Large-scale recirculations systems for storage of imported bivalves as a means to counteract introduction of cysts of toxic dinoflagellates in the coastal waters of the Netherlands

Systèmes de recirculation à grande échelle pour le stockage de bivalves importés comme moyen d'empêcher l'introduction de dinoflagellés toxiques dans les eaux côtières des Pays-Bas

RENGER DIJKEMA
Netherlands Institute for Fisheries Research (RIVO-DLO), BP77, 4400 AB Yerseke, Netherlands

Abstract

In the coastal waters of the Netherlands and in the German and Danish Wadden Sea, at present no PSP-producing toxic dinoflagellate blooms occur. This is in contrast with the situation in a number of countries from which Dutch traders import mussels (*Mytilus edulis*) and oysters (*Ostrea edulis*). Especially bulk imports of mussels entail a risk of the introduction of cysts of toxic dinoflagellate species. These can be present within the bivalves but, when dredged mussels are concerned, particularly in accompanying mud and sand. Such cysts can be released into the coastal waters when the imported bivalves are kept in open storage basins or relaid on plots on the sea bottom. To prevent introduction, a ban is in vigour on the immersion into Dutch coastal waters of any bivalve shellfish, originating from other areas than the Wadden Sea (figure 1). To enable the shellfish industry to import bivalves in periods of low national production, the Netherlands Institute for Fisheries Research (RIVO-DLO) has designed recirculating storage systems in which mussels and oysters can be kept in quarantine. Up to 50 tonnes of mussels can be kept in good condition during at least three days and flat oysters can be stored for several weeks. To ensure elimination of any cells and cysts of dinoflagellates present, the effluent of the systems must pass filters which retain at least 99.9% of the particles larger than 20 µm, the minimal diameter of potentially dangerous cysts. Eight of these quarantine systems for mussels and 18 for oysters were in operation by the end of 1992.

Résumé

Les efflorescences algales de dinoflagellés toxiques produisant des toxines de PSP n'existent pas actuellement dans les eaux côtières des Pays-Bas ni dans les eaux allemandes et danoises de la mer des Wadden. Ceci contraste avec la situation de nombreux pays d'où proviennent les moules (*Mytilus edulis*) et les huîtres (*Ostrea edulis*) importées par les négociants hollandais. Les importations de moules en vrac comportent notamment un risque d'introduction de kystes d'espèces toxiques de dinoflagellés. Ceux-ci peuvent être présents à l'intérieur des mollusques, mais également dans la vase et le sable qui accompagnent les moules récoltées à la drague. Ces kystes peuvent ensuite être relargués dans les eaux côtières lorsque les bivalves importés sont transférés dans des bassins de conser-

vation en eau ou reparqués sur des concessions en mer. Pour éviter cette introduction de kystes, une mesure d'interdiction est en vigueur, interdisant dans les eaux côtières hollandaises l'immersion de tout coquillage bivalve provenant de zones autres que la mer des Wadden (figure 1).

Pour permettre à l'industrie conchylicole d'importer des bivalves en période de faible production nationale, l'Institut hollandais de recherche sur la pêche (RIVO-DLO) a conçu des systèmes de stockage à recirculation dans lesquels les moules et les huîtres sont mis en quarantaine. On peut ainsi conserver jusqu'à 50 tonnes de moules dans de bonnes conditions pendant un minimum de trois jours, et les huîtres peuvent être stockées pendant plusieurs semaines. Afin de s'assurer de l'élimination de toutes cellules et tous kystes de dinoflagellés éventuels, les effluents provenant de ces systèmes passent dans des filtres qui retiennent un minimum de 99,9 % des particules de granulométrie supérieure à 20 µm, correspondant au diamètre minimum de kystes potentiellement dangereux. En fin 1992, huit de ces systèmes de mise en quarantaine étaient en exploitation pour les moules et 18 pour les huîtres.

