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Feasibility of Active Biological Monitoring (ABM) of phytoplankton toxins with suspended mussels (*Mytilus edulis*) in the Dutch coastal area.

by

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Abstract

During toxic blooms, an equilibrium between intake and excretion of phycotoxins causing Paralytic Shellfish Poisoning (PS)P, Diarrhetic Shellfish Poisoning (DSP) and Amnesic Shellfish Poisoning (ASP) in bivalve shellfish is generally reached within 10 days. Accumulation and excretion of phycotoxins appear mainly to be determined by the toxin concentration in the phytoplankton and, notably in ASP and PSP, by the concentration of toxic cells. Depuration of PSP, DSP and ASP toxins at 10°C occurs within 3, 10 and 0.5 days respectively in clean water. For PSP and ASP it can hence not be excluded that a short term contamination could be missed during ABM at a fortnightly sampling frequency. PSP toxins of, for instance, *Alexandrium spp* or *Gyrodinium* spp. in bloom densities have been reported to cause physiological stress in mussels, resulting in, among other things, decreased filtration rate. This could obscure a possible relationship between algal cell concentration and toxicity. As prime material for ABM, medium-sized mussels with a meat yield of 20% - 25%, should be used. Cultured mussels have a more homogeneous meat yield, age and size than wild mussels. Mussels should be collected by hand from a bottom location; gentle handling is important to avoid stress and breakage. Experiences in other countries with ABM of phycotoxins are generally positive. It is concluded that ABM of phycotoxins with suspended mussels is technically feasible, and that a practical experimental phase is necessary.

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1. INTRODUCTION

The occurrence of harmful, (i.e. toxic for men, toxic for marine organisms or secondary oxygen-depleting) phytoplankton in marine waters is, in Europe, dealt with from two different points of view. As far as public health in connection with edible bivalves is concerned, the Directive 91/492 of the European Union obliges member states to monitor commercial bivalve shellfish production areas for phycotoxins as well as for the presence of potentially toxic algal species. Besides, the Oslo-Paris convention (OSPARCOM) supervises the monitoring programmes in the allied countries, aimed at protecting the marine environment from harmful algal blooms. Algal blooms are, in the first place, detected and followed by measuring of cell concentrations.of potentially toxic species. When increased concentrations of (potentially) harmful species are found, which means: concentrations known to be causative for toxicity in bivalve shellfish, generally an "alert" phase starts, during which sampling frequency is intensified and preventive measures may be taken.

High concentrations of algal toxins in bivalves do not always go together with high cell concentrations of toxic algal species. On the other hand, low cell concentrations have been reported to occur in combination with toxicity. Depending on the physiological condition of the algal cells, and possibly also on the presence of bacteria (Kodama, 1993), also higher toxin concentrations may occur than could be expected on basis of cell concentrations alone. An increase of PSP toxicity was found after a toxic bloom of *Alexandrium tamarense* had already disappeared (Sakamoto et al.., 1993). Also it was found that toxicity was caused by toxic cysts which were ingested by shellfish after having been resuspended from the sediment in which they had accumulated (White & Lewis, 1982). Also water temperature can influence the concentrations of toxins in shellfish, as is the case in *Dinophysis acuminata*. Few answers have hitherto been found on the question which conditions make that phytoplankton species start to produce toxins. At the Scottish east coast, recurring PSP-toxicity started only after increased cell concentrations of *Alexandrium tamarense* had already been present for a number of years (Waldock, 1991). *Alexandrium tamarense* produced much less PSP toxins under Nitrogen limitation than during the growth phase of the culture (Boyer et al.., 1987). This was also corroborated by results in cultures of *A. fundyense, A. tamarense* and *A.*spp (Anderson et al.., 1990). On the other hand, it appeared that Nitrogen or Phosphorous limitation did not cause an increase of PSP toxicity in a culture of *A. minutum*. Under experimental conditions, only Nitrogen limitation, followed by a repeated addition of Nitrogen, resulted in a strong increase of PSP production (Flynn et al., 1994).
Sakamoto et al., (1993) found that free-living bacteria, in association with *Alexandrium tamarense*, were able to produce PSP toxins. It did not become clear if *A. tamarense* is able to produce toxins in absence of such bacteria.

As it has not yet been possible to cultivate *Dinophysis* spp., environmental conditions which can induce production of DSP toxins cannot be studied under laboratory conditions. From field studies, only the stability of the water column has appeared to be an important factor for the development of blooms of this genus. Nitrogen and Phosphorous concentrations do not seem to play a role (Delmas et al., 1992). DSP concentrations of 0.07 pg/cell are mentioned (Subba Rao et al., 1993).

For the different *Pseudonitzschia spp* which are able to produce ASP, the occurrence of blooms in late autumn does not make N- or P- limitation very probable. Also in these algae, the highest toxin production seems to occur during the stationary phase, resulting in about 7 pg/cell domoic acid (Bates et al., 1993).

From the development of DSP in the Dutch coastal waters, it has appeared that uptake of DSP toxins from *Dinophysis acuminata* by bivalve shellfish can occur very rapidly: in about one day. Detoxification, however, may take several weeks. It is, therefore,

possible, that toxic algae have already disappeared from a sampling location while the toxin is still present in intoxicated shellfish (Kat, 1983). A timely detection of toxins produced during algal blooms is therefore essential for a realistic prognosis of possible effects of harmful algae on men or on the ecosystem. The lack of a clear correlation between algal cell concentrations and toxin levels, both in algae and in shellfish, makes a prognosis of effects impossible when suspiciously high concentrations of potentially harmful algae are found.

2. THE PHYCOTOXINS AND THEIR METHODS OF ANALYSIS

2.1 PSP toxins

The complex of PSP toxins contains about eighteen different toxins, produced, among others, by dinoflagellates of the genus *Alexandrium*, (Sullivan et al., 1985).
The best known, but certainly not the most generally occurring PSP toxin is saxitoxin, isolated from the clam *Saxidomus giganteus*, occurring in North America and Canada. The "classical" detection method for PSP toxins is the mouse-bioassay, following McFarren (1959). An advantage of the bioassay method is that the tocicity of the PSP toxins present is measured directly, regardless of the composition of the mixture of toxins. Unfortunately, this test is very animal-unfriendly. which meets ethical and political resistance in the Netherlands and some other countries. Modern determination methods use HPLC techniques, converting the toxins before (Laurence, 1991) of after (Sullivan, 1985) column-chromatographic separation into substances which can be detected using fluorescence techniques. A considerable practical problem at this stage is, that only a small proportion of the PSP toxins are available as standards. This gives the HPLC method the character of a screening test.

