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Department: Aquaculture

Report: AQ 91-01

Hazards of provoking toxic dinoflagellate blooms in the Dutch coastal waters through immersion of imported bivalves, originating from red tide areas.

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Project: 60.032

Datof publishing: December, 1991

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# 1 SUMMARY

At the present time, no PSP-producing phytoplankton blooms occur in the coastal waters of the Netherlands and the German and Danish Wadden Sea. This is in contrast with the situation in the coastal waters of a number of countries from which the Dutch mollusc shellfish industry imports mussels (Mytilus edulis) and oysters (Ostrea edulis). Especially bulk imports of mussels are considered to entail a risk of introduction of cysts of toxic dinoflagellate species. Cysts may not only be entrapped within bivalves, but also in large amounts in the mud and sand, mixed up with dredged mussels. Introduction of these cysts into the Dutch coastal waters can be expected when imported bivalves are stocked on rewatering plots on the sea bottom. On basis of literature data it is calculated that 1.8 million cysts per ton of imported mussels or, extrapolated, 18 billions of cysts per year, should be released and accumulate in a quiet,coastal embayment. There is evidence in literature that accumulating cysts may form "seed beds" for toxic dinoflagellate blooms. In order to prevent possible introductions of these cysts, a ban is in vigour on any direct contact with the coastal waters of bivalve shellfish, originating from other areas than the Wadden Sea.

# 2 OCCURRENCE OF TOXIC BLOOMS

Toxic blooms of dinoflagellate species causing Paralytic Shellfish Poisoning (PSP), in this case mainly the dinoflagellates Alexandrium tamarense (Protogonyaulax tamarensis), A. minutum, Gymnodinium breve and Gyrodinium aureolum, have hitherto never been found in the Dutch coastal waters and the Wadden Sea. Toxic blooms of these species have been reported from most European countries, like France (Belin el al., 1989), the Mediterranean, Ireland, UK, the Danish Limfjord and the Baltic Sea. A. tamarense in low cell densities has been encountered in phytoplankton samples from the central North Sea ) in 1990 (Tripos, 1991), and 1991 (L. Wetsteyn (1991), pers. comm.) respectively, but no toxicity has been reported. There is a report on the occurrence of A. tamarense in the Ho- Bight (Danish Wadden Sea) in august 1991 (1000 cells.$l^{-1}$) from the Danish Ministry of Fisheries, (Emsholm, 1991, pers. comm.), although the determination of the species has not yet been confirmed through exact imagery such as e.g. Scanning Electron Microscopy.

The reasons for the absence of PSP-causing dinoflagellate blooms in the Dutch coastal waters are unknown. Anyhow, it is thought unlikely that the environmental conditions in the area should preclude the development of blooms of PSP-causing species. Introductions of large amounts of cells or of living dormant stages of dinoflagellate species, mostly named cysts, hypnozygotes or resting spores, are reported to have caused the development of toxic blooms in other areas. This, for instance, happened in Tasmania, where the toxic species Alexandrium catenella and A. tamarense were introduced with the ballast water of ships, arriving from Europe and Japan (Hallegraeff & Bolch, 1990). Besides, cysts are toxic themselves (Dale et al., 1978) and can, occurring in sufficient density, cause toxicity in bivalves, even if no blooms occur (Yentsch & Mague, 1979).

## 3 INTRODUCTION MECHANISMS

Apart from ballast water, molluscan shellfish are supposed to be a vector for dinoflagellate cysts (Anderson & Wall, 1978, Anderson, 1984, Hallegraeff et al., 1990). Exploitable stocks of molluscs often occur in coastal areas with low current velocities, an environment where conditions for formation and sedimentation of cysts are favourable and where cysts can be found in the sediment in high concentrations. Anderson & Keafer, (1985) and Tyler et al. (1982) found densities near 1,000 cysts per $cm^3$ in bottom sediments in Chesapeake Bay. Moreover, mussels are able to concentrate suspended cysts (diameter: 20 - 60 $\mu m$) from the water by filtration (the filtration capacity of one 40 mm mussel is about 2 - 3 liters per hour at temperatures of 10 $^oC$ and higher). These cysts, most of which have a multi-layered, 2 - 5 $\mu m$ thick wall consisting of calcium carbonate and/or highly resistent polysaccharids like cellulose or sporopollenin (Anderson, 1985), can resist highly toxic $H_2S$ and can therefore be considered capable of passing the intestinal tract of the bivalves unaffected by digestive enzymes. Cysts are then excreted either with the faeces or, directly, with the pseudofaeces of the bivalves. Especially in areas with low current and wave energy, most of these biodeposits accumulate as a sediment layer underneath the mussel bed. If environmental conditions are not favourable for the germination of the cysts, these are capable to remain viable in the sediment for long periods, up to 6 years or more (Dale, 1979 & 1983). There they can stay dormant, even under the toxic and anoxic conditions, prevailing inside the sediment (Anderson et al., 1978).