INTRODUCTION

Imports of bivalves

In the Netherlands, 3,460 people are directly and indirectly employed in the culture and fishery of mussels, oysters, cockles, *Spisula* and in their processing and trade. In the township Yerseke in the province Zeeland (figure 1), over 2000 of its 6000 inhabitants live off the molluscan industry (Dijkema, in press). In 1990, the export value of molluscan shellfish and its commodities amounted to HFL 300 million (US$ 158 million). Much effort is invested by the industry in monitoring and control of sanitary and chemical water quality and toxic phytoplankton species in the coastal waters. A summary of the dutch bivalve production is given below:

SPECIES	PRODUCTION (in metric tons)	WHOLESALE VALUE (in HFL*10^6)
Mytilus edulis	60,000 - 120,000	100 - 250
Ostrea edulis	400 - 800	10 - 20
Crassostrea gigas	700 - 1,000	4 - 7
Cerastoderma edule	20,000 - 60,000	40 - 120
Spisula subtruncata	1,000 - 2,000	0.6 - 1.2

(*) 1 HFL = 0.53 US$ (1993)

About 70% of the dutch production of mussels and 90-95% of that of oysters and cockles is exported. The production of these three species shows large variations from year to year, which are mainly caused by the effect of storms in the shallow western Wadden Sea and by variations in recruitment. Figure 2 shows the landings of cultivated mussels. In years of low production, import of mussels and oysters is necessary to ensure the export trade. Particularly in the period 1987-1993, the mussel and cockle production have decreased drastically, due to an exceptional combination of heavy storm losses, low recruitment, and intensive predation on the remaining stocks by eider ducks (*Somateria mollissima*).