2.2 DSP toxins

The complex of DSP toxins contains two main components: Okadaic Acid (OA) and DTX1, and besides a number of acylic-derivatives of DTX1. Recently, also a toxin, named DTX2 has been isolated (Tingmo et al., 1993). DSP toxins are all fat-soluble polyethers which can be produced by dinoflagellates of the genus *Dinophysis* and probably by two species of the genus *Prorocentrum*. Okadaic acid has also been found in the sponge Halichondria okadai, which explains its name. Just like for PSP toxins, mouse bioassay has been proposed as a detection method for DSP toxins (Yasumoto, 1978). As also this test is based on the death of the test animals, it can be considered as an animal-unfriendly method which, in the Netherlands, where monitoring for of DSP is carried out on a regular basis, is not acceptable. Instead, since 1961, the much more animal-friendly (only feces structure and eating behaviour are recorded) and even slightly more sensitive rat-bioassay method, described by Kat, (1983), is in regular use in the Netherlands. In either test, the toxicity of all toxins present is determined. Also, for the determination of DSP toxins, the HPLC method has made his appearance (Lee, 1987). This method is, however, still very sensitive for errors, which makes that it can hitherto only be used for confirmation.

2.3 ASP toxins

The most important toxin responsible for ASP-intoxication is domoic acid (DA), produced by different diatoms of the genus *Pseudonitzschia*. It is a readily water-soluble aminic acid, earlier detected in Japan, on the macro-alga *Chondria armata*, which bears

the Japanese name of "Domoi". For determination of DA, the customary HPLC techniques for determination of aminic acids are used (Quillam et al., 1989).

3. ACCUMULATION AND RETENTION OF PHYCOTOXINS BY MUSSELS AND OTHER BIVALVES

3.1 Accumulation and retention of PSP

Mussels appear to be good accumulators for PSP toxins. The highest concentrations of PSP toxins accumulate in the intestinal tract of mussels, exposed to toxic algae. At a temperature of 16°C, the highest equilibrium level of 450 mg/kg saxitoxin-equivalents was reached after 12 - 13 days of exposure to 150 000 - 250 000 cells/l of *Alexandrium tamarense* (Bricelj et al., 1990). They found that, under these conditions, the tolerance of bivalve shellfish for PSP toxins of 0.8 mg/kg was exceeded already after one hour of exposure. The composition of the PSP- complex in the intestinal tract of the mussels (more or less in accordance with that of *A. fundyense*) differed markedly from that in the rest of the mussel tissue, i.e. that it contained much more saxitoxin. Excretion of PSP toxins appeared to be strongly influenced by the presence of toxic cells: in young mussels this excretion decreased exponentially with increasing cell numbers of *A. fundyense* from 50 to 700 cells/l (Bricelj et al., 1990). Also Chebib et al.(1993) found that chronical exposure to PSP blooms can influence accumulation, detoxification and biotransformation of PSP toxins. Mussels from a clean environment accumulated less toxins, contained larger amounts of the more toxic PSP toxins and detoxified more rapidly than mussels which had been exposed beforehand. Accumulation was rapid, in the order of magnitude of days, whereas the half-life of excretion was about 3 days. Under the given circumstances (St Lawrence bay, August), the PSP- toxins level in the mussels decreased within two weeks until below 10% of the maximal value (i.e. a half-life of also three days). An impression of the difference in accumulation rate between oysters (*Crassostrea gigas*), mussels (*Mytilus edulis*), and clams (*Tapes japonica = Ruditapes philippinarum*) may be obtained from the results of a study by Akusawa (1963) into a case of PSP-intoxication in Hiroshima Bay. In the respective species he found 31, 215, and 20 mouse-units of PSP-toxins per gram. Mussels appeared to accumulate PSP toxins much more rapidly than oysters. Giant scallops (*Placopecten magellanicus*) from the Bay of Fundy (Canada), when fed with toxin-free phytoplankton, lost about 90% of their PSP-contents after one year of storage in clean sea water at temperatures between - 0.2 and 14°C (Waywood et al., 1995), which indicates a half-life of about 3 months. It appeared that the excretion-time of these shellfish can be considerably longer than in mussels, which might also be influenced by their size.

In the Dutch coastal waters, no PSP has been recorded so far, although incidentally, low concentrations (< 10 cells/l) of suspect *Alexandrium spp.* are reported (Koeman, et al., 1992). In the most suspect area in this respect, NW of the island of Terschelling, Peperzak et al., (1995) carried out an experiment, using ABM with suspended mussels.

Shumway (1990b) gives an extensive review of retention periods for phycotoxins in bivalves, in which *Mytilus edulis* profiles itself as a "rapid release" species, excreting toxins quicker than other bivalve species. Shumway (1988) reported that mussels in the state of Maine (USA) showed signs of PSP intoxication about one week earlier than soft shell clams (*Mya arenaria*). Desbiens et al (1990) compared accumulation of PSP toxins in wild and cultured mussels. They did not find any differences in toxicity

For *Mytilus edulis,* retention periods for toxins of *Alexandrium tamarense* are mentioned, varying between 10 days (Oshima et al., 1982) and 50 days (Gilfillan et al., 1976). For toxins of *Gonyaulax excavata,* periods of 2 - 3 weeks are mentioned (Gaard & Poulson, 1988), for those of *Gonyaulax catenella* 4 weeks (Sharpe, 1981) and 11 weeks (Quayle,

1965). Cembella et al. (1990) studied uptake and detoxification of PSP toxins in *Mytilus edulis* and *Mercenaria mercenaria* under experimental conditions, fed with two species of *Alexandrium* with different toxicity per cell and a different toxin composition. A saturation level of 450 mg/kg saxitoxin-equivalent was reached in the mussel tissue after exposure to a cell concentration of 250 000 cells of *A. fundyense* per /l, which was considered realistic. Detoxification occurred in two phases after feeding of toxic algae was stopped: the toxin concentration decreased within 24 hours, particularly in the viscera, followed by a gradual decrease over several weeks. Particularly during the first phases of the experiment, the profile of the accumulated toxins was about equal to that in the algae.

