing tank for fertilizer and intake of fjord-water. "a" to "e" are sampling points in the poll.

Both oyster broodstock and spat have been moved between different areas (fig 1A) without previous examinations, and spat and juveniles have been distributed to several ongrowing sites along the Norwegian western coast. At present, the southern and western coast of Norway thus has to be considered as one zoosanitary zone. One of the main production units is located in Espevik at Tysnes outside Bergen (fig 1B & 1C). This unit combines the traditional production in a seawater bassin or "poll" (this term is defined in i.e. Matthews and Heimdal 1980) with indoors hatchery and nurseries. In Espevik, oysters of different origins have been stocked within the fascilities, and this place was therefore chosen as sampling site. Occasionally oysters were also received from Vågsstranda (Tab 1, fig 1A) which is the largest production unit in Norway..

As filter feeders bivalve molluscs will allways inhabit commensals, mutualists or even opportunists. The aim of the examinations were thus not to describe the total microfauna in the oysters, but to examine for the presence of known or potentially disease-causing microorganisms, particularly focusing on those listed by the OIE (De Kinkelin et al. 1990).

For fish, a sample size of 150 correspond to the number of individuals requested to detect latent carriers at a minimum of 2 % prevalence on a 95% confidence level. This number was chosen for two samplings of from Espevik (Table 1). In additional biannual examinations 30 - 40 specimens were sampled.

Bivalve molluscs are considered as carriers and vectors of various pathogen agents, probably also including fish pathogen viruses (Hill 1982, Mortensen et al. 1992). It is obvious that introduction of such viruses with shipments of live bivalve molluscs are unacceptable. Infectious pancreatic necrosis virus (IPNV) has been isolated from both scallops, *P. maximus* (Mortensen et al. 1990), and mussels, *Mytilus edulis*, (authors, unpublished) in Norway. A virological assay was therefore performed for each species at each sampling.

MATERIALS AND METHODS

SAMPLING AND OBSERVATIONS ON THE SITE

In general the sampling was performed by the author, and mainly from animals located in or in the vicinity of the hatchery, or in the effluent, thus filtrating water which had passed through the fascilities (fig 1C).

Samplings were performed every spring and autumn since autumn 1989. The points of time, sampling sites and the number of specimens sampled are listed in table 1. In addition to the numbers listed in table 1, 20 - 40 extra specimens were opened for the observation of the gross morphology of soft-parts.

The poll (fig 1C) was inspected by diving in August 1989, August 1990 and March 1992.

Table 1: Samplings of *Ostrea edulis* in Espevik and Vågstranda, Western Norway from 1989 to 1992 ("h.s = heart smears).

	1989	1990		1991		1992
	AUTUMN	SPRING	AUTUMN	SPRING	AUTUMN	SPRING
ESPEVIK	50 50 h.s	150 20 h.s	30	150	40	50
VÅGSTRANDA	35 35 h.s		40			40 50 h.s

GROSS MORPHOLOGY

Shells and soft parts of all specimens were observed individually for the presence of ectoparasites, as well as deformities, erosions, lesions etc.

HISTOLOGICAL ASSAY

Cross sections were cut according to standard procedures (Howard and Smith 1983), fixed in buffered 4 % formol, embedded in paraffin, sectioned at 5µm, stained with hematoxylin / eosin / saffron (HES), and examined in light microscope (screened at 40 or 100 x, details examined at 400 or 1000 x magnification). Each preparate was observed for 6-12 minutes

Hemolymph smears (see table 1) were stained in Diffquick (Baxter Dade AG) and observed in light microscope at 1000 x magnification.

VIROLOGICAL ASSAY

The Chinook Salmon Embryo (CHSE-214) cell line was used in virological assays. Cells were cultured in the Earles modification of the Minimum Essential medium (EMEM) (Flow) supplied with 10 % foetal bovine serum (Flow), 1 % non essential amino acids (Flow) and 100 mg Gentamicin (Schering) per liter.

At each sampling hepatopancreas tissue was dissected from 10 animals, pooled, diluted 1:1 in EMEM and pounded in a Stomacher Lab-Blender 80 (Seward Lab.). Homogenates were diluted 1:50 and filtered through 0.2 µm disc filters. Filtrates (2 ml) were inoculated onto CHSE cell culture monolayers in 25 cm^2 Nunclon cell culture bottles and incubated at 20°C for 7 days. Supernatants were passed onto new cell cultures twice, each after one week intervals.

RESULTS

OBSERVATION ON THE SITE

No mortality of *O.edulis* could be observed by inspection in the poll.

