

Validation of a method for age determination in the razor clam *Ensis directus*

with a review on available data on growth, reproduction and physiology

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Colophon

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Background

Sand dredging in the North Sea causes not only the removal of organisms in dredged areas but also the release of large amounts of silt in the water column. This release of silt reduces light in the water column and leads to an increase in silt deposition. As a result, a decrease in food quality and quantity may occur. To evaluate the effects of increased silt concentrations and decreased primary production in the water for the benthic fauna, a research program was proposed, embedded in an extensive monitoring programme of RWS (National Institute for Waterways and Public Works of the Department of Infrastructure and Environment) and LaMER Foundation (a foundation of water constructors). In this research program, focus is put on the most common bivalve species along the Dutch coast, the razor clam *Ensis directus*. Two main questions are made:

- What are the effects of silt increase in the water column and the resulting decrease in algal concentration on growth of *E. directus*?
- When does food limitation occur as a result of changes in silt and algal concentrations?

To answer these questions, several projects are currently running: a) development of a DEB (Dynamic Energy Budget) model for *E. directus* to predict growth in the field under different environmental conditions; b) growth of *E. directus* in the field in relation to environmental conditions; c) development and use of a shell position monitor which monitors the reaction of the shell (opening and closing of valves) in relation to environmental conditions, namely different concentrations of silt and algae; and d) adaptation of the DEB model to an ecosystem model including water, silt, algae and benthos. The study presented in this report falls under project a), which involves different tasks: 1) analysis of growth of *E. directus* in the field, 2) compilation of data from literature, 3) lab experiments and 4) development of a DEB model for *E. directus*. In this report we present results dealing with tasks 1) and 2). In task 1), the seasonality in shell growth lines of *E. directus* was validated using stable isotopes. To analyse growth in the field, accurate age determination is important. Data on growth in the field (age-at-length relationships and Von Bertalanffy growth rates) is necessary for comparing DEB model predictions with field data. In task 2), a review of the available published and unpublished literature on growth, reproduction and physiology of *E. directus* was done with the aim of finding information useful for the estimation of DEB model parameters or for comparison of model predictions with field observations. The results of this study, together with the results of the lab experiments on growth, respiration and filtration rate, will be used to estimate the DEB parameters for *E. directus*. This particular report represents Part B2 and B4 of the evaluation programme Sand-mining (Ellerbroek et al. 2008).

1. Validation of the seasonality in shell growth lines in *Ensis directus* using stable isotopes - a method to accurately determine age and growth

1.1. Introduction

The shell of bivalves grows through deposition of successive layers of carbonate material. This calcification process in the shell is usually a seasonal event mainly related to temperature and food conditions, with highest calcification rates during spring-summer and lowest in autumn-winter. The deposition of growth bands can usually be used to determine age, which is important for an accurate analysis of growth. However, since other varying environmental conditions could also influence the chemical composition of the shell, the seasonality of growth marks on the shell needs to be validated. For many species and areas such validation has been done. Growth marks identified as periodic features (e.g. annual lines) have been used to determine age, seasonality and growth rates of various bivalve species such as mussels *Mytilus edulis*, soft-shell clams *Mya arenaria* and the bivalve *Spisula subtruncata* (e.g. Rhoads and Lutz 1980, Richardson et al. 1993, Brousseau and Baglivo 1987, Maximovich and Guerassimova 2003, Cusson and Bourget 2005, Cardoso et. al 2007). This task aims to validate a method for accurate age determination of *Ensis directus*. Age and Von Bertalanffy growth rates are important life history characteristics used to compare output results of DEB modelling with field data. Therefore, accurate age determination is very important.

A commonly used method for validation of the seasonality of growth bands in bivalve shells is the determination of oxygen and carbon isotope ratios ($^{18}\text{O}/^{16}\text{O}$ and $^{13}\text{C}/^{12}\text{C}$) along the longitudinal profile of the shell. The precipitation of calcium carbonate during shell formation is done in isotopic equilibrium with surrounding water and, therefore, mollusks shells record habitat conditions during growth (Wefer and Berger 1991). The ratio of stable oxygen isotopes ($^{18}\text{O}/^{16}\text{O}$, also referred to as $\delta^{18}\text{O}$) in the shell reflects the interaction of water temperature and water isotopic content (Epstein et al. 1953, Grossman and Ku 1986). Because water $\delta^{18}\text{O}$ has an almost-linear relationship with salinity, the above relationship may also be understood in terms of salinity. In marine environments, water $\delta^{18}\text{O}$ and salinity are generally assumed to be constant during the life of an organism, so changes in shell $\delta^{18}\text{O}$ record mainly changes in water temperature, where higher $\delta^{18}\text{O}$ (enriched in ^{18}O) corresponds to colder water, and lower $\delta^{18}\text{O}$ (depleted in ^{18}O) indicates warmer water. In areas with variable salinity, it is important to know the relationship between water $\delta^{18}\text{O}$ and salinity to be able to correctly analyse the $\delta^{18}\text{O}$ record in the shell.

Carbon stable isotope ratios ($^{13}\text{C}/^{12}\text{C}$, also referred to as $\delta^{13}\text{C}$) values in carbonate shell materials are influenced by metabolic factors and environmental conditions, and, therefore, the profiles of carbon isotope ratios in shells are less clear than oxygen isotope ratios (Wefer 1985, Kalish 1991). Early work suggested that skeletal carbon originates directly from dissolved inorganic carbon (DIC) in seawater (Mook and Vogel 1968, Killingley and Berger 1979, Arthur et al. 1983). Since the stable carbon isotopic composition of the DIC

($\delta^{13}\text{C}_{\text{DIC}}$) is related to salinity (Gillikin 2005), $\delta^{13}\text{C}$ of the shell can give an insight into the salinity in which the shells grew. However, $\delta^{13}\text{C}_{\text{DIC}}$ is also influenced by anthropogenic carbon inputs, productivity, and respiration, which may mask the relationship between $\delta^{13}\text{C}_{\text{DIC}}$ and salinity (Gillikin 2005). Nevertheless, since metabolism is mostly related to temperature and food conditions, which vary usually in an annual cycle, a seasonal pattern for this isotope could be expected as well.

Isotope ratio profiles can, therefore, be used to validate whether or not identified growth bands in the shell of bivalves are formed at regular (annual) intervals. This approach will be used in this study to identify the growth patterns of *E. directus* in the field. *E. directus* is a razor clam with an aragonitic shell (Kahler et al. 1976). A detailed analysis of the isotope ratios in the shell of this species will allow an identification of time of deposition of growth bands and will allow a separation of visual annual bands from those produced by non-annual events.

1.2. Materials and Methods

1.2.1. Selection of shells for analysis

Shells for analysis were collected in the framework of the research program BWN (Buiding with Nature) and the Monitoring Programme of RWS LaMER, off the coast of Egmond aan Zee (Fig. 1). This is an area with freshwater influence and therefore salinity is variable. Only shells with no damage on the valve were selected for analysis.



Fig. 1. Map showing the locations where shell BWN1, BWN3 and BWN4 were sampled, off the coast of Egmond aan Zee.