Cysts may either be entrapped within the intestinal tract or the intervalvular cavity of bivalves, or can be present in the sediment which is caught in the dredge together with the bivalves. This sediment is present in transports of dredged bivalves which, as is mostly the case, have not or insufficiently been rinsed prior to transport. Shipments of imported mussels, when they arrive in the Netherlands, almost invariably contain sand, mud, shell debris etc., up to 30 - 50% of their total weight, as appears from import statistics. Although no concrete proof is available, we are convinced that, apart from cysts in the shell cavities and guts of bivalves, cysts of dinoflagellates can be introduced into the Netherlands via this sediment pathway.

## 4 RELEASE OF CYSTS AND GENERATION OF TOXIC BLOOMS IN DUTCH COASTAL WATERS, A RISK CALCULATION.

There is increasing evidence from literature that accumulating resting stages of A. tamarense are able to form "seed populations". These populations can give rise to blooms when the cysts germinate, induced by environmental stimuli like changes in temperature (Anderson & Morel, 1979, Anderson & Keafer, 1985), light or oxygen concentration (Anderson et al., 1978). Once introduced in a certain area, toxic blooms are, in a number of instances, known to re-appear regularly, causing great damage to the molluscan industry: Toxic blooms of A. tamarense are now found annually at the English/Scottish North Sea coast, where they first appeared in 1968, with toxicities varying from year to year (Waldock et al., 1991). Another example can be found in the U.S. in Falmouth, Mass. in 1976 (Anderson & Wall, 1978). A similar situation is feared to arise for the Oosterschelde estuary. The local economy of the township Yerseke (Fig. 1), is highly dependent on the production, processing and export of mussels, oysters and cockles from the Dutch coastal waters. About 2,000 of the 6,000 inhabitants of this village are directly employed in the molluscan shellfish industry (Dijkema, 1988).

Cysts of toxic dinoflagellates can easily be introduced into the Netherlands. Imported bivalves generally arrive by lorry and are then transferred to the mussel and oyster storage facilities, located in the Oosterschelde estuary in and around the township of Yerseke, where they are relaid on plots or stored in basins, connected with the sea (Fig.1). After a re-watering period of 15 days, the mussels are dredged and rinsed. Dinoflagellate cysts, re-suspended from the sediment on the storage plots or in the effluent of washing installations, are subsequently released in the adjacent part of the Oosterschelde, a shallow, sheltered embayment of about 100 $km^2$. The low current velocities (V max = 0.10 - 0.30 $m.s^{-1}$) which prevail in this area since the Oosterschelde was semi-closed with a permeable flood barrier in 1986 , favour sedimentation of the resuspended cysts. This could lead to the building-up of a seed population of cysts, with the attached hazard of the eventual emergence of toxic dinoflagellate blooms.

The following, tentative calculation gives an order of magnitude of this risk. Based on measurements in mussel shipments, 30% of tare can be assumed to be present in the lots of imported mussels, 10% of this tare is biodeposited mud and silt (Dijkema, unpublished data). Further, conservatively assuming a concentration of 200 cysts per $cm^3$ of sediment (as many as 1,000 per $cm^3$ were found by Anderson and Kaefer, 1985, and Tyler et al., 1982), imported shipments of mussels can be calculated to contain potentially 1.8 million cysts per metric ton of mussels (45 millions per shipment). According to import statistics, 675 tons of mussels and 114 tons of oysters have been imported into the Netherlands from potential "red tide" areas in the period between November 18 and December 2, 1991, by two importing firms. The number of firms with quarantaine installations since then increased to four by December 1991. Extrapolated, in the period from November 1991 until March 1992, 100 tons of imported mussels per working day are expected to arrive in the Netherlands. This corrresponds with the introduction of about 18 billions of cysts over the entire mussel season. These figures are of the same order of magnitude as the introductions reported by Hallegraeff et al. (1990): 300 million cysts in the water of one ship's ballast tank, which led to outbreaks of toxic dinoflagellate blooms in Tasmania.

## 5 BAN ON THE IMMERSION OF IMPORTED BIVALVES

In order to protect the Dutch coastal waters from PSP-causing phytoplankton, a prohibition is in vigour on the immersion into the coastal waters of any imported bivalves, originating from other areas than the Wadden Sea. Storage of these bivalves is only permitted when these are sold directly to the consumer or are kept separated from the coastal water. They are only allowed to be kept in closed quarantine systems, duly isolated from the sea. Effluent water from these systems is only allowed to be discharged back into the sea if cells or cysts of dinoflagellates are appropriately removed or killed. Solid waste from the bivalves: sand, silt and shell debris, has to be disposed of on land.

