

Bonamia ostreae infections in flat oysters (*Ostrea edulis*) from Lake Grevelingen, The Netherlands, 15 years after introduction

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Introduction

Lake Grevelingen, an enclosed salt water lake in the South-West of the Netherlands, is the main centre of flat oyster (*Ostrea edulis*) culture in the Netherlands (Figure 1). In 1988 the protozoan parasite *Bonamia ostreae* was detected for the first time in the flat oyster population of Lake Grevelingen. The introduction of *B. ostreae* resulted in a dramatic decline of the flat oyster population in the Netherlands.

In 1980 a routine monitoring programme has been started to determine, on a yearly base, the prevalence of *B. ostreae* by means of histopathology. Recently, an *in situ* hybridisation (ISH) technique specific for *B. ostreae* was implemented as a confirmatory method.

In this study data on *B. ostreae* infections in Lake Grevelingen since 1988 were analysed. Oyster samples of 2001 and 2002 were also analysed on *B. ostreae* with ISH.

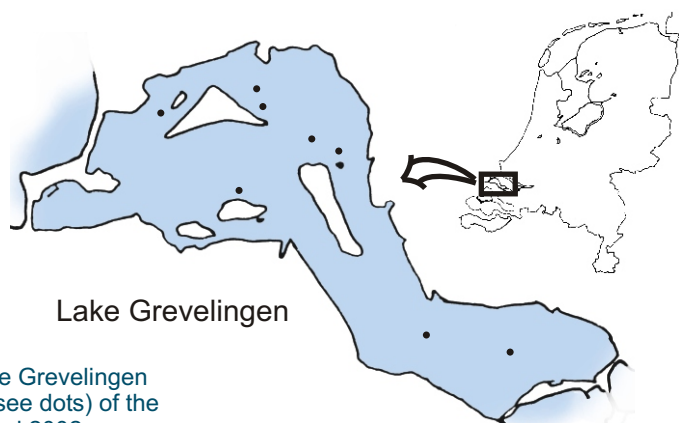


Figure 1. Map of Lake Grevelingen with sampling sites (see dots) of the monitoring in 2001 and 2002.

Material and Methods

In the period 1988-2002 flat oysters were sampled each year, in spring and autumn, at 6 sites in Lake Grevelingen. At each site a sample of 25 flat oysters was taken. The oysters were fixed in Davidson fixative and embedded in paraffin. Sections were stained with haematoxylin and eosin (H&E) and screened for *B. ostreae* infection by light microscopy. An ISH for detection of *B. ostreae* was used to analyse *B. ostreae* suspected oysters from the 2001 and 2002 monitoring. The ISH was performed according to Cochennec *et al.*, 2000 (*J Invert Pathol* 76: 26-32).

Results

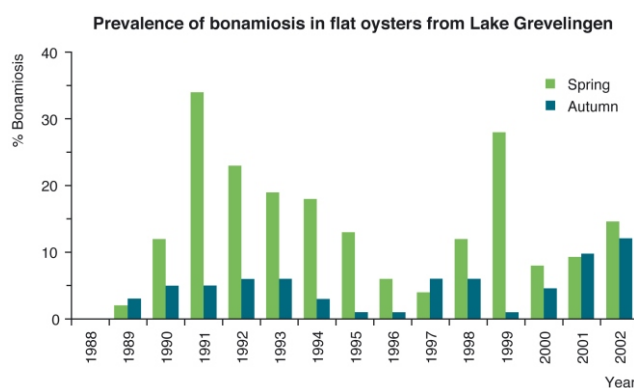


Figure 2. Average percentage of oysters infected by *B. ostreae* from Lake Grevelingen in the period 1988-2002. Overall, the percentage of infected oysters was higher in spring than in autumn. In the recent years a tendency is observed to an increasing number of infected oysters in autumn.

Table 1. *Bonamia ostreae* positive samples were classified as light (+), moderate (++) and severe (+++) by histology (H&E score). Subsequently, for each class the presence of *B. ostreae* was estimated in each tissue type by means of ISH (absent -, low +, intermediate ++ and high ++++) (ISH score).

H&E score	ISH score						
(Overall)	Gills	Mantle	Epithelium skin	Gonads	Dig. divert.	Connective tissue	Epithelium dig. tract
+	+	-	-	-	-	-	±
++	++	+	-	-	+	+	+
+++	++	++	±	±	+	+++	++

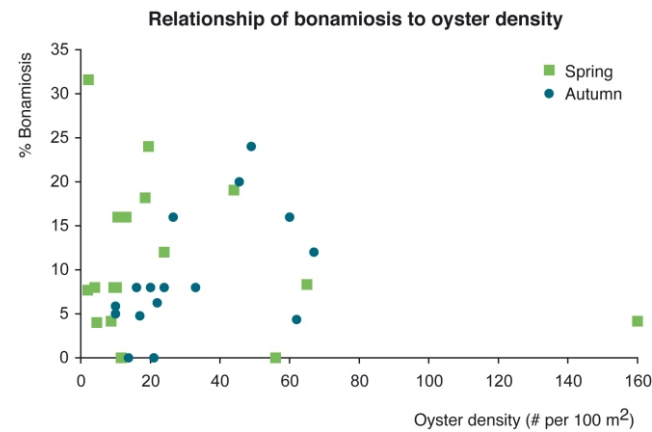


Figure 3. Density of oysters at the sampling sites (2001-2002) with the accompanying average percentage of *B. ostreae* infected oysters at those locations. There was no correlation between the percentage infected oysters and the density of oysters at a site.

