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THE RISK OF PROVOKING TOXIC DINOFLAGELLATE BLOOMS IN THE DUTCH COASTAL WATERS THROUGH IMMERSION OF IMPORTED BIVALVES, ORIGINATING FROM RED TIDE AREAS

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

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Abstract

Until the present time, no Paralytic Shellfish Poisoning (PSP)-causing phytoplankton blooms have occurred in the coastal waters of the Netherlands and in the German and Danish Wadden Sea. Only DSP-causing blooms occur now and then. The absence of PSP-causing blooms is in contrast with the regular appearance of this kind of blooms in the coastal waters of a number of European countries from which the Dutch molluscan shellfish industry imports mussels (*Mytilus edulis*) and oysters (*Ostrea edulis*). The Dutch authorities fear that immersion into the cosatal waters of bivalve molluscs, imported from these countries increases the risk of introduction and outbreak of PSP-causing blooms in the Dutch coastal waters

The majority of dinoflagellate blooms occur in shallow coastal areas, an environment where wild and cultured mussel and oyster beds are generally found. Suspended resting stages (cysts) of dinoflagellates can be filtered out of the water by bivalves and can accumulate in (pseudo)faeces biodeposits, for instance underneath mussel beds. When the mussels are dredged up and are exported, cysts may particularly be present in the mud and silt mixed up with them. The risk of introduction of such cysts into the Dutch coastal waters is real as imported mussels and oysters are stored in an area where hydrographic conditions favour sedimentation and accumulation of cysts. Introduction and successive outbreaks of PSP-causing blooms would severely affect the Dutch shellfish industry. Besides, introduction of exotic phytoplankton species is undesirable. The fear of introduction of cysts and outbreak of toxic blooms is based on evidence in literature that introduced cysts of toxic dinoflagellates accumulated on the see floor form "seed beds", which can trigger blooms. In Tasmania, such an introduction has led to outbreak of recurrent toxic blooms. To prevent this way of introduction of toxic dinoflagellate blooms, since 1987 a ban is in vigour on immersion in the Dutch coastal waters of bivalve shellfish, originating from other waters than the Dutch, German and Danish Wadden Sea.

On basis of literature data and with conservative assumptions, it is calculated that 2.5 million viable cysts of toxic dinoflagellates can be present in one tonne of mussels, imported from "red-tide areas". Taking into account the amounts of mussels imported into the Netherlands, annually $1.05 * 10^{10}$ dinoflagellate cysts can be introduced into the Dutch coastal waters. This number is of the same order of magnitude as the amounts of cysts annually introduced with ballast water into Australia, which have caused toxic dinoflagellate blooms in Tasmania.

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1 INTRODUCTION

Toxic blooms of dinoflagellate species which cause Paralytic Shellfish Poisoning (PSP), for instance *Alexandrium tamarense* (*Protogonyaulax tamarensis*), *A. minutum*, *Gonyaulax polyedra*, *Gymnodinium breve* and *Gyrodinium aureolum*, have until now not been reported from the Dutch coastal waters and the Wadden Sea. Only DSP-causing blooms of *Dinophysis acuminata* occur with a frequency of about once in four years. More or less regular PSP-causing blooms, whether or not toxic, are reported from most European countries, for instance France (Belin el al., 1989), the Mediterranean, the UK (Balch 1983, Waldock et al., 1991), the Danish Limfjord and the Baltic Sea. The presence of *Gonyaulax sp* or *Alexandrium sp* in low cell densities has been demonstrated in phytoplankton samples from the central North Sea in 1990 (Tripos, 1991), and in 1991 (L. Wetsteyn, 1991, pers. comm.) respectively, but no toxicity has been reported. There is also a report of the occurrence of *A. tamarense* in the Ho- Bight (Danish Wadden Sea) in August 1991 (1000 cells.l^{-1}) by the Danish Ministry of Fisheries, (H. Emsholm, 1991, pers. comm.), but the exact species determination could not be confirmed. In Ireland, non-toxic blooms of *Gonyaulax* sp and *Gyrodinium aureolum* have occurred in 1988 on the south coast. Reid (1972) reported the presence of cysts of toxic dinoflagellates in the sediment of embayments on the Irish west coast and in the Irish Sea in near shore environments.