GROSS MORPHOLOGY AND HISTOLOGICAL EXAMINATIONS

The screening did not reveal any sign of serious pathogen agents, neither by observation of gross morphology nor by microscopical examination of sections and smears.

The shells of a few specimens revealed boring sponges (*Cliona* sp.). A general feature was meagre specimens, frequently with pale digestive glands. Microscopical examinations revealed dilated digestive tubules with relatively low epithelial layers.

Protozoans of approximately 35 µm length were observed on gills or in the lumen of digestive diverticula of maximum 10% of the specimens (fig 2b). These cells were observed in specimens from both Vågsstranda and Tysnes. The intensity was maximum 15 per section, and no pathological changes were observed.

Polymorphic granulation resembling inorganic crystals or precipitations were observed in specimens from Tysnes. The granulations were normally neglectable to moderate, but moderate to massive in 93 % of the specimens sampled in spring 1991 (fig 2c). Granulations were localized in connective tissue surrounding the stomach and intestine, frequently in the stomach and intestine epithelia, and occasionally in, and surrounding, the digestive gland tubules. The granulations were often surrounded by massive hemocytic infiltrations. In some specimens precipitates were also observed in the stomach, causing erosion of the ciliated epithelium (fig 2d). As the poll was fertilized with silicate during spring and summer until 1991, oysters sampled in i March 1992 were collected on different locations in the poll, in different distances from the silicate dozer (fig 1C). Granules were observed in a few specimens. In most specimens hemocytic aggregations were observed in the connective tissue surrounding the stomach.

VIROLOGICAL ASSAY

No cytopathic effect was observed on the CHSE-214 cell culture inoculated with homogenates from any sample.

DISCUSSION

The introduction of the intrahemocytic protozooan (Ascetospora) parasite *Bonamia ostreae* (Pichot et al. 1980, Comps et al. 1980) into new regions are in general followed by massive mortalities of flat oysters, *O. edulis*. Also other flat oyster species, like *Ostrea puelchana*, *Tiostrea chilensis* and *Ostrea angasi* have been proven to be susceptible (Pascual, pers. comm., Bougrier et al. 1986, Dinamani et al. 1987, Grizel et al. 1983, Hine 1991). There have been no known introductions of flat oysters into Norway since *B. ostreae* was introduced in Brittany, France (Elston et al. 1986). The examinations of flat oysters support the previous hypothesis that Norwegian flat oysters are free from *Bonamia* sp.

As summarized by Fries et al. (1991) the presence of *Rickettsiales*-like organisms (RLO) has been recorded from different tissues of a variety of bivalve molluscan species. Structures resembling Rickettsial colonies observed in the oysters, support the presumption that these procaryotes are widely distributed in the aquatic environment and occur in a variety of marine species.

Smal, droplet-shaped protozooans of approximately 9 µm length appeared on the gill filaments of maximum 10% of the specimens. These cells were observed in specimens from both sites, and appeared to be attached to the gills and palps. The intensity was low, and no pathological changes were observed.

Dense, finely granular, basophilic inclusions were observed in the epithelia of digestive gland tubules of specimens from both sites (fig 2a). The prevalence was maximum 12 % and the intensity maximum 5 per section. No inflammation was observed, indicating that the inclusions were intracytoplasmic. The inclusions presumably represent colonies of *Rickettsiales*-like organisms.