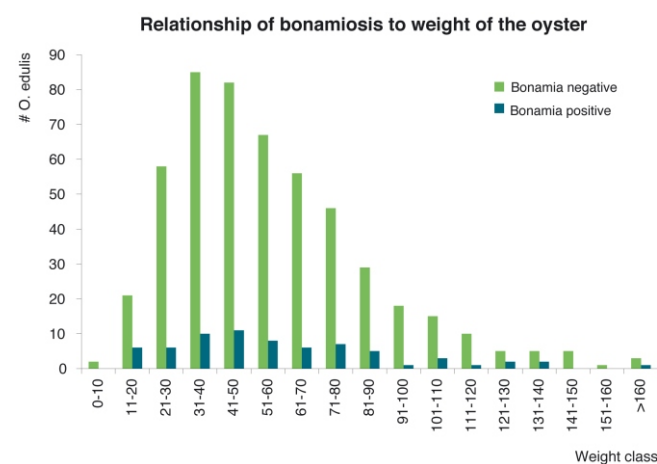


Figure 4. Non-infected and *B. ostreae* infected oysters from the 2001 and 2002 monitoring categorised by weight class. In all weight classes approximately 10% of the total number of oysters was infected with *B. ostreae*.

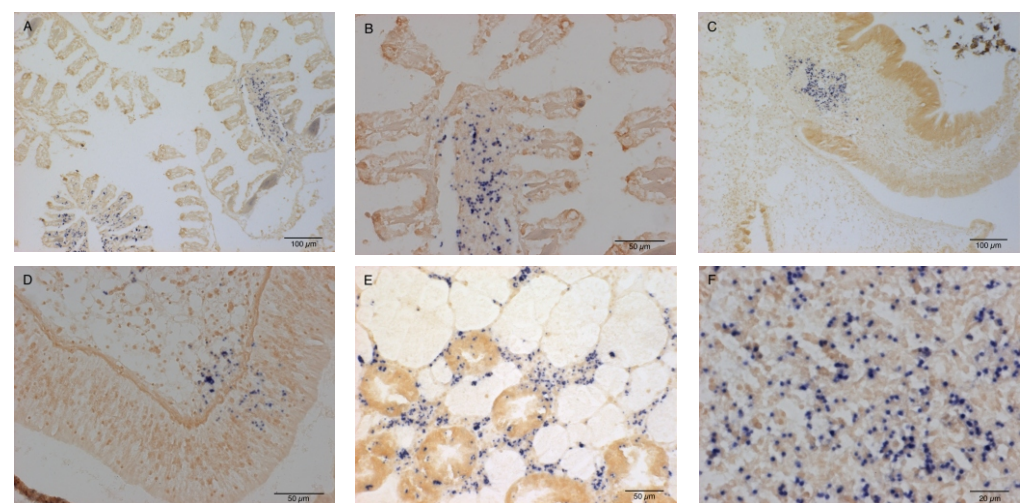


Figure 5. ISH of *B. ostreae* infected flat oyster with a DIG-probe specific for *B. ostreae* 18S ribosomal RNA (purple-black) and counterstained with Bismarck Brown Yellow. (A) Gill tissue, (B) gill tissue, (C) mantle, (D) epithelium digestive tract, (E) digestive diverticulum and connective tissue and (F) inflammatory tissue.

Conclusions

- Despite earlier attempts to eradicate the parasite from the Netherlands, *B. ostreae* is now an endemic parasite of the flat oyster in Lake Grevelingen.
- In general, over the period 1988-2002 the prevalence of *B. ostreae* is higher in spring than in autumn. In recent years an increase of prevalence is seen, especially in autumn.
- *B. ostreae* infections in 2001 and 2002 were not correlated to oyster density or weight or length (data not shown) of the oyster.
- Compared to standard H&E screening, ISH facilitates the detection of *B. ostreae* at low levels of infection and enables precise topographical localisation of *B. ostreae* in the oyster.
- In light infections *B. ostreae* was predominantly present in inflammatory tissue in the gills and in epithelium of the digestive tract, suggesting these tissues to be prime targets for entrance of the parasite.
- In heavy infected oysters *B. ostreae* was detected in virtually all screened organs.

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