Recycling of macronutrients from sea to land using mussel cultivation

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Abstract: The presence of diarrheic shellfish toxins (DST) has been the main obstacle to mussel cultivation in Sweden. Monitoring of DST concentrations in mussels by HPLC for 12 years has shown great geographical and seasonal differences. Furthermore, individual mussels on the same farming strip may differ by a factor as great as 16. DST levels are usually low in summer, rise in September, remain high in the winter and vanish after the start of the spring bloom. A part of the Bohuslän fjord system north of the island Orust, which is secluded from the open sea by shallow sills, regularly shows low DST concentrations. This basin is among the most eutrophicated parts of the Bohuslän coastline. Cultivation of mussels in this area has been calculated to reduce the eutrophication, and results of field-tests support this view. As a consequence, a strategy for sea-gardening is being developed in which the presence of nutrients with primary production in the sea and cultivation of mussels are planned to balance one another.

Keywords: diarrheic shellfish toxins, eutrophication, mussels, sea-gardening.

Reference to this paper should be made as follows: Edebo, L., Haamer, J. Lindahl, O. Lars-Ove, L. and Piriz, L. (2000) 'Recycling of macronutrients from sea to land using mussel cultivation', *Int. J. Environment and Pollution*, Vol. 13, Nos. 1–36, pp. 190–207.

1 Introduction

Increased loads of nutrients in coastal areas are a serious environmental problem almost worldwide. The principal sources of nutrients are municipal wastewater, agriculture, forestry and intensive livestock-farming. In recent years, even larger sea areas, for example the Kattegat-Skagerrak and the Baltic Sea, have suffered from this problem. In areas where eutrophication has been observed, higher algae production and oxygen consumption have been registered, which are thought to be the cause of structural and functional changes to coastal ecosystems. In Swedish waters, such changes have been shown to have a negative effect on biodiversity, fish-egg survival, and recruitment and foraging of commercially important fish species. This situation is playing havoc with human activities, e.g. to fishery by clogging nets, and to recreation through beach pollution and unpleasant odours.

The international goal of reducing nitrogen and phosphorus from anthropogenic land-based sources to the sea by 50% between 1985 and 1995 (Oslo-Paris Convention, 1992) has not been reached, and there is still a need for innovative efforts to achieve this. Sweden, for example, has set a national target to halve the nitrogen losses from arable land by the year 2000. This is to be achieved by a combination of measures covering legislation, advisory services, grants and levies. In addition to source-related strategies, environment-related management strategies are envisaged to restore affected areas. Following this line, two initiatives have been suggested in Sweden to recycle nutrients from sea to land: the cultivation of filtering organisms, such as blue mussels, and the harvesting of short-lived opportunistic macro-algae. The experimental studies for the former are to be funded by the Swedish Foundation for Strategic Environmental Research (MISTRA), and the latter is receiving support from the European Union's LIFE programme.

One development towards sustainable food production requires increased recycling of the macronutrients (N and P) contained in the food consumed in coastal cities. Thus, it is of strategic importance to investigate the possibility to use the sea in a recycling process similar to the ancient discovery of the usefulness of fields to recycle manure into corn and other crops. The suggestion here is to use mussel (*Mytilus edulis*) farms as the recycling unit for increased primary production in coastal waters.

The blue mussel is the most farmed marine organism in Europe (more than 500 000 tons a year), and there is an increasing demand from consumers. However, the demand is largely met by import from the Far East. Part of the reason for this is that mussel harvesting in Europe has for long periods repeatedly suffered from the presence of marine biotoxins, particularly diarrheic shellfish toxins (DST).

A fortunate situation exists on the Swedish west coast: a fjord basin north of Orust, which is secluded from the sea by shallow straits, contains both one of the most heavily *eutrophicated waters* of the Bohuslän coast and nearly permanently *DST-free mussels*. Intensified mussel cultivation in this basin should yield both permanent access to non-toxic mussels and less eutrophicated coastal waters. Available data indicate that DST contamination is mainly brought to the Swedish coastal waters from the open sea, rather than synthesized *in situ*. Thus mussel cultivation in the fjord basin might be treated like continuous cultivation in liquid media in a giant culture vessel with nutrients supplied as natural primary production (plankton), where a large part of the nutrient salts originates from anthropogenic sources. Biological recycling of nutrients by cultivation of non-toxic mussels will accomplish not only a steady supply of food of attractive taste and high nutrient value but, furthermore,

- The diffusive supply of nutrients to the sea from non-point sources, such as domestic waste, agriculture, intensive livestock farming, forestry, and down-fall may be recycled to land;
- Low-cost waste treatment in sparsely populated areas along the coast can be arranged in a decentralized way satisfying present hygienic demands. Such a strategy may favour the avoidance of water toilets. Thus the need for sewage pipes is reduced and rocks and money are saved. Low concentrations of nutrients in *e.g.* washing water may be recycled in the sea;
- Fertilization of fields from deposits below mussel farms will recycle nitrogen without passing through the atmospheric stage, saving much energy;
- Shells from cultivated mussels can be used to neutralize acid lakes and farmland;
- The oxygen concentration in deep water will increase and animal and plant life will return to the sea-floor of the basin as putrefaction of the primary production disappears.

There is a great environmental and economic potential in using the enormous synthesizing capacity of mussels as a link in the recycling of nutrients from sea to land. The main obstacle to such a development is the appearance of natural phytoplankton toxins and contagious organisms from human and animal sources. However, certain sites are rich in nutrients and low in toxins. Hypothetically, sheltered estuarine waters are relatively free from DST, and such sites might be exploited for mussel cultivation. Because such waters often are close to human settlements, more knowledge is needed about the efflux of infectious agents, their survival in the sea and in vectors, as well as uptake and depuration mechanisms in mussels.

