WAGENINGEN
UNIVERSITY & RESEARCH

Postbus 68 | 1970 AB IJmuiden

De Rijke Noordzee
t.a.v. Mevr. Kelder
Postbus 1578
3500 BN Utrecht

Author(s): Pauline Kamermans

Assignment

In spring of 2022 de Rijke Noordzee (DRN) placed 1000 flat oysters originating from Tralee Bay in Ireland at the Luchterduinen windfarm. To get an impression of oyster reproduction Wageningen Marine Research (WMR) and Bureau Waardenburg (BuWa) were requested to carry out water sampling to detect the presence of oyster larvae.

Materials and Methods

On 12 July 2022 monitoring of oyster larvae was carried out by WMR. To this end, water samples were collected at three locations (EL10, EL4 and EL5). At each location, in total three water samples were taken by pumping 200L water with a pump (provided by BuWa) over a fine mesh netting (100 µm mesh size). The samples collected in the plankton net were transferred to separate vials and preserved in 98% ethanol for analysis in the lab. Each sample was used to count the number of flat oyster larvae (*Ostrea edulis*) and other shellfish larvae under the microscope at WMR. For this three subsamples of the total sample were counted. Subsequently the same sample was used to identify the presence of flat oyster larvae and quantify the amount using qPCR analysis by Wageningen Environmental Research (WEnR). qPCR is a DNA-based technique to identify specific species.

Results and discussion

The results show no detection of *O. edulis* by microscope, but positive values for all samples using the qPCR technique. This is unexpected. Since the same samples were used the difference cannot be caused by actual differences between samples. Larvae of other shellfish species were observed with the microscope. Thus, an explanation can be that *O. edulis* larvae were present in such low numbers that they did not end up in the subsamples. Or the larvae were wrongly identified as other shellfish species. Another explanation can be that the equipment used in the lab was contaminated with *O. edulis* DNA giving the impression that *O. edulis* was present in the sample while it was not.

DATE
November 2th 2022

SUBJECT
letter report

OUR REFERENCE
2218255-PK-lcs

POSTAL ADDRESS
Postbus 68
1970 AB IJmuiden

VISITORS' ADDRESS
Haringkade 1
1976 CP IJmuiden

INTERNET
www.wur.nl/marine-research

CoC NUMBER
09098104

HANDLED BY
Dr. Pauline Kamermans

TELEPHONE
+31 (0)317 48 7032

EMAIL
marine-research@wur.nl

Table 1. Abundance of European flat oyster larvae (O. edulis) determined microscopically (number per 100L) and with qPCR analysis (molecules per microliter reaction volume).

	O. edulis		Other shellfish species	
Location	mic	qPCR	mic	
EL 10-11 1	0	31.50	123	
EL 10-11 2	0	21.45	136	
EL 10-11 3	0	3.20	110	
EL 10-11 surface	0	104.50	20	
EL 4 1	0	20.45	81	
EL 4 2	0	0.35	48	
EL 4 3	0	8.20	83	
EL 5 1	0	0.36	76	
EL 5 2	0	2.45	139	
EL 5 3	0	5.15	103	
Concentrations	mic	Number per 100L		
	qPCR	Molecules per microliter reaction volume		

To evaluate this unexpected result further it is advised to compare the WMR/WEnR results with the results of the eDNA samples collected by De Rijke Noordzee (DRN) and analysed by Wageningen University - Marine Animal Sciences (WU-MAE). When those samples do not indicate any presence of *O. edulis*, contamination of the larval samples may have occurred in the lab and the microscopic analysis is correct. When, however, eDNA analyses did detect *O. edulis* this can either come from adult oysters that were placed at Luchterduinen in the spring of 2022 or from larvae that were present and wrongly identified with the microscope.

Quality Assurance

Wageningen Marine Research utilises an ISO 9001:2015 certified quality management system. The organisation has been certified since 27 February 2001. The certification was issued by DNV.

Justification

Project Number: 431310194

The scientific quality of this report has been peer reviewed by a colleague scientist and a member of the Management Team of Wageningen Marine Research

Approved:	Dr. Linda Tonk Researcher
Signature:	
Date:	02 November 2022

Approved:	Drs. J. Asjes MT member Integration
Signature:	
Date:	02 November 2022