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# Observations on bonamiasis in the stock of the European flat oyster, *Ostrea edulis*, in the Netherlands, with special reference to the recent developments in Lake Grevelingen

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ABSTRACT

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The stock of the oyster *Ostrea edulis* in the Netherlands is located in an estuary on two sites, Yerseke Bank and Grevelingen. The Yerseke Bank became infected with the protozoan oyster pathogen *Bonamia ostreae* in 1980, as a result of importations of infected oysters from France. Due to bonamiasis, the oyster production of the Yerseke Bank suffered increasing losses and finally ceased because of lack of commercial possibilities. The oyster stock of the Grevelingen has been checked since 1980 and was found to be free of bonamiasis until 1988. In the summer of that year the first presence of bonamiasis was observed. A rapid spread, with increasing prevalences, followed, both in wild stock and in commercial stocks. In 1989 maximum prevalence levels of 48% and maximum prevalences of dead oysters up to 80% were reached. The bonamiasis situation is considered serious for Dutch *Ostrea edulis* production in the coming years. A 5-year research programme has been started to monitor the spread of bonamiasis in the wild stock of the Grevelingen and to select the best management strategy for commercial production of *O. edulis* despite bonamiasis. From the first results it was concluded that bonamiasis was introduced into the Grevelingen by transport of infectious material, probably by ships. Further, it was concluded that the level of prevalence and development of bonamiasis is related in some way to (fishery) stress factors of the oysters and to environmental factors, but stocking density does not appear to affect the prevalence of bonamiasis.

## INTRODUCTION

The protistan *Bonamia ostreae*, pathogenic for the European flat oyster *Ostrea edulis*, was introduced in 1980 with imported lots of oysters from France to the Dutch oyster area Yerseke Bank (Van Banning, 1982). The oyster area Yerseke Bank is located in an estuary system in the south of the Netherlands (Fig. 1, inset map), an area open to the sea and therefore with tidal flows. Another oyster area, the salt water Lake Grevelingen (Fig. 1), is part of the

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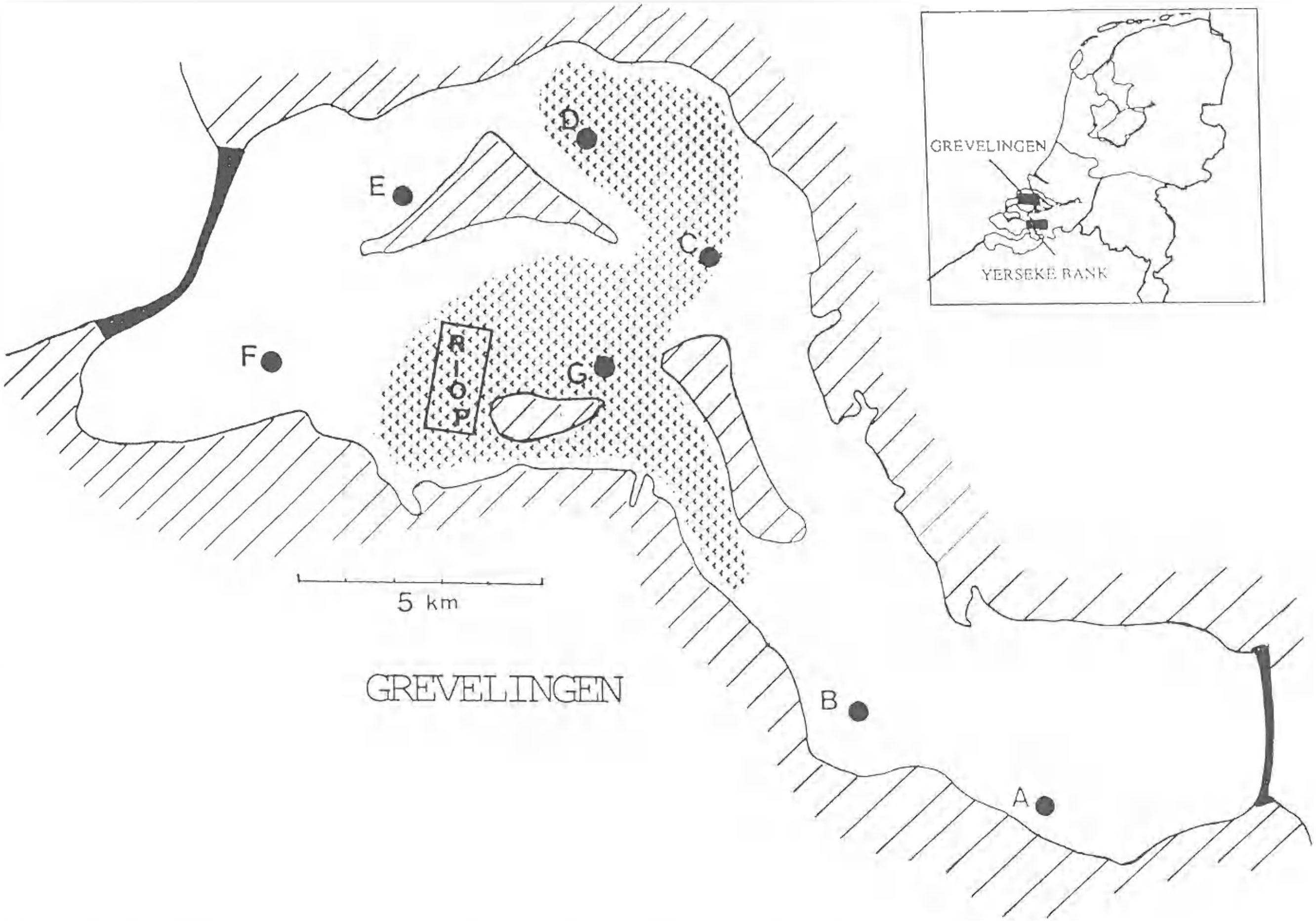