It is well known that the problem of marine eutrophication is due to the diffuse nature of the sources and the dispersion patterns in the sea, which are more complex to investigate than other types of pollution of a point-source type. A critical scenario could be a case where the main origin of the problem (*e.g.* nutrient sources in urban areas), its consequences (poor water quality for recreation and coastal fisheries in the archipelagos), and the site having advantages for mussel culture (the Orust fjord basin) do not coincide geographically. In such a case, local people from the selected site will be willing to accept mussel culture only if it renders clear benefits to their lives. Therefore, it is important to understand present patterns of resource use at the selected site and to avoid unintended conflicts that the establishment of musssel culture may create. The feasibility of any management technique, e.g. mussel culture, to combat and mitigate problems of this nature, will not only vary with the nature of the environment, as recently mentioned is the case of the Orust fjord system, which has particular biophysical advantages. Its success will also depend on other aspects that are perceived only when we regard the environment in a more comprehensive way, making connections within and between the biophysical and cultural (human) level more evident.

2 Mussels in recycling

Shellfish is becoming more attractive as food in Europe, and there is an increasing demand on the productivity e.g. for mussels. The main producers of mussels in Europe, Holland and Spain, have, however, decreased their production owing to increased appearance of toxic events in the farming areas. Thus, the demand for canned and frozen mussels is largely met by import from China, which developed large-scale mussel cultivation fairly recently and now has become the main producer in the world (more than 500 000 tons per year). Globally, mussel cultivation has become the fastest growing, extensive 'non feeding' aquaculture activity, with an annual increase of about 5%.

Rationalization of agriculture and migration of people to cities along the coasts is expected to result, by the turn of the century, in 75% of the world's population living in coastal areas. In order to assist the development towards sustainable food production, there is a challenge to increase the recycling of the macronutrients (N and P) emanating from human activities in coastal areas rather than to succumb to nutrient pollution of the coastal waters. Similarly to the ancient invention of using manure to improve the harvests from the fields, the coastal waters may be used for food production in a recycling process (Figure 1).

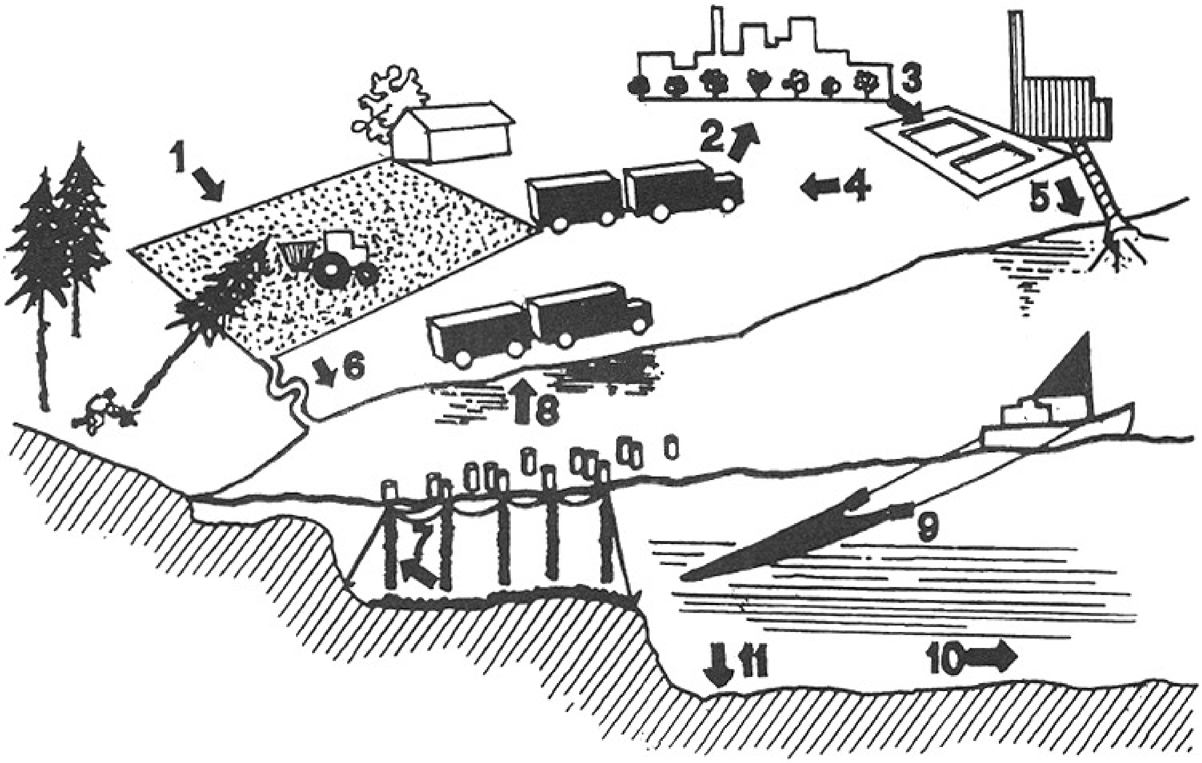

Figure 1 Schematic drawing of the flow of nutrients in coastal waters. Mussel cultivation will retain nutrients in the recycling process by expanding the connection between sea and land (Haamer, 1977). (1) Fertilization of fields. (2) Food to urban areas. (3) Refuse to sewage plants. (4) In Sweden, 2% of the nutrients may return to farms from sewage plants. (5) Nutrients in wastewater pouring into the sea. (6) Nutrients reaching the sea from downfall and fields. (7) Mussel faeces can be used for field fertilization. (8) Nutrients in mussel meat in harvested. (9) A small amount of nutrients is landed with fish. (10) Nutrients are brought to the deep sea mainly by currents. (11) Nutrients become absorbed in the sediment and disappear from the recycling process.