Shumway (1990 b) compared the toxicity of PSP toxins (*Alexandrium tamarense*) in samples of the European Flat Oyster (*Ostrea edulis)* and mussels (*Mytilus edulis*) taken simultaneously at the same location in the field. The mussels were toxic to very toxic (up to 35 mg/kg saxitoxin-equivalent, the oysters were not to a little toxic, at most just above detection level. The oysters became toxic about three weeks after the mussels. On the other hand, Lesser & Shumway (1993) mention that juvenile *Ostrea edulis* became toxic prior than any other species under field conditions, and that this species showed a very high clearance rate for toxic *Alexandrium tamarense* compared with other species. In both mussels and oysters, the toxin level appeared to decrease at the same rate. In the Netherlands in 1994, DSP toxicity was found in mussels (*Mytilus.edulis*) in Lake Grevelingen, whereas it was not detected in oysters (*Ostrea. edulis*) (Dijkema, unpublished data).

In a review article, Shumway & Cembella (1993) mentioned that, in contrast with *Mytilus edulis,* the scallop species *Placopecten magellanicus* and *Patinopecten yessoensis* were able to maintain their toxicity during a very long period: between a few months and two years, particularly in the hepatopancreas and the mantle tissue. In *Patinopecten yessoensis,* toxicity decreased from an initial value of 340 mg/kg saxitoxin equivalents during the first days of purification in clean water, until 40 mg/kg, afterwards. It increased again until 104 mg/kg and afterwards stayed below 20 mg/kg in the tissue, even after 5 months storage in outdoor tanks (Oshima et al., 1982). Several causes were indicated by these authors:

1 A slow basal metabolism and reduced filtration rate, particularly at low water temperatures and a low food supply.
2 Conversion of toxins to still more toxic derivatives, causing an increase of net toxicity, even when the original toxicity was decreasing.
3 Hidden toxic blooms in deeper water layers.
4 Ingestion of faecal pellets during the die-off phase of old blooms.
5 Ingestion of resuspended toxic cysts accumulated in the sediment.

Surf clams *Spisula solidissima* from Georges Bank, on the other hand, retained PSP toxins until more than two years after intoxication. Highest toxin levels were initially found in the digestive gland, but later in the year the relative levels of toxin in gill and mantle tissue increased. Variation in PSP toxicity between individual clams was large (Shumway et al., 1994). Responses of juveniles of *Ostrea edulis, Mytilus edulis, Spisula solidissima, Crassostrea virginica* and *Placopecten magellanicus* on PSP toxins from *Alexandrium tamarense* and toxins from *Gyrodinium aureolum* appeared to be very species-specific (Lesser & Shumway, 1993) and were more intensive in spring (10°C) than in winter (5°C). Effects of *Gyrodinium* toxins appeared reversible after returning the shellfish to clean sea water (Widdows et al., 1979, Errard-le Denn, 1990). Cembella et al., (1993) studied the distribution of PSP toxins over different organs of sea scallops (*Placopecten magellanicus*)and surf clams (*Spisula solidissima*). They found that the toxin profile in the bivalves differed considerably from that in the algae. Toxin levels between tissues showed a large variability in both species. Levels were highest in the digestive gland, followed in order by the mantle, the gills, the gonads and the adductor muscle.

3.2 Accumulation and retention of DSP toxins

Like PSP toxins, DSP toxins especially accumulate in the hepatopancreas of bivalves. In an environment intoxicated with DSP, all of the DSP toxins which accumulated in oysters (*Crassostrea gigas* and *Ostrea edulis)* and scallops (*Patinopecten yessoensis* and *Chlamys nipponensis)* appeared to be present in the hepatopancreas, and in mussels (*Mytilus edulis)* almost all DSP toxins. Scallops contained about the same amounts of DSP as mussels, oysters about one third of this (Yasumoto et al., 1978). Accumulation of DSP in mussels apears to be strongly dependent on the concentration of DSP-producing *Dinophysis* spp. and on water temperature. Whereas, at temperatures above 20°C, a concentration of hundreds of cells of *Dinophysis* spp/l did not result in detectable toxicity in mussels, at temperatures between 5 and 15°C , some tens of cells per liter already resulted in detectable DSP-toxicity (Kat, 1989a). In sea water with a temperature of 17°C, mussels accumulated over 2 mg/kg DSP toxins in the hepatopancreas after 10 days, at a concentration of 3000 *Dinophysis* cells/l. The human health closure norm for shellfish areas is 0.4 mg/kg OA-equivalents in the hepatopancreas. The accumulation rate under these conditions is high: within 2 - 3 days an equilibrium concentration is reached. In the warmer (17°C) upper layer of the water, accumulation appeared to be slower than in the colder (14°C) water below the thermocline (Marcaillou-le Baut et al., 1993). Reguera et al., (1993) found a time lag of one week between a maximum in the density of *Dinophysis acuminata* and a peak in the concentration of Okadaic Acid in mussels on a raft in a Spanish Ria, which is an indication of accumulation time. Detoxification of OA took one week at a depth of 15 m, and two weeks at depths of 5 - 7 m. They could not demonstrate a good relationship between cell counts and OA concentrations. They mentioned diurnal vertical migration by *Dinophysis* spp as a possible cause.
Excretion of DSP toxins by mussels in the Dutch Wadden Sea showed a half-life of about 12 days. Laboratory experiments in clean sea water at 13°C showed a half-life for mussels of up to 9 days (Kat, 1989b). During studies in Sweden by Edebo et al., (1988), a reasonable excretion of DSP-toxins appeared to exist at sea water temperatures of 1.4 - 3°C, as long as phytoplankton was still available. This could mean that the causes of the strong temperature-dependency of DSP accumulation in mussels have to be sought in a higher toxicity per *Dinophysis* cell at lower temperatures.
Transplantation experiments in Sweden with mussels from a toxic location to a location with a lower toxicity level, showed at 8°C a half-life of 3 - 4 days (Haamer, 1990), which was considerably faster than in the Dutch situation described above. Apparently, the concentrations of phytoplankton present are most important in these conditions. However, complementary information is needed before a firm conclusion can be made. Remarkable are also the differences in DSP-toxin concentration between mussels at different depth in the same area and in areas very close to each other, mentioned by several authors (Kat, 1987, Haamer et al., 1990b).