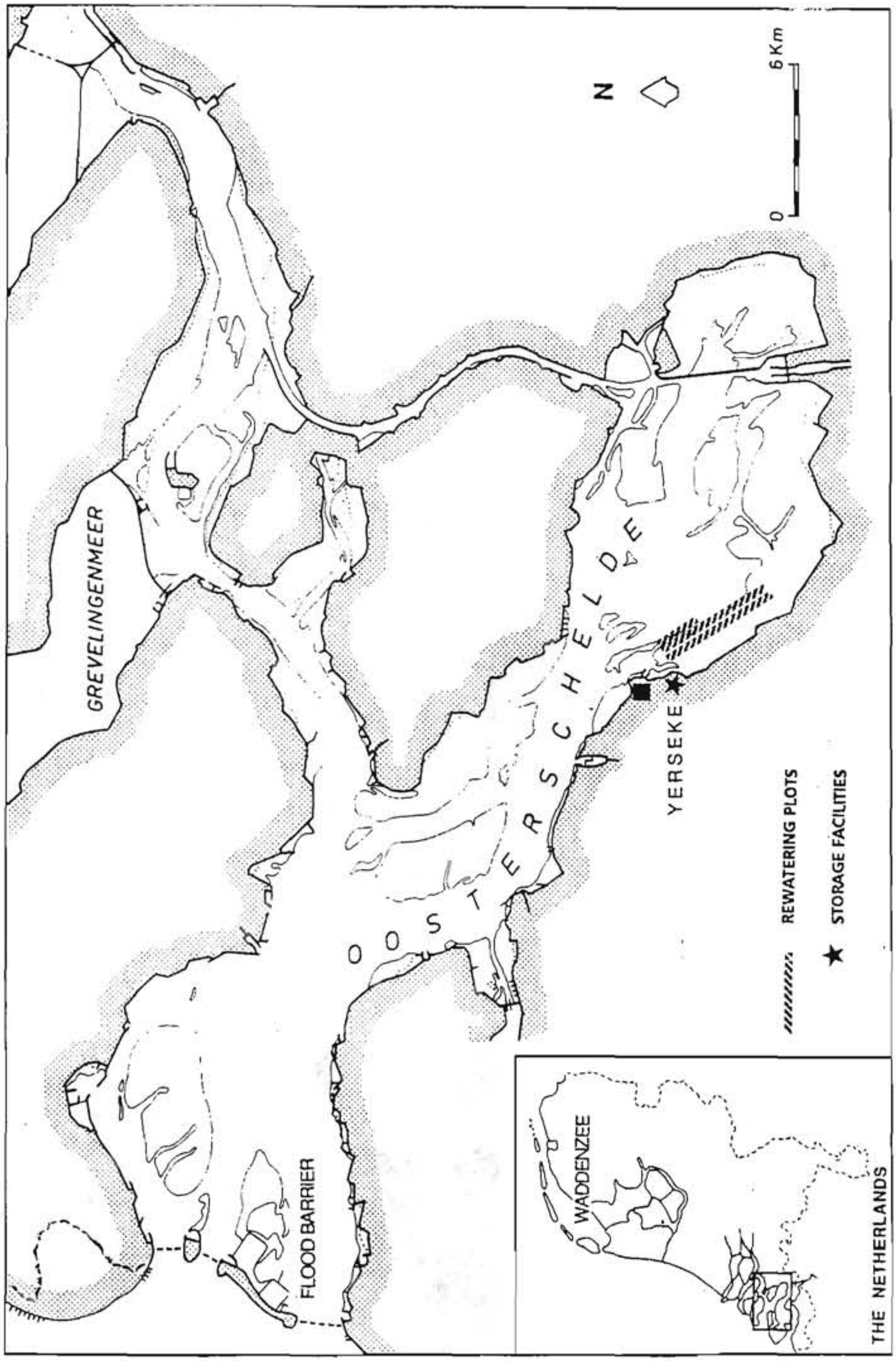

Figure 1: Oosterschelde estuary, showing Yerseke, the centre of molluscan processing and trade, with mussel rewatering plots and on-land storage facilities for mussels and oysters. Insert: the Netherlands, showing the Wadden Sea.

Figure 3 shows the production and the increased imports of mussels during the last decades. Most of these come from the German and the Danish Wadden Sea. An increasing number is imported from the United Kingdom, Ireland, the Limfjord and Baltic Sea in Denmark 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 tend to be more and more restricted by measures to protect nature and wildlife.

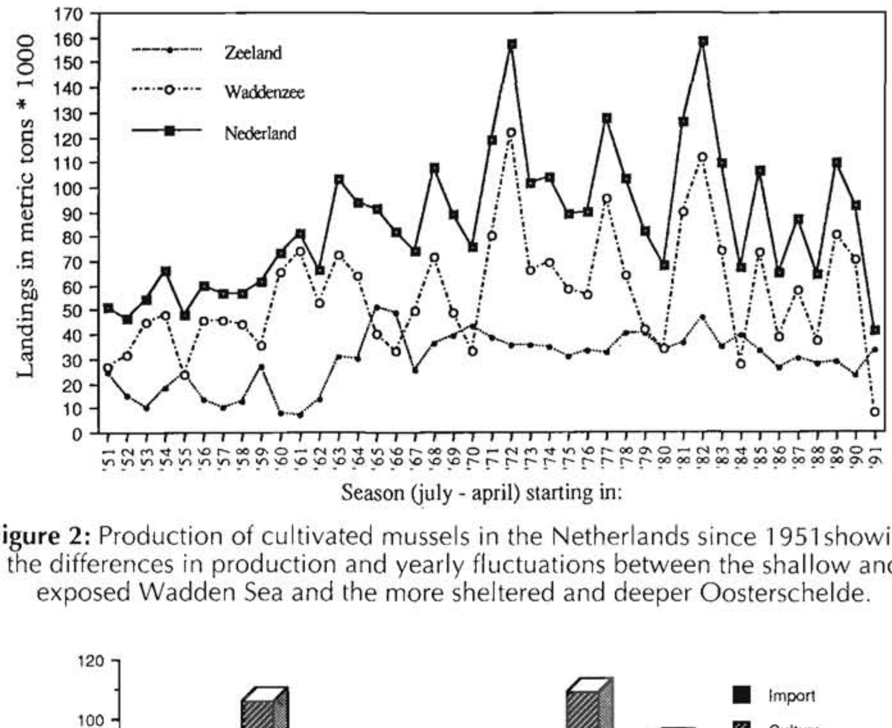

Figure 2: Production of cultivated mussels in the Netherlands since 1951showing the differences in production and yearly fluctuations between the shallow and exposed Wadden Sea and the more sheltered and deeper Oosterschelde.

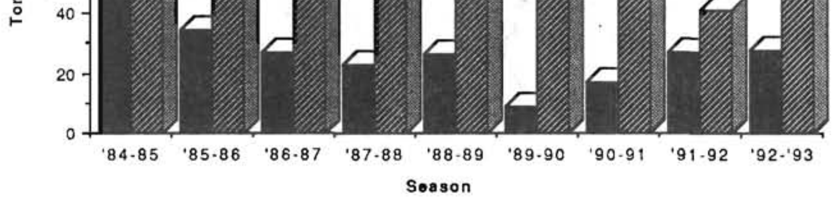

Figure 3: Comparison between the production of cultivated mussels and mussel imports in the Netherlands during the period 1984-1993.