1.2.2. Preparation of shell blocks and valves

For growth band analysis on the shell, shell length of the animals was measured, shells were opened and all soft parts removed. In each animal, the number of macroscopically visible external bands was recorded. Right valves were filled with epoxy resin so that they were strong enough to be drilled for isotope analysis of carbonate material. Left valves were placed face down in a plastic mould and embedded in epoxy resin. Once hardened, the blocks with

the left valves were sectioned longitudinally through the hinge, in the form of slices of about 5 mm thick (Fig. 2). The surface of the cross-sections was then ground flat under successively finer grit (600, 800, 1200 and 4000 μm) and wet polished with commercial polishing compounds until adequate thickness and texture were reached.



Fig. 2. Cross-section of an embedded *E. directus* shell from which an acetate peel is made.

Photographs of polished sections were made under a binocular microscope. The number and position of internal bands were analysed and compared to the records for the external bands. Acetate peels of the shell-cross sections were prepared by submerging the polished shell sections in 1% HCl solution for about 20 seconds. By using this solution, the organic parts of the carbonate matrix are conserved and the carbonate parts are dissolved, resulting in a relief on the shell surface. This pattern was transferred to a 0.1 mm thick sheet of cellulose acetate by covering the shell surface with drops of acetone and laying the acetate sheet over it. Acetate peels were put on a microscope slide and pictures were taken under a microscope (Fig. 3a). From the composite pictures (Fig. 3b), the areas to be analysed for isotopic composition of calcium carbonate powder were selected.

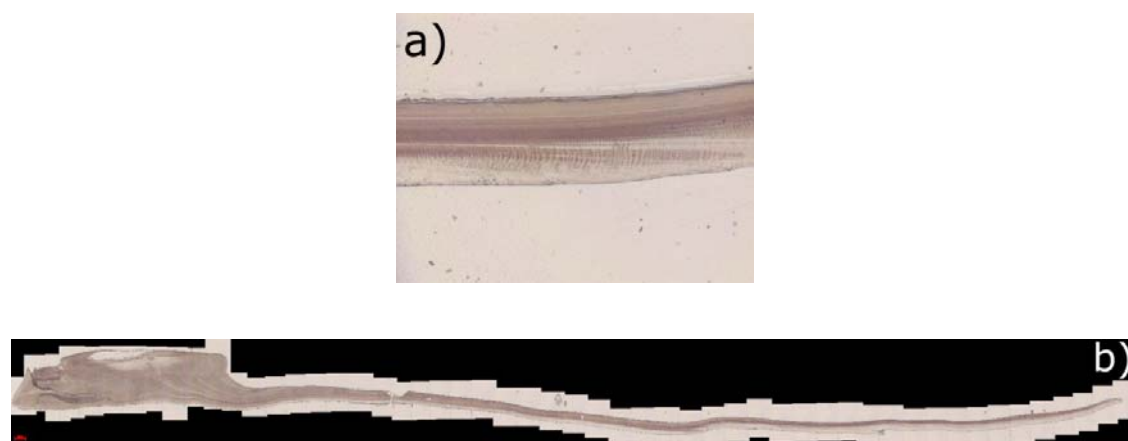


Fig. 3. Example of a photograph of an acetate peel section (a) and of a composite image (b). Image 2b was broadened to improve the visualization of the section.

1.2.3. Analysis of isotopic composition of calcium carbonate powder

Prepared shell valves described previously were drilled to collect calcium carbonate powder for determination of carbon and oxygen isotopic composition. Up to know two shells of *E. directus* were drilled and analysed (two more are ready to be analysed). From each shell, calcium carbonate powder was sampled along the valve in a dorso-ventral series, following the growth lines, using a small dental drill (bit size 0.5 mm) in equally spaced (~1 mm) intervals (Fig. 4). The dental drill sampled each defined line independently and powder was collected from each line separately. Some lines did not result in enough powder for analysis and in that case two lines were pooled. Powder was analyzed for oxygen and carbon isotopic composition using a Thermo Finnigan MAT253 mass spectrometer coupled to a Kiel IV carbonate preparation device. The seasonal pattern in isotope profiles along the valve was then analyzed.

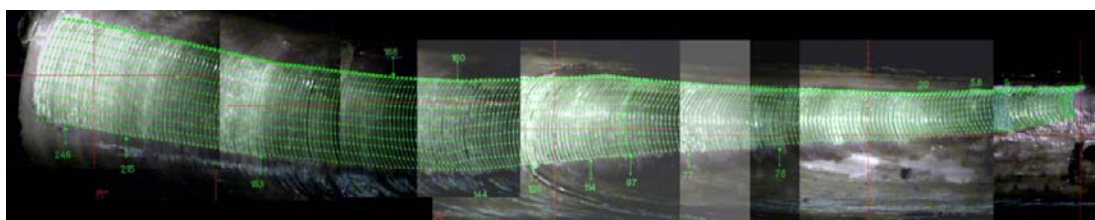


Fig. 4. Lines drilled out on the valve of *E. directus* shell BWN4 (in green).

1.3. Results

1.3.1. Analysis of external and internal shell growth lines

On the valve of shell BWN4, 1 line was considered to be a winter line as it was the only clearly visible line which could be followed along the shell (Fig. 5a). On the acetate peel of the section also 1 line was seen in the chondrophore and 1 coming out on the valve which could be followed from the hinge (Fig. 5b).



Fig. 5. a) One line was considered to be a winter line on shell BWN4 (arrow); b) Composite image of the acetate peel of the cross-section of the valve of shell BWN4. For better visualization the image 4b was broadened. The red arrow indicates the line visible in the chondrophore; the white arrow shows the line visible in the cross-section of the valve.

On the valve of shell BWN3, many lines could be seen by eye on the external side of the valve (Fig. 6a). However, only four lines were considered to be year lines (i.e. lines formed

during winter) as they could be followed along the shell. These matched the lines observed in the cross-section and the chondrophore (Fig. 6b).

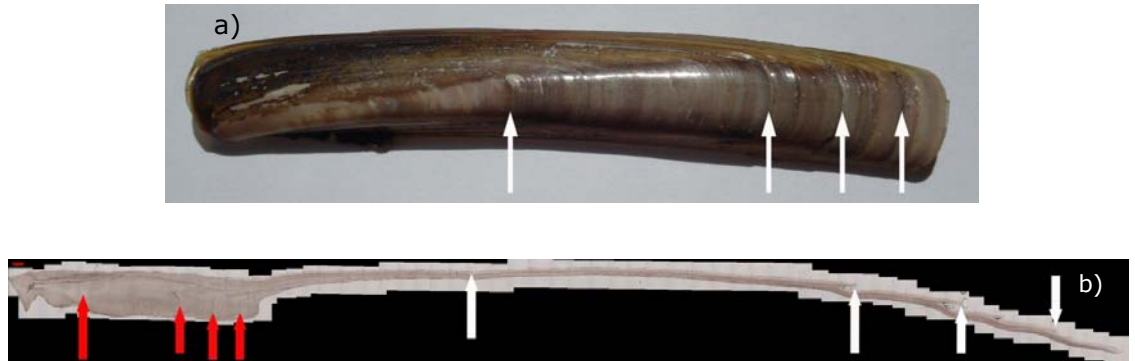


Fig. 6. a) Lines visible by eye on the external part of the valve of shell BWN3 which were considered to be winter lines (arrows); b) Composite image of the acetate peel of the cross-section of the valve of shell BWN3. For better visualization the image 5b was broadened. Red arrows indicate lines visible on the hinge, white arrows show lines visible on the valve.