The reasons for the non-occurrence of PSP-causing dinoflagellate blooms in the Dutch coastal waters and the Wadden Sea are unknown. It seems not likely that the environmental conditions in this area should preclude their development. There is a constant risk that toxic dinoflagellates, or their cysts, are introduced and accumulate until sufficient quantities to trigger blooms with a recurrent behaviour. For this reason the Dutch Government in 1987 has issued a decree which is meant to protect the Dutch coastal waters from introductions of PSP-causing phytoplankton species. It bans immersion into the coastal waters of any imported bivalves originating from other areas than the Dutch, German and Danish Wadden Sea. Import of these bivalves is only possible when they are either sold directly to the conserve industry or the consumer or when they are kept outside the coastal water. They are therefore only allowed to be stored in closed quarantine systems, duly isolated from the sea. The purpose of this paper, which is rather a literature study with some attached calculations than a presentation of research results, is to serve as a motivation for this measure.

2 THE BIVALVE MARKET SITUATION, IMPORTS

Export of all kinds of shellfish has been the mainstay of he Dutch molluscan industry for more than a century. In order to stabilise the supply and to compensate for periods of low production, large quantities of mussels, oysters and cockles are regularly imported. The wide fluctuations in the production of mussels (fig 2), mainly a result of storm mortality in the main production area, the Wadden Sea, increasingly make imports of mussels necessary. Fig 3 shows the production and the increased amounts of mussels imported per year during the last years. Most of these come from the German and the Danish Wadden Sea. An increasing number is imported from the United Kingdom, Ireland, the Danish Limfjord and Baltic area and, to a small extent, from Canada, Spain and France. The share of imported mussels originating from other waters than the Wadden Sea is expected to become more important in the future, as bivalve culture and fishery in the entire Wadden Sea will be more and more restricted by measures to protect nature and wildlife.

Imports of the European flat oyster (*Ostrea edulis)* have taken place since the 18-th century. They have recently become of vital importance after, as in most western European countries, the Dutch production of flat oysters was decimated as a result of the introduction and subsequent outbreak of the oyster disease bonamiasis. The Dutch oyster growers and traders, who always have exported 80 - 90% of their production, now resort to imports to satisfy the demand. Oysters are imported from Ireland, the UK, Greece and Turkey, the United States, Canada and even Chile.

3 INITIATION OF TOXIC DINOFLAGELLATE BLOOMS

3.1 Cyst accumulation, resuspension and repetition of blooms

Toxic blooms often re-appear in the same areas. This is ascribed to the formation of hibernating stages by the motile phytoplankton cells (Anderson et al., 1982 b). These stages, named cysts, hypnozygotes or resting spores, are formed when environmental conditions change. They rest dormant on the sea bottom and germinate under certain conditions to initiate later blooms. They can help to disperse phytoplankton species through currents, sediment transport etc. (Anderson et al., 1982). Cysts of toxic species can be toxic themselves, even more toxic than motile cells (Dale et al., 1978, White & Lewis, 1982). When accumulated in sufficient quantities they can cause toxicity of bivalves when ingested following resuspension from the sediment. Balch et al., (1983) studied the distribution of dinoflagellate cysts in the shallow and turbulent Tamar estuary (southern Great Britain). They concluded that, in the mouth of the estuary, sedimentation and resuspension by tidal mixing, river currents and waves caused an active interchange of cysts between water column and sediment. Ingestion of resuspended cysts is supposed to cause toxicity in bivalves in periods in which no blooms occur (White & Lewis, 1982, White, 1988, Yentsch & Mague, 1979).

4 MAN-CAUSED INTRODUCTION OF TOXIC BLOOMS

In at least one case, anthropogenic introduction of large amounts of cells or cysts of dinoflagellates has been reported to have caused the development of toxic blooms in other areas. This was in Tasmania, where cysts are introduced with the ballast water and the mud, accumulated in the ballast tanks of cargo ships arriving from Japan. Every year, about 60 million tonnes of ballast water are discharged in Australian ports, on average 25,000 tonnes per ship. About 300 million cysts were found in the tank of one ship and 6% of 80 ships examined contained cysts of toxic dinoflagellates (Hallegraeff & Bolch, 1990, Hallegraeff et al., 1988). A rough, tentative calculation learns that per year approximately $4.3 * 10^{10}$ cysts of toxic dinoflagellates are introduced into Auatralian waters. In Tasmania, where only a part of this ballast water is discharged, this is assumed to have led to repeated outbreaks of toxic blooms of the species *Alexandrium catenella* and *A. tamarense.*