Imports of the European flat oyster have been necessary after years of low recruitment since the 18th century. They have recently become of vital importance after the production of flat oysters was decimated following the introduction and subsequent outbreak of the oyster disease bonamiasis in 1980. The Dutch oyster growers and traders now resort to imports to satisfy the demand. *Ostrea edulis* is imported from Ireland, the UK and, to a lesser extent, from Greece and Turkey, the United States, Canada and even Chile. The Pacific oyster *Crassostrea gigas*, indigenous in the Dutch waters since 1964 (Dijkema, in press.), is not imported.

Development of toxic algal blooms

Toxic blooms of the dinoflagellate *Dinophysis acuminata,* causing Diarrhetic Shellfish Poisoning (DSP), occur in the Dutch coastal waters about once per 4 - 6 years. They rarely pose problems to the bivalve industry, thanks to the existence of an intensive monitoring and control programme. Blooms of dinoflagellate species which cause Paralytic Shellfish Poisoning (PSP) have hitherto never been observed in the Dutch coastal waters and the Wadden Sea. In other European countries, toxic or non-toxic blooms of species like *Alexandrium tamarense* (*Protogonyaulax tamarensis*) *A. minutum, Gymnodinium breve* and *Gyrodinium aureolum* have been reported or occur regularly. Figure 4 gives an overview of the reported blooms of potentially PSP-causing dinoflagellates in Europe. Why PSP-causing dinoflagellate blooms do not occur in the Dutch coastal waters and the Wadden Sea is unknown. There is no evidence that the environmental conditions in this area preclude their development. It must therefore be taken into account that the introduction of sufficient quantities of cysts could induce the development of blooms. There is increasing evidence in literature that accumulating resting stages of *A. tamarense* can form "seed beds" (Anderson and Keafer, 1985; White and Lewis, 1982), which initiate recurrent blooms when the cysts germinate, induced by environmental stimuli like changes in temperature (Anderson and Morel, 1979; Anderson and 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 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 (Waldock *et al.*, 1991). In the Unites States, recurrent blooms have been reported after first outbreaks: in 1985 in the 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.* 1982a; Anderson and Wall, 1978; White and Lewis, 1982).

Possible transfers of cysts

In Tasmania, repeated blooms of the dinoflagellates *Alexandrium catenella* and *A. tamarense* are supposed to have been caused by cysts, introduced with the ballast water of ships arriving from Europe and Japan (Hallegraeff & Bolch, 1990). Apart from ballast water, molluscan shellfish transports are considered to be potential vectors for dinoflagellate cysts (Anderson and Wall, 1978),