Consequently, the problem of the eutrophication of coastal waters may be transformed into a resource with farms of filter feeders. This possibility has been discussed by several authors (Ryther *et al.*, 1972; Haamer, 1977). Also, the important role of spontaneously established filter feeder communities in relation to eutrophication has been described (Kautsky, 1981; Cloern, 1982; Loo and Rosenberg, 1983, 1989; Rosenberg and Loo, 1983; Loo, 1991). Decreased eutrophication effects on suspension-feeding bivalves have been described for San Francisco Bay (Cloern, 1982; Nichols, 1985), for estuaries in North Carolina, USA (Officer *et al.*, 1982) and in Laholm Bay (Loo and Rosenberg, 1989). The full food production chain in the sea is, however, more complicated. It is also more vulnerable to contamination than food production on land, in the same way that contamination of liquid media by micro-organisms leads to more extensive and complicated consequences than contamination of solid substrates.

3 The Orust fjord system

There is a fjord system with relatively small water exchange with the sea north of the island Orust (Figure 2), which is nearly always free of DST, even when places only 1 km away but connected to the open sea show very high levels. During an observation period of over eight years (Edebo *et al.*, 1991; Haamer, 1995), there has continuously been a gradient of increasing phycotoxin concentrations from the fjords north of Orust towards the open sea.

In this fjord system there is a negative long-term trend showing decreasing deep water oxygen concentrations, which has to change in order to restore the benthic ecosystem (Rosenberg, 1990). Consequently, there is a need for evaluation of quantitative data on the amount of carbon fixed by the phytoplankton and the proportion removed from the fjord system, when mussels are grown and harvested, and how this influences the oxygen concentrations at different sites and depths. The total primary production consists of two parts, the 'new' and the recirculated production, depending on whether the nutrients are used for the first time, or if they are regenerated. The ratio between new and regenerated production depends on a number of different processes and is of great interest when the effects of the mussel farming are studied. Such results can be achieved by combining measurements of total primary production (^{14}C-technique *in situ*) and of sedimentation of particulate material (sediment traps) (Wassman, 1990). So far, the only measurements on primary production in the Byfjord and the Kalvö Fjords were made in 1973 by Söderström and Rex (1974). The present knowledge of the dynamics and amounts of phytoplankton primary production is thus limited. For a comparison, the relationship between primary production, sedimentation and oxygen consumption has been studied in the nearby but hydrographically very different Gullmar Fjord (Lindahl, 1987, 1988). The observation period on primary production in the Gullmar Fjord is at present 12 years long (Lindahl, 1995, unpubl.).

Several factors affect the amount of seston, including phytoplankton, in the fjord system: (1) the specific growth rate of phytoplankton; (2) the water residence time in the bay, including export; (3) the grazing of zooplankton; (4) the feeding activity of benthic animals; and (5) sedimentation and resuspension processes.