Not only ballast water but also molluscan shellfish are supposed to be potential means for transport (Anderson et al., 1982, Anderson & Wall, 1978, Anderson, 1984, Hallegraeff et al., 1990). Exploitable stocks of molluscs mostly are found in coastal areas where, when toxic blooms occur, conditions for formation and sedimentation of cysts are favourable and where cysts can accumulate in the sediment (Anderson & Keafer, 1985, White & Lewis, 1982). When these cysts become re-suspended by turbulence (waves or tidal currents), bivalves concentrate them from the water column by filtration. The filtration capacity of one 40 mm mussel, for instance, is about 2 - 3 liters per hour at temperatures above 10 °C (Bayne, 1976). This means that 1 m^2 of a mussel bed with a density of 5 kg/m^2 can ingest and concentrate the cysts from 0.6 - 1 m^3 of sea water per hour. Balch et al. (1983) found an average concentration of 9.2 $cysts.l^{-1}$ in the water column in the mouth of the Tamar estuary in southern England, occurring during about 6 weeks per year. At that concentration, 6 - 9 million cyst would be filtrated from the water column per m^2 of mussel bed per year. The ingested cysts can be found in the intestinal tract or in the intervalvular cavity of the bivalves. They are finally excreted with the faeces and pseudofaeces. In the case of mussels, a thick sediment layer forms underneath the bed when current speeds are not too high. In areas with regular occurrence of red tides where cysts are formed, mussel beds must be assumed to show much higher concentrations of cysts than the surrounding area and to act as "traps" for cysts. For other bivalve species like oysters, which have a lower filtration rate and do not accumulate such large quantities of mud as mussels, this way of accumulation can be considered less important.

Dinoflagellate cysts generally have a diameter between 20 and 65 µm. Most of them have 2 - 5 µm thick, multi-layered walls, consisting of calcium carbonate and/or highly resistant polysaccharids like cellulose and/or sporopollenin (Anderson, 1985). They have been found to be resistent to acetolysis and concentrated sulphuric acid (Anderson and Wall, 1978) and apparently are able to survive the toxic (H_2S) and anoxic conditions within the sediment layer for long periods (Anderson et al., 1978). Dale (1979 & 1983) even mentions dormancy periods of 6.5 - 16.5 years. Thanks to this resistance, cysts can be expected to be capable of passing the intestinal tracts of bivalves without being affected by their digestive enzymes and of remaining dormant in the bio-accumulated sediment for many years. As appears from the foregoing, beds of bivalve molluscan species can have increased amounts of dinoflagellate cysts in their sediment in areas where red tides regularly occur. In the case of mussels, they can then be expected in the sediment which is dredged up with them. This sediment is also found in transported lots of dredged bivalves which, as is mostly the case, are not or poorly rinsed previously. This appears from Dutch import statistics, which show that in shipments of imported mussels, when they arrive in the Netherlands, almost invariably up to 30 - 50% of the total weight is taken by sand, mud, shell debris etc. Although no research has been done into the abundance of cysts of dinoflagellates in shipments of bivalves, it can be assumed from the foregoing that cysts are introduced into the Netherlands via this pathway.

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

5.1 Initiation of toxic blooms

There is increasing evidence in literature that accumulating resting stages of *A. tamarense* can form "seed beds" (Anderson & Keafer, 1985, White & Lewis, 1982), which initiate recurrent blooms when the cysts germinate, induced by environmental stimuli like changes in temperature (Anderson & Morel, 1979, Anderson & Keafer, 1985), light intensity or oxygen concentration (Anderson et al., 1978). Once introduced in a certain area, toxic blooms have been reported to re-appear regularly, causing great detriment to the molluscan shellfish industry. A number of instances is documented: Toxic blooms of *A. tamarense* are now found annually at the English/Scottish North Sea coast, where they first appeared in 1968, after which toxicity varied from year to year (Waldock et al., 1991). In the Unites States, recurrent blooms have been reported after first outbreaks: in 1985 in he state of Maine (Hurst, 1975), and later in 1972 in the states of New Hampshire and Rhode Island and in Massachusetts in the Cape Ann and Cape Cod region (Anderson et al. 1982 a, Anderson & Wall, 1978, White & Lewis, 1982).

