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Flat oysters in the Eijerlandse Gat, Wadden Sea

PROGRAMMA NAAR EEN RIJKE WADDENZEE

Flat oysters in the Eijerlandse gat, Wadden Sea

Results of a survey in September 2017

This report presents the results of a short survey of flat oysters (*Ostrea edulis*) in the Western Wadden Sea. Ten sites were visited and flat oysters were found on nine locations in the Eijerlandse gat. Empty cockleshells and live and dead Pacific oysters provided the main settlement substrate. The presence of larvae was detected in water samples by microscopic inspection and DNA analysis at several locations in the Eijerlandse gat. The results show that flat oysters have returned to the Wadden Sea after its extinction in the last century, but still occurs at low densities.

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Preface

Programma naar een Rijke Waddenzee (PRW, *Program towards a Rich Wadden Sea*) has taken the initiative to explore the conditions for the return of the flat oyster to the Wadden Sea. Recent observations of flat oysters near Texel prompted PRW to commission a flat oyster survey in 2017 to Bureau Waardenburg in cooperation with Wageningen Marine Research and Altenburg & Wymenga.

Contact person for Programma naar een Rijke Waddenzee was M. Firet.

The project team consisted of dr. T.M. van der Have (project leader), drs. M. Teunis and P. Snoeken (Bureau Waardenburg) and dr. E. M. van der Zee (Altenburg & Wymenga), who carried out the fieldwork. dr. P. Kamersmans (Wageningen Marine Research) processed the water samples in cooperation with A. de Groot (qPCR, Wageningen Ecological Research).

We thank J. Hottentot and P. Slik for their assistance in the fieldwork and provided transport with their boat.

E. Menkveld and E. Boot (Natuurmonumenten) provided advice and licence for the field work in the nature reserve De Vlakte van Kerken of Natuurmonumenten.

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M. Teunis commented on previous versions of this manuscript.

The authors thank everyone who has contributed to this report.

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Inhoudsopgave

Summary

A short survey of flat oysters in the litoral and sublitoral of the Eijerlandse Gat, Wadden Sea, was carried out in July and September 2017. In total 10 sites with Pacific oyster beds were surveyed within the Eijerlandse Gat tidal basin. On 9 locations flat oysters (*Ostrea edulis*) were observed (51 flat oysters in total). The density in two sites was very low, 46 and 27 per ha, in Jack IJst en Lange Gat, respectively. Most flat oysters were found along two tidal creeks: Lange Gat and Jack IJst. One flat oyster was found in the sublitoral zone by scuba divers. In the long-term shellfish-monitoring programme in the Wadden Sea (ongoing since 1995), several flat oysters were observed recently (2014, 2017) in the sublitoral zone, also in another tidal basin between Vlieland and Terschelling.

Dead bivalve shells, predominantly cockles, were abundant in most areas where flat oysters were found (along the tidal creeks Lange Gat and Jack IJst) and nearly absent in two locations (Foksdiep, one flat oyster, Gasboeiengat, no flat oysters). Flat oysters were slightly more abundant along the tidal creeks Lange Gat and Jack IJst.

The settlement substrate included dead shells of Pacific oyster *Magellana gigas*, mussel *Mytilus edulis*, cockle *Cerastoderma edule*, fossil Atlantic surf clam *Spisula subtruncata* and the soft shell clam *Mya* arenaria. A few individuals settled on live Pacific oysters. The majority of the flat oysters along the Lange Gat settled on live Pacific oysters (53%), most flat oysters along Jack IJst settled on dead cockle shells (47%). These differences are mainly determined by the availability of suitable substrate. Along Jack IJst many dead shells are present of predominantly cockles, but also *M. arenaria*, mussels and Pacific oysters. Along Lange Gat several sites with Pacific oysters are covered with silty sediment and less small shells are available. At these sites, several flat oysters were found at the top of a Pacific oyster, which was standing upright in the sediment.

The presence of *O. edulis* DNA in water samples from various sites in the Eijerlandse gat was determined using a newly developed qPCR method. The method detected *O. edulis* DNA in the Zwin and Jack IJst tidal channels. Microscopic analysis of a limited number of samples identified *O. edulis* larvae at Foksdiep. Confirmation by microscopic analysis was limited due to budget constraints and was positive for 1 out of 2 samples. The mismatch is most probably caused by difficulties with the DNA extraction of the field samples. The results show that: (1) the strongest DNA signal is observed in the area with the highest number of flat oysters (Jack IJst), and (2) that flat oysters are currently reproducing in the Eijerlandse Gat.

The observation that cockles are suitable settling substrate for flat oysters is an important factor for the restoration ecology of flat oysters, because there is large, natural supply of shells available in the North Sea coastal zone and Wadden Sea tidal inlets. Sediment, which is rich in bivalve shells, is essential recruitment habitat for flat oysters provided that larvae are present and other conditions are also favourable.

These results suggest that the flat oyster in the Wadden Sea has recovered from its extinction. There are indications that the flat oysters, which were found in this study in the Wadden Sea between Texel and Vlieland, originate from an aquaculture experiment on Texel in the seventies of the last century. The spread within the Eijerlandse gat tidal basin

and just outside it near Vlieland suggests that suitable conditions for flat oyster recovery are currently present in the Wadden Sea. A wider distribution in the Wadden Sea is expected, in particularly in the sublitoral zone, because of the species' preference for deeper locations.

(1) It is recommended (1) to carry out a survey of flat oysters in all tidal basins in the Wadden Sea by combining field surveys and sampling for eDNA and larval abundance; (2) to study the population composition and settlement substrate of flat oysters elsewhere in the Wadden Sea; (3) to monitor the presence of hard substrate provided by bivalve shells (“shelliness”) in the Wadden Sea, both in the litoral and sublitoral zone, and the associated organisms (4) to enhance the production of oyster larvae by distributing flat oysters, which originate from a hatchery of Wadden Sea flat oysters, at locations with suitable substrate and thereby facilitating natural recruitment.

Nederlandse samenvatting

Een korte survey van platte oesters *Ostrea edulis* in het litoraal en sublitoraal van het Eijerlandse Gat werd uitgevoerd in juli en september 2017. Tien locaties met Japanse oesterbanken werden in het Eijerlandse Gat bezocht en op negen locaties werden platte oesters gevonden (51 exemplaren in totaal). De meeste platte oesters werden langs de kreken Jack IJst en Lange Gat tussen Texel en Vlieland. De dichtheid was erg laag, 46 en 27 per hectare, respectievelijk langs Jack IJst en Lange Gat. Eén platte oester werd in het sublitoraal bij Texel gevonden door duikers. Een aantal platte oesters zijn onlangs (2014, 2017) bij Vlieland in een ander getijdebasin gevonden het kader van het lange-termijn schelpdier monitoring programma in de Wadden Zee (programma gestart in 1995).

Dode schelpen van tweekleppigen, voornamelijk kokkels, zijn talrijk aanwezig in gebieden waar platte oesters werden gevonden (Jack IJst, Lange Gat). Slechts in twee gebieden waren lege schelpen afwezig en hier werden minder platte oesters gevonden (Foksdiep, 1 platte oester; Gasboeiengat, geen platte oesters).