On the valve of shell BWN1, 4 lines were considered as winter lines (Fig. 7a). The same amount of lines was seen in the cross-section and chondrophore (Fig. 7b).

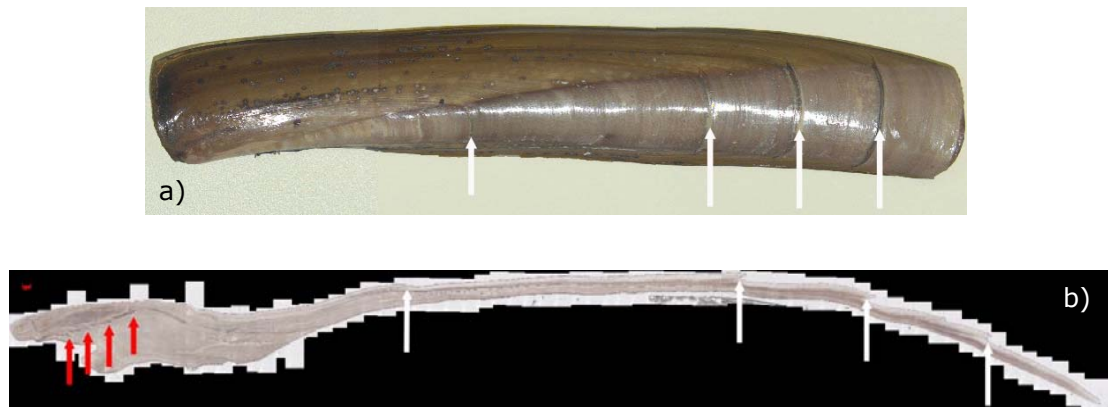


Fig. 7. a) Lines visible by eye on the external part of the valve of shell BWN1 which were considered to be winter lines (arrows); b) Composite image of the acetate peel of the cross-section of the valve of shell BWN1. For better visualization the image 6b was broadened. Red arrows indicate lines visible on the hinge, white arrows show lines visible on the valve.

Micro-growth rings, which could for e.g. represent daily lines, were not observed in any of the shells.

1.3.2. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ profiles in shell BWN4

In total, 238 lines have been drilled in shell BWN4 (Fig. 4). However, isotope determination was not possible for all lines since in some cases not enough powder could be collected. Therefore, some lines were pooled resulting in 221 data points of isotope data.

Oxygen isotope composition along the right valve of shell BWN4 is seen in Figure 8. Before drilling the shell, a drawing of the shell valve and the growth lines visible by eye was made and compared to the drawing of the internal shell lines visible in the acetate peel of the cross-section of the left valve. These were matched with the isotope profile (Fig. 8). There is a clear pattern in oxygen isotope ratios along the shell. Samples with high isotopic values (corresponding to low temperatures) were collected at a shell length of about 6.5 cm, nearby and on the growth line visible by eye and in the cross-section. This suggests that this growth line was formed during winter.

BWN4 showed 2 spring/summer periods and 2 autumn/winter period, indicating that this shell is beginning its 3rd year of growth. Because it was collected in April 2010, the last isotope cycle cannot be from the current year but must be from 2009, indicating that the animal was born in 2008.

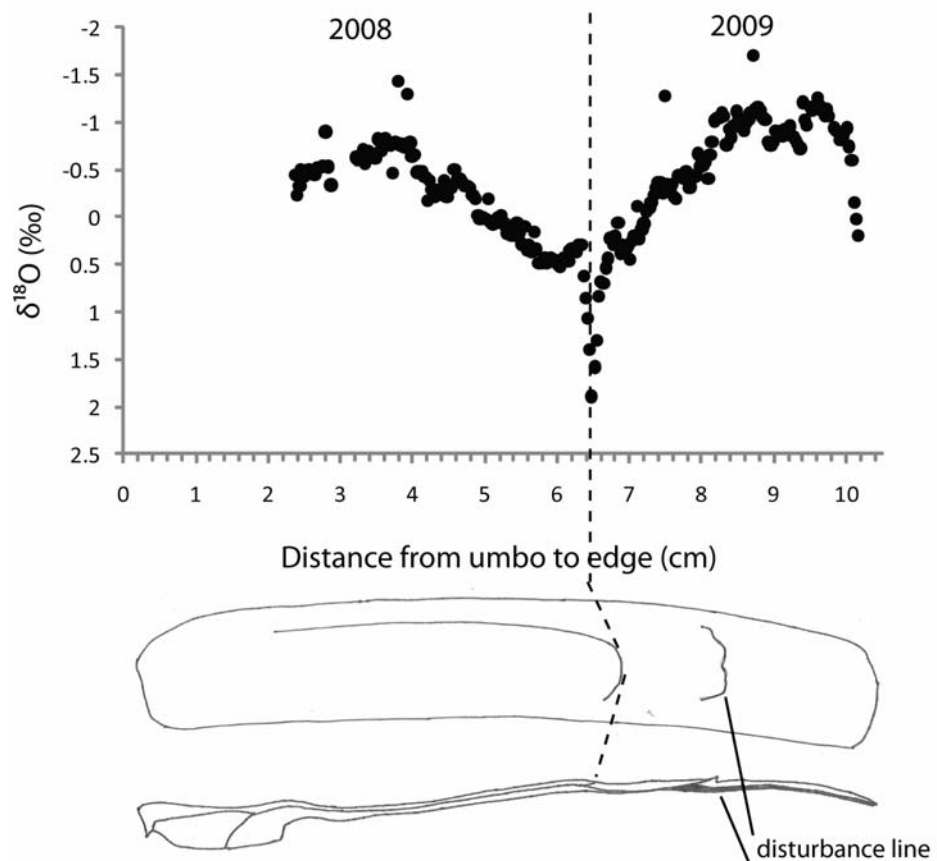


Fig. 8. Oxygen isotope ratios ($\delta^{18}\text{O}$, ‰) along the shell of *E. directus* BWN4 (10.5 cm shell length, collected in April 2010) with drawings of cross-section and valve showing the position of the growth line. Note that the y-axis scale is inverted.

The highest $\delta^{18}\text{O}$ value (corresponding to the winter line) was of 1.88‰ and the lowest values (corresponding to the summer) were -1.44‰ in one year and -1.71‰ in the other year. What was considered a disturbance line corresponded to low isotope values

indicating that it was formed in spring, between January/February (coldest months) and April (collection date), when the shell was already growing. It is therefore not a winter line.

Carbon isotope profile along the valve of BWN4 is seen in Figure 9, in combination with the drawings of valve and acetate peel. This profile does not seem to show any clear pattern as there are several periods of decrease in carbon isotopes which do not show any straightforward relationship with the growth line. The lowest isotopic value occurred at about 2.5 cm shell length, increasing gradually up to about 6 cm, after which values stayed relatively constant except for two low isotopic values at around 6.5 and 8.5 cm shell length.