Blooms of *Dinophysis* spp. appear to develop preferably during quiet weather conditions in stratified water. Along the French Atlantic coast, maximal counts of 5000 cells/l of *Dinophysis* spp. were reported (Lassus et al., 1988), along the Dutch coast up to 20 000 cells/l (Kat, 1983, 1987). Maximal numbers of *D. sacculus, D. norvegica* and *D. acuminata* occur along the French coast in June, the latter species also in July and August (Lassus et al., 1988). Along the Dutch coast, blooms of *D, acuminata* occur in August and September (Kat, 1989a). In Sweden a maximum of *D. acuminata* even occurs as late as October (Haamer, 1990). This indicates later appearance of toxic *Dinophysis*-blooms at higher latitudes.

Blanco (in press) used for his model calculations a toxin concentration of 6 pg OA per *Dinophysis* cell, a calculated toxicity limit for closure of mussel culture areas of 0.4.mg OA per kg hepatopancreas, and a depuration rate for OA of 20% per day. This value was 30 - 100% higher than that found by Marcaillou-Le Baut et al. (1993), which brought

him to the conclusion that little information is available about the influence of the nutritional quality and quantity of the seston on the detoxification process in mussels. Just like Masselin et al. (1992) he found a higher toxin concentration per cell at high densities in net samples. He ascribed this to a possible detoxicating influence of other phytoplankton species present in the sample. Einar Dahl (pers. comm.) reported that in Norway DSP toxicity starts occurring at cell concentrations of *Dinophysis* spp. between 500 and 1000 cells/l.

Sampaio et al. (1990) mention a number of cases with PSP toxicity at cell concentrations of 100-200 cells/l of *Dinophysis acuta* and *D. sacculus,* and sometimes even the presence of toxins in absence of algae, at temperatures of 16 - 22°C. Also they concluded that the quantity of non-toxic algae present can influence the degree of toxicity of the DSP-toxins.

Dahl & Yndestad (1985) found high concentrations of DSP toxins, produced by *Prorocentrum lima* at a concentration of 100 cells per litre. Dahl et al. (in prep) found a large variation in DSP toxicity per *Dinophysis* cell. During a period with a peak concentration at a depth of 3 m in June, 1987 of 20 000 cells *D. acuminata* per liter, toxicity in mussels was the same or even lower than at a depth of 15 - 20 m at a cell concentration of 200 - 100 cells/l. They concluded that it would complicate the monitoring of algal concentrations when this should be the only means for surveillance of mussel toxicity.

3.3 Accumulation and retention of ASP toxins

Also the toxin responsible for ASP, domoic acid (DA), accumulates mainly in the hepatopancreas of mussels. As small mussels have a relatively large hepatopancreas, the DA concentration in small mussels is therefore higher than in large mussels (Novaczek et al., 1992).
In the coastal waters of California, the maximal concentrations of domoic acid from *Pseudonitzschia australis* in November - December 1991 in mussels, oysters, and clams was. 47, 2, and 29 mg/kg respectively. Also in this case, the mussel seems to be a very suitable indicator species (Langois et al., 1993). High concentrations of *Nitzschia* spp. cells seem to inhibit the excretion of domoic acid, which can result in very high concentrations of ASP in bivalve shellfish (Silvert and Subba Rao, 1991). For this reason, it seems doubtful if a linear relationship can be expected between cell counts of toxic algae and the ASP concentration in mussels, used in ABM.

The excretion of DA by different bivalve species is generally relatively rapid. Laboratory studies with mussels (*Mytilus edulis*) and Atlantic scallops (*Placopecten magellanicus),* showed that the uptake efficiency of domoic acid from a culture of *Nitzschia pungens f. multiseries* was less than 10%, that scallops accumulate DA faster and more efficiently than mussels, but also that the half-life of OA in mussels is about 8 hours at 15°C (Wohlgeschaffen et al., 1992). This short half-life was confirmed by Novaczek et al. (1992), who found that at 6°C in clean sea water, 50% of the DA is excreted within 24 hours. After 72 hours, the DA level had fallen below 10% of the original value of 50 mg/kg. Excretion seems to be somewhat faster in smaller mussels (45 - 55 mm) than in larger mussels of 60 - 70 mm. The excretion of DA at 11°C. was about twice as fast as at 6°C, which also confirms the half-life of 8 hours at 15°C mentioned before. There seemed not to be any influence of salinity or food content of the water on the excretion rate of OA.

Evidence has, in the meantime, been presented for the presence of potentially DA-producing *Nitzschia* spp. in the Dutch coastal waters. In a culture of *Nitzschia pungens f. multiseries* from the Dutch Wadden Sea, Vrieling et al. (unpublished data) demonstrated up to 19 pg/cell domoic acid. ABM could be instrumental in studying the possible production of DA by other *Nitzschia* spp occurring in the Dutch coastal waters. For this

purpose, the present detection limit of the DA analysis of 0.5 mg/kg (the public health norm is 20 mg/kg) should be lowered, also taking into account that the present public health norm is 20 mg/kg.

Accumulation and retention of DA by mussels (*Mytilus edulis*) and Bay scallops (*Argopecten irradians*) was studied by Scarratt (1991). In a series of experiments he kept mussels in a suspension of *Nitzschia pungens* of 1 000 000 cells/l, with a DA concentration of 0.897 µg/l. The concentration in the hepatopancreas of the mussels, after an exposure period of 84 hours, was about 60 mg/kg, decreasing until 5 mg/kg after 48 hours in clean sea water. The accumulation rate in the scallops was about half of that in mussels.

MacKenzie (1993) found a relatively short retention time for DA in the greenshell mussel (*Perna canaliculus*) in new Zealand. After the start of feeding with toxic *Nitzschia pungens*, a toxicity peak of 3 mg OA/kg fresh weight occurred after two days. After changing to a non-toxic species, the DA concentration decreased, again within two days, until zero value. The water temperature was 17°C.

In the following overview, accumulation, retention and excretion rates of phycotoxins in mussels found in literature, are summarised.

Type of toxin	Toxic cells (cells per liter) * 1000	Accumulation until equilibrium (days)	Equilibrium concentration (mg/kg hepatopancreas)	Half-life of the excretion (days
PSP	150 - 250	12 - 13 (10°C)	450 (10°C)	3 (10°C)
DSP	3	2 - 3 (17°C)	2 (17°C)	4 (17°C) 3-5 (16°C) 7 (13°C) 9 (10°C) 12 (8°C)
ASP	1000	4 (10°C)	60 (10°C)	0,3 (15°C) 0,5 (11°C) 0,7 (10°C) 1,0 (6°C)

4. THE SUITABILITY OF MUSSELS AS INDICATORS FOR PHYCOTOXINS

4.1 Reactions of mussels on phycotoxins

Although the toxins dealt with in this paper mainly affect vertebrates, a number of authors also mention that functions of bivalve species can be influenced by phycotoxins.