De platte oesters waren gevestigd op de (dode) schelpen van verschillende soorten schelpdieren, waaronder Japanse oester *Magellana gigas*, mossel *Mytilus edulis*, kokkel *Cerastoderma edule*, fossiele halfgeknotte strandschelp *Spisula subtruncata* en strandgaper *Mya arenaria*. Enkele platte oesters waren gevestigd op levende Japanse oesters. De meeste platte oesters (47%) langs de kreek Jack IJst waren gevestigd op dode kokkelschelpen, langs de kreek Lange Gat vooral op levende Japanse oesters (53%). Deze verschillen worden vooral bepaald door het aanbod van geschikt substraat. Langs Jack IJst bestond het schelpmateriaal vooral uit kokkelschelpen, en kleinere aantallen schelpen van strandgapers, mosselen en Japanse oesters. Langs de Lange Gat kreek zijn op verschillende locaties de wadplaten meer bedekt met slibbig sediment en zijn minder lege schelpen beschikbaar. In deze locaties zijn een aantal platte oesters gevonden die gevestigd waren op de top van een Japanse oester, die verticaal uit het sediment stak.

Een nieuw ontwikkelde qPCR methode toonde de aanwezigheid aan van platte oester DNA in watermonsters uit het Eijerlandse Gat. Deze methode detecteerde de aanwezigheid van platte oesters in de kreken Zwin en Jack IJst. De hoogste concentratie werd gevonden op de locaties waar ook de meeste platte oesters gevonden zijn (Jack IJst). Microscopische analyse van watermonsters toonde platte oesterlarven aan in een van de twee bekeken monsters. De resultaten laten zien dat: (1) het sterkste DNA signaal werd waargenomen in het gebied met de meeste platte oesters (langs de Jack IJst kreek); en (2) dat platte oesters zich voortplanten in et Eijerlandse Gat.

De waarneming dat kokkels een geschikt substraat vormen voor de vestiging van platte oester larven is belangrijk voor de ecologie van het herstel van platte oester populaties. In de Noordzeekustzone en de binnendelta's van de Waddenzee is het natuurlijke aanbod aan lege schelpen (voornamelijk kokkels en fossiele halfgeknotte strandschelpen) erg groot. Sediment dat rijk is aan lege schelpen is belangrijk voor de recrutering en aanwas van platte oesters, indien voldoende larven aanwezig zijn en ook andere omgevingsfactoren geschikt zijn.

Deze resultaten geven aan dat de platte oester is teruggekeerd in het Eijerlandse Gat na eerder te zijn uitgestorven in de Waddenzee. Er zijn aanwijzingen dat de platte oesters die in deze survey gevonden zijn tussen Texel en Vlieland afkomstig zijn van een kweekexperiment op Texel in de jaren zeventig van de vorige eeuw. De uitbreiding in het Eijerlandse Gat getijdebasin en net daarbuiten bij Vlieland suggereert dat geschikte condities aanwezig zijn in de huidige Waddenzee. Een grotere verspreiding in vooral het sublitoraal van de Waddenzee is waarschijnlijk, omdat de platte oester een voorkeur heeft voor de diepere delen van der Waddenzee.

(2) Het wordt aanbevolen om (1) een platte oester survey uit te voeren in alle getijdebassins in de Waddenzee door veldbezoeken te combineren met het nemen van watermonsters voor het bepalen van eDNA met qPCR en het tellen van platte oesterlarven; (2) onderzoek te doen naar de populatieopbouw en vestiging-substraat van platte oesters in de hele Nederlandse Waddenzee; (3) het aanbod van hard substraat in de vorm van lege schelpen *("shelliness"*) en de daarop gevestigde organismen te monitoren in zowel het litoraal als het sublitoraal van de Waddenzee; (4) de productie van oesterlarven te verhogen door het uitzetten van platte oesters, afkomstig uit een hatchery van Wadenzee platte oesters, op locaties met geschikt substraat en daardoor de natuurlijke aanwas te vergroten. .

1 Introduction

1.1 Flat oysters in the Wadden Sea

Flat oyster beds (*Ostrea edulis*) were common in the Wadden Sea until the beginning of the 20th century (Gercken & Schmidt, 2014; Smaal et al., 2015), but became extinct around the mid of 20th century due to overexploitation, habitat disturbance, diseases and cold winters. Recovery of flat oyster populations in the Delta area (e.g., Sas et al., 2016) motivated PRW to commission a feasibility study of flat oyster recovery in the Dutch Wadden Sea (van der Have & van der Zee, 2016). These studies also made clear that recent ecological information on flat oysters in the Wadden Sea is completely lacking. During the preparation of the report, flat oysters were reported by Pacific oyster gatherers in the Eijerlandse Gat and confirmed by a short field visit. These observations prompted PRW to commission a flat oyster survey of the Eijerlandse Gat in 2017.

1.2 Objectives

The objectives for this survey are:

(1) Carry out a short survey of flat oysters at litoral locations which were identified as suitable in van der Have & van der Zee (2016);
(2) Further explore the sublitoral zone in the Vlakte van Kerken for the presence of flat oysters;
(3) Develop a quick survey method to detect the presence of flat oysters in the Wadden Sea by combining field surveys in the litoral and sublitoral zone with eDNA and larval abundance sampling;
(4) Provide photographic / video material of flat oysters with associated organisms.

2 Materials and method

2.1 Litoral survey

The tidal basin between Texel and Vlieland of the Eijerlandse Gat was visited on three occasions, 4, 18 and 19 September 2017 (Table 2). Several sites were selected based on the experiences of the Pacific oyster gatherers and the results of recent monitoring surveys of Pacific oyster beds in the Wadden Sea in the litoral (Brummelhuis et al., 2012; van den Ende et al., 2016) and sublitoral zone (Brummelhuis et al., 2012).

Seven litoral sites were visited: Jack IJst 1 & 2 (southern side of the Hengst intertidal area), Hengst / Lange Gat (northern edge of the Hengst), Foksdiep, Gasboeiengat, Lange Gat and Cupido's Gaatje (Figure A1). Jan Hottentot and Pieter Slik assisted in the fieldwork and provided transport with their boat.

The time window available for the litoral survey was approximately one hour before and one hour after low tide. At each site 0,25 – 0,5 ha (as determined by a set of GPS waypoints) was inspected with 2-3 persons by slowly walking over the mudflats. The search areas were situated at the edge of Pacific oyster beds, preferably where many dead bivalve shells were present. The resulting densities are considered as minimal densities because some flat oysters could have been overlooked.

2.2 Sublitoral survey

Scuba divers surveyed the sublitoral zone of 'het Kiltje', a tidal creek within the Vlakte van Kerken, near Texel, on 22 September 2017 (Table 2, Figure A1). A large Pacific oyster bed is present on the surrounding litoral mudflats and extends into the sublitoral of the creek. The soft sediments around the Pacific oyster bed were visually inspected around low tide. The area searched was very limited due to the very low visibility.

2.3 Shellfish monitoring programmes

Additional information was acquired from several shellfish monitoring programmes in the Dutch Wadden Sea and North Sea coastal zone by Wageningen Marine Research in the period 1995 – spring 2017 (including mussels and Pacific oysters, e.g., Brummelhuis et al., 2012; van den Ende et al., 2016).

2.4 Size and settlement substrate

A handheld GPS marked the position of the observed flat oyster. Flat oyster size was measured as the longest axis of the concave (left) valve with calipers to the nearest milimetre, that is the distance between the anterior and posterior tip (width). Length was measured as widest distance perpendicular to the width.

The settlement substrate was determined by visual inspection of the umbonal area (dorsal tip) of the concave (left) valve. The dead shell (complete or fragmented) attached to the flat oyster was identified to the species level. After inspection the flat oyster was placed back into its original position.