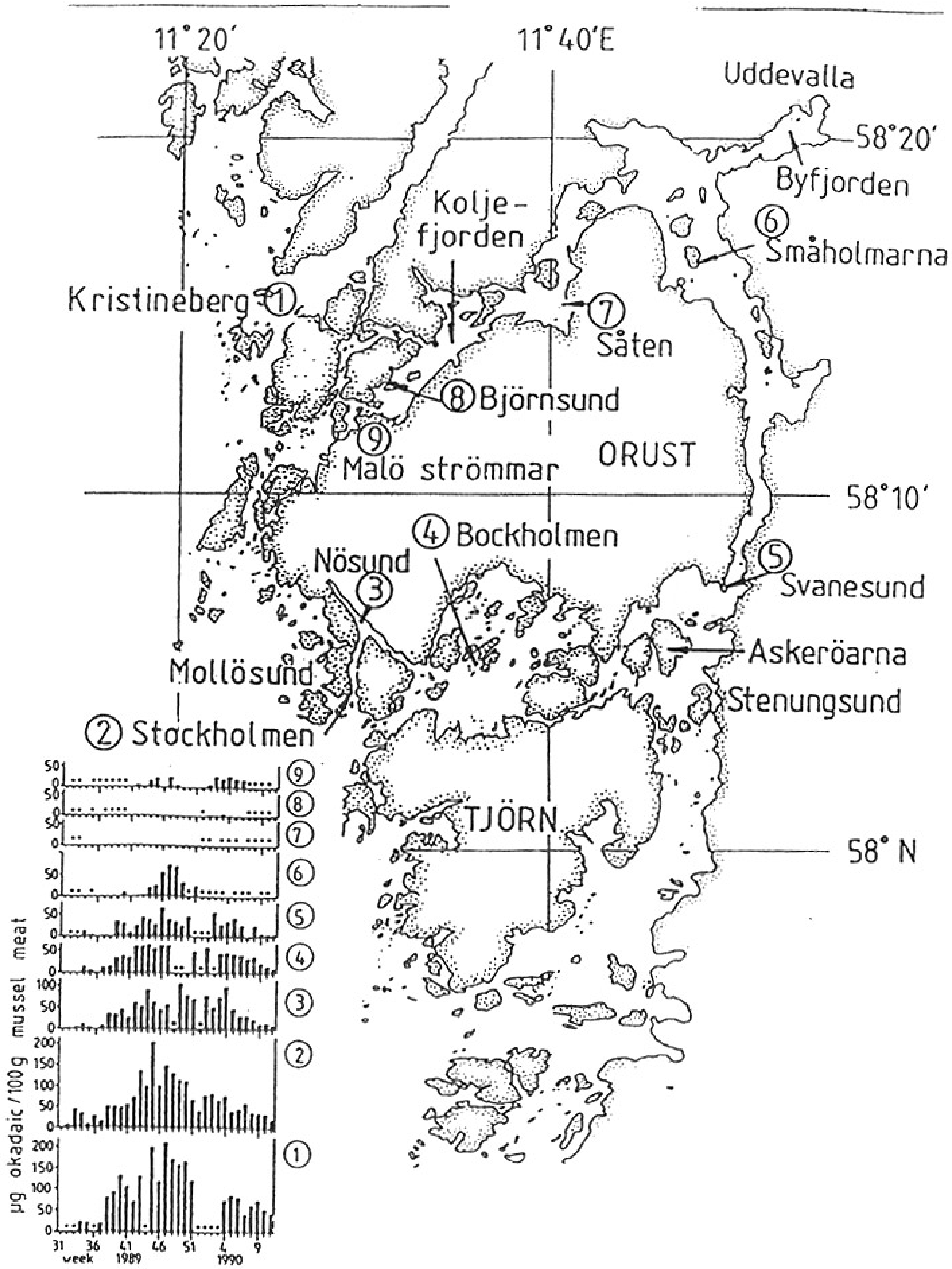

Figure 2 Map showing the fjord system, where weekly monitoring of OA in mussels was performed at nine sites (1–9) during the winter 1989–1990, when the OA levels were high along the Swedish west-coast (e.g. site 1 and 2). There is an anti-clockwise net current in the fjord system, and nutrients are added to the water from the towns Stenungsund and Uddevalla. Consequently, the fjords north of the island Orust have the highest nutrient levels and are low in oxygen. The lowest OA values in mussels were found here (sites 7 and 8). Dots on the bar charts indicate that the weekly sample was not collected.

We have estimated that an annual production of ca. 14 000 tons of mussels in the fjord system would reduce the nitrogen concentration of the effluent water by more than 20% (Figure 3; Haamer, 1995). The mussel farms required would cover 1–3% of the fjord area, the amount depending on the farming method chosen. The nitrogen flow-rate has been calculated from the measured average concentrations in the area and the retention effect of the mussel farms on nitrogen concentrations in mussels and mussel mud. Preliminary calculations indicate possibilities of influencing the ecosystem with minor intervention. We are in the process of quantifying these variables.

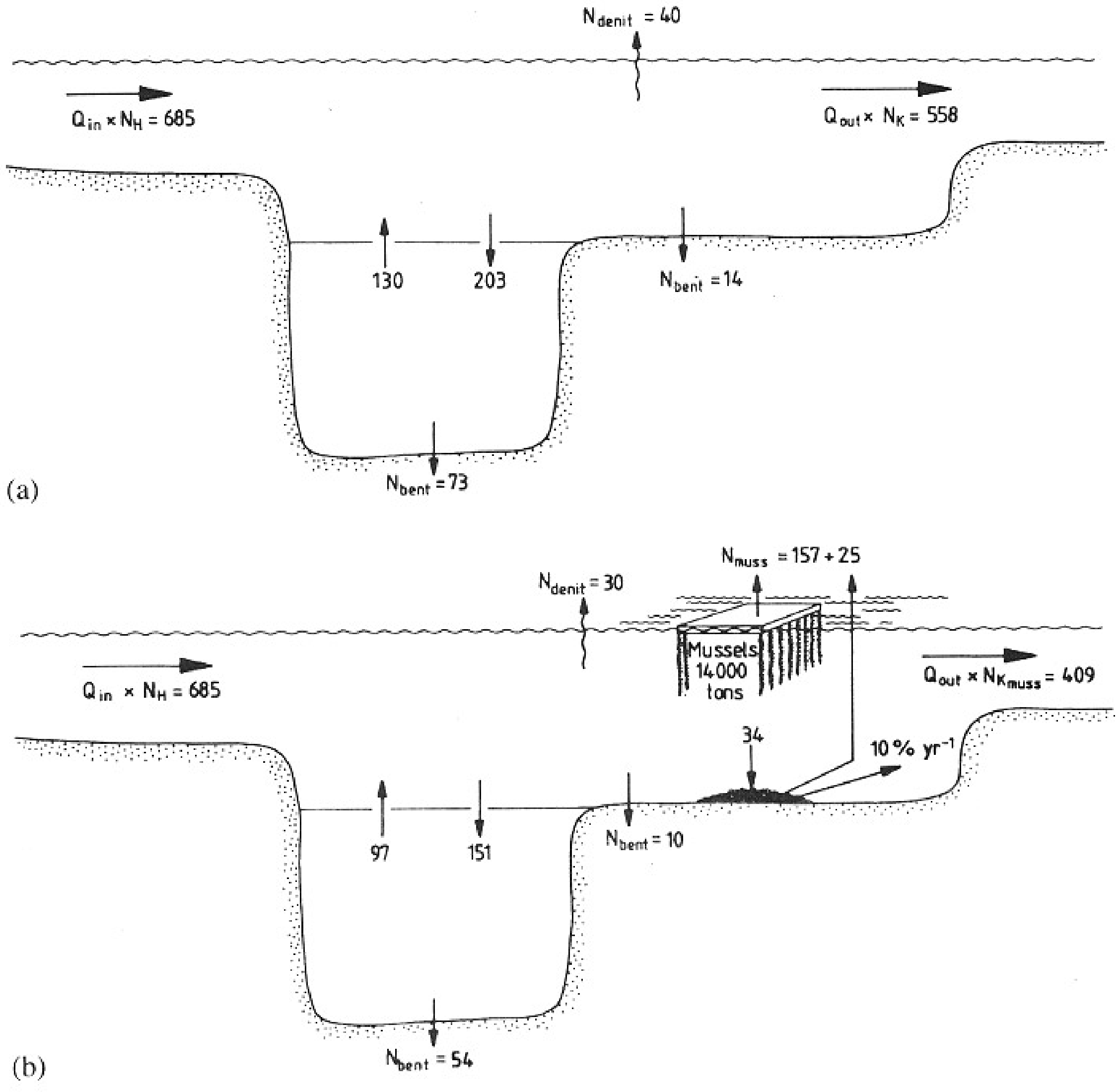

Figure 3 The flow of nitrogen through the fjord system (a) without and (b) with mussel farms. Q_{in} = water flow (m^3 year^{-1}) into the fjords; Q_{out} = water flow out of the fjords; N_H = DIN (dissolved inorganic nitrogen concentration, μM $\times 14 \times 10^{-9}$) of the ingoing water; N_K = DIN of the outgoing water; N_{bent} = N accumulated in sediment (tons year^{-1}); N_{denit} = N lost to the atmosphere; N_{muss} = N accumulated in mussels and mussel sediments (tons year^{-1}); N_{Kmuss} = DIN in the outgoing water with the planned number of mussel farms (Haamer, 1995).