5.2 The situation in the Netherlands

A situation similar to the cases cited above is feared to arise in the Netherlands, where imported molluscs are relaid in the Oosterschelde estuary. There, the economy of the adjacent township Yerseke (Fig. 1) is largely dependant on production, processing, import and export of bivalves (Dijkema, 1988). Imported bivalves generally arrive by lorry and are then transferred to the mussel and oyster storage facilities in and near Yerseke. Mussels are relaid there on rewatering plots in the Oosterschelde, whereas oysters are stored in on-shore flow-through basins, discharging into the Oosterschelde (Fig.1). After a re-watering period of about 15 days, the mussels are dredged up, cleaned, packed or processed. Dinoflagellate cysts from the water in which mussels and oysters are kept and from the washed-out sediment from among the imported mussels, can accumulate on the mussel rewatering plots and near effluent discharge places of washing installations and storage basins. The inland section of the Oosterschelde forms a shallow, 4 - 10 m deep and sheltered embayment of about 100 km^2 east of Yerseke (Fig. 1). The low current velocities (V max = 0.10 - 0.30 $m.s^{-1}$) in this area and shelter from waves from westerly directions favour sedimentation and accumulation of fine sediment. Lewis (1988) and Anderson et al. (1982 b) demonstrated that accumulation of cysts is associated with the occurrence of fine sediments. This section of the Oosterschelde offers conditions for the accumulation of cysts, comparable with those in areas in New England, described by Anderson et al. (1982), and Anderson & Keafer (1985).

5.3 Abundance of cysts in the sediment, literature data

Literature provides fairly consistent information on concentrations of cysts in the sediment of areas where accumulation takes place: White and Lewis (1982) found 2000 - 8000 cysts of *Gonyaulax excavata* per cm^3 of wet sediment in an area of 2000 km^2 in the Bay of Fundy. The average density of cysts they found in the principal area of accumulation was 2233 per cm^3. Anderson et al. (1982 b) found 700 viable cysts of *A. tamarense* in shallow (1.5 m) and enclosed Perch Pond (Massachusetts) and 250 cysts of *Gyrodinium uncatenum* in the lower reaches of Potomac River (Chesapeake Bay). Anderson and Keafer (1985) and Tyler et al, (1982) found up to 1000 cysts of *Alexandrium tamarense* per cm^3 in the top 6 cm of sediment in Chesapeake Bay. Lewis (1988) found an average of 2136 viable cysts of *Gonyaulax polyedra* per cm^3 in the top layer of the sediment in Loch Creran (W. Scotland). Reid (1972) found 1600 dinoflagellate cysts per gram of sediment in the eastern Irish Sea and in enclosed Cardigan Bay on the west coast of Ireland. Balch et al (1983) found an average concentration of 914 cysts per cm^3 in the flocculent layer of the sediment of the mouth of the Tamar estuary. In fine sediments they found concentrations of 2000 - 3000 cysts per cm^3. All these cases concern accumulation of cysts exclusively by sedimentation. On basis of the filtration of cysts from the water column by bivalves described earlier, it can be assumed that a higher degree of accumulation of cysts would occur in bivalve beds in these areas. The amounts of cysts, present in other bivalves such as oysters, could not be quantified due to lack of concrete information.

Authors	Location	Species	Density
White and Lewis (1982)	Bay of Fundy	G. excavata	$2233.cm^{-3}$
White and Lewis (1982)	Gulf of Maine	G. excavata	$3220.cm^{-3}$
Anderson et al. (1982 b)	Perch Pond	A. tamarense	$700.cm^{-3}$
Anderson c.s (1982b)	Potomac river	Gyrodinium unc.	$250.cm^{-3}$
Anderson /Kaefer (1985)	Chesapeake Bay	A. tamarense	$1000.cm^{-3}$
Tyler et al. (1982)	Chesapeake Bay	A. tamarense	$1000.cm^{-3}$
Lewis (1985)	Scotland	G. polyedra	$2000.cm^{-3}$*
Lewis (1988)	Scotland	G. polyedra	$2136.cm^{-3}$
Reid (1972)	Irish Sea + West coast	G. polyedra and others	$1454.cm^{-3}$
Balch et al. (1983)	S. England	8 spp.	$914.cm^{-3}$

Table 1: Summarised densities of dinoflagellate cysts in sediment, reported in literature. Not all data give numbers of viable cysts. In a number of cases, 40-60% of the cyst appeared to be viable. The figures have been converted, if necessary, from numbers per gram dry sediment on basis of 0.40 g dry matter per cm^3 of wet sediment.
* = approximation

5.4 Estimation of the numbers of cysts introduced with mussels

To establish an order of magnitude of the amounts of cysts which could be introduced into the Dutch coastal area, a tentative estimate has been made of the amounts of cysts, potentially present in dredge-captured mussels from "red-tide" areas. This estimate is based on the following information and assumptions:

Information:
During samplings in mussel shipments, an average of 30% of tare has been found present in loads of imported mussels. Of this tare, 10% is biodeposited mud and silt (Dijkema, unpublished data).