2.5 Presence of larvae

Water samples were taken at 10 locations in the Eijerlandse Gat (Table 1, Figure 1) for DNA analysis (qPCR) and counts of oyster larvae. Five samples were taken at locations with litoral Pacific oyster beds (Kiltje, Jack IJst, Engelsvaarwater and Foksdiep). Flat oysters have been found at two locations (Kiltje, Jack IJst), the other three locations (Foksdiep 1 and 2, Engelsvaarwater) are sites with high potential. Five samples (Zwin 1-5) were taken as a reference at sites without Pacific oyster beds in the Zwin, a relatively deep tidal channel.

Table 1. List of 10 sampling locations for DNA and larval abundance in the Eijerlandse Gat, Wadden Sea, 11 July 2017, with date, time, time of high water (HW), depth and coordinates.

Location	Datum	time	HW	depth (m)	LONG	LAT
Kiltje	11-07-17	12:30	10:56	3	4,90395	53,14809
Zwin 1	11-07-17	13:10	10:56	8	4,89920	53,16633
Zwin 2	11-07-17	13:20	10:56	4	4,90792	53,16633
Zwin 3	11-07-17	13:30	10:56	4	4,92066	53,16499
Zwin 4	11-07-17	13:40	10:56	4	4,93329	53,16527
Zwin 5	11-07-17	13:55	10:56	4	4,95744	53,16707
Jack IJst	11-07-17	14:10	10:56	3	4,97219	53,18134
Engelsvaarwater	11-07-17	14:45	10:56	3	5,02994	53,19346
Foksdiep 1	11-07-17	16:25	10:56	1,5	5,01280	53,15398
Foksdiep 2	11-07-17	16:55	10:56	1,5	5,01280	53,15398

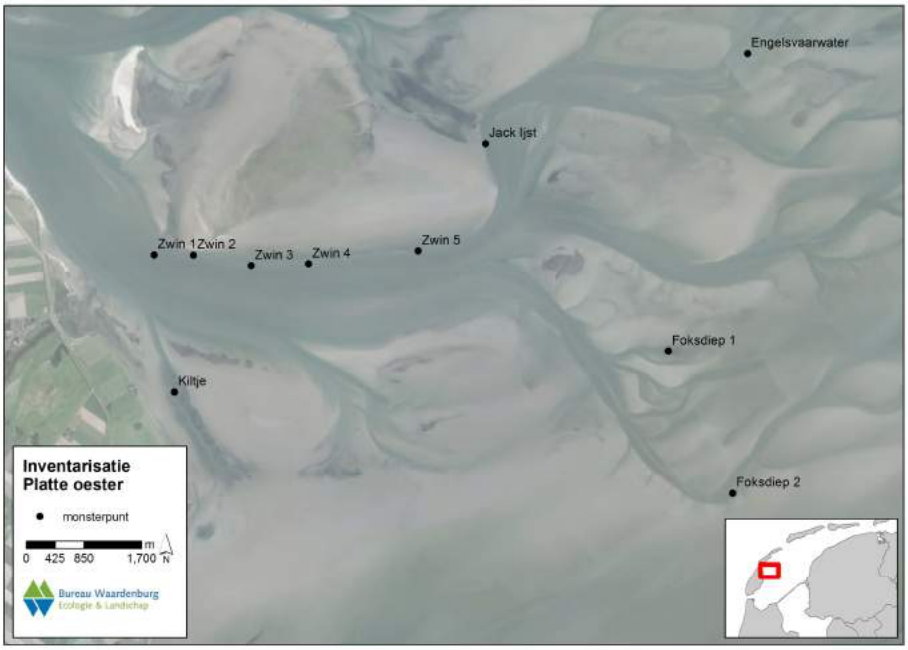

Figure 1. Sampling locations for DNA and larval abundance in the Eijerlandse Gat, Wadden Sea, 11 July 2017. Five are located near Pacific oyster beds (Kiltje, Jack IJst, Foksdiep 1, Foksdiep 2, Engelsvaarwater), five are control sites within a tidal creek (Zwin 1-5).

At each location 100 liters seawater was sampled from the surface and sieved through the plankton net (100 µm mesh size) into a beaker. The filtrate was collected and immediately conserved with 96% ethanol. The beaker and plankton net were cleaned thoroughly with fresh water after each sample. In the laboratory of Wageningen Ecological Research, DNA was extracted from the filtrate and analysed with qPCR as part of a separate KB research programme of WUR (Arjen de Groot). The aim of that project was to develop a qPCR assay to detect *O. edulis* larvae. For *O. edulis* an assay is not available in the literature. The assay was developed based on DNA sequences of the target species and several related species that are available in the online databases (Genbank; https://www.ncbi.nlm.nih.gov/). The assay replicates a fragment of <100bp of the ribosomal DNA. With this assay several samples were analysed for the presence of flat oyster larvae. Two samples were checked microscopically to confirm presence or absence of *O. edulis* larvae.

2.6 Identification of live oysters

Oyster shells in general, and Pacific oysters specifically, are variable in form and size, which depends highly on the growth conditions. The main discriminating characters between *Ostrea* and *Crassostrea* oysters, the colour of adductor scar and presence or absence of chominata (hinge structure), are only visible inside the shell (van der Have & van der Zee, 2016). All investigated oysters were placed back into their original location and, therefore, were identified alive. Identification based on the shell exterior is straightforward. Flat oyster is usually round (equally long as wide) and rather flat, with 20-30 fine ribs (Figure 2). Pacific oyster is usually elongated (much longer than wide) and curved with 8-12 ribs and an

irregular, curved edge of the shell (Figure 2). In soft sediments, they are usually positioned vertically and stick out of the sediment (or vertically in a mussel bed). Flat oysters are usually positioned horizontally on the sediment with the flat valve above.

Figure 2. Flat oyster (FO) compared to Pacific oyster (PO), found on an intertidal mudflat in the Eijerlandse Gat (18 September 2017). (A) Flat oyster with its flat (right) valve upward, surrounded by several young Pacific oysters in various positions; (B) flat oyster with its curved (left) valve upward compared to a Pacific oyster with the curved (left) valve upward.

3 Results

Table 2. List of 8 locations (7 litoral, 1 sublitoral) visited during the Eijerlandse Gat survey, with date, habitat type and number of flat oysters found.

Location	date	depth	habitat type	# flat oysters
Jack IJst 1	04-09-17	litoral	Pacific oyster bed, sandy, many shells	24
Jack IJst 2	18-09-17	litoral	Pacific oyster bed, sandy, many shells	8
Foksdiep	18-09-17	litoral	Pacific oyster bed, large mussels, cockles, no shells	1
Gasboeiengat	18-09-17	litoral	Pacific oyster bed, silty, many *Lanice conchilega*, no shells	0
Hengst/Lange Gat	18-09-17	litoral	Pacific oyster bed, silty, many *Lanice conchilega*, shells partly covered with sediment	12
Lange Gat	19-09-17	litoral	Pacific oyster bed, silty	1
Cupido's gaatje	19-09-17	litoral	Pacific oyster bed, sandy, shells	4
Kiltje	22-09-17	sublitoral	Pacific oyster bed, sandy, many shells	1
Total				51

3.1 Litoral survey

51 flat oysters were found during the short, litoral survey of seven locations in the Eijerlandse Gat, Wadden Sea, on 4, 18 and 19 September 2017 (Table 2, Figure A1). Pacific oyster beds were present at all locations, but differed in type of sediment and availability of dead bivalve shells ("shellines").