4 Farming methods and cultivation capacity

Modern mussel farming has been developed in Sweden since 1971. The technology to farm mussels in suspended cultures is used commercially, and about 2500 tons of mussels are produced annually (Ackefors and Haamer, 1987; Gosling, 1992). The farming method used in Sweden is the long-line system (Figure 4), and tests have been initiated with farming also from rafts. The farming capacity of an average Swedish long-line farm is about 200 tons of mussels in two years, and each farm occupies a water surface area of 2000 m^2. Fresh mussel consists of 40% shell, 40% meat and 20% juice. The amount of dry protein is about 5% of the total weight.

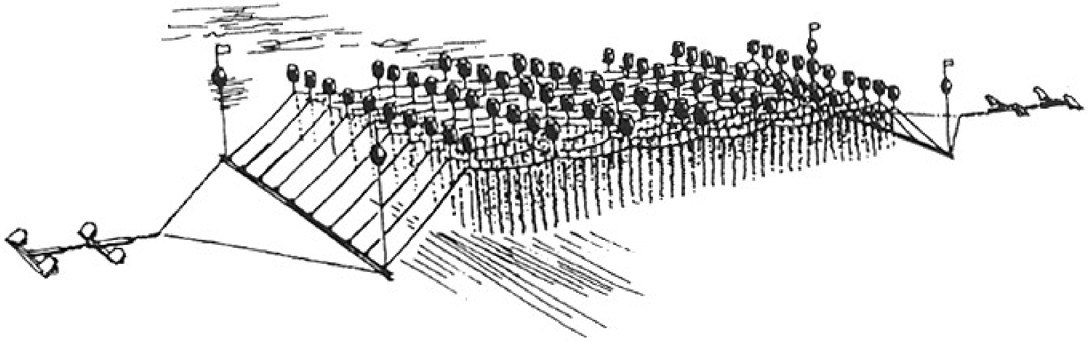

Figure 4 The Swedish long-line system with the dimensions 10 × 200 m. The long-lines can carry 20 000 m of farming strips with a production capacity of about 200 tons in two years. The mussel operation consists of 10 wires each about 200 m long, which are supported by 200 buoys each about 200 l. The mooring is done with two 10 m long rails and four 200 kg anchors. Drawing: Terrence Florell (Haamer, 1994).

The average production of mussels per occupied water areain a Swedish farm is 400 tons/hectare/year, which gives 20 tonnes of dry protein. The food and the protein composition is of high quality. The primary production in the Swedish west coast waters is about 8 kg m^{-2} $year^{-1}$ (200 g C m^{-2} $year^{-1}$) and the conversion efficiency of mussels is about 20%. This gives a theoretical production of 1.6 kg of mussels m^{-2} $year^{-1}$. In the most developed mussel farming district in Europe (Ria Arosa in Spain) 160 000 tons of mussels are harvested each year from an area of 250 km^2, which gives 0.64 kg of mussels m^{-2} $year^{-1}$ The Orust and Tjörn fjord system, where this experiment is planned to take place, has a total area of 230 km^2.

We have calculated for a production of 14 000 tonnes of mussels a year, which is the amount of canned mussels imported annually to Sweden, mainly from Denmark and China. It is also a suitable mass for a modern production line for canned or frozen mussels. Today, a factory like that would cost about 2.5 MECU and keep 30 workers busy with a turnover of 5 MECU a year at full production. A modern mussel factory with that capacity existed in Sweden in 1984 (Musselina AB) but went bankrupt, when DSP hit the industry.

5 Future possibilities

Our goal is to develop a farming and harvesting system that will include offshore farming. According to farming tests made on the Swedish west coast (Haamer, 1977) the supply of mussel larvae and mussel growth is also sufficient some kilometres out of the archipelago in the water influenced by the Baltic current. If these nutrient-rich offshore waters were used for mussel farming, an annual production of several million tons of mussels in Swedish waters might be possible. Offshore farming of mussels seems to be an almost unlimited renewable resource. In that way the anthropogenic outlets of nutrients from the European continent to the Skagerrak–Kattegat area (400 000 tonnes of N and 35 000 tonnes of P a year) might to some extent become recirculated to land (North Sea Task Force, 1993).

6 The advantage for mussels to be suspended in the water column

Loo and Rosenberg (1995) have compared secondary production of the dominant suspension feeders with the primary production and energy budgets in four different areas. The primary production of the four compared habitats varied between 177 and 247 g C m^{-2} $year^{-1}$. Despite the rather small difference in primary production, large differences in secondary production were seen, which emphasizes the importance of water exchange as a significant factor for growth of shallow water suspension feeders. The populations studied were the dominant macrofaunal species in their respective habitats, all suspension feeders. First, a population of cultured *Mytilus edulis*; second, shallow semi-exposed populations of *Cerastoderma edule* and *Mya arenaria*; third, shallow exposed populations of *C. edule* and *M. arenaria*; and fourth, a deeper (40 m) population of *Amphiura filiformis*.

The production of a *M. edulis* culture exceeded primary production by about 6.25 times, whereas semi-exposed shallow water populations of *C. edule* and .*M. arenaria* had a production equal to primary production. In an exposed area, the latter species had a secondary production to primary production ratio of 0.15:1, whereas for a deeper (40 m) living *A. filiformis* population this relation was 0.0034:1. *M. edulis* followed by *C. edule* and *M. arenaria* in the semi-exposed habitat had the highest absorption to respiration ratios, and the highest production to absorption ratios. This result emphasizes the ecological importance of horizontal advective processes for energy transfer from the pelagic to the benthic system. Energy budgets of the suspension-feeding populations studied in the four habitats resulted in great differences. The sites differed in hydrodynamic regime, food availability and depth. The *M. edulis* culture had a production 6.25 times higher than the primary production, which shows the high production capacity of such a system. This high secondary production was a result of spatial distribution of high densities among the mussels, and the fact that the growth efficiency of the cultured mussels (P:R = 1:1.5) was equal to their potential rate (Fenchel and Finlay, 1983), suggests that the hydrodynamic environment was indeed favourable (turbulent currents).