A significant but very weak positive relationship was found between $\delta^{13}\text{C}_s$ and $\delta^{18}\text{O}_s$ in shell BWN4 (Regression, $r^2 = 0.01$; $p = 0.05$).

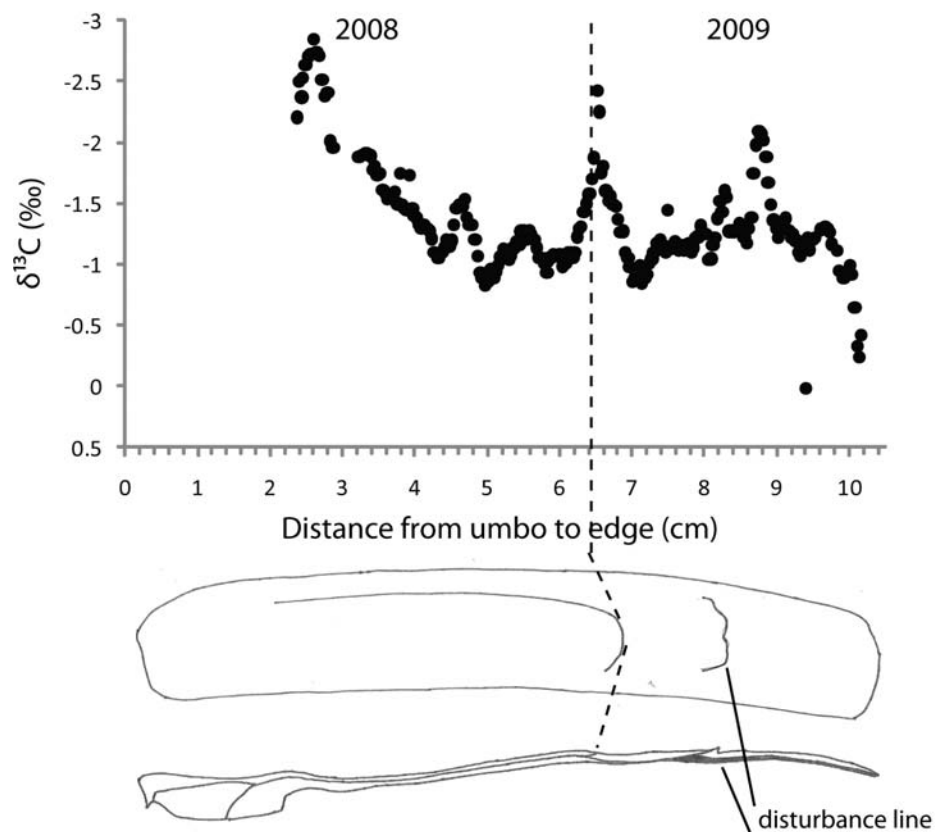


Fig. 9. Carbon isotope ratios ($\delta^{13}\text{C}$, ‰) along the shell of BWN4 (10.5 cm shell length, collected in April 2010) with drawings of cross-section and valve showing the position of the growth line. Note that the y-axis scale is inverted.

1.3.3. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ profiles in shell BWN3

In shell BWN3, 240 lines have been drilled (Fig. 10). Isotope determination was possible for all lines, except for 3 lines which gave unreliable isotope values.

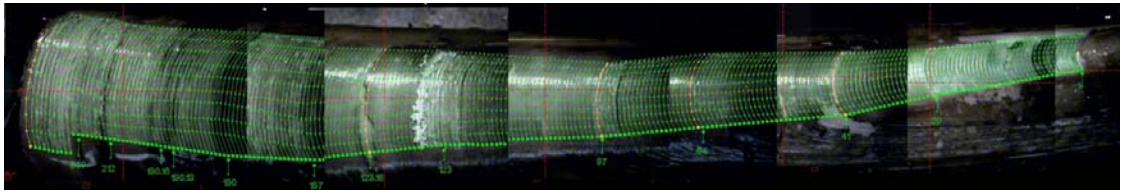


Fig. 10. Lines drilled out on the valve of shell BWN3.

$\delta^{18}\text{O}$ along the right valve of shell BWN3 is seen in Figure 11. There is a clear pattern in $\delta^{18}\text{O}$ along the shell. Samples with high isotopic values (corresponding to low temperatures) were collected at shell lengths of 5.3, 8.5, 9.5 and 10.3 cm. These high values corresponded to areas where a year growth line was seen to occur on the valve and in the cross-section. The isotope results support the idea that these lines were formed during winter.

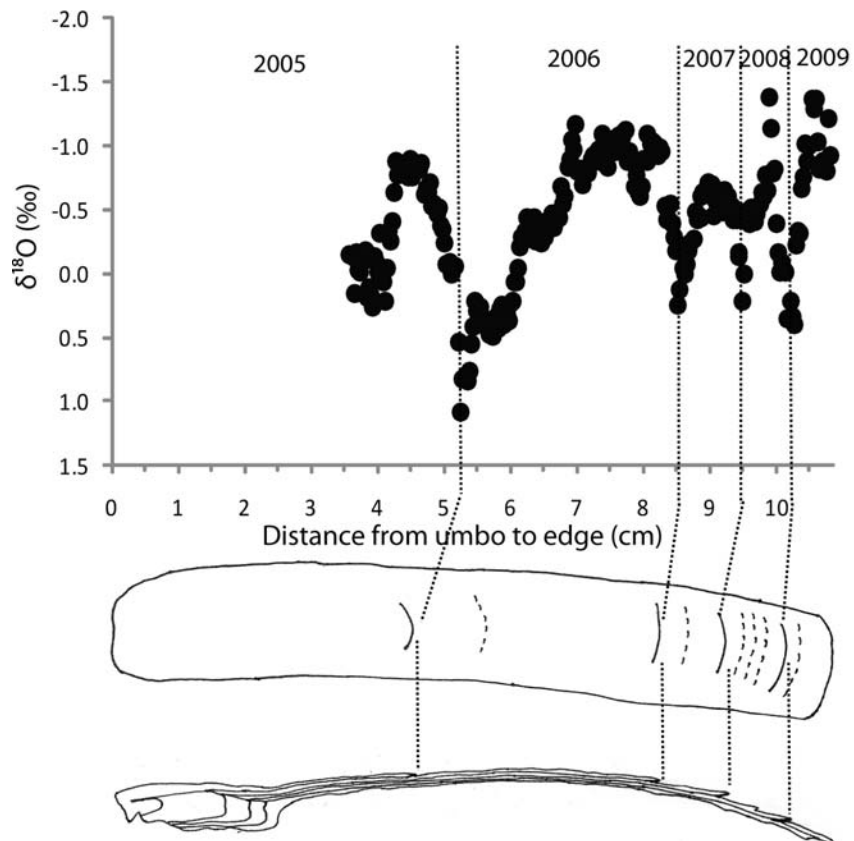


Fig. 11. Oxygen isotope ratios ($\delta^{18}\text{O}$, ‰) along the shell of *E. directus* BWN3 (10.8 cm shell length, collected in April 2010) with drawings of cross-section and valve showing the position of the growth lines considered to be year lines (solid lines). Lines visible on the shell surface which were not considered to be year lines are also shown (broken lines). Note that the y-axis scale is inverted.