Jack IJst

A large area with Pacific oyster beds is present on Jack IJst. Relatively sandy areas with a high abundance of dead bivalve shells occur on the surrounding mudflats (Figure A3). This location was visited twice (4 September, Jack IJst 1; and 18 September 2017, Jack IJst 2), due to the relatively large number of flat oysters found. At both occasions the outline of the search area was defined by GPS waypoints for an estimate of minimal density. The observed densities are very low: in Jack IJst 46 ha^{-1} and in Lange Gat 14 ha^{-1}. The flat oysters were settled on various bivalve species (Figures A4-A5).

Foksdiep

Pacific oyster bed with many mussels and cockles and relatively silty sediment, but no dead bivalve shells available. One flat oyster was found, which was settled on a Pacific oyster.

Gasboeiengat
Pacific oyster bed is present but declining due to siltation and possibly bioturbating activity of Lanice. No flat oysters were found.

Hengst / Lange Gat
Pacific oyster bed is present, but with silty sediment and bioturbating activity of Lanice (Figure A6). Many Pacific oysters are partly covered in sediment. 12 flat oysters were found attached to several species of bivalves.

Lange Gat
Extensive Pacific oyster bed is present but possibly declining because of siltation (Figures A7-A8). One flat oyster was found attached to the top of a live Pacific oyster.

Cupido's gaatje
Extensive Pacific oyster bed is present with sandy sediment and abundant dead bivalve shells in the surrounding area (Figure A9). Four flat oysters were found attached to live Pacific oysters including one with a young flat oyster on it (Figure A9).

General observations
51 flat oysters were found in total. 9 out of 10 visited locations with Pacific oyster beds within the Eijerlandse Gat tidal basin contained flat oysters. The density in two sites was very low, 46 and 27 ha-1. Most flat oysters were found along two tidal creeks: Lange Gat and Jack IJst.

Dead bivalve shells, predominantly cockles, were abundant in most areas where flat oysters were found (along Lange Gat and Jack IJst) and nearly absent in two locations (Foksdiep, one flat oyster, Gasboeiengat, no flat oysters).

3.2 Sublitoral survey

Scuba divers inspected the sublitoral of the Kiltje, a tidal creek in the Vlakte van Kerken, on 22 September 2017 around low tide. The visibility was very low (30-70 cm), which limited the search area to less than 0,1 ha in total. One large flat oyster was found alive, as well as several empty large flat oyster shells (including a doublet). The empty flat oysters shells were of very recent origin.

3.3 Additional surveys

Wageningen Marine Research has monitored the litoral and sublitoral zone of the Dutch Wadden Sea since 1995 as part of various shellfish monitoring programmes (e.g., as part of the “Wettelijke Onderzoekstaak”, WOT, including mussels and Pacific oysters, Brummelhuis et al., 2012; van den Ende et al., 2016). All sampling locations in the period 1995 – spring 2017 are presented in Figure 3. Flat oysters were found only in recent years: in the Vliesloot near Vlieland (one 2014, two 2017) and on the Hengst / Lange Gat between Vlieland and Texel (one 2017). The flat oysters in the Vliesloot belong to the (unofficial) small tidal basin between Vlieland and the sand flat the Richel, which is part of the large tidal basin between Vlieland and Terschelling. Extensive Pacific oyster beds are found on sublitoral areas around the Vliesloot.

3.4 Size distribution and settlement substrate

Flat oyster were slightly more abundant along two tidal creeks: Lange Gat and Jack IJst. The composition of these flat oyster populations also differed slightly. Larger oysters are more common along the Lange Gat (mean length 6,3 cm) and smaller size oyster are somewhat more common along Jack IJst (mean length 5.2 cm, Figure 4), but the mean sizes are not significantly different (t-test, $P>0.05$).

The settlement substrate could be identified in most flat oysters and included shells of Pacific oyster *Magellana gigas*, mussel *Mytilus edulis*, cockle *Cerastoderma edule*, and soft shell clam *Mya arenaria* (Table 3). A few individuals were settled on live Pacific oysters. There was a clear difference between the populations along the Lange Gat and Jack IJst (Figure 5). The majority of the flat oysters along the Lange Gat were settled on Pacific oysters (53%), most flat oysters along Jack IJst were settled on dead cockle shells (47%). These differences are mainly determined by the availability of suitable substrate. Along Jack IJst many dead shells are present of predominantly cockles, but also *M. arenaria*, mussels and Pacific oysters. Along Lange Gat, several sites with Pacific oysters were covered with silty sediment and less small shells were available. In these sites several flat oysters were found at the top of a Pacific oyster, which was standing upright in the sediment.

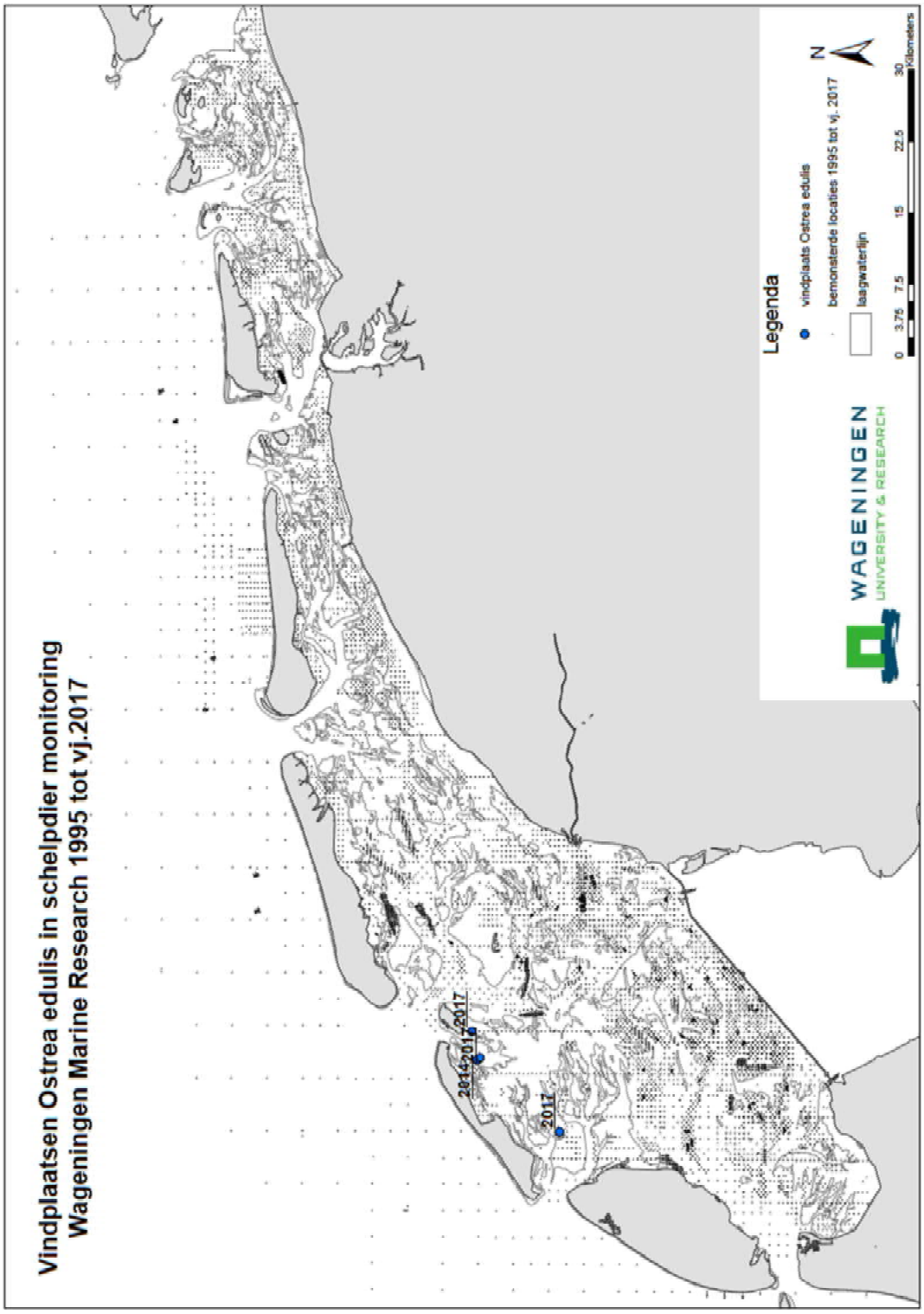

Figure 3. Observations of flat oysters (blue circles) in shellfish monitoring programmes in the Dutch Wadden Sea and North Sea coastal zone 1995 – spring 2017 (Wageningen Marine Research). Each black dot represents a sampling location both in the litoral and sublitoral zone.