4.1.1 Reactions on PSP-toxins

Gainey & Shumway, (1988a) made an extensive review of possible effects of PSP-toxins on bivalve molluscs. The reactions of different bivalve species on PSP-toxins can differ widely. Shumway & Cucci (1979) found that, out of 15 mussels, three specimens did not react on administered doses of *Alexandrium* and *Gonyaulax* toxins GT429, whereas the remainder reacted with at least partial valve closure. They found that the filtration rate increased in one case, and decreased in another. Toxin was excreted in both the faeces and the pseudofaeces, which means that from a part of the phytoplankton, no

toxins were accumulated in the mussels. Gainey & Shumway (1988) found that GT429 caused significant heart beat decrease and death in *Mytilus edulis* from Maine. Marsden & Shumway (1992) found that oxygen consumption and grazing rate increased, the shells were opened during a long period and byssus production was impaired in the mussel species *Perna canaliculus*, after exposure to the toxin GT492 produced by *Alexandrium tamarense* in a concentration of 1 000 000 cells/l and a toxin concentration of 13 mg/kg saxitoxin-equivalents in the tissue. The norm for human safety is 0.8 mg/kg. The authors did not report increased mortality, and they concluded that the intoxication had rather caused a general physiological stress condition than an inhibition of specific functions. All these effects seemed to become evident particularly, or stronger, in mussels which had not been in contact with PSP toxins earlier. Shumway et al. (1987) found also in *Mytilus edulis, Modiolus modiolus* and *Geukensia demissa* a decrease of byssus production after exposure to GT429. Bricelj et al., (1993) exposed juvenile mussels of 9 - 13 mm without PSP history to the toxic, *Alexandrium fundyense* clone GTCA29 in concentrations of 164 000 and 182 000 cells/l GTCA29. Shell growth did not show differences, tissue growth, however, was retarded by 26% in the experiment with toxic algae, compared with a control experiment in which only the non-toxic diatom *Thalassiosira weissflogi* was fed to the mussels. The number of ingested cells per unit body weight was kept equal during the entire experiment. The toxicity of the *Alexandrium fundyense* used was relatively low: 9.5 pg saxitoxin-equivalents per cell. Shell growth of the mussels appeared not to be inhibited, but growth of the soft tissues was lower with the GtCA29 diet than with the non-toxic diet. Weight-specific ingestion rate of algal cells did, however, not differ between the two diets, averaging 1.94 x 10^6 cells d^{-1} g body wet $weight^{-1}$ The difference in tissue growth might, according to the authors, also be ascribed to a difference in food quality between the dinoflagellates and the diatoms. It was found striking that relatively large amounts of living *A. fundyense* cells were found in the faeces of the mussels. The gut passage time of the toxic *A. fundyense* was 48% longer than with *T. weissflogi.*

Twarog & Yamaguchi, 1947 found that *Mytilus edulis* was not sensitive for saxitoxin (STX) in concentrations lower or equal to 0.1 mM. Nielsen and Stromgren (1991) found a reduction in shell growth of 60 - 70% in mussels which were exposed to initial concentrations of 4.5, 110.9, and 120 million cells/l of respectively the species *Alexandrium ostenfeldii, Chrysochromulina polylepis, Gyrodinium aureolum* and *Gymnodinium galatheanum.* The effects did not show in the filtrate of the algal suspension, which brought the authors to the conclusion that endotoxins must be concerned. After the mussels had been placed back in clean sea water, the growth rate recovered in those mussels affected most within 2 - 4 days. The growth reduction was not attributed to lack of food, but to the toxins present. No further effects, such as the decrease in filtration rate, reactions of the siphons and during opening and closure of the shells, mentioned by other authors, were found. *Gyrodinium aureolum* is mentioned to be able to cause mortality among mussels (Dahl et al., 1982, Heinig & Campbell, 1992, Errard-Le Denn et al. 1990). In an experiment described by the last authors, they used cell concentrations which can occur in natural conditions during blooms (18 000 000 cells/l). A special case is mentioned by Parry et al.,(1989) in Port Philip Bay (Australia). They found mortality, growth rate reduction and bitter taste in mussels (*Mytilus edulis planulatus)*, scallops (*Pecten alba*) and flat oysters (*Ostrea angasi*), caused by an unknown toxin and in presence of large numbers of the diatom *Rhizosolenia chunii.*

4.1.2 Reactions on DSP toxins

Little information is available on the reactions of mussels on DSP toxins. Haamer et al. (1990a) found that *Mytilus edulis* accumulated much less toxins during strong toxicity of the phytoplankton than could be expected on basis of filtration rate and phytoplankton toxicity alone. They concluded that there must have been a reaction which inhibited accumulation of the toxins, but they did not correlate this with loss of other functions.

4.1.3 Reactions on ASP toxins

Silvert and Subba Rao (1990) mentioned that a concentration up to 1000 mg/kg of DA found in *Mytilus edulis* can hardly be explained by a model made by them of toxin fluxes in mussels. They concluded that, during the peak of the *Nitzschia* bloom, the excretion rate by the mussels decreased.

4.1.4 Reactions on non toxic algae

Possibly independently of the presence of toxins, shellfish can react negatively on ingestion of certain algal species, for instance by closure of the shell, starvation or death. The case of the dinoflagellate *Prorocentrum minimum* is complicated. Luckenbach et al.(1993), found that ingestion of *P. minimum* from monospecific cultures caused a mortality of 43% - 100% in juvenile oysters within 14 and 22 days respectively and at 33% and 100% respectively of the bloom density (10 000 - 100 000 cells/l). They did not find out if toxicity was concerned or not. In another strain of *P. minimum*, named EXUV, growth inhibition in juvenile oysters was ascribed to poor nutritional quality of these algae (Wickfors et al., 1993). In France, Lassus & Barthomé (1988) found mortality among old oysters caused by *Prorocentrum minimum.* Nakazima (1968) credited *P. minimum* with causing outbreaks of shellfish poisoning in *Tapes japonica* (Gmelin), which have been lethal to humans. No specific toxin was, however, mentioned. Also from other species negative influences on the growth of bivalves are mentioned. Slime-producing algae, such as *Phaeocystis pouchetti* can clog the gills of bivalves and thus inhibit ingestion of food and gas exchange. Small, thick-walled dinoflagellates with a mucous cover, such as *Synechococcus spp, Aureococcus anophagefferens en Pardococcus anorexus.*, at bloom densities, caused "Brown tides", with anorexia and widespread mortality of *Mytilus edulis* in Long Island Sound (USA) (Tracey et al., 1988).