BWN3 showed 5 spring/summer periods and 5 autumn/winter periods, although the last one (2009/2010) is not captured in the isotope profile. Since it was collected in spring 2010, back calculating the year of birth indicates that it is a shell from the year class of 2005.

Growth during the year of 2010 is not yet visible in the shell. $\delta^{18}\text{O}$ values varied between 1.1‰ (winter line of the 4th year) -1.4‰ (summer line of the 4th year).

$\delta^{13}\text{C}$ profile along the valve of BWN3 is seen in Figure 12, in combination with the drawings of valve and acetate peel. Although during the first year of life $\delta^{13}\text{C}$ seems to follow a similar pattern as $\delta^{18}\text{O}$, the variability is very large and along the rest of the shell no clear pattern is seen. The lowest $\delta^{13}\text{C}$ value occurred at was -2.5‰ and occurred at a shell length of about 4 cm while the highest value was -0.5‰ and occurred at a size of about 8 cm.

A significant but very weak negative relationship was found between $\delta^{13}\text{C}_s$ and $\delta^{18}\text{O}_s$ in shell BWN3 (Regression, $r^2 = 0.05$; $p < 0.001$).

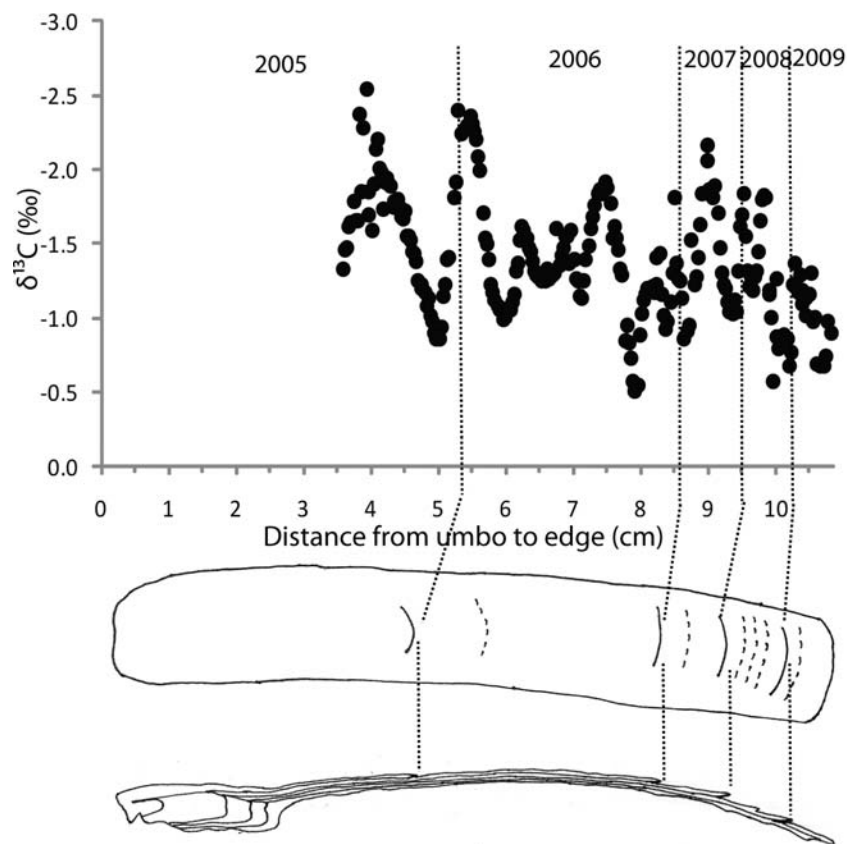


Fig. 12. Carbon isotope ratios ($\delta^{13}\text{C}$, ‰) along the shell of *E. directus* BWN3 (10.8 cm shell length, collected in April 2010) with drawings of cross-section and valve showing the position of the growth lines considered to be year lines (solid lines). Lines visible on the shell surface which were not considered to be year lines are also shown (broken lines). Note that the y-axis scale is inverted.

1.3.4. $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ profiles in shell BWN1

In total, 312 lines have been drilled in shell BWN1 (Fig. 13). Isotope determination was possible for all lines.

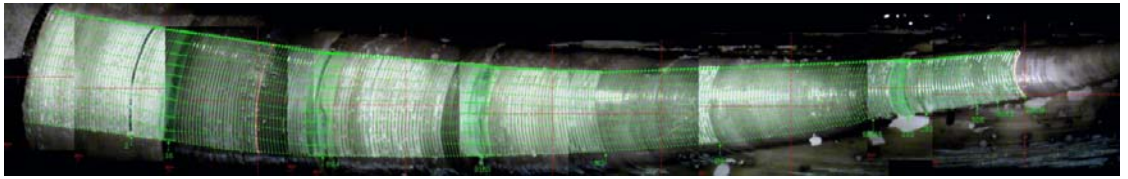


Fig. 13. Lines drilled out on the valve of shell BWN1.

Similar to the other shells, there is a clear pattern in $\delta^{18}\text{O}$ along the shell BWN1 (Fig. 14). Samples with high isotopic values corresponded to areas where a year growth line occurred and were collected at shell lengths of 5.1, 9.2, 10.8 and 12.3 cm. High values are also observed at the shell edge, just before sampling date, and therefore, the last isotope cycle must be from 2009.

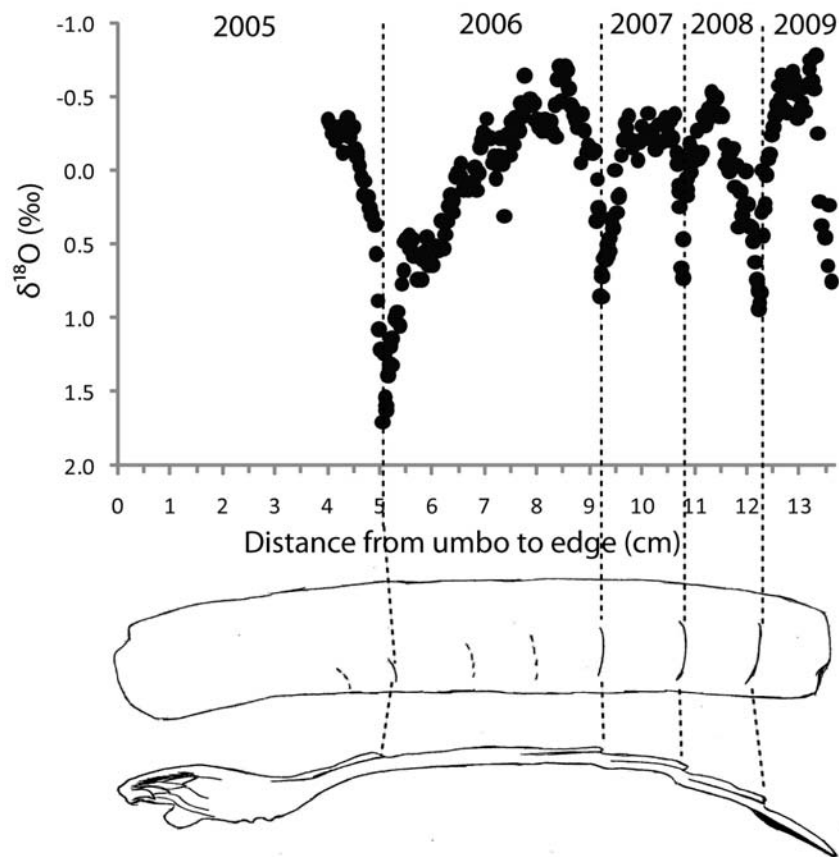


Fig. 14. Oxygen isotope ratios ($\delta^{18}\text{O}$, ‰) along the shell of *E. directus* BWN1 (13.7 cm shell length, collected in April 2010) with drawings of cross-section and valve showing the position of the growth lines considered to be year lines (solid lines). Lines visible on the shell surface which were not considered to be year lines are also shown (broken lines). Note that the y-axis scale is inverted.