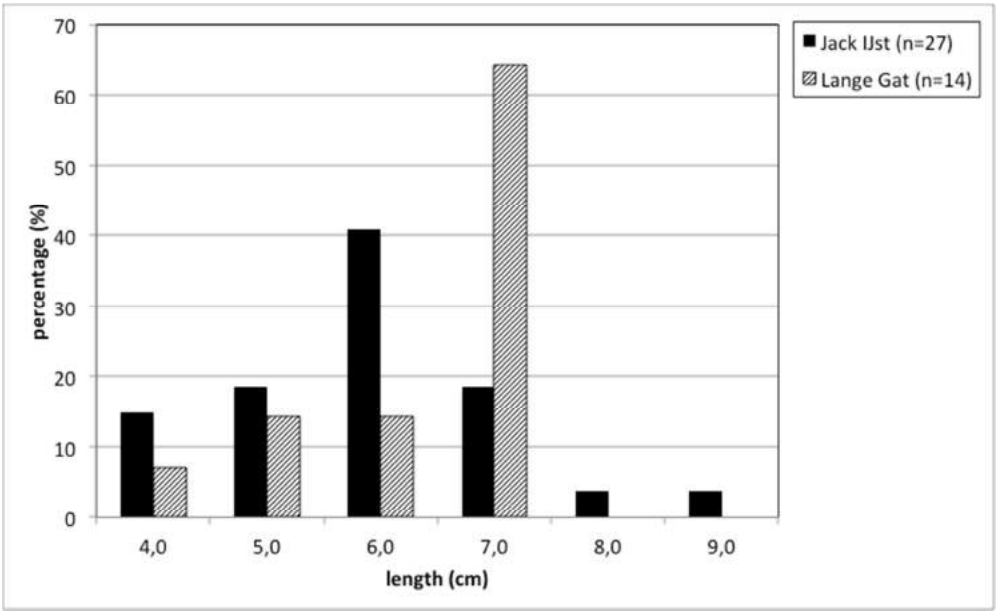

Figure 4. Frequency distribution of flat oysters (shell length, cm) found on litoral mudflats at two locations in the Dutch Wadden Sea (September 2017).

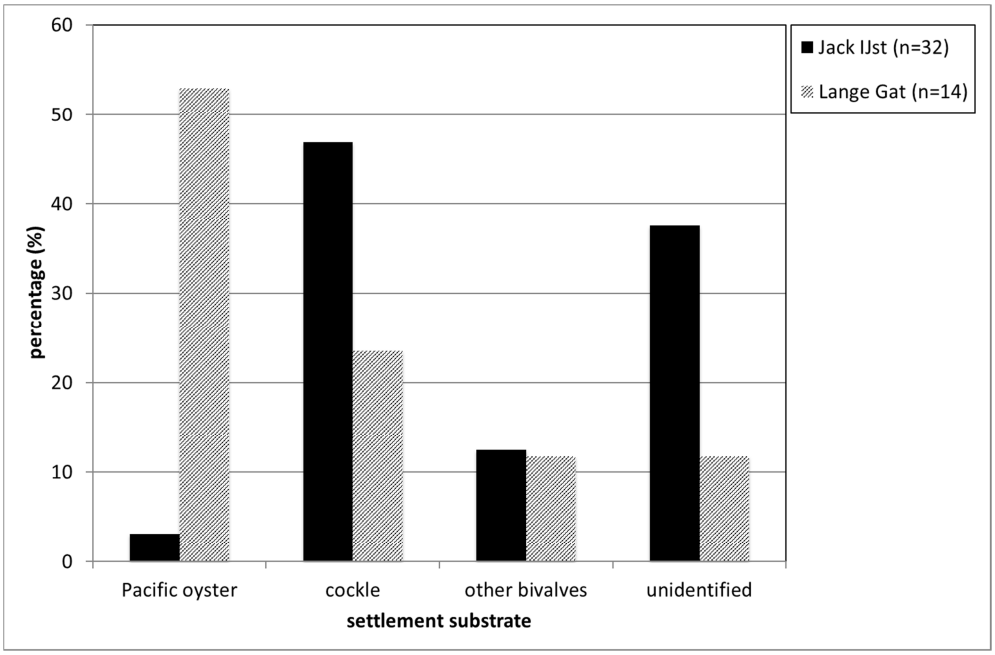

Figure 5. Settlement substrate of flat oysters found on litoral mudflats at two locations in the Dutch Wadden Sea (September 2017). Pacific oysters (M. gigas) included both live and dead shells. The other bivalves included (dead) shells of mussels (M. edulis) and soft shell clams M. arenaria. Most unidentified items included shell fragments.

Table 3. List of 51 flat oysters sampled during a litoral survey (nrs 1-50) and a scuba diving survey (nr 51) in the Eijerlandse Gat, Wadden Sea, September 2017 with measurements (length, width, in cm) and settlement substrate.