Fig. 1. The Grevelingen situation of 1989, with the positions of the sites A–F for the monitoring of bonamiasis of the wild oyster stock, the position G of the first observed presence of bonamiasis in 1988, and the position of the RIOP experimental area. The dotted area indicates the area of spread of bonamiasis in 1989, based on the data of commercial and experimental oyster beds.

same estuary system, but is isolated by land and dikes from the Yerseke Bank area. This makes the Grevelingen a stagnant water, sometimes with currents driven by the winds.

Experiments were carried out in the Yerseke Bank area in the period 1981–1986, with yearly cleaning of the infected sites followed by one-season replantings of bonamiasis-free *O. edulis*. The bonamiasis-free oysters used for these replantings on the Yerseke Bank came from the Grevelingen, which was free of bonamiasis in that period. The experimental cleaning and replanting actions resulted finally in a very low (nearly zero) prevalence for the Yerseke Bank in 1986 (Van Banning 1988). This situation led to a first trial of free commercial replanting in March 1988. Three months later the unexpected fact was established that bonamiasis had returned in most of the replanted commercial oysters of the Yerseke Bank, with prevalence levels in the range 8–71% for the different sites. Analyses of this unexpected development showed that the oysters used by the oyster-growers for the replantings were in nearly all cases stored for several weeks or months in oyster basins for commercial purposes. Such treated, and probably stressed, oysters showed the highest prevalence of bonamiasis after replanting on the outside beds, and consequently suffered high mortalities in the autumn harvesting period (August–November). It was remarkable that nearby oysters, planted on the Yerseke Bank directly after fishing of the Grevelingen (so without first a long-term storage in basins), showed no signs of bonamiasis or mortality over the same period. It was considered that storage, handling or other stressing situations for *O. edulis* can favour or boost the development of bonamiasis. The return of bonamiasis with high prevalence in a short period discouraged the Dutch oyster growers, and no further replantings of *O. edulis* on the Yerseke Bank were made after 1988. Consequently, the commercial production of *O. edulis* in the Netherlands remained totally dependent on the oyster stock of the Grevelingen.