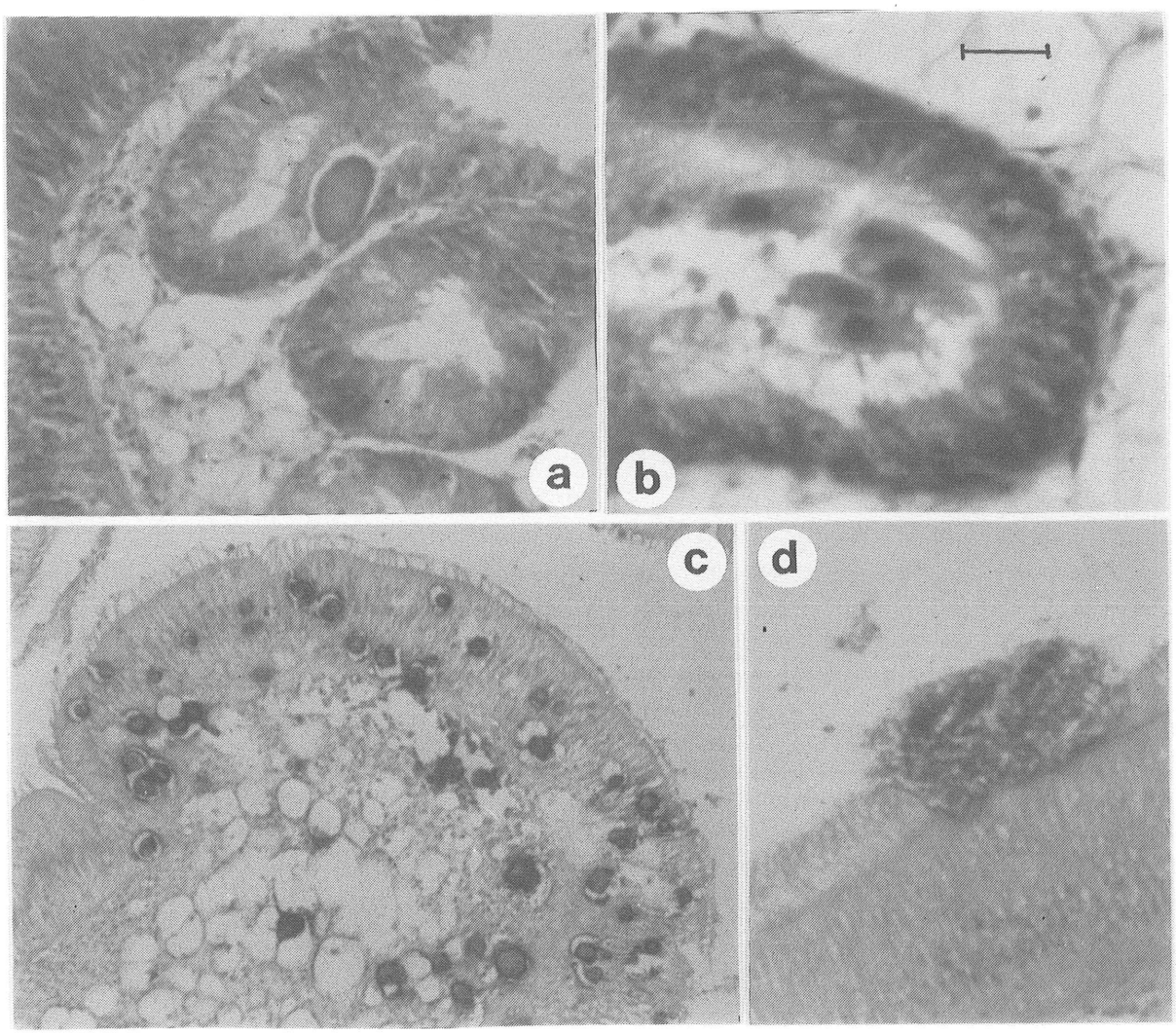

Figure 2. a: Structure in flat oyster *O. edulis* hepatopancreas tubule epithelium resembling a colony of *Rickettsiales*-like organisms. Original magnification = 200 x. b: Five ciliates in the lumen of a digestive diverticula, Bar = 20 µm, orig. magn. = 400 x. c: Polymorphic presumably crystalline structures in stomach epithelium and underlying connective tissue. Orig. magn. = 100 x. d: Granulous or flocculated material attached to ciliated epithelium in the stomach. Orig. magn. = 200 x.

Extremely meagre animals was a striking feature in several samplings, and the animals were in many cases in poor physiological state. The oysters might for certain periods of time not suffered from limited food availability. As starved and weakened animals generally have increased susceptibility to diseases, attention should be paid to stocking density of bivalves in these fascilities.

A variety of protozoans are normally found in the digestive system of bivalve molluscs. Fencel (1965) reported 47 morphologically different ciliates from bivalve molluscs in Scandinavia, most of which are probably commensals being more or less specifically adapted to their host. In the oyster sampled, the prevalence and intensity were low, and no pathological changes were observed. The ciliates, resembling an *Ancistrocoma* sp. were thus considered a part of the oysters' microbial fauna.

Aggregations of hemocytes indicated that the observed crystallization or granulations on the tissues represented a stress to the animals. Absence of granulations but presence of hemocytic aggregations in the last sample could indicate that the material was removed by mobile hemocytes. If fetilization of the poll continues, further investigations, as well as experimental work, is recommended to elucidate the observed phenomenon.

Conclusively , until now, no serious pest or parasite have been recorded in stocks of commercially exploited oysters in Norway. However, this investigation included relatively few specimens from few sampling points, and a contiuous, organized survey, satisfying international standards is thus strongly recommended.