nr	location	date	time	length	width	settlement substrate
1	Jack IJst	4-09-17	13:35	5,9	6,7	Pacific oyster live
2	Jack IJst	4-09-17	13:55	6,6	7,6	unidentified fragment
3	Jack IJst	4-09-17	14:01	8,7	9,3	*M. edulis*
4	Jack IJst	4-09-17	14:07	6,6	6,2	*M. arenaria*
5	Jack IJst	4-09-17	14:13	5,9	7,2	*M. edulis*
6	Jack IJst	4-09-17	14:15	4,5	5,1	Cockle
7	Jack IJst	4-09-17	14:23	7,0	7,4	unidentified fragment
8	Jack IJst	4-09-17	14:29	5,5	6,6	unidentified fragment
9	Jack IJst	4-09-17	14:34	6,5	7,2	Cockle
10	Jack IJst	4-09-17	14:36	4,1	4,6	unidentified fragment
11	Jack IJst	4-09-17	14:41	7,7	8,2	unidentified fragment
12	Jack IJst	4-09-17	14:50	5,6	6,5	unidentified fragment
13	Jack IJst	4-09-17	14:52	4,2	4,9	Cockle
14	Jack IJst	4-09-17	14:55	5,5	6,4	*M. edulis*
15	Jack IJst	4-09-17	15:00	4,4	4,2	Cockle
16	Jack IJst	4-09-17	15:01	7,0	8,1	unidentified fragment
17	Jack IJst	4-09-17	15:02	6,1	7,0	Cockle
18	Jack IJst	4-09-17	15:03	6,2	7,1	unidentified fragment
19	Jack IJst	4-09-17	15:05	4,2	4,5	Cockle
20	Jack IJst	4-09-17	15:09			unidentified fragment
21	Jack IJst	4-09-17	15:10			Cockle
22	Jack IJst	4-09-17	15:15			unidentified fragment
23	Jack IJst	4-09-17	15:16			Cockle
24	Jack IJst	4-09-17	15:17			Cockle
25	Jack IJst	18-09-17	13:48	5,8	6,4	unidentified fragment
26	Jack IJst	18-09-17	13:52	4,7	5,2	Cockle
27	Jack IJst	18-09-17	13:54	5,7	6,2	unidentified fragment
28	Jack IJst	18-09-17	13:56	5,5	5,5	Cockle
29	Jack IJst	18-09-17	13:57	5,7	5,6	Cockle
30	Jack IJst	18-09-17	13:58	5,3	5,5	Cockle
31	Jack IJst	18-09-17	14:06	4,8	4,9	Cockle
32	Jack IJst	18-09-17	14:24	4,7	5,2	Cockle
33	Lange Gat	19-09-17	15:12	6,8	7,4	Pacific oyster
34	Hengst, Lange Gat	19-09-17	14:18	5,6	6,4	Cockle
35	Hengst, Lange Gat	19-09-17	14:26	4,6	5,5	Pacific oyster
36	Hengst, Lange Gat	19-09-17	14:29	5	5,9	*M.edulis*
37	Hengst, Lange Gat	19-09-17	14:59	6,7	7,2	unidentified fragment
38	Hengst, Lange Gat	19-09-17	15:04	3,9	4,1	Cockle
39	Hengst, Lange Gat	19-09-17	15:07	6,8	8,8	Cockle
40	Hengst, Lange Gat	19-09-17	15:13	5,5	7,1	*Crepidula* (possibly)
41	Hengst, Lange Gat	19-09-17	15:23	6,8	8,2	Pacific oyster
42	Hengst, Lange Gat	19-09-17	15:29	6,9	8,4	unidentified fragment
43	Hengst, Lange Gat	19-09-17	15:31	6,7	8,2	Pacific oyster
44	Hengst, Lange Gat	19-09-17	15:35	6,5	7,6	Pacific oyster
45	Hengst, Lange Gat	19-09-17	15:36	6,8	7,7	Cockle
46	Cupido's gaatje	19-09-17	16:35	6,7	7,3	Pacific oyster
47	Cupido's gaatje	19-09-17	16:11			Pacific oyster
48	Cupido's gaatje	19-09-17	16:01			Pacific oyster
49	Cupido's gaatje	19-09-17	16:01			Pacific oyster
50	Foksdiep	18-09-17	15:14			Pacific oyster
51	Kiltje	22-09-17	10:43	9,4	10,2	unidentified fragment

3.5 Presence of larvae

The qPCR method detected *O. edulis* DNA at Zwin and Jack IJst (Table 4). Microscopic analysis showed *O. edulis* larvae at Foksdiep. Confirmation by microscopic analysis was limited to two samples due to budget constraints. One analysis confirmed the qPCR results, while the other did not. The mismatch is most probably caused by difficulties with the DNA extraction of the field samples.

The results show that: (1) the strongest DNA signal is observed in the area with the highest number of flat oysters (Jack IJst), and (2) that flat oysters are currently reproducing in the Eijerlandse Gat.

Table 4. Presence of O. edulis larvae detected with a newly developed qPCR method and checked microscopically in two samples.

Location	Date	Estimated O. edulis DNA concentration	Microscopic check	O. edulis in microscopic check (#/100L)
Kiltje	7/11/2017	0	yes	0
Zwin 1	7/11/2017	0.5	no	
Zwin 2	7/11/2017	0	no	
Zwin 3	7/11/2017	0	no	
Zwin 4	7/11/2017	0	no	
Zwin 4	7/11/2017	0	no	
Zwin 5	7/11/2017	0.7	no	
Zwin 5	7/11/2017	0.5	no	
Jack IJst	7/11/2017	11.2	no	
Jack IJst	7/11/2017	10.5	no	
Engels Vaarwater 6	7/11/2017	0	no	
Foksdiep 13	7/11/2017	0	yes	29
Foksdiep FD BR	7/11/2017	0	no	

4 Discussion

The short survey shows that flat oysters are widely distributed in the tidal basis of the Eijerlandse Gat, both in the litoral and sublitoral zone, although at very low densities. Recently, shellfish monitoring programmes also observed flat oysters in the tidal basins between Vlieland and Terschelling on shallow sublitoral Pacific oyster beds. In addition, oyster larvae are also present, which suggests that flat oysters are reproducing locally.

The search strategy was focused on Pacific oyster beds and this proved to be successful. Despite the widespread occurrence of individual flat oysters, no flat oyster beds, which are defined as areas with a density of more than 5 oysters per m^{-2} (Kennedy & Roberts, 2006), were found. The observed densities were three orders of magnitude lower. However, the sublitoral zone, where these beds are primarily expected (van der Have & van der Zee, 2016), was surveyed unsuccessfully due to the low visibility and the fact that only one location was visited. Most of the locations, where flat oysters were present, were indicated as suitable in the suitability map in van der Have & van der Zee (2016). For example, the presence of flat oysters in the Lange gat/Hengst location was somewhat unexpected. This may be related to the fact that no recent information on the depth range of flat oysters in intertidal areas is available.

Settlement substrate is an important component in the flat oyster life history and crucial for successful recruitment (e.g., Sas et al., 2016 and). In most of the visited locations in the Eijerlandse Gat there is a good supply of hard substrate provided by dead bivalve shells ("shelliness") and live Pacific oysters. Information on the "shelliness" in other areas in the Wadden Sea is unknown. Pacific oysters also depend on hard substrate to settle and their local presence in the western Wadden Sea possibly also suggests that local conditions with respect to settlement substrate are favourable.

The observation that dead cockleshells are suitable settling substrate for flat oysters is an important factor for the restoration ecology of flat oysters, because cockleshells are very common in the North Sea coastal zone and tidal inlets of the Wadden Sea (Reijngoud, 2001; Ministerie van Verkeer en Waterstaat, 2004). Sediment, which is rich in bivalve shells, is essential habitat for flat oysters provided that larvae are present and other conditions, such as salinity, depth and bottom shear stress are also favourable (van der Have & van der Zee, 2016). Dankers & van Moorsel (2001) have explored the importance of bivalve shells as a habitat in the Wadden Sea.

These results suggest that the flat oyster in the Wadden Sea has recovered from its extinction but still occurs in low densities. There are indications that the flat oysters, which were found in this study in the Wadden Sea between Texel and Vlieland, originate from an aquaculture experiment on Texel in the seventies of the last century (A.C. Smaal, pers. comm.). Dispersal from the Delta area is considered unlikely because the dispersal range is very limited (up to a few kilometres, Smaal et al., 2015, van der Have & van der Zee, 2016, and references therein).

The spread within the Eijerlandse Gat tidal basin and just outside it near Vlieland suggests that suitable conditions for flat oyster recovery are currently present in the Western Wadden Sea. A wider distribution in the Wadden Sea is expected, in particularly in the sublitoral, because of its preference for deeper locations (up to 40 m in the North Sea, Smaal et al, 2015; van der Have & van der Zee, 2016 and references therein).

5 Recommendations

It is recommended

(3) To carry out a survey of flat oysters in other tidal basins in the Wadden Sea by combining field surveys and sampling for DNA and larval abundance.

(4) To monitor the presence of hard substrate provided by bivalve shells (“shelliness”) in the Wadden Sea, both in the litoral and sublitoral, and the associated organisms (cf. Dankers & van Moorsel, 2001).