The Grevelingen has been monitored for bonamiasis since 1980, with a yearly check based on a spring and autumn sampling and histological research. The results showed that the Grevelingen could be considered free of bonamiasis. To prevent introduction of bonamiasis from the infected Yerseke Bank, regulations were drawn up in 1980: the oyster firms must clean their oyster/mussel ships thoroughly of remaining organisms and sediments before entering the Grevelingen, different dredges had tó be used for the two areas, and any transplanting of shellfish to and in the Grevelingen was forbidden. Despite these regulations, the bonamiasis-free status of the Grevelingen ended in July 1988, with the observation of *Bonamia ostreae* in a sample of *O. edulis* from the location Stampersplaat (Fig. 1, position G). This location belongs to an area of the Grevelingen with commercial fishery activities in the period 1987–1988. With the first observation of bonamiasis, research was started in the Grevelingen with two programmes: a monitoring programme to estimate the spread and prevalence of bonamiasis in the wild stock, and a special management programme, named RIOP (=RIVO Oester Project, translation: Netherlands Institute for Fishery Investigations Oyster Project). The purpose of the RIOP programme is to estimate the commercial losses and to study the possibilities of management for continued commercial oyster production under circumstances of bonamiasis.

## MATERIALS AND METHODS

The monitoring sampling of the wild oyster stock of the Grevelingen was carried out in 1988 on six sites (A–F, Fig. 1) in April, June and August. The sites and the periods were chosen to cover most of the geographical area of the existing wild oyster stock and to cover the seasonal differences in development of bonamiasis.

The RIOP programme of 1989 involved application of two fishery techniques and a comparison of two planting densities (5 and 10 oysters/$m^2$). To study the effects of different fishing techniques on the development of bonamiasis, two systems were compared: the generally used tow dredge and a new suck dredge system. Each variation was tested on a different oyster bed, planted with 340 000–570 000 oysters per bed to reach the planting densities mentioned and to reach a commercial scale. The RIOP oyster beds were cleaned before the experiments started. The oysters were planted in June on the experimental beds and were collected with the two fishery methods at a site of the Grevelingen with known bonamiasis infection (prevalence 1–8%). Before replanting on the RIOP beds, experimental oysters in a size class of 65 mm and over (consumption class) were selected by mechanical sieving. The experiments were finished in November and the experimental oyster beds were cleaned with suck fishery.

Each experimental bed was sampled at monthly or 2-monthly intervals for histological research ($n=50$) and for the prevalence of recently dead oysters ($n=100$–$150$). The latter is based on empty shells showing an interior without any traces of fouling, i.e., with a clean white interior. For the histological research, small parts of the hepato-pancreas were fixed routinely in Davidson's solution (Shaw and Battle, 1957). Fixed tissues were dehydrated in 2-dimethoxypropane (De Ruiter et al., 1981) and stained in Mayer's haematoxylin, with phloxine as counterstain. The sections were checked with a light microscope for the presence of *Bonamia ostreae* and for pre-signs of infection, such as increased infiltration of haemocytes into the connective tissue.

## RESULTS

The results of the monitoring samplings in the wild stock in April, June and August, showed that in 1989 bonamiasis had already reached the area with the wild oyster stock in the Grevelingen. The disease was found on two sites, C and D, reaching maximum prevalences of 8% and 18% respectively (Table 1). The other sites, A, B, E, and F, showed no bonamiasis in 1989.

The results of the 1989 RIOP oyster plantings (Table 2) showed a considerable increase of bonamiasis prevalence to a level of 20–48% over the period June/July to October/November (Table 2). The increased prevalences occurred with both fishing techniques, the tow dredge and suck fishing (Table 2). The total loss of oyster production in 1989 in the RIOP experiments reached levels of 21–49% at the end of the growing season (October/November), estimated by the total number of recently dead oysters in the final samplings (Table 2).

The commercial oyster beds sampled at different locations in the central part of the Grevelingen, showed bonamiasis prevalence levels up to 80% (Table 3).

TABLE 1

Prevalence of bonamiasis in the wild oyster stock of the Grevelingen, sampled at six sites (A–F, see Fig. 1)

| Period 1989 | Location | A | B | C | D | E | F |
|---|---|---|---|---|---|---|---|
| April | | 0 | 0 | 8% | 0 | 0 | 0 |
| June | | 0 | 0 | 8% | 4% | 0 | 0 |
| August | | 0 | 0 | 4% | 18% | 0 | 0 |