7 The DST case and other harmful microbes

7.1 *Acute and long-term effects of DST*

In Europe, DST has caused large outbreaks of diarrheic shellfish poisoning (DSP) and for periods has locked up the harvesting of mussels in several mussel-growing areas, particularly since 1980. DSP in Norway was described by Thesen in 1901. Not until recently, however, have the composition of the toxins (Yasumoto, 1978; Yasumoto and Murata, 1993) and their distribution been known, and control measures been in operation. In western Europe, okadaic acid (OA) is the dominant toxin, but dinophysistoxin-1

(DTX-1) and DTX-2 have also been found repeatedly. DST is often found in mussels at several locations at concentrations below that considered risky with respect to acute toxicity.

When the mucosa of a rat ileal ligated loop is exposed to OA, bud-like protrusions appear within 5 min on the surface of the villi. After 30 min, the enterocytes have become distended and have started to leave the basement membrane. There is a resulting massive exodus of enterocytes into the gut content, such that goblet cells will make out the mucosal surface (Edebo *et al.*, 1988a; Lange *et al.*, 1990). Furthermore, OA is a tumour-promoting agent which enhances the effect of chemical carcinogens, such as dimethylbenzanthracene, on mouse skin and rat gastric epithelium (Suganuma *et al.*, 1988; Fujiki and Suganuma, 1993). Also virus-induced cell transformation with papilloma virus (Tsang, 1991) or HIV (Thévenin, 1990) are stimulated by OA. However, OA may also antagonize the cell transformation by certain oncogenes (Sakai, 1989). It is not known whether OA will be discharged from the mucosa with the enterocytes of the small intestine and possibly reabsorbed further down the intestinal tract. This is of potential importance, because tumours are extremely rare in the small intestine but very common in the colon and rectum.

The effects of OA are mediated to a large extent by the inhibition of protein phosphatase 1 and 2A (Cohen, 1990). Thus, OA has a general cell-stimulating activity by inhibition of deactivation. Other cell-activation substances, such as phorbol esters and bacterial lipopolysaccharides (LPS), which increase protein phosphorylation, show synergistic effects in some *in vitro* test systems but not with respect to tumour promotion (Fujiki and Suganuma, 1993). LPS is extremely abundant in the colon and rectum, and might thus influence the effect of OA.

Okadaic acid is a lipophilic and very stable substance that can withstand boiling for 2 h (Edebo *et al.*, 1988b). Thus, it may survive in the food chain, and small quantities have been found in herbivorous and carnivorous fish (Edebo *et al.*, 1992). In preliminary experiments on the stability in liver homogenates from mussels, fish and mammals, no conspicuous breakdown was seen after seven days (Edebo, unpublished). Thus it is of great importance with respect to the consumption of marine food in general and mussels in particular to investigate the fate of DST, because shellfish toxins seem to appear generally in the marine food chain (Shumway, 1995).

7.2 *Analytical methods for DST*

For years, mussel DST-control has been done by feeding mussel hepatopancreas to rats and observing diarrheic tendencies of their faeces (Kat, 1983) or mouse MLD (minimum lethal dose) tests (Yasumoto, 1978). The high-performance liquid chromatographic (HPLC) method developed by Yasumoto's group (Lee *et al.*, 1987) spares animals and has greater precision. In Sweden, it has been used since 1987 for surveillance and control. As far as we know, no DSP has occurred when our standard rules have been obeyed. In most other countries, the control of mussels is done by animal tests. The precision of the animal tests is lower than that of HPLC, and there is a desire to minimize the use of animals for toxicity testing. However, the HPLC method is technically difficult – in the BCR (Community Bureau of Reference of the EC) *2nd Intercomparison Study on Okadaic Acid (OA) in solutions and in contaminated hepatopancreas extracts*, only some of the laboratories showed reproducible results (van Trijp *et al.*, 1994). Furthermore, it has been argued that animal tests cover marine 'toxins', which have been detected by the animal tests but have not been recognized to cause intoxication, e.g. yessotoxin. Thus there is a need to develop better analytical methods and systems. Recently, an HPLC-MS method for determination of DST as 4-bromomethyl-7-methoxycoumarin (BrMmc) dericatives has been described (Hummert *et al.*, 1993), which might become standardized more easily. Furthermore, HPLC peaks indicating the presence of DTX-1 have appeared in mussels at times without showing toxicity in rat tests. Such peaks seem to appear at certain locations and disappear more rapidly than the OA peaks. Further investigations chemically (HPLC-MS) as well as rat tests should help to explain these observations.

Recently, receptor-based analytical methods employing the capacity of DST to block the enzymic activity of protein phosphatase-2A have been developed. A colorimetric method using paranitrophenyl phosphate as substrate detected 40 pg OA per ml (100 ng OA per g mussel tissue) in a cuvette with a semipurified protein phosphatase PP2 A extract (Simon and Vernoux, 1994), or 63 pg/ml in microtiter plate using commercial phosphatase (Tubaro *et al.*, 1996 a,b). A fluorescent enzyme inhibition assay using 4-methylumbelliferyl phosphate and fluorescein diphosphate as substrates resulted in a detection limit of 12.8 ng OA per g hepatopancreas (Vieytes *et al.*, 1997). The coefficient of variation was, however, fairly high, 18.8–37.9%. Another interesting, innovative, biosensor-type model uses the inhibition of acid phosphatase from potato. This effect is quantified amperometrically as the inhibition of glucose phosphate hydrolysis, as glucose is determined by subsequent oxidation with glucose oxidase (Stacchini *et al.*, 1996).

Enzyme-blocking methods have now begun to be tested in surveillance tests against intoxication. They seem to offer advantages compared with HPLC to cover the entire DST complex, whereas standard HPLC detects OA, DTX-1 and DTX-2. The precision is lower, however, but presumably better than that of animal tests, which pick up 'toxins' other than the DST complex that might not be harmful to humans by the oral route. In parallel tests with HPLC, PP2A-blocking tests have not failed to detect mussel samples containing OA, DTX-1 and DTX-2.