At the shell edge, a small darker area could also be seen by eye on the surface of the valve confirming that there is a growth line at the shell edge. Since high isotope values correspond to low temperatures, results suggest that year growth lines were formed during winter.

BWN1 showed 5 spring/summer periods and 4 autumn/winter periods. Since it was collected in spring 2010, back calculating the year of birth indicates that it is a shell from the year class of 2005. $\delta^{18}\text{O}$ values varied between 1.7‰ (1st winter line) -0.8‰ (last summer). Other deeper lines observed on the valve were confirmed to be disturbance lines since they were not the result of growth stop in winter but they were formed in spring and summer.

$\delta^{13}\text{C}$ profile of shell BWN1 is shown in Figure 15. In three of the years, low values of $\delta^{13}\text{C}$ appear to occur just after an annual growth line is observed. However, this does not seem the case in the last year and. Besides variability is quite large and two to three cycles of high and low values are seen within each year of growth. The lowest $\delta^{13}\text{C}$ value (-2.3‰) occurred at shell lengths of 5.1 and 9.5 cm, while the highest value (-0.12‰) occurred just before collection date at a length of about 13.5 cm.

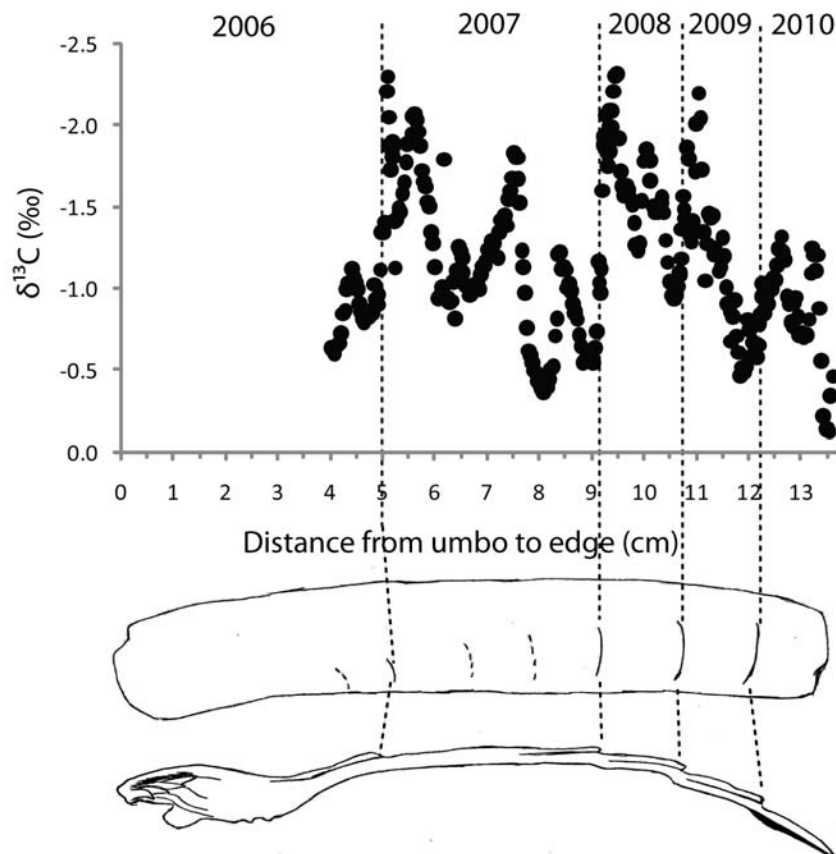


Fig. 15. Carbon isotope ratios ($\delta^{13}\text{C}$, ‰) along the shell of *E. directus* BWN1 (13.7 cm shell length, collected in April 2010) with drawings of cross-section and valve showing the position of the growth lines considered to be year lines (solid lines). Lines visible on the shell surface which were not considered to be year lines are also shown (broken lines). Note that the y-axis scale is inverted.

A significant but very weak negative relationship was found between $\delta^{13}\text{C}_s$ and $\delta^{18}\text{O}_s$ in shell BWN1 (Regression, $r^2 = 0.01$; $p < 0.001$).

1.4. Discussion

$\delta^{18}\text{O}$ along the shell of *E. directus* showed periods of low values followed by periods of steep increase, suggesting seasonal variation. The most positive $\delta^{18}\text{O}_s$ values coincided with the growth lines considered as annual on the surface of the valve and in the cross-section. Therefore, these lines were considered to be annual growth lines formed due to periods of growth cessation. These growth cessations cause the narrow positive peaks in the isotopic record. Because high $\delta^{18}\text{O}$ values correspond to low water temperatures, annual growth lines in the analysed *E. directus* indicate winter growth cessation. Since annual lines in the external surface of the valve were confirmed to be formed yearly (by isotope results), we can conclude that counting the annual lines on the valve leads to an accurate estimation of age and growth of an individual. Age and Von Bertalanffy growth rates are important life history characteristics used to compare output results of DEB modelling with field data.

Being able to determine individual age from the analysis of the growth lines on the shell is a fast and non-destructive method to further estimate growth rates of populations in the field. However, reading the number of annual lines on the surface of the valve may not be as easy as it seems. In this study, we have considered annual lines dark and well-marked lines which could be followed along the shell. Analysing and counting annual lines should be done by an observer with experience in bivalve growth and analysis of shell lines, to be sure that annual lines are identified in a systematic and realistic way. Analysing shells collected along the year may help identifying annual lines as at a certain moment in the year (after winter, when the shell starts to grow again) a line should be seen to appear near the shell edge. Seeing how this line looks like may help identifying other lines as being annual or not.

1.4.1. $\delta^{18}\text{O}$

Overall, results showed that there is a relationship between stable oxygen isotope ratios and the formation of growth lines in *E. directus*. Such relationship has also been found in other bivalve species such as the sea scallop, *Placopecten magellanicus* (Krantz et al. 1984), the ocean quahog *Artica islandica* (Witbaard et al. 1994) and the Antarctic bivalve *Laternula elliptica* (Brey and Mackensen 1997).

Nearby the sampling location, a distinct annual temperature cycle is observed, with mean temperatures fluctuating between 5 °C in February and 19 °C in August. Salinity variations are minimal, varying between 31.4‰ and 32.8‰, thus not exceeding 1.4‰ variability (Fig. 16).