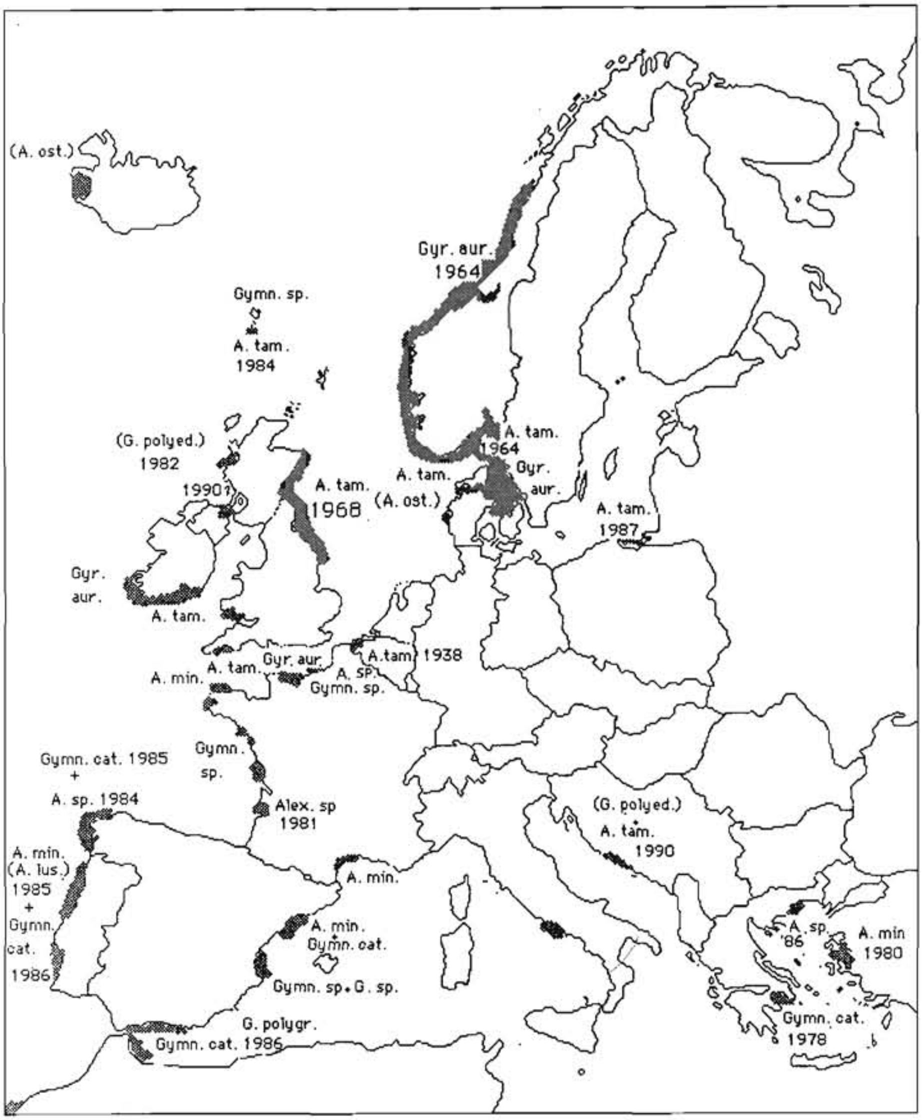

Figure 4: Overview of toxic or non-toxic blooms of the species *Alexandrium tamarense* (A. tamar.) *Alexandrium minutum* (A. min.), *Alexandrium ostenfeldi* (A. ost.), *Alexandrium lusitanicum* (A. lus.), *Gonyaulax polyedra* (G. poly.), *Gonyaulax polygramma* (G. polygr.), *Gymnodinium catenatum.* (Gymn, cat.), and the years in which these were first reported.

Anderson, 1984; Hallegraeff *et al.*, 1990). Areas with high abundances of dinoflagellate cysts are often found in sections of coastal waters where depth and low current velocities increase sedimentation (Anderson and Keafer, 1985). Exploitable stocks of bivalves often occur in such areas. (Re)suspended cysts in the water column will be ingested and concentrated by the filtrating activity of the bivalves (1 m^2 of wild mussel bed is able to filter 6-9 million cysts per year (Dijkema, 1992). Cysts can either be entrapped in the intestinal tract or the intervalvular cavity of dredged bivalves or can be present in the mud and sand which often accumulates underneath shellfish beds, particularly of *Mytilus edulis.* A part of these bio-deposits are eventually dredged up together with the mussels and, as these are generally not or poorly rinsed before they are exported, are found in the shipments of mussels which are imported into the Netherlands. According to import statistics, shipments of imported mussels almost invariably contain 25% - 50% of tare, 10% of which consists of sand and silt, the other half of shells, stones, dead crabs, starfish etc. (Dijkema, unpubl. data). On basis of literature data it could be estimated that as an average 1000 cysts are present in one cm^3 of sediment from "red-tide areas". A rough and conservative estimation learned that, in this way, 2.5 million cysts are imported per tonne of mussels per year, resulting in a possible annual introduction of about 10^{10} cysts of toxic dinoflagellates into the Oosterschelde (Dijkema, 1992).

Motives for the development of land based quarantine systems

Introduced cysts can be transferred to mussel and oyster storage facilities in the Oosterschelde estuary (figure 1). There they are expected to accumulate in a shallow and quiet area of about 100 km^2 with current velocities from 0.10 - 0.30 $m.s^{-1}$. There is increasing evidence in literature that accumulated cysts of *A. tamarense* can thus form "seed populations", capable of initiating toxic blooms when they germinate collectively (Anderson and Wall, 1978). This risk motivated the governments of Australia and New Zealand to issue respectively consider measures to prevent the introduction of exotic dinoflagellate cysts with ballast water or imported bivalves (Alexander, 1992). In the Netherlands, such a situation is feared to arise in the shallow part of the Oosterschelde off Yerseke (figure 1). Although no concrete evidence is available, the risk that cysts of toxic dinoflagellates are introduced is considered to be realistic by the dutch authorities (Dijkema, 1992). One could, at the other hand, also wonder why toxic blooms have not been induced earlier in history, considering long-existing pathways of possible introduction of cysts such as the imports of mussels and oysters which already exist for a long time in the Netherlands and also the ballast water which is discharged in large seaports in the vicinity such as Rotterdam, Antwerp, Hamburg and Bremen.