7.3 Variation, sampling, toxification and depuration

It has been shown that the OA concentrations in individual neighbouring mussels may in extreme cases differ by a factor of 4.0–6.7, and between mussels on the same farming strip at different depths (surface and 6 m down) as much as 16-fold (Edebo *et al.*, 1988b). The mechanisms of this variation are not known. Differences in filtration rate based on opening periods of the bivalves might be one reason, but it has not been tested. Alternatively, certain *Dinophysis* cells may be capable of OA synthesis also after being filtered off by the mussels, or the efficiency of depuration may vary. As a consequence of this great variation, regular sampling in Sweden is performed by taking four mussels each from the surface, the middle, and the bottom of the farming strip. The mussels are chucked, the meat weighed, the hepatopancreas dissected out, weighed and homogenized in a tissue press at ca. 4 MPa (40 kp cm^{-2}), the homogenate mixed, and then 1 g is taken for extraction with methanol, etc. (Lee *et al.*, 1987; Edebo *et al.*, 1988b).

At times, there are high concentrations of OA-producing dinoflagellates in the seawater filtered by mussels, which show up as high concentrations of OA in mussel hepatopancreas (Haamer *et al.*, 1990). It has been claimed (Pillet and Houvenaghel, 1995) that mussels exposed to toxins reduce the filtering flow. However, extended experiments have shown that mussels can feed on toxic *Prorocentrum lima* only which produce OA and can be cultured in vitro (Pillet *et al.*, 1995). The OA is accumulated in the hepatopancreas. No data are available to show redistribution to other organs or biotransformation. As shellfish and fish feed on mussels and on plankton etc., OA also appears elsewhere in the food chain. Thus OA has been found in cod fed toxic mussels and in canned anchovies but at lower concentrations (Edebo *et al.*, 1992).

Owing to the great spontaneous variation in OA concentrations between individual mussels, it is also difficult to study the mechanism of depuration. When OA-containing mussels were fed different bacteria, yeasts or process water (from the fish industry) in laboratory experiments to accomplish depuration, there was no conspicuous decrease in 14 days. Rather there seemed to be an increase, indicating a state of infection of the mussels or the aquarium by *Dinophysis* with capacity to synthesize OA (Edebo, unpubl.). Also when depuration experiments were run in large tanks, there was an initial increase (Haamer *et al.*, 1990). Regarding the possible tumour-promoting effect, it is of great interest to elucidate the synthesis and elimination of also lower concentrations of OA in mussels.

7.5 Fate of pathogenic bacteria and viruses in mussels

Estimation of the contamination of seawater and mussels with bacteria and viruses of faecal origin is usually performed with traditional thermotolerant *E. coli* enumerations. However, *E.coli* does not usually thrive in seawater and is killed in mussels used as food (Birkbeck and McHenery, 1982) such that viable counts tend to underestimate faecal contamination. Thus, alternative indicators of faecal contamination should be sought, such as the more salt-tolerant enterococci or the marker substance 3-hydroxymyristic acid, which is characteristic of *Enterobacteriaceae* lipopolysaccharide (LPS) – being a stable cell-wall constituent – in relation to shorter hydroxy-fatty acids, which appear in the LPS of aquatic bacteria (Mattsby-Baltzer *et al.*, 1989). Estimation of the contamination with enteric viruses has been performed with F-specific RNA bacteriophages as markers of faecal viruses (Havelaar *et al.*, 1993), since more than 100 types of human pathogenic virus may be present in faecally polluted water and only few can be determined by presently available methods. Since the DNA of *E.coli* filtered by mussels is rapidly degraded (McHenery and Birkbeck, 1985), analysis for the microbial DNA in mussels by PCR (polymerase chain reaction) might become a sensitive method to study contamination.

7.6 Determination of paralytic shellfish toxins (PST) and amnesic shellfish toxins (AST)

PST and AST are so far not known to have caused intoxications in Sweden. During the years 1989–1997, weekly mussel samples have been analysed for PST from a few sites along the coast by the mouse test, from April to August. Only few tests have shown the presence of PST. In late April and early May 1992, they showed PST at sites located far from each other reaching a maximum of 222 MU per 100 g mussel meat (one MU equals 0.183 μg ±0.022 μg PST; 'saxitoxin equivalents'). In early May 1994 and 1995, they showed PSP on one occasion and at one site each, the sites being different (200 and 209 MU per 100 g). In April–June 1997, PST was found in mussels from several sites, maximum 842 MU per 100 g, the highest level in Sweden recorded so far. The Orust fjord system has been tested for PST all the year round from 1992 to 1995 and never showed any presence of PST. Since our local Ethical Committee of Laboratory Animals in Göteborg presently opposes the Nordic PST mouse test protocol adopted by the National Food Administration, there is an urgent need for alternative methods, such as the neuroblastoma cell culture PST testing method, in order to escape the conflict (Gallacher and Birkbeck, 1992, 1993; Jellett *et al.*, 1992). We have begun to use it and we expect it will soon become internationally accepted (Manger *et al.*, 1995).

Amnesic shellfish poisoning (ASP) and the diatom *Pseudonitzschia pungens* producing the amnesic shellfish toxin (AST) domoic acid, have been thoroughly investigated in Canada (Quilliam and Wright, 1989). Since the diatom has been found in Swedish waters, and diatoms are favoured by silicates, which are abundant in certain Swedish waters, mussels should be analysed for domoic acid (HPLC), particularly when *P. pungens* is appearing. So far no domoic acid has been found.