## 6 THE MARKET SITUATION, IMPORTS

The Dutch domestic production and import figures in 1989 and 1990 are given in the table below. Large declines in the landings of mussels and flat oysters, especially the last 2 years, force the molluscan shellfish trading industry to resort to imports in order to satisfy the demand and not to see its market channels dry up. Molluscs are regularly imported from Denmark, Ireland and the UK (mussels) and Ireland, the UK, the United States, Canada, Greece and Turkey (oysters). Mussel imports are expected to increase in 1992.

| | 1989 | 1990 |
|---|---|---|
| Mussels, production: | 99,688 (68,489) | 98,843 (101,700) |
| Mussels, imports: | 10,273 ( 4,331) | 15,895 ( 7,556) |
| Oysters, export | | 116 ( 12,000) |
| Oysters, imports: | | 248 ( 2,600) |

Production and export figures of bivalve molluscs and mollusc products. The inland consumption of mussels is 15 - 20% of the production. _Weights in metric tons and values between brackets in DFL. * 1000 (1 DFL = US$ 0.56).Source: Yearly report 1990 of the Commodity Board for Fish and Fish products (PVV), Rijswijk.

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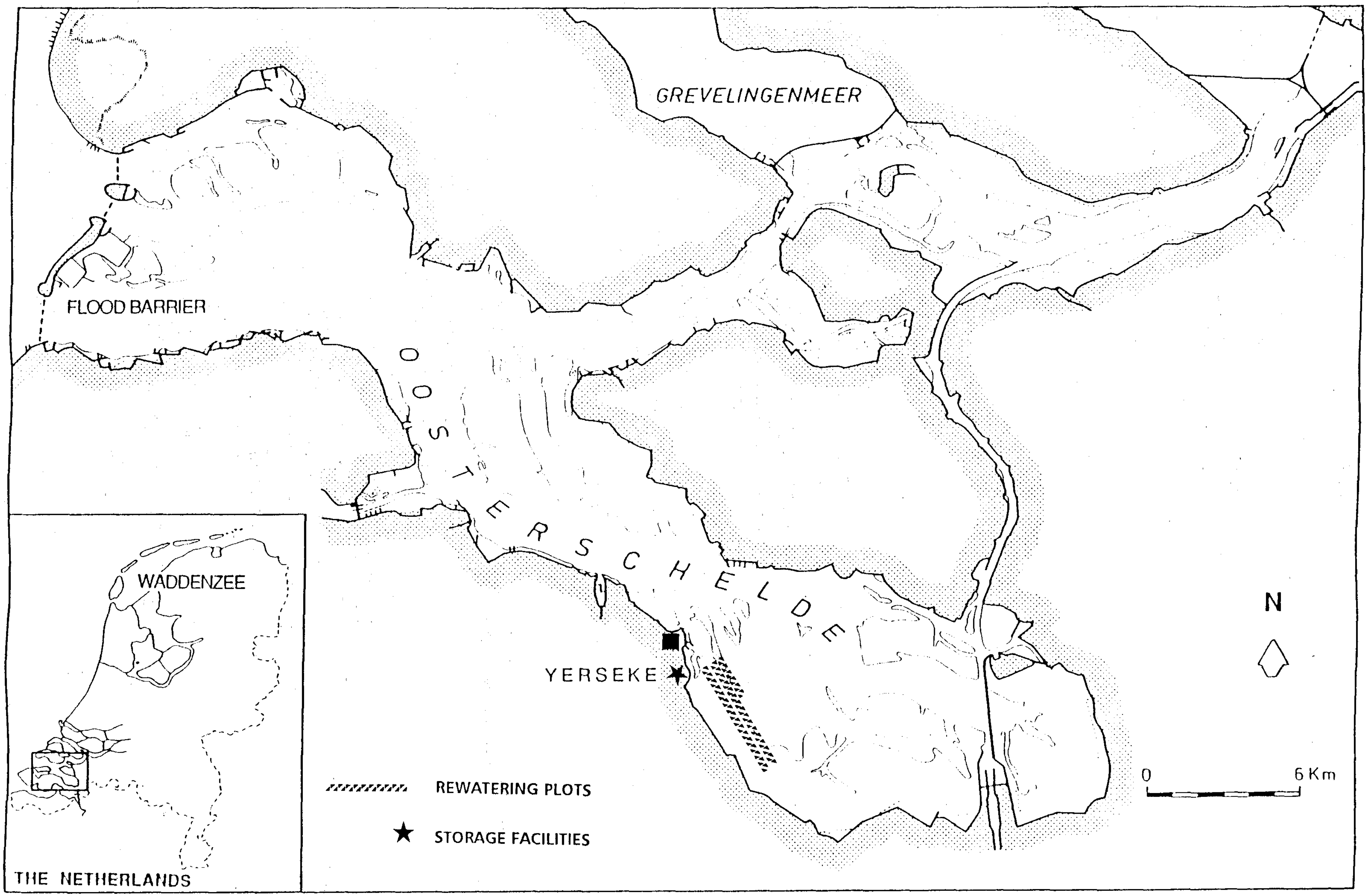


Fig.1: Oosterschelde estuary with location of flood barrier, mussel rewatering plots and on-shore storage facilities for bivalves .