In the season 1991-1992, 4200 metric tonnes of mussels and 330 metric tonnes of flat oysters (*O. edulis*), originating from countries with "red-tide areas", were imported into the Netherlands.

Assumptions:

On basis of literature data cited above it is assumed that 1000 viable cysts are present in one cm^3 of sediment, imported with mussels dredged in "red tide areas".

Not all bivalves which are imported from countries with "red tide areas" are exactly dredged in areas where toxic blooms occur. For this reason it is assumed here that 10% of the mussel and oyster shipments from countries with "red-tide" areas actually contain cysts.

6 CONCLUSIONS

One metric tonne of imported mussels from an area where accumulation of cysts occurs can contain $25 .10^6$ cysts of toxic dinoflagellate species.

Taking into account that cysts are present in only 10% of the shipments of mussels imported from countries where toxic phytoplankton blooms occur, potentially $2.5 * 10^6$ cysts are imported per metric tonne of mussels.

Without taking into account possible introductions of cysts with imported oysters, this means that annually $1.05 * 10^{10}$ cysts of toxic dinoflagellates may end up in the sediments of the Oosterschelde.

This figure is of the same order of magnitude as the $4.3 * 10^{10}$ toxic dinoflagellate cysts, which may be introduced annually into the whole of Australia, and which has caused outbreaks of toxic dinoflagellate blooms in Tasmania..

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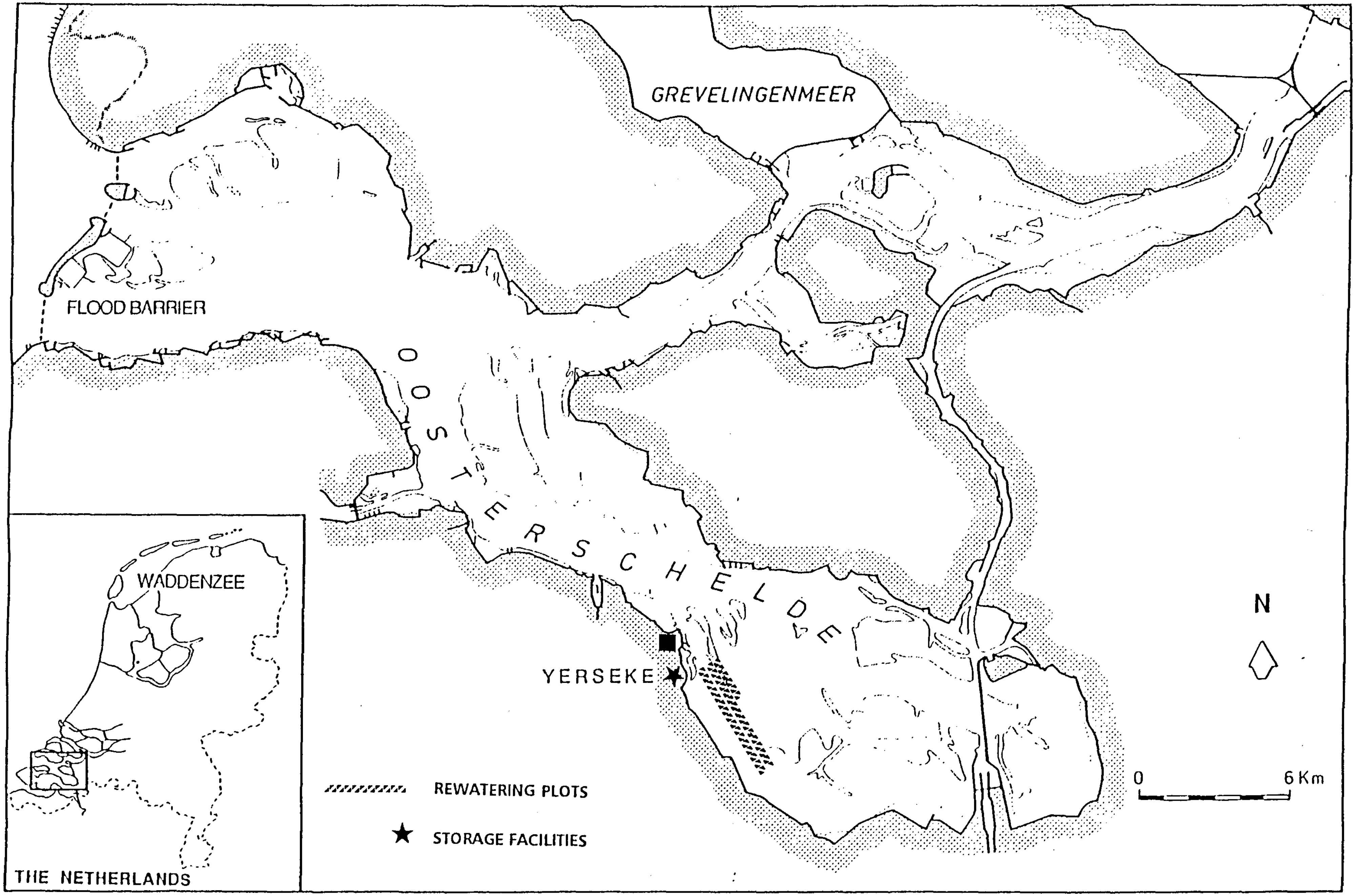