In order to at least eliminate the possibility of introduction of toxic blooms through imported shellfish, the Dutch Commodity Board for Fish and Fish products, the competent authority in this field, in 1987 has issued a decree which bans any immersion into the coastal waters of imported bivalves originating from other areas than the Dutch, German and Danish Wadden Sea. Import of these bivalves is possible, but only when the bivalves are either sold directly to the conserve industry or the consumer, or when they are kept strictly separated from the coastal water. They are therefore only allowed to be stored in holding facilities which are duly isolated from the sea. To enable the mollusc trading and processing industry to import mussels and oysters in compliance with the import regulations mentioned above, closed "quarantine" systems had to be developed. To keep the costs of investment as low as possible, initially a communal system was envisaged, in which all of the about 20 mussel traders in Yerseke could participate, cleansing and storing their own mussels but sharing a system for intake, distribution, circulation, filtration and discharge of water. A pre-design was made in 1990 by RIVO-DLO and the civil engineering consultant Grontmij n.v. for two alternatives with a storage capacity of 250 tonnes of mussels: the first option was a semi-recirculated system with a recirculation flow of 400 m^3. The effluent flow was 60 m^3/h. The second option was a flow-through system with a flow of 2500 m^3/h. The effluent of both systems in that stage was planned to be sterilised with UV radiation. The estimated investment costs were about HFL 6.4 million for the recirculated system and HFL 15 million for the flow-through system. The high costs of the latter were caused by a pipe line, necessary for continuous intake of clean water. The industry found these investment costs too high and a number of the mussel traders started to construct their own quarantine storage systems. They asked the Netherlands Institute for Fisheries Research to make designs suitable for their particular conditions. The systems had to meet the following requirements:

For the mussel industry:

– Sufficiently large to contain 25-40 tons (at least one truck-load of 25 tons) of mussels in bulk.

– Suitable to keep mussels in good condition during at least 72 hours.

– Suitable to be used for normal storage and de-sanding of locally produced mussels in years of little import.

For the import regulations:

– The systems must be isolated from the coastal waters, no wash-water, effluent, leaking water, spray or foam may be discharged into the sea before 99.9% of the cysts or cells of potentially toxic algae have been killed or removed.

– Processing (rinsing, de-clumping, de-byssing and packing) of imported mussels must be carried out in installations, likewise isolated from the coastal waters.

– Solid waste from the systems, mainly shells, must be collected and disposed of on inland refuse dumps or treated in such a way that cysts are killed.

Designing of the recirculating systems

Elimination of cysts in large flows of effluent is difficult and costly. For this reason, recirculating systems with partial recirculation of sea water were envisaged. To keep mussels in a good condition during a period of at least 3 days, a minimal dissolved oxygen level of 70 % saturation and a maximal ammonium concentration of 10 mg NH_4-N.l^{-1} were taken as acceptable limits for the water quality in the systems. The systems generally comprise 4-8 containers for storage of the mussels, a tank for stocking and aeration of the water, pumps for circulation and for intake and discharge of sea water and effluent. Make-up water is pumped up from the adjacent Oosterschelde estuary, into which the treated effluent is finally pumped back. The systems have sealed floors and are surrounded with spray-screens or walls to prevent that leaking water, foam or spray reaches the coastal water. For separation and collecting of solid waste: shells, stones, dead starfish, crabs, mussels, etc., sieves respectively by containers are used.