Due to the disease problems in the production of bivalve molluscs in Europe, the availability of disease free specimens for use as broodstock, and for ongrowing in intensive production, might represent a valuable resource. The risk of introducing pathogen agents must thus not be ignored when considering any introduction of live bivalve molluscs into Norway.

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REFERENCES

Anonymous, 1991, Council Directive of 28 January 1991 (91/67/EEC), concerning the animal health conditions governing the placing on the market of aquaculture animals and products. Official Journal of the European Communities, No L 46/l.

Bougrier, S., Tige, G., Bachere, E. and Grizel, H., 1986. *Ostrea angazi* acclimatization to French coasts. Aquaculture 58, 151-154.

Comps, M., Tige, G. and Grizel, H., 1980. Recherches ultrastructurales sur un protiste parasite de l'huitre plate *Ostrea edulis* L. C.R.Acad. Sci., 290 (D), 383-384.

De Kinkelin,P., Håstein,T., Krecek,J., Chen,S.N. and Hill,B.J., 1990. The role of the OIE (Office International des Epizooties) in improving awareness and control of international transfers of fish and shellfish diseases. Bull.Eur.Ass.Fish Pathol, 10 (1), 4-6.

Dinamani, P., Hine, P.M and Jones, J.B., 1987. Occurrence and characteristics of the haemocyte parasite *Bonamia* sp. in the New Zealand dredge oyster *Tiastrea lutarica*. Dis. Aquat. Org. 3, 37-44.

Elston, R.A., Farley, C.A. and Kent, M.L., 1986. Occurrence and significance of bonamiasis in European flat oysters *Ostrea edulis* in North America. Dis. Aquat. Org. 2, 49-54.

Fenchel, T., 1965. Ciliates from Scandinavian molluscs. Ophelia 2 (1), 71-174.

Fries, C.R., Grau, S.B. and Tripp, M.R., 1991. Rickettsiae in the cytoplasm of gill epithelial cells of the soft-shelled clam *Mya arenaria*. J. Inv. Pathol. 57, 443-445.

Grizel, H., Comps, M., Raguenes, D., Leborgne, Y., Tige, G. and Martin, A.G., 1983. Bilan des essais d'acclimatation d' *Ostrea chilensis* sur les cotes de Bretagne. Rev. Trav. Inst. Pêches marit. 46 (3), 209-225.

Gaarder, T. and Bjerkan, P., 1934. Østers og østerskultur i Norge. J. Grieg Boktrykkeri, Bergen, 96 p. (in norwegian)

Helland-Hansen, B., 1908. Die austernbassins in Norwegen. Internationale revue der gesamten hydrobiologie und hydrographie. 1, 553.

Hill, B.J. 1982. Infectious pancreatic necrosis virus and its virulence. p 91-114. In: Roberts, R.J. (ed.), Microbial diseases of fish, Academic press, London, ISBN 0 12 589660 3, 305 p

Hine, P.M., 1991., The annual pattern of *Bonamia* sp. in New Zealand flat oysters, *Tiostrea chilensis*. Aquaculture, 93, 241-251.

Howard, D.W. and Smith, C.S., 1983. Histological Techniques for Marine Bivalve Mollusks. NOAA Technical Memorandum NMFS-F/NEC-25, Woods Hole, Mass, USA.

Matthews, J.B.L. and Heimdal, B.R., 1980. Pelagic productivity and food chains in fjord systems, pp 337-397. In: Freeland, H.J., Farmer, D.M. and Levings, C.D. (Eds). Fjord oceanography, Plenum Press, New York.

Mortensen, S.H., Bachere, E., Le Gall, G. and Mialhe, E. 1992. Persistence of Infectious Pancreatic Necrosis Virus (IPNV) in scallops (*Pecten maximus*). Dis. Aquat. Org. 12, 221-227.

Mortensen, S.H., Hjeltnes, B., Rødseth, O, Krogsrud, J. and Christie, K.E., 1990. Infectious pancreatic necrosis virus, serotype N1 isolated from Norwegian turbot (*Scopthalmus maximus*), halibut (*Hippoglossus hippoglossus*) and scallops (*Pecten maximus*). Bull. Eur. Ass. Fish Pathol, 10(2), 42-43.

Pichot, Y., Comps, M., Tige, Grizel, H. and Rabouin, M.A., 1980. Recherches sur *Bonamia ostreae* gen. n., sp. n., parasite nouveau de l'huitre plate *Ostrea edulis* L. Rev. Trav. Inst. Pêches Marit. 43, 131-140.