7.7 Privileged sites

It has become established in western Europe that toxic plankton appears in the open sea and that mussels more exposed to the sea get higher DST concentrations than mussels growing in waters secluded from the sea (Edebo *et al.*, 1988b, 1991; Lassus *et al.*, 1991). It was reported recently that blooms of *Dinophysis acuta* and *D. acuminata* originate at the thermocline in an area of weak circulation in the northwestern Celtic Sea south of Ireland. From there the toxic phytoplankton are transfered by currents into the bays (McMahon *et al.*, 1997). There is a basin north of the island Orust made up from the Kalvö, Borgila and Koljö Fjords and secluded from the open sea by shallow sills at Nötesund and Malö Strömmar. This basin shows the lowest concentrations of OA in mussels. The fortunate situation appears that freedom from toxins coincides with almost constant eutrophication (Figure 2; Edebo *et al.*, 1988b, 1991). Thus, mussel cultivation in this area would accomplish both toxin-free mussels and improved coastal waters. Low levels or absence of DST have now been found in weekly controls for ten years, with the exception of 1998, when high DST levels prevailed during this very rainy summer even in this basin. These results stress the multifactoral dependence of the presence of DST and the importance of being quantitative.

Initially, it was hypothesized that the privileged condition of the fjords north of Orust was a consequence of the dominating anti-clockwise currents around Orust entering by Hake Fjord and pouring out by Malö Strömmar, thereby gradually losing its content of DST-containing plankton stemming from the open sea (Edebo *et al.*, 1991). To this interpretation is added the recent discovery (Haamer, 1995) that the water of the fjord system holds relatively high concentrations of silica. Dinoflagellates, which contain toxic species, such as *Dinophysis* spp., are favoured by the increasing supply of N to the sea (Officer and Ryther, 1980; Smayda, 1990; Egge and Aksnes, 1992) at the expense of the rarely toxic diatoms which, in turn, depend on silica, which shows some decrease over the years. Actually, when different sites along the Bohuslän coast were compared, the relationship between silica in water and OA in mussels was conspicuous (Haamer, Figure 5). The N:Si ratio differences are explained by the fact that there is no Si in the anthropogenic supply of nutrients to the sea. The supply of Si is mainly by the rivers. This observation may aid the mussel farmers in the selection of optimum sites with respect to the cultivation of mussels and in the improvement of the coastal waters. It also directs attention to the Northern Göteborg archipelago where the Göta and Nordre Rivers, which carry large amounts of silica, discharge into the sea. This area has been poorly analysed with respect to DST so far. It also opens up the possibility to favour growth of non-toxic plankton by supplementing silica to the fjord basin. When the mechanisms of *Dinophysis* growth and DST-production are understood the mussel cultivation, harvest, and depuration strategy might be changed accordingly.

The unusually high levels of OA during the autumn–winter–spring seasons of 1993 and 1994 in traditionally cultivated mussels caused a serious blow to the mussel industry. Two ways have been used to escape the toxicity, both exploiting the sanctuary of the fjord basin: transplantation of toxic mussels to and harvesting of wild mussels from the fjord basin. Both procedures have been successful and have kept the industry surviving. They are laborious, though. A further strategy is planned to contain increased capacity of culture in the fjords. However, mussel cultivation is not always so successful at all fjord sites, because poor mussel growth and settling of competing organisms (*e.g.* ascidians) on the mussels occur. The moving of long-lines with mussels still attached from cultivating farms farther out to sea, where settling and most of the mussel growth is good, into the fjord basin for harvest at strategic moments has been used successfully.

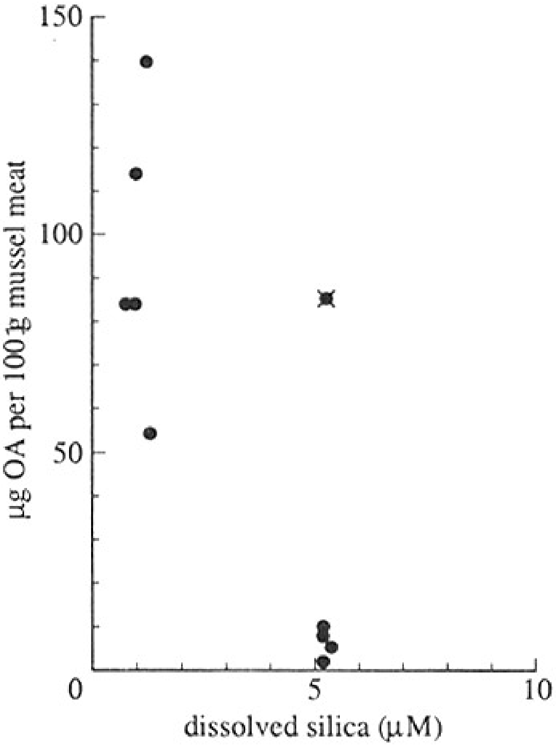

Figure 5 Average surface dissolved silicate concentrations in the surface water for the period Sept–Oct 1990–1992 in the investigated area compared with OA concentrations in mussels collected close to the hydrographic stations in mid October 1993. The value with the cross is from the mouth of the Orust fjord system, where offshore and fjord water mix and the silica concentration has had very little time to influence the plankton community.

Dinophysis spp. have not yet been successfully cultivated *in vitro*. They do not seem to prefer water with high light intensity, and electron micrographs show food vacuoles indicating heterotrophic or mixotrophic feeding (Granéli *et al.*, 1995; Maranda, 1995). In a study in 1994–95 (Lindahl and Andersson, 1995) there was a decreasing gradient of dinoflagellates along the anti-clockwise current around Orust, agreeing with the OA concentration in mussels. Furthermore, *Dinophysis* died off in water from the fjord basin, whereas they multiplied in water from the nearby open-ended Gullmar Fjord (Norén, 1997). Furthermore, diatoms increased both in the fjord along the current and *in vitro*.

Thus, the water of the fjord basin seems to be selectively unfavourable for *Dinophysis* and possibly other dinoflagellates, but the mechanisms are not understood. Certain bacteria may favour their growth, whereas other microbes or substances might be antagonistic.

8 Acknowledgement

This work was supported by a grant from the Swedish Foundation for Strategic Environmental Research, MISTRA.

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