TABLE 2

Prevalence of bonamiasis and dead oysters on RIOP oyster beds with different management experiments on a commercial scale, 1989

| Period 1989 | Prevalence range of bonamiasis (%) | Prevalence range of recently dead oysters | Type of experiment |
|---|---|---|---|
| June/July | 2– 4 | 12–14 | 10 $m^2$, |
| Oct./Nov. | 20–22 | 40–42 | suck dredge |
| June/July | 4– 6 | 4– 6 | 10 $m^2$, |
| Oct./Nov. | 40–42 | 39–41 | tow dredge |
| June/July | 4– 8 | 9–11 | 5 $m^2$, |
| Oct./Nov. | 36–48 | 47–49 | suck dredge |
| June/July | 2–6 | 11–13 | 5 $m^2$, |
| Oct./Nov. | 22–26 | 21–23 | suck dredge |
| June/July | 1– 2 | 1– 3 | undisturbed wild |
| Oct./Nov. | 26–38 | 31–33 | oyster bed |
| June/July | 4–12 | 3– 5 | 1 × fishery disturbed |
| Oct./Nov. | 42–44 | 38–40 | oyster bed |

TABLE 3

Prevalences of bonamiasis in five commercial oyster beds of the central part of the Grevelingen, sampled in the period Sept./Oct. 1989

| Prevalence range of bonamiasis (%) | Prevalence of recently dead oysters (averaged) (%) |
|---|---|
| 14–32 | 78 |
| 18–28 | 80 |
| 32–46 | 27 |
| 26–34 | 35 |
| 34–36 | 51 |

## DISCUSSION

The results show a rapid and serious increase of bonamiasis in the oyster population of the Grevelingen. The wild stock, mostly near the infected commercial oyster beds in the central part of the Grevelingen, showed an infection extending to site D. It is remarkable that the eastern part (sites A and B) and the western parts (sites E and F) are still free of bonamiasis. This geographical situation indicates that the infection probably started in the central part of the Grevelingen. This is the part with the most commercial oyster fishing activities. It is considered that infectious material (e.g. organisms, mud or water) was probably carried by boats not thoroughly cleaned, coming from the infected Yerseke Bank area. The spread of the disease in 1989 to the wild oyster stock of site D might indicate that bonamiasis could extend further to the eastern and western wild stock of the Grevelingen in the coming years. The RIOP experiments show an increase of bonamiasis prevalence when oysters are stressed by fishing and replanting actions, as is observed with the different disease levels in October/November between undisturbed wild oysters (prevalence range 26–38%, mortalities up to 33%, Table 1) and RIOP fishery-stressed oysters (prevalence range 42–44%, mortality up to 40%, Table 2) of the same area. A similar characteristic was observed with the Yerseke Bank experiments of 1988: oysters stressed by fishery and/or basin storage showed increased prevalences of bonamiasis compared with non-disturbed oysters over the same period. An explanation of this 'stress effect' might involve the presumptive life cycle of *Bonamia ostreae*, in which the gonadal cycle and maturity of the (female) oyster is considered to be related in some way to the development of bonamiasis (Van Banning, 1990). Furthermore, stressing circumstances might have an effect on the gonadal maturity of the oyster and consequently on the development of the parasite.

The experiments with two planting densities (5 and 10 oysters/$m^2$ Table 2) gave the unexpected result that the higher planting density showed a lower prevalence of bonamiasis (20–22%) than the lower density (at which different prevalences were observed: 22–26% and 36–48%). These results suggest that oyster planting density is not of major importance for bonamiasis development, but probably other environmental factors are involved. Such environmental factors, sometimes ruled by very local situations, can probably differentiate the condition and maturity stage of the oysters, and this can be reflected in different prevalence levels of bonamiasis in a seemingly uniform oyster stock.

The RIOP experiments of 1989 have shown that the commercial losses due to bonamiasis under commercial replanting circumstances can be high in the Grevelingen, even in one growing season. This means that a serious reduction of commercial oyster production can be expected in the Grevelingen for the next years, and this could be a preview of a serious situation for the Dutch commercial *O. edulis* oyster stock and consequently for Dutch oyster fisheries.

## CONCLUSIONS

The pathogen *Bonamia ostreae* of the European flat oyster *Ostrea edulis* can be spread to other areas by ships having contact with material from infected areas. The levels of development and prevalence of bonamiasis can be different within a similar uniform area or oyster stock, and depend on stressing factors or local environmental factors acting differentially according to the condition and maturity characteristics of the oysters.

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