4.1.5 Individual variability in PSP and DSP toxicity in mussels

White et al. (1993) studied, by means of mouse bioassay, the variation in toxicity between individuals in a sample in a number of shellfish species. The variation coefficient varied between 19% and 99%, and averaged 48.5%. In a sample of 10 specimens of *Mytilus edulis* Medcof et al. (1947) found a variation coefficient of 35%. While studying DSP toxicity in mussels (*Mytilus edulis*), Haamer et al., (1990a) found, at a sample size of 12 mussels a considerable difference in toxicity between individuals. De Kock & Kramer (1994) suggest a sample size of 50 - 100 mussels on account of the large variation in the levels of contaminants between individual mussels. RIVO-DLO uses in its monitoring programme as a norm that at least 20 gram hepatopancreas tissue is necessary for a reliable analysis result. At a mean weight of 0.5 grams of a fresh hepatopancreas for a 50 mm mussel, this corresponds well with a sample size of the 50 specimens.

4.2 Suspending and sampling of mussels for ABM

4.2.1 Suitability according to origin, size and age

Mussels of different origin and size have been used for ABM in the Netherlands for a number of years. In this chapter, the suitability of wild and cultured mussels of different size and age is compared, based on experience and general knowledge of properties of wild and cultured mussels. Especially in wild mussels, a clear length/age relationship is difficult to establish, due to large differences in growth rate of mussels, even at the same location. Although to a lesser extent, also cultured mussels can differ considerably in

growth rate, depending on food supply, stocking density and culture method. Cultivated mussels, however, show a much more homogeneous age and length distribution than wild mussels.

Mussels of different size differ in a number of respects. In the first place, small mussels have a higher pumping rate and hence may accumulate more phytoplankton per unit body weight than larger mussels. In the second place, small mussels have a relatively larger hepatopancreas than large mussels, which may cause differences in the proportional amounts of toxins accumulated. It is known from experience that large mussels tend to decrease in condition index and to loose shell liquor more easily under less favourable conditions than medium-sized or small mussels. For these reasons, we recommend to use medium-sized (35 - 50 mm), cultivated mussels, and to make sure that no mussels of different year-classes are mixed.

4.2.2 Condition index

A practical and widely used type of condition index is the meat yield of mussels: the weight of the cooked meat in relation to the total fresh weight. Advantages of this method are that it is quick and is also used in mussel culture and trade. The condition index reflects the relative changes in the amount of food reserves in the mantle tissue and the weight of the gonads. It fluctuates under influence of the reproduction cycle, the water temperature and food supply. It is, therefore, not possible to dispose of mussels of the same condition index throughout the year. Wild mussels on the bottom generally have a lower condition index than bottom-cultivated mussels, whereas mussels growing or cultured off-bottom on ropes, poles, buoys etcetera. have a higher index than those on the bottom. Besides, cultured mussels show less individual variation in meat colour and meat weight. This means that, in order to keep variation as low as possible, sample size for wild mussels should be larger than than for cultivated mussels. The meat yield of cultured mussels in the Netherlands varies between 12% (16% is the minimally allowed commercial value) and over 40% (rope-cultured mussels). The seasonal cycle of the meat yield of bottom-cultured mussels is as follows: meat yield is minimal just after spawning, in April-May: 12-15%. At the beginning of the commercial season, it rises to 25 - 35% in mid-July - November, then declines as phytoplankton concentrations drop and gametogenesis sets in, to 15-18% in March-April. In other countries, autumn spawning or diffuse spawning throughout the year can make these cyclic changes less distinct than in the Netherlands, and may cause a larger individual variability in meat yield and body composition. Especially large mussels with a high meat yield (25 - 40%) may show a sudden drop in meat yield (e.g. from 35% to 20%) when exposed to environmental stress (such as abrupt changes in temperature or a drop in food supply or in the composition of the ingested seston), or to mechanical stress (such as caused by dredging, handling, processing, and re-seeding). A part of their glycogen reserves is then resorbed within a few days, which may influence the quantities of accumulated toxins. Mussels with a low meat yield may also not have sufficient glycogen reserves when transplanted to areas with poor food supply. For these reasons, it is recommended to use mussels with an average meat yield: between 20% and 25%, for ABM.

4.2.3 Resistance against handling

A sturdy shell is a prerequisite, as ABM practice requires a relatively large number of manipulations of the mussels before they are eventually analysed in the laboratory. During these manipulations, mussels may be physically damaged, causing them to loose shell liquor and, subsequently, to dry out and die. Mechanical shocks weaken the mussels, prolong the adaptation period after transplantation and may cause decrease of meat yield in larger mussels. For these reasons, it is recommended not to obtain mussels from the regular trade channels, as these have generally been processed and have undergone a series of stressful treatments like de-clustering and de-byssing. Mussels

destined for ABM should preferably be collected by hand from a location where they have naturally grown or, at least, have been left in peace for at least two weeks. During the entire ABM-process, a gentle treatment is imperative, including working swiftly, avoiding shocks, not pulling out but cutting byssus with a pair of scissors, avoiding excessive heat or cold etcetera.

Mussels from off-bottom culture generally possess a high meat yield (35 - 40%) which they maintain during a large part of the season. They tend to loose shell liquor earlier when stressed or stored, and their shelf-life is shorter than that of bottom-cultured or wild mussels. Their shells are relatively fragile and their byssus strong. These mussels are less suitable for ABM-use than bottom-grown mussels.