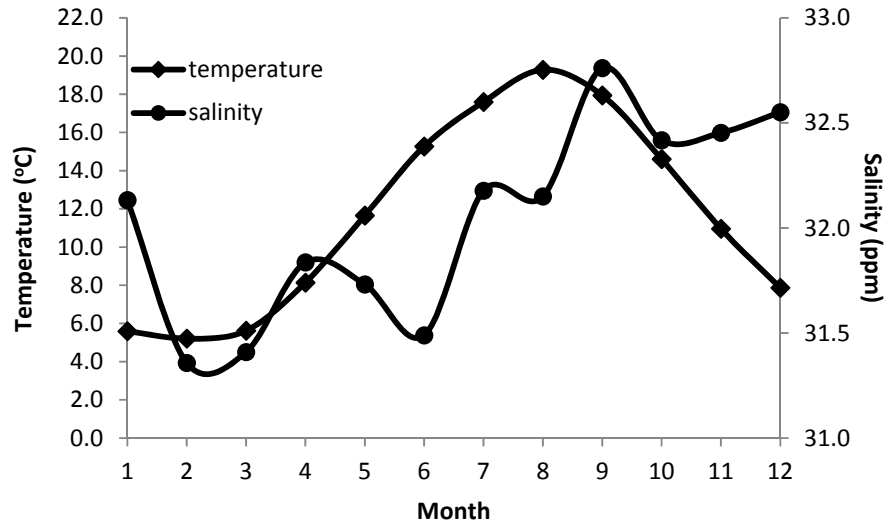


Fig. 16. Mean water temperature (°C) and salinity (ppm) per month (Jan-Dec) off the coast of Noordwijk from 2000-2006 (obtained in <http://live.waterbase.nl/>).

The expected maximum annual variation in shell $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{shell}}$) can be calculated using the equation for biogenic aragonite by Grossman and Ku (1986):

$$1000 \ln(a) = 2.559 (10^6 T^{-2}) + 0.715 \quad [1]$$

where T is temperature in Kelvin and a is the fractionation factor between water and aragonite described by the equation:

$$a = (1000 + \delta^{18}\text{O}_{\text{Aragonite}}) / (1000 + \delta^{18}\text{O}_{\text{water}}) \quad [2]$$

whereby $\delta^{18}\text{O}_{\text{Aragonite}}$ is the $\delta^{18}\text{O}$ of the shell ($\delta^{18}\text{O}_{\text{shell}}$) (VSMOW) and $\delta^{18}\text{O}_{\text{water}}$ is the $\delta^{18}\text{O}$ of the seawater (VSMOW). Because $\delta^{18}\text{O}_{\text{shell}}$ values are usually reported relative to VPDB (Vienna Pee Dee belemnite), $\delta^{18}\text{O}_{\text{shell}}$ values calculated in terms of VSMOW (Vienna Standard Mean Ocean Water) are converted to VPDB using the equation of Gonfiantini et al. (1995).

Using the empirical relationship between water $\delta^{18}\text{O}$ ($\delta^{18}\text{O}_{\text{water}}$) and salinity developed for North Atlantic waters (Ganssen unpublished in Witbaard et al. 1994)

$$\delta^{18}\text{O}_{\text{water}} = -14.555 + 0.417 * \text{salinity}, \quad [3]$$

the expected range of $\delta^{18}\text{O}_{\text{water}}$ values during the year due to the variation in salinity can be calculated for the sampled area.

Based on eq. [3], $\delta^{18}\text{O}_{\text{water}}$ is expected to vary between -0.89‰ and -1.48‰ with an average of -1.19‰. At a constant temperature, this salinity variation is responsible for a 0.59‰ variation in $\delta^{18}\text{O}_{\text{shell}}$ (based on eq. [1]). While the expected annual variation in $\delta^{18}\text{O}_{\text{shell}}$ due to the temperature differences observed in the studied area would be 3.1‰. The

observed maximum variation is between 3.5‰ in shell BWN4 and 2.5‰ in shells BWN3 and BWN1. The fact that in shells BWN3 and BWN1 maximum variation is lower than expected suggests that that carbonate is deposited during only a fraction of the annual temperature cycle. Higher observed variation than expected in shell BWN4 could be due to the fact that only mean temperature values are used or that temperature data are not representative of the location where this specimen was collected. Nevertheless, expected and observed variation values are very similar.

The predicted water temperatures ($T_{\delta^{18}O}$) during shell growth can be estimated based on the measured $\delta^{18}O$ values in the shell and on the observed $\delta^{18}O$ in the water. $T_{\delta^{18}O}$ were derived using eq. [1] solved for temperature, using the adjustment of Dettman et al. (1999) for correcting water $\delta^{18}O$ VSMOW values to the VPDB scale:

$$T_{\delta^{18}O} = 20.6 - 4.34 (\delta^{18}O_{shell} - (\delta^{18}O_{water} - 0.27)), \quad [1]$$

where $T_{\delta^{18}O}$ (°C) is the predicted water temperature, $\delta^{18}O_{shell}$ (‰) is the $\delta^{18}O$ measured on the shell carbonate material and $\delta^{18}O_{water}$ (‰) is the $\delta^{18}O$ in the water where the shell grew.

Because, at the moment, it was only possible to retrieve mean monthly salinity data, only the minimum and maximum predicted temperatures may be estimated and not a predicted profile of temperatures along the shell. To be able to predict water temperatures during shell growth $\delta^{18}O$ in the water must be calculated first. This average water $\delta^{18}O$ was then used to estimate predicted minimum and maximum temperatures experienced by the animal in the field.

In shell BWN4, the mean minimum $\delta^{18}O_{shell}$ value (considering the 2 years of growth) was around -1.5‰, while the maximum was 1.9‰. The reconstructed water temperatures using these values (eq. [1]) result in a mean maximum temperature of 21.0°C and a minimum temperature of 6.1°C. For shell BWN3, mean reconstructed temperatures were between 12.2 and 19.0°C (mean minimum and maximum $\delta^{18}O_{shell}$ values of -2.38 and 0.48‰, respectively). For shell BWN1, mean minimum and maximum $\delta^{18}O_{shell}$ values were respectively -0.6 and 1.03‰ and reconstructed temperatures between 9.8 and 16.8°C. Results are presented in Table 1.

Table 1. Minimum and maximum measured $\delta^{18}O_{shell}$ in each analysed *E. directus* and respective minimum and maximum predicted temperatures experienced during shell growth.

Shell	Minimum $\delta^{18}O_{shell}$ (‰)	Maximum $\delta^{18}O_{shell}$ (‰)	Minimum Temperature (°C)	Maximum Temperature (°C)
BWN4	-1.5	1.9	6.1	21.0
BWN3	-2.4	0.5	12.2	19.0
BWN1	-0.6	1.0	9.8	16.8

The mean temperature cycle (2000-2006) varied between 5.2°C in February and 19.3°C in August (Fig. 16). Maximum predicted temperatures in shells BWN4 and BWN3 are similar to the observed mean maximum temperature. However, mean maximum temperature retrieved from shell BWN1 is lower than the observed temperature (16.8 vs. 19.3°C). Shell BWN1 was collected in a different location, within the same area, which may have different environmental properties (in terms of depth and water current) than the location where the other shells were collected (see Fig. 1). Therefore, animals may have been exposed to different temperature or salinity cycles.

