

## ***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.

Since 1980 a routine monitoring programme is in practice to determine the prevalence of *B. ostreae* by means of histopathology. Recently, an *in situ* hybridisation (ISH) 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.

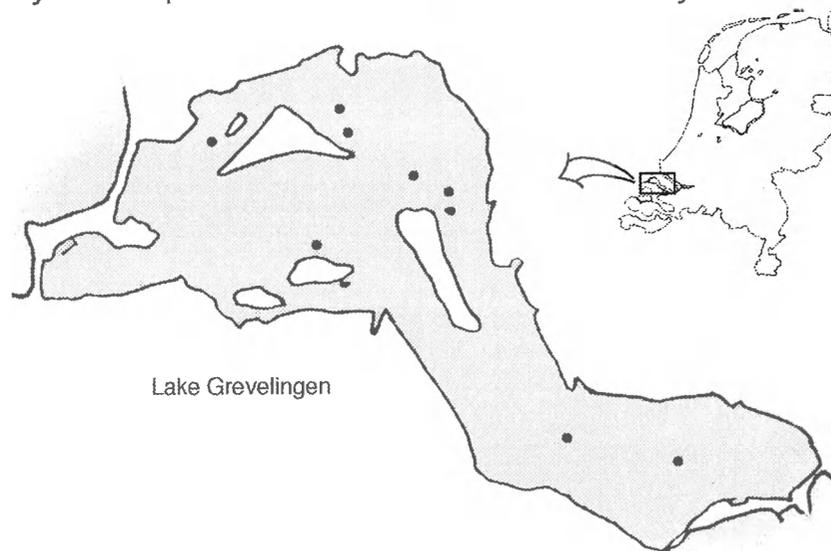


Figure 1. Map of Lake Grevelingen with sampling sites of the monitoring in 2001 and 2002 (dots).

### **Material and Methods**

In the period 1988-2002 flat oysters were sampled annually, 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 Cochenec *et al.* (2000; *J Invert Pathol* 76: 26-32).

## Results

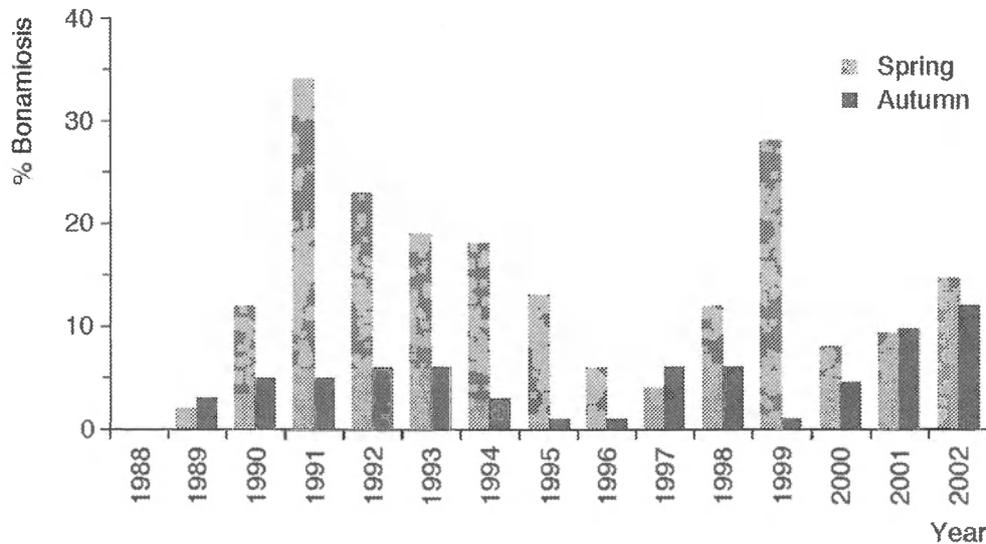


Figure 2. Prevalence of bonamiosis in the flat oyster population from Lake Grevelingen in the period 1988-2002. Average percentage of oysters infected by *B. ostreae* in spring (grey) and autumn (black). Overall, the percentage of infected oysters was higher in spring than in autumn and increasing in the recent years.