4.3 Changes in condition and food supply and ABM

During an experiment in the North Sea at a distance of 135 km North West of the Dutch island of Terschelling, Peperzak et al. (1955) found that mortality of suspended mussels was less than 2%. Shell length increased gradually in the course of the experiment (0.5 mm per month), but much less than the value of 4-5 mm per month found by Page and Hubbard (1987) and the 6 mm per month found in cultured mussels by Van Stralen (1988) in the Oosterschelde. The condition index increased in the course of the experiment, which the authors ascribed to the fact that the condition index of the mussels had been very low at the start of the experiment. The chlorophyll-a content of the sea water in both years was between 0.3 and 5 µg/l, which is lower than regular values in the near shore coastal water. Peeters et al. (1993) found at a location at 10 km off Terschelling (fig. 1) a chlorophyll-a concentration of 5 - 10 µg/l. At another location, 20 km off Noordwijk (fig 1), spring values were even higher. From this, it can be concluded that food availability within 20 km off the Dutch coast is much more favourable for ABM that at 135 km offshore. Condition loss of suspended mussels at a distance within 20 km from the shore seems unlikely, provided that mussels with a not too high meat yield are used. Taking into account the experience that suspended mussels may have a higher meat yield than those grown on the bottom, relatively lean mussels might even increase in meat yield during ABM, especially during bloom periods.

5. EXPERIENCES WITH MUSSELS FOR MONITORING OF PHYCOTOXINS

5.1 Experiences in the Netherlands

Accumulation of phycotoxins in mussels has routinely been carried out since 1971 for DSP, since 1988 for ASP and since 1993 for PSP in the Dutch coastal waters. This is done in the national programme of sanitary monitoring of bivalve shellfish production areas for mussels, cockles and oysters. The possible presence of phycotoxins is determined in hepatopancreas tissue. For bacteriological sanitary monitoring of mussel areas, carried out following EU Directives 91/429 and 79/923, mussels are used. When no wild or cultured mussels are available, mussel samples are also suspended for a period, long enough to accumulate bacteria from the water. During the experiment by Peperzak et al. (1995) 135 km NW of Terschelling, mussels were analysed for the presence of DSP and PSP toxins every 2 - 3 weeks with with rat-bioassay and HPLC respectively. DSP-toxins were detected twice , the first time while no *Dinophysis* spp were present, the second time at a cell count of 130 cells of *Dinophysis acuminata*/l. Suspended mussels are further routinely used for monitoring contaminants during several monitoring programmes in fresh and brackish water (with zebra mussels (*Dreissena polymorpha*) and in the marine environment (with *Mytilus edulis)* in behalf of the Dutch water quality management.

5.2 Experiences in other countries, an enquiry

Foreign colleagues, involved in monitoring programmes with ABM were asked for their experiences with ABM of phycotoxins. Eleven of them, from the United States, Canada, Sweden, Denmark, Norway, Spain, Portugal, Great Britain and Scotland, reacted on the enquiry. Those reactions containing relevant information are represented below.

USA, Canada
Dr John Hurst (Department of Marine Resources, Maine, USA) in his reaction considered mussels very appropriate for monitoring phycotoxins in aquaculture areas. The 3000 miles long coast of Maine, however, posed logistic and financial constraints to such a monitoring-programme, notably in selecting suitable locations and replacing mussel samples that were lost due to predation or theft. For these reasons he judged an ABM-programme at the coast of Maine not to be cost-effective. In Boothbay Harbor, suspended mussels offer a good early warning against development of PSP toxicity.

Spain, Portugal
Dr Beatriz Reguera (Instituto Espagnol de Oceanografía) reported that, in the Rias of Galicia, rafts with cultured mussels are used to study uptake and detoxification of phycotoxins. Rope-culture rafts are moved between toxic and clean areas (Reguera et al., 1993).

Norway, Denmark, Sweden
Dr Einar Dahl (Flødevigen Marine Research Station of the Institute of Marine Research Norway), Dr Helle Emsholm (Ministry of Fisheries,Kopenhagen) and Dr Pontus Elvingson (National Food Administration, Uppsala, Sweden), reported that in their countries, mussels were used for monitoring of DSP and PSP toxins. Locally collected wild or cultivated mussels were used, however, and no suspended mussels.

United Kingdom, Scotland
Dr David Alderman (MAFF, Weymouth) reported that biological monitoring of DSP and PSP toxins in England and Wales is common, but that no suspended mussels are used, as sufficient mussels are available at the sampling locations.
Dr Geodfrey Howard (Marine Laboratory, Aberdeen) reported that in Scotland, suspended mussels are regularly used for monitoring of DSP and PSP toxicity in aquaculture areas, particularly where no wild or cultured mussels are available. According to his experience, accumulation and detoxification took place at the same rate as in wild mussels. Storm damage and vandalism sometimes were a problem, even at locations that were difficult to reach.

6. DISCUSSION AND CONCLUSIONS

6.1 Discussion

Experiences with ABM of phycotoxins in other countries are generally positive, except practical problems such as theft and the accessibility of locations. Such problems are expected to be less relevant in the Dutch situation, where offshore suspending/sampling stations are envisaged. Reactions of mussels on DSP and ASP toxins are hardly mentioned in literature, relatively many authors report that PSP toxins of, for instance, *Alexandrium* or *Gyrodinium* in bloom densities may cause general physiological stress, resulting in growth inhibition, decreased filtration rate etcetera. A decrease in filtration rate could obscure a relationship between cell concentration and toxicity. This relation is, however, also made less obvious by other factors, such as the relative abundance of non-

toxic algal species, water temperature, the presence of bacteria, and still unknown toxins. Aimed studies using suspended mussels are will have to demonstrate whether such relationships exist in the dutch coastal waters.

Monitoring of exotoxins, produced by e.g. *Gyrodinium*, known to be potentially harmful for fish and shellfish stocks, is also important, but still difficult because no toxic cells are ingested by the shellfish, but detectable levels of toxins must be reached in another way, before toxic levels in the water are reached. ABM could perhaps offer possibilities to develop alternative concentration methods for such toxins, which make early detection possible.

Their high filtration rate, capability to adapt to new environments relatively rapidly and their relatively easy availability, make mussels appropriate for ABM of phycotoxins. As these toxins accumulate predominantly in the hepatopancreas of mussels, this seems to be the most appropriate organ for detection of phycotoxins in ABM. For analysis of PSP an ASP toxins, the available HPLC techniques should be used. Analysis of DSP-toxins can, for the time being, best be performed by means of rat bioassay. Small mussels have a proportionally larger hepatopancreas than large mussels, which results in a different accumulation rate for phycotoxins. This makes the use of equally-sized mussels important. No differences can be expected in this respect between wild and cultivated mussels.