(5) To study the population composition and settlement substrate of flat oysters elsewhere in the Wadden Sea.

(6) To enhance the production of oyster larvae by distributing flat oysters, which originate from a hatchery of Wadden Sea flat oysters, at locations with suitable substrate and thereby facilitating natural recruitment.

6 Literature

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Appendices

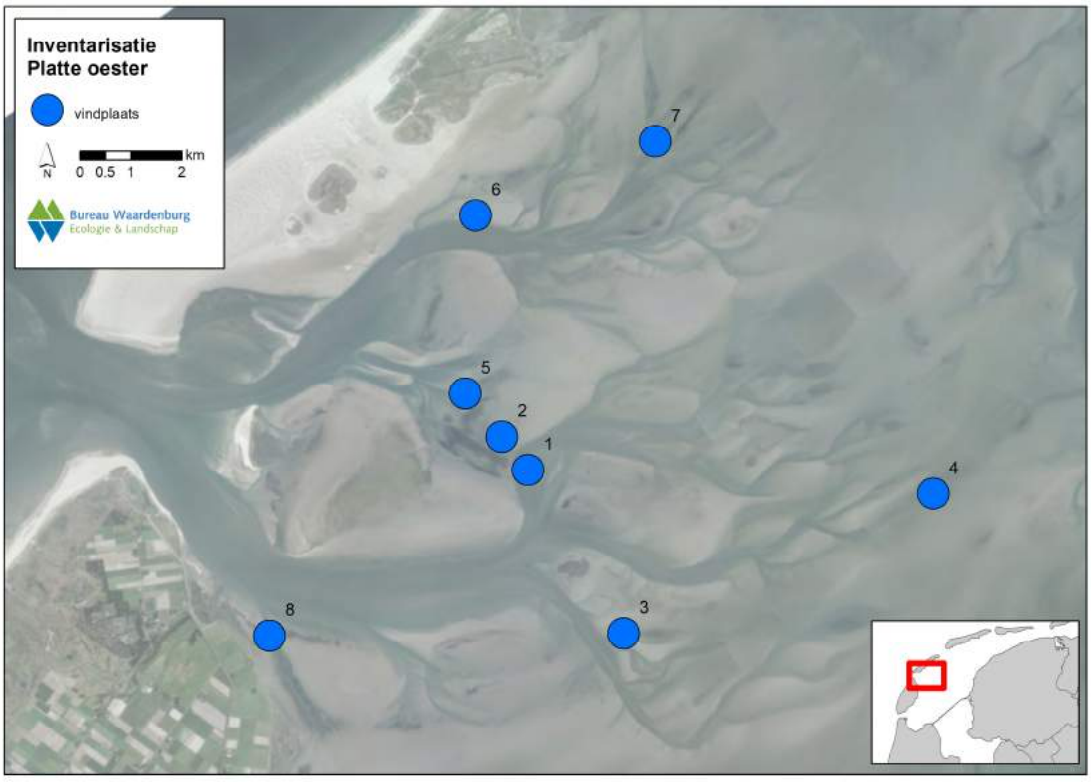

Figure A1. Locations visited during a survey of the Eijerlandse gat, Wadden Sea, September 2017. (1) Jack IJst 1; (2) Jack IJst 2; (3) Foksdiep; (4) Gasboeiengat; (5) Lange Gat / Hengst; (6) Lange Gat; (7) Cupido's gaatje; (8) 't Kiltje. Flat oysters were observed at all locations except in (4) Gasboeiengat.

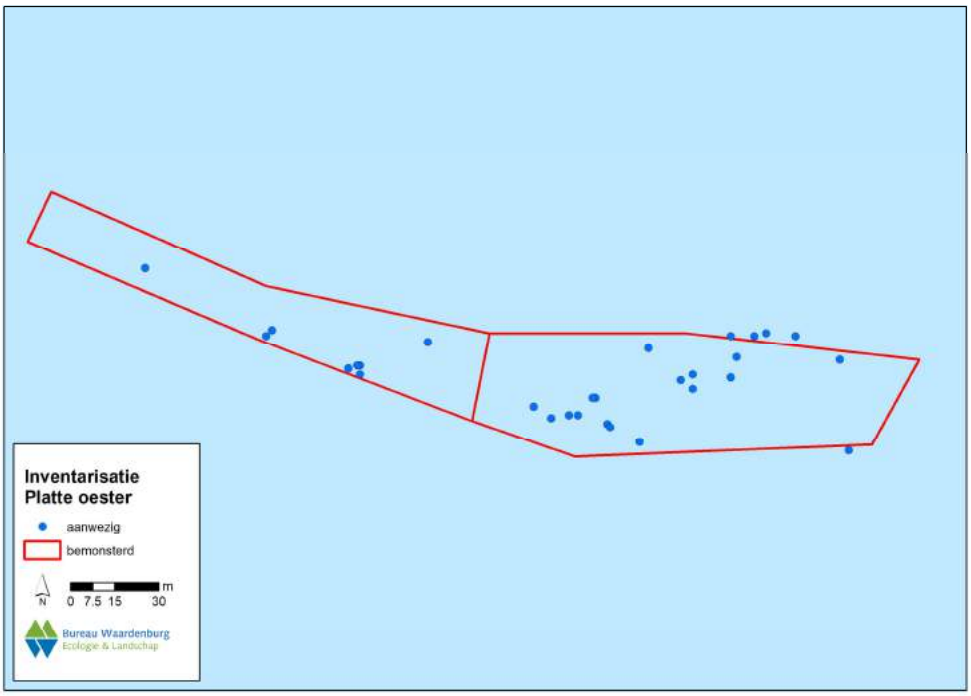

Figure A2. Observations of flat oysters made at the Jack IJst location with the search area on 4 September 2017 (right) and 18-19 September 2017 (left).

Figure A3. Flat oysters along the Jack IJst tidal creek (19 September 2017): (A) flat oyster (in front) just before low tide with a large Pacific oyster bed at the horizon; (B) searching for flat oysters along the edge of a large Pacific oyster bed; (C) two flat oysters among individual Pacific oysters and shells (mainly cockles); (D) one flat oyster left of centre among Pacific oysters an shells.

Figure A4. Flat oysters along the Jack IJst tidal creek on various settlement substrates (19 September 2017): (A) live Pacific oyster; (B) same individual in natural position; (C) subfossil Atlantic surfclam Spisula subtruncata; (D) empty mussel shell; (E) Mya arenaria; (F) cockle Cardium edule (inside the shell); (G) cockle (attached to the outside of the shell).

Figure A5. Flat oysters along the Jack IJst tidal creek in their original position on the sediment. Most oysters are with the concave (right) shell attached to the substrate, except for one (lower, left).