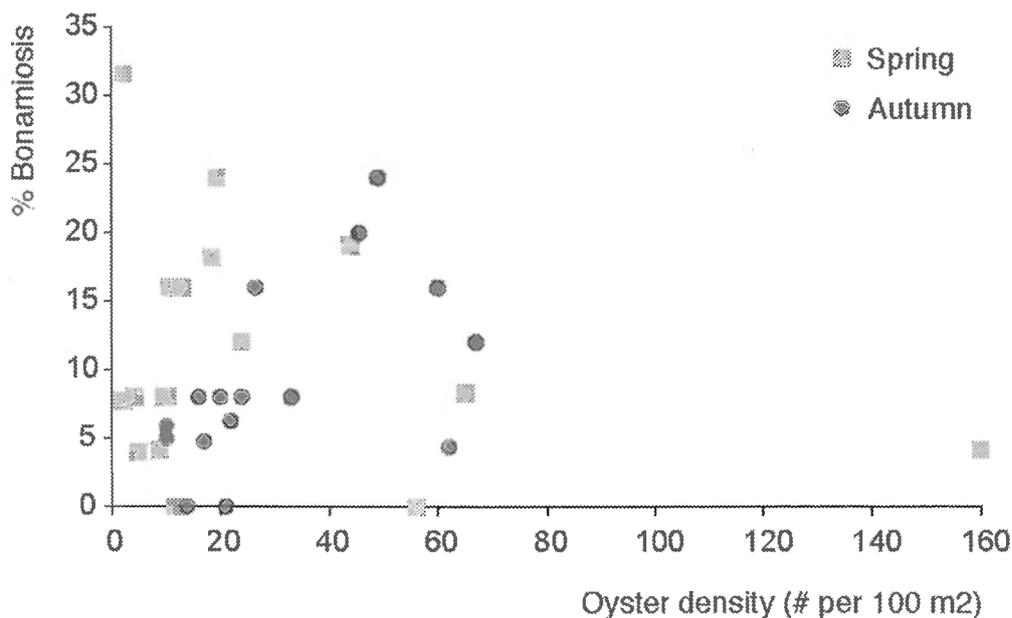


Figure 3. Density of oysters at the sampling sites (2001-2002) with the accompanying percentage of *B. ostreae* infected oysters at that location. There was no correlation between the percentage infected oysters and the density of oysters at a site.

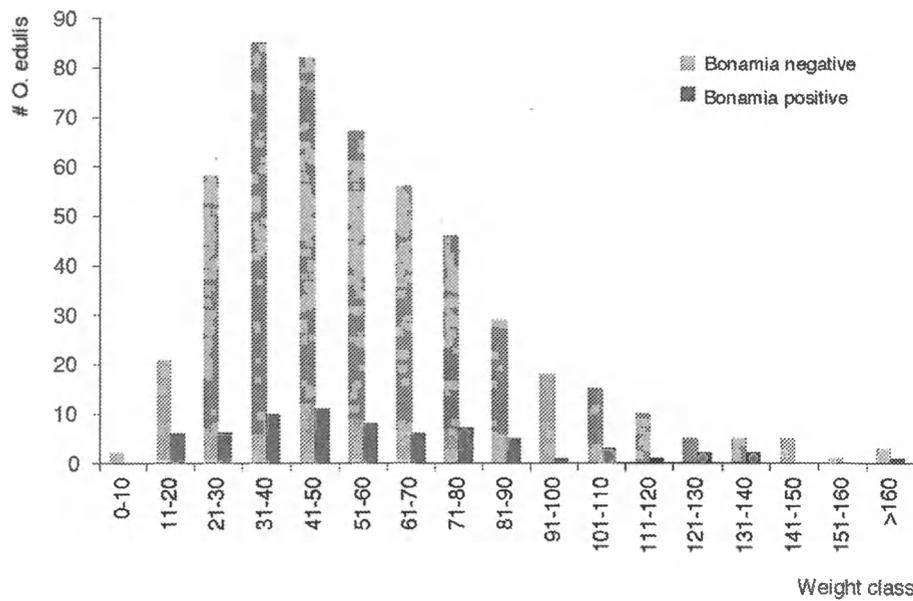


Figure 4. Frequency of weight classes of non-infected (grey) and infected oysters (black; 2001-2002). In all weight classes approximately 10% of the total number of oysters was infected with *B. ostreae*.