The movable containers, already in use by the mussel industry for more than ten years for storage, de-sanding and ship-to-shore transport of mussels are also deployed in most quarantine systems. Imported mussels are loaded into the containers with grabs after arrival by truck. These containers are then placed in the quarantine systems. They generally measure 6 x 2.5 x 1.2 m and can hold 5-8 tons of mussels in layers of 80 -100 cm thick. The mussels are lying on a perforated double-bottom plate. Water pours into the containers through one or two pipes, generally from a height of some decimeters, for additional aeration. The water then percolates down through the layer of mussels at a variable rate, adapted to the thickness of the mussel layer, the water temperature and other factors. When the water temperature lies above 20 °C, the available amount of dissolved oxygen in the water is low and the oxygen consumption rate by the mussels is high. Also because the vitality of the mussels is then generally at a low level, a thinner layer of mussels and an increased flow rate are recommended. To keep the mussels submerged in the container, a fixed water level is maintained with one or two standpipes.

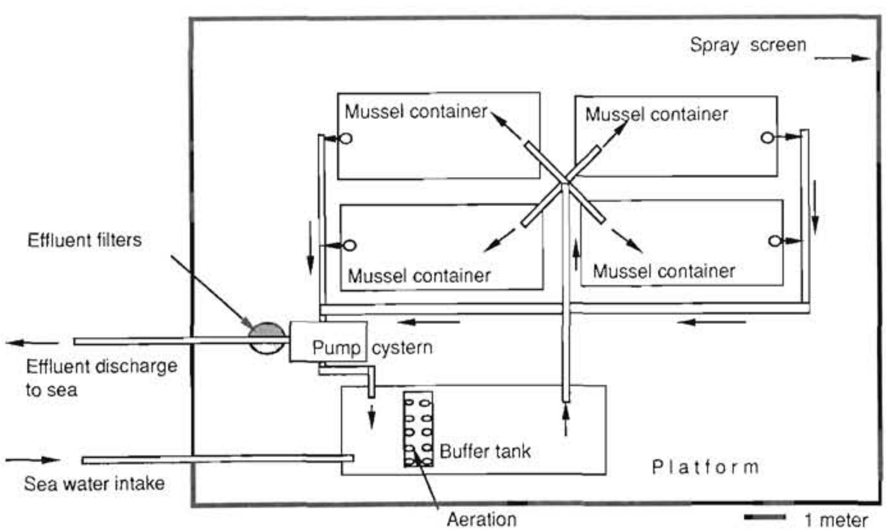

Figure 5: Layout of an on-land recirculating storage system with a capacity for storage of 25 tonnes of mussels. The systems are generally situated at the plants of mussel and oyster processors and exporters, lying at the edge of the Oosterschelde.

The circulating water leaves the containers by gravity via the standpipes and is then collected in a pump-cystern, from where it is pumped into an elevated buffer tank. This tank with a volume of 40 - 60 m^3 serves for intermediate stocking of the water during emptying of the containers for loading and unloading. If necessary, oxygen is suppleted in this tank by means of aerators. From the buffer tank, the water flows back to the containers by gravity. In a number of systems, the buffer tank is positioned underneath the containers and the water is pumped into the containers. The water in the systems is continuously refreshed with sea water at a rate of 10-15% of the system volume per hour. This corresponds with effluent flow rates between 15 and 25 $m^3.h^{-1}$. Ammonium levels in the effluent of a working system appeared not to exceed 2 $mg.NH_4\text{-}N.l^{-1}$. The highest levels were measured during the first hour of storage. Figure 5 shows the layout of a recirculating storage system for mussels.

Selection of a method to kill or eliminate cysts

A method for eliminating cysts from the effluent had to be selected from the following options: killing by heat treatment, by chemical means, by irradiation with UV or by mechanical removal: sedimentation, swirl-separation or filtration. Unfortunately, it has not been possible to try out methods experimentally.