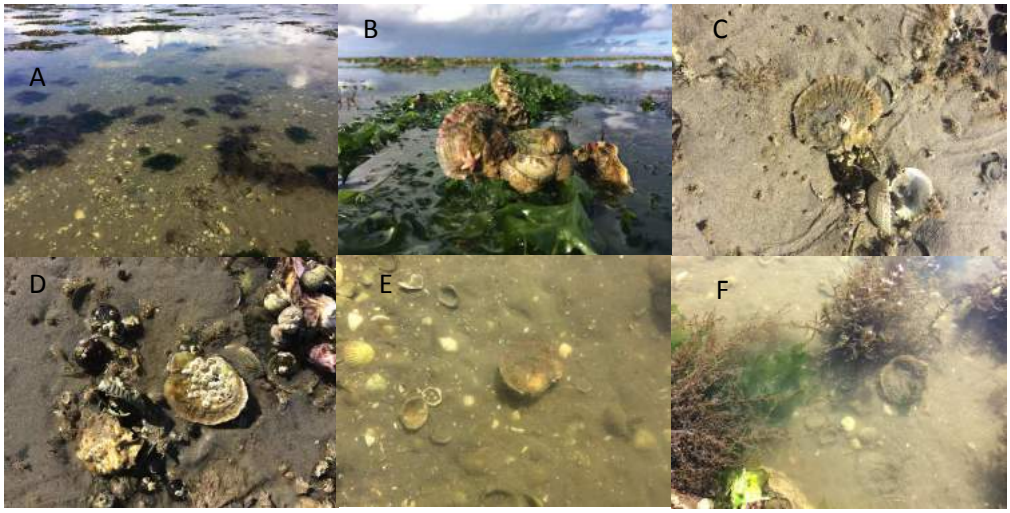

Figure A6. Flat oysters along the Lange Gat tidal creek: (A) area with low density of Pacific oysters and many dead bivalve shells; (B) flat oyster (left) attached to Pacific oyster (right) with slipper limpet (Crepidula fornicata, middle); (C – F) flat oysters in their original position on the sediment.

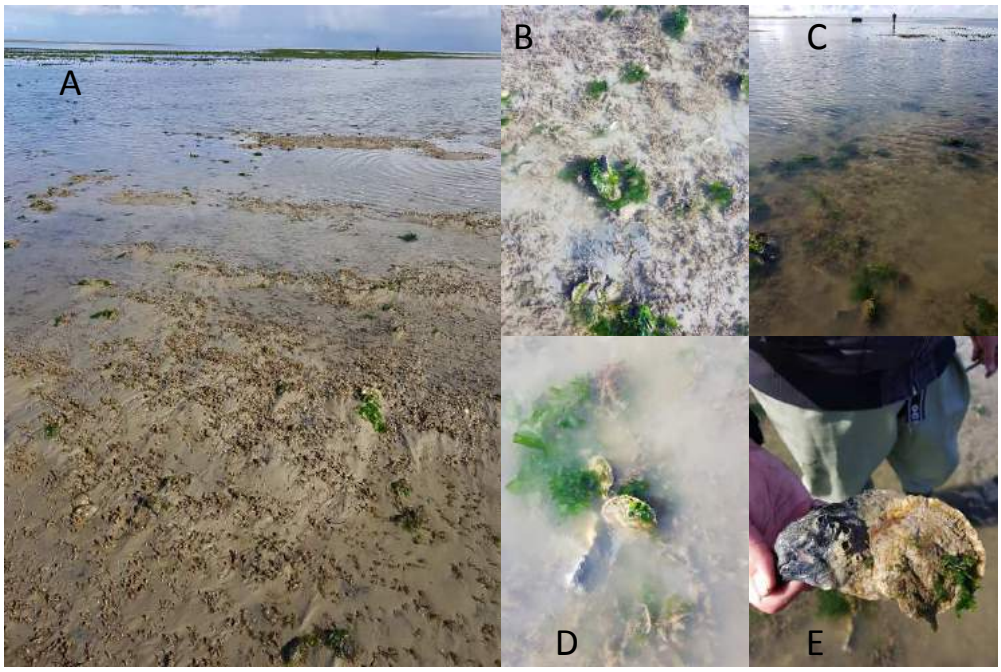

Figure A7. Flat oysters along the Lange Gat tidal creek: (A) mudflat dominated by Sand mason worm Lanice conchilega with a large Pacific oyster bed in the background en few dead bivalve shells; (B) area with low density of Pacific oysters (with sea lettuce Ulva lactuca attached to it) and Lanice dominated mudflats in between; (C) mudflat with low density of Pacific oysters c. one hour after low tide; (D) flat oyster attached to Pacific oyster in original position; (E) flat oyster (same individual) attached to Pacific oyster, which was partly buried in the sediment (black colouration)..

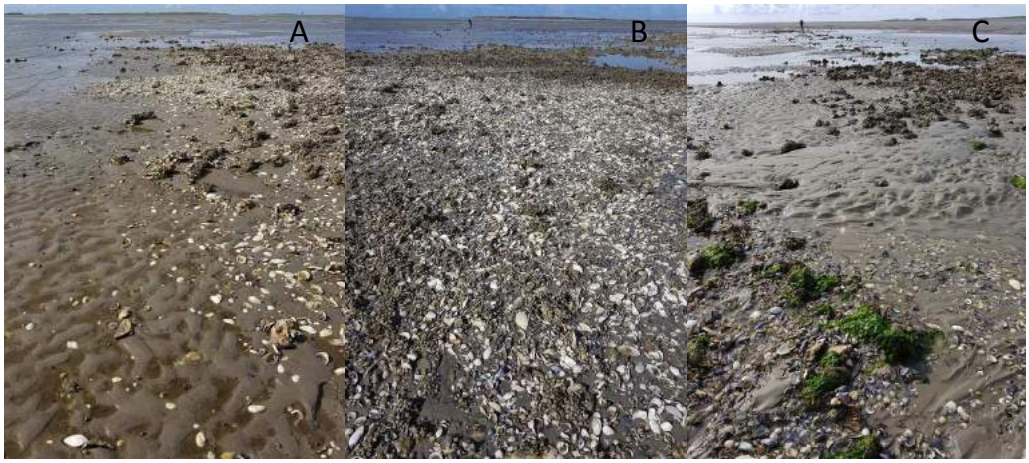

Figure A8. Mudflats situated along the Lange Gat tidal creek with low density of Pacific oyster beds and limited (left and right) to high abundance (middle) of dead bivalve shells.

Figure A9. Extensive Pacific oyster beds with abundant dead bivalve shells situated at the end of the Lange Gat tidal creek (Cupido's gaatje, above) and three flat oysters in their original position attached to live Pacific oysters (below). The white area on the flat oyster in the middle is an empty valve of a young flat oyster.

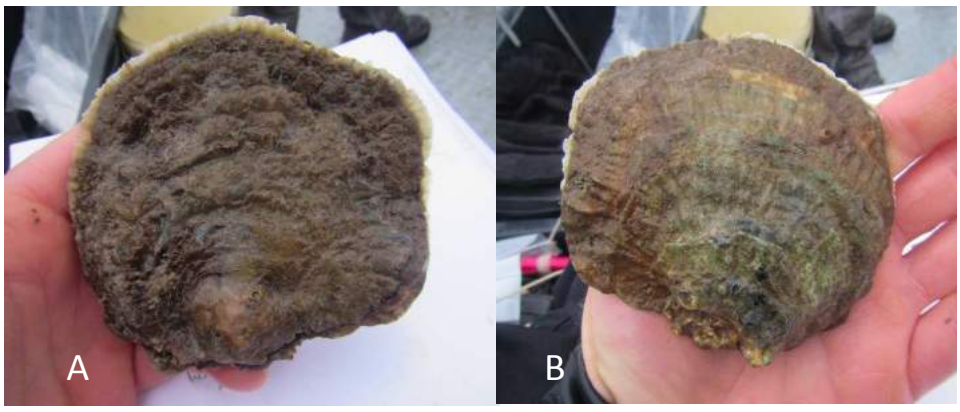

Figure A10. Large flat oyster found in the tidal channel 't Kiltje, Texel: (A) flat (right) valve; (B) curved (left) valve (22 September 2017). The pale edge of the shell suggests it is actively growing.

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