Fig. 1: Oosterschelde with the flood barrier, the sheltered section east of Yerseke with mussel rewatering plots and land-based storage facilities for molluscs.

Fig. 2: Total annual production of cultivated mussels in the Netherlands and in the production areas Zeeland and the Wadden Sea.

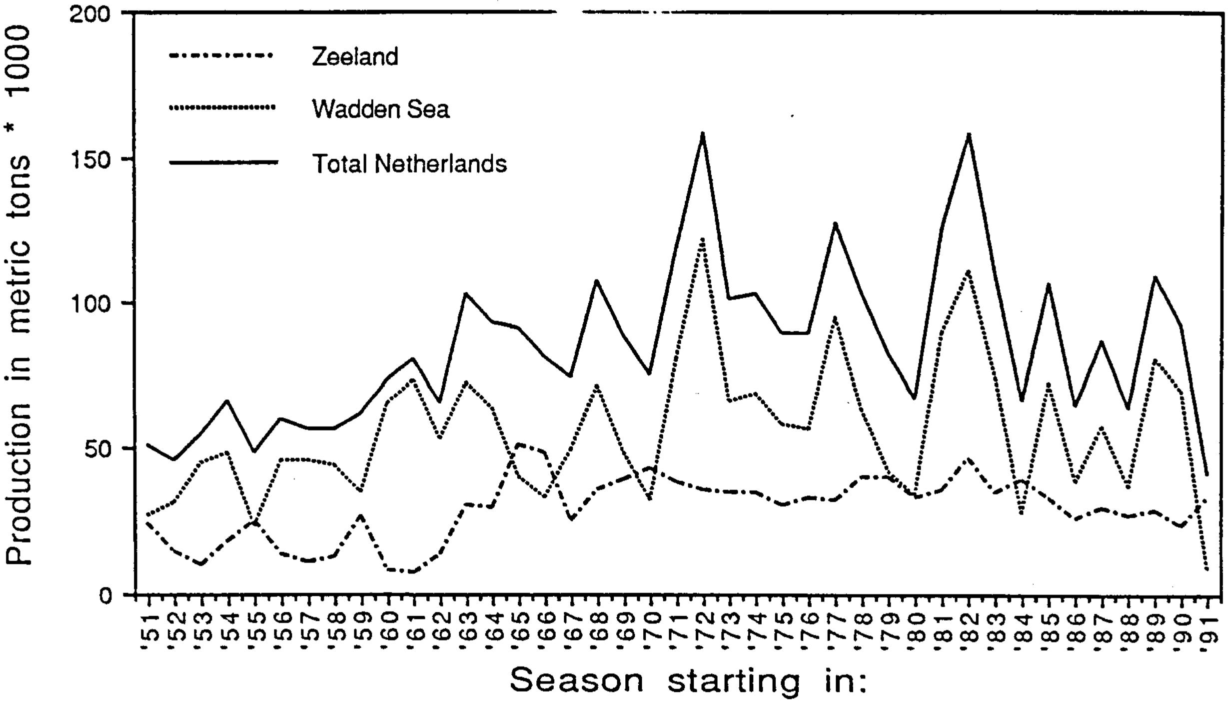

Fig. 3: Comparison between mussel imports into the Netherlands and the production of mussel cultivation in the period 1970 - 1992.