Accumulation and excretion of phycotoxins generally proceeds rather rapidly. Generally within about 10 days, an equilibrium is reached between intake and excretion of PSP, DSP and ASP toxins. The level of accumulation is determined by the toxin concentration in the phytoplankton, on the other hand, especially in ASP and PSP, inhibition of the excretion by the numbers of toxic cells has been mentioned. When placed in clean water, depuration of PSP, DSP and ASP toxins at 10°C may be expected within respectively 3, 10 and 0.5 days. For PSP and ASP it can, for this reason, not be excluded that a short-term, medium strength contamination in the Dutch coastal waters could easily be missed during an ABM-programme with a, for instance, fortnightly sampling frequency. Such short-term peaks would, however, only be relevant for the few bivalve shellfish species which are fished in the offshore monitored area. They are less relevant for the production areas, which are situated in inshore waters.

At a distance until 20 km off the Dutch coast, the risk of loss of condition of mussels due to starvation seems to be considerably lower than further offshore.

As material for ABM, medium-sized mussels of homogeneous age and size composition, with a meat yield between 20% and 25%, seem to be most appropriate. It is recommended to use bottom-cultured mussels, which have a strong shell, a relatively high and less variable meat yield, and a more homogeneous age and size composition. They should be collected by hand from a bottom location. Gentle and quick handling of the mussels during the whole ABM procedure is important.

6.2 Summary of the possibilities for mussels as ABM indicator

Reactions on phycotoxins:	Probably only with PSP toxins in high concentrations. Needs further research.
Individual variation and sample size:	Variation is large, samples of more than 50 specimens are required, in wild mussels possibly more.
Optimal size range:	35 - 50 mm
Optimal meat yield range:	20 - 25%

Sample composition:	Size and age as homogeneous as possible
Resistance against handling:	A sturdy shell is required
Origin:	Preferably from bottom culture, not from suspended culture
Treatment:	Preferably to be fished manually, not pulling out byssus, handling gently and quick, short periods out of the water, cool, constant temperature, no shocks.

6.3 Conclusions

Active Biological Monitoring of phycotoxins with suspended mussels as an early warning system for phytoplankton toxicity in the Dutch coastal water seems, on basis of information from literature and from experience, to be feasible. A preliminary, practical experimental phase during which a cost estimate can be done, is deemed necessary. This phase is also important to get insight in relationships between cell numbers and toxicity level.

Due to a relatively fast accumulation and excretion, short-term peaks in PSP or ASP toxins could be missed when fortnightly sampling is practised.

Hand-collected, bottom-grown, cultivated mussels of 3.5 and 5 cm, and a meat yield of 20 - 25% are most suitable as prime material for ABM of phycotoxins. Off-bottom cultured mussels seem less appropriate.

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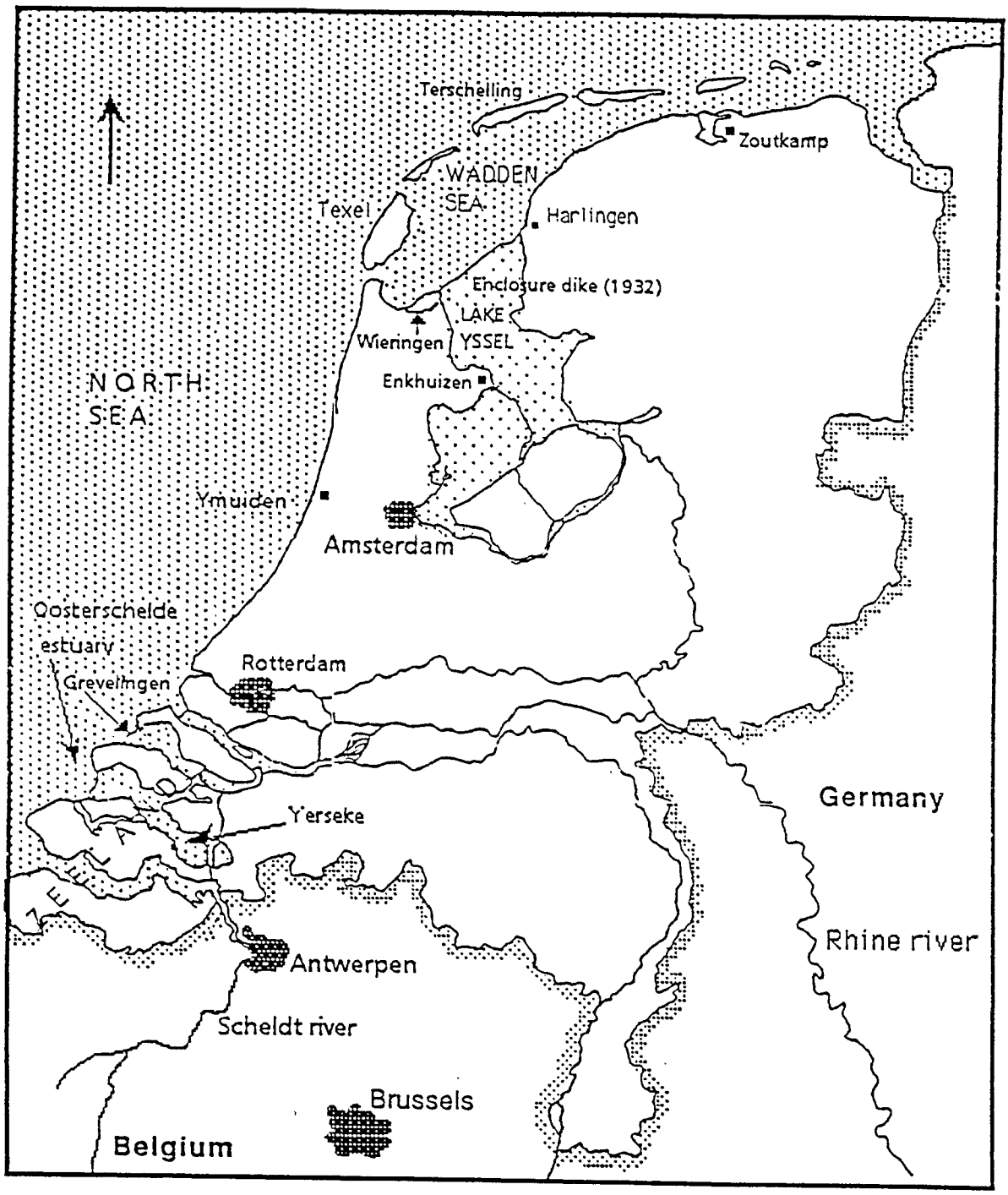

Fig. 1. **The Netherlands with the shellfish production areas.**