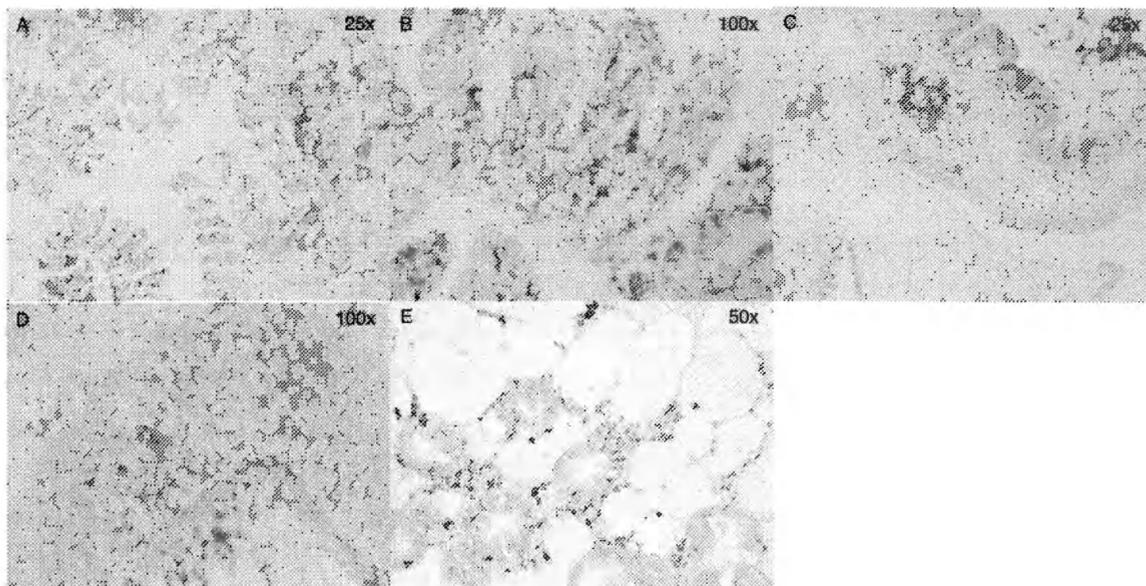


Figure 5. ISH of *B. ostreae* infected flat oyster with a DIG-probe specific for *B. ostreae* 18S ribosomal RNA (purple-black) and counterstained with Bismarck Brown Yellow. (A) Gill tissue, (B) Gill tissue, (C) Mantle, (D) Epithelium digestive tract and (E) Digestive diverticulum and connective tissue.

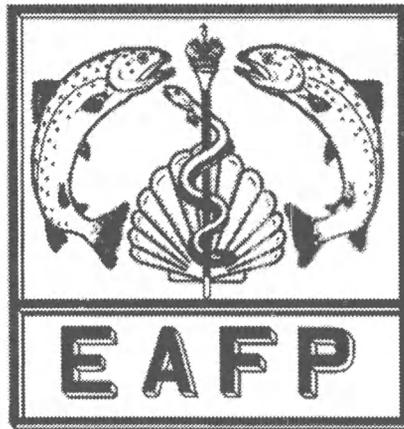
Table 1. *Bonamia ostreae* positive samples were classified as light (+), moderate (++) and severe (+++) by histology (H&E score). Subsequently, for each class the presence of *B. ostreae* was estimated in each tissue type by means of ISH (absent -, low +, intermediate ++ and high +++) (ISH score).

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

## 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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