Dinoflagellate cysts are created to survive and possess a number of properties which make them particularly difficult to kill. Their cell walls are multi-layered and 2-5 μm thick and mostly consist of Calcium carbonate and/or highly resistant polysaccharids such as cellulose or sporopollenin (Anderson, 1985). The fact that cysts are able to survive the adverse conditions in the sediment, which are anoxic and toxic due to high concentrations of Hydrogen Sulphide (H_2S), during periods over 6 years (Dale, 1979 ; 1983), implies that extremely high dosages of toxins such as chlorine, formaldehyde, malachite green or ozone would be necessary to kill them. The relatively high concentrations of organic matter and suspended clay in the effluent would further increase the effective doses of these agents due to reduction and adsorption. Apart from expected negative environmental effects, especially connected to the application of halogens or ozone, this would render the costs of these methods prohibitive. The option of irradiation with UV was considered impracticable for two reasons: first the minimum lethal dosage for most vegetal cells appeared to be 180 times as high as those for viruses and bacteria (Hoffman, 1974). This would render the necessary investments and energy costs extremely high. Secondly, the high extinction rates of the relatively turbid effluent of the systems would considerably increase the necessary dosage. The option to remove cysts by means of sedimentation or swirl-separation was rejected because the difference in specific density between dinoflagellate cysts (Anderson *et al.*, 1985) and sea water was found insufficient to make this kind of separation feasible. To kill cysts by means of heat-treatment of the effluent requires the heating of water flows of 15-25 $m^3.h^{-1}$ until a temperature of minimally 90°C during a certain period. This requires transfer of large amounts of heat and can only be considered economically feasible when the applied heat can efficiently be recovered. This method has not been evaluated further. Boiling is used by some processors to treat solid waste such as shells, peat and stones from cleansing installations, to make dumping into the Oosterschelde possible.

Effluent filtration

A realistic option appeared to be filtration. To ensure the required 99.9% elimination of the cysts or cells of potentially dangerous toxic dinoflagellate species, the effluent has to pass a filter unit which separates all particles, equal to or larger than the minimal diameter of these cysts. Dale (1979), Marasovic (1989) and Anderson and Wall (1985) give a minimum diameter of 20 µm for the relevant dinoflagellate cysts. Filtration is a stochastical process and absolute, 100% removal of particles of 20 µm can only be achieved at much smaller nominal porosities. Reliable functioning of such filters requires a guaranteed and duly documented selectivity rating and robust construction. Control of proper use requires that the elements used can be identified. Only a few types of filters appeared to meet this requirement and are now allowed.

A problem arose due to the high concentrations of suspended matter in the effluent, which can reach values up to 180 $mg.l^{-1}$ and incidentally even higher. This suspended matter mainly consists of clay, naturally present in the local coastal water and arriving with the landed mussels, but also of micro-algae, particularly in late summer. It was assumed that suspended material would be concentrated and separated by the filtration by the mussels. This, however, appeared not to be true, probably because the faecal pellets of the mussels disintegrate due to the high turbulence in the systems. Suspended matter appeared to clog the 20 µm filters within 20 minutes. It was necessary to keep the mud-loaded effluent from the mussel washing installations apart and to remove excessive silt by sedimentation. Additionally, pre-filtration of the effluent appeared necessary to spare the terminal filter. This could successfully be achieved with different types of filters, such as woven stainless steel mesh filter mats, exchangeable bag filters or continuous sand filters, sometimes used in combination. The 20 µm final filters now generally stand for up to two weeks before they need to be replaced or cleaned. To prevent the use of unauthorised filter materials, the filter housings are sealed and regularly checked by inspectors of the Commodity Board.

Quarantine systems for oysters

Development of quarantine systems for imported oysters proved to be less complicated for two reasons: the first is that almost no mud and sand adhere to the oysters. Consequently, the water in the storage systems is relatively clean. Besides, under normal conditions, little accumulation of ammonium and nitrite appeared to take place in the stagnant oyster storage basins. During a preliminary experiment, the concentrations of NH_4-N and NO_2-N in a basin with a stocking density of 81 kg *Ostrea edulis* per m^3 did not exceed 1 and 2 $mg.l^{-1}$ respectively after 25 days. This is attributed to nitrification due to the formation of a film of nitrifying bacteria on the shells of the oysters, the trays and the walls of the basin (Dijkema, unpubl. data). The water refreshment rate in these basins can consequently be very low. These two factors make that quarantine storage of imported flat oysters is possible in the existing stagnant basins which have been used for post-harvest stocking of cultivated and wild-caught oysters for more than a century. To be used for quarantine storage, these basins must meet the requirements of the import regulation. This implies that they must be duly isolated from the coastal water and that provisions must be made that no water can leak or spray into the adjacent Oosterschelde. Due to the cleanliness of the water in the basins, pre-treatment of the effluent preceding filtration at 20 µm is not necessary. Generally, the basins measure 25-50 m^2. They are often built in pairs, so that the water can be stored in one of them, whilst the other is drained for collecting or cleaning of the oysters. By December 1992, about 18 oyster growers and traders were using this type of quarantine systems.

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