The fact that in all shells minimum predicted temperatures are higher than observed suggests that carbonate is deposited during only a fraction of the annual temperature cycle. However, this minimum predicted temperature varied between shells and different years. Growth stop during winter may be influenced not only by temperature but also by food availability (Wefer 1985, Wefer and Berger 1991) and the combination of these two aspects may lead to year-to-year differences in growing period. The lowest reconstructed minimum temperature was 6.1°C, corresponding to the months March/April (Fig.16), suggesting that shell started to grow only after this period. At this time in the year, gonadal tissue is at its maximum and spawning takes place, after which growth in somatic tissue occurs (Cardoso et al. 2009a). This suggests that reproductive cycle may also influence the start of shell growth.

In two of the studied shells (BWN4 and BWN1), an increase in $\delta^{18}\text{O}_{\text{shell}}$ values was observed at the growing edge of the shell (just before sampling date in April). This supports the idea that shells growth in the area starts only in beginning of spring (March/April) and the high values observed at the edge are part of the annual growth line of the previous winter. In shell BWN1, a small dark area is visible at the edge supporting the existence of a growth line. However, since the shell edge is quite thin, the growing edge may be damaged and growth lines at the edge may be missed and will not be retrieved in isotope profiles. This is the case of the other two shells. This aspect must be considered when ageing shells by taking in account the collection date and the period of growth.

1.4.2. $\delta^{13}\text{C}$

The interpretation of $\delta^{13}\text{C}$ is much more difficult than for $\delta^{18}\text{O}$ mainly because the effect of temperature on $\delta^{13}\text{C}$ values of aragonitic carbonate is uncertain. It seems that temperature has a small effect or even no effect at all on $\delta^{13}\text{C}$ values (Kalish 1991, Romanek et al. 1992) and that metabolism is much more important in determining variation in $\delta^{13}\text{C}$ values (Wefer 1985, Wefer and Berger 1991). However, since metabolism is related to temperature and food, which also vary in an annual cycle, a seasonal pattern for this isotope could be expected as well. Because an increased metabolism leads to more negative $\delta^{13}\text{C}$ in the shell (Lorrain et al. 2004), higher metabolic rates from either spawning or seasonally increased growth (caused by an increase in temperature and food supply) would also result in a more negative shell $\delta^{13}\text{C}$. Therefore, similarly as observed for $\delta^{18}\text{O}$, high $\delta^{13}\text{C}$ values would correspond to lower temperatures. $\delta^{13}\text{C}$ ratios in the analysed shells did not show clear

patterns. Results of $\delta^{13}\text{C}$ values in bivalve shells are very variable. In some species, such as *Lampsilis cardium*, *A. islandica* and *Crassostrea virginica*, profiles follow a more or less sinusoidal trend similar to the seasonal variation of $\delta^{18}\text{O}$, suggesting that seasonal factors influence variation in $\delta^{13}\text{C}$ (Witbaard et al. 1994, Surge et al. 2001, Goewert et al. 2007). Others do not show clear patterns and any apparent seasonality is lacking, as observed in *Spisula sachalinensis* and *Mactra chinensis* (Khim et al. 2000). For *E. directus*, $\delta^{13}\text{C}$ ratios do not seem a suitable tool to validate growth lines.

1.5. Conclusions

Oxygen isotope ratios confirm that some of the lines observed on the surface and in the cross-section of the valve of *E. directus* are formed during winter and can be considered annual growth lines. By visual analysis of the lines in the valve and cross-section, annual growth lines are marked lines which can be followed from the hinge or chondrophore to the ventral margin. If such lines are easily distinguishable by eye, then counting the growth lines on the valve is an accurate method to determine age in *E. directus*.

In addition, daily temperature data from the area where the shells were sampled is available and will be retrieved in the near future. Temperature and isotope profiles can then be matched in order to add a detailed calendar axis to $\delta^{18}\text{O}_{\text{shell}}$ values. Since the distance between sampled lines on the shell is known, knowing the time frame will also allow an estimation of growth rates.

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2. Review of published and unpublished data on growth, reproduction and physiology of *Ensis directus*

2.1. Introduction

The American razor clam *Ensis directus* (Conrad, 1843) (also known as *E. americanus* Binney, 1870) is a suspension-feeding bivalve, common along the Atlantic coast of North America, from Labrador (in Canada) to Florida (Abbot and Morris 2001). In European waters, it was first observed near the German North Sea coast in 1979 and it is thought to have been introduced in Europe shortly before by larval transport in ballast waters of ships that crossed the Atlantic (Von Cosel et al. 1982). Since then, *E. directus* has spread along the Wadden Sea and North Sea coasts, and is now found from France to Norway (including Britain) and the west coast of Sweden (Fig. 17; Beukema and Dekker 1995 and references therein, Hopkins 2001, Minchin and Eno 2002, Dauvin et al. 2007).



Fig. 17. Distribution and spreading of *E. directus* in European waters (obtained from VLIZ Alien Species Consortium 2008).

Both in its native area as well as in Europe, *E. directus* occupies low intertidal and shallow subtidal areas (Stanley 1970, Swennen et al. 1985, Beukema and Dekker 1995) although it also occurs in deeper subtidal areas (Von Cosel et al. 1982, Dörjes 1992, Kenchington et al. 1998, Armonies and Reise 1999, Daan and Mulder 2006, Dauvin et al. 2007). The species shows a patchy distribution and appears to prefer sandy sediments with low silt content but it can also be found in mud and gravel (Stanley 1970, Von Cosel et al. 1982, Dörjes 1992, Kenchington et al. 1998). It is most abundant in areas exposed to winds and currents, characterized by mobile and turbulent sediments (Drew 1907, Stanley 1970, Kenchington et al. 1998, Beukema and Dekker 1995). Such areas are usually poorly occupied by native macrozoobenthos (Beukema 1976, 1988; Swennen et al. 1985) and, therefore, may present an "empty" niche for *E. directus*.

In Dutch waters, the first well identified *E. directus* specimen was found in the Ems estuary in 1981 (Essink 1985), and by 1982 the species had spread to the western Dutch Wadden Sea (Beukema and Dekker 1995, Essink 1985). In 1984/85, the species was observed along the Dutch North Sea coast (Luczak et al. 1993). Rapid and successful invasion took place despite variable recruitment. High overwintering mortality and events of mass mortality are common phenomena in *E. directus* (Mühlenhardt-Siegel et al. 1983, Beukema and Dekker 1995, Cadée et al. 1994, Armonies and Reise 1999). Nevertheless, due to the high reproductive capacity, *E. directus* has managed to build up a strong population in Dutch waters over the last decades (Dekker and Waasdorp 2007, Perdon and Goudswaard 2007).

In this task, we aim to review available literature on growth, reproduction and physiology of *E. directus* necessary for the estimation of DEB model parameters or for comparison of model predictions with field observations.

2.2. Reproductive cycle

Not much information is available on the reproduction of *E. directus*. In the Dutch Wadden Sea, gametogenesis starts early in the year (January/February), along with the increase in seawater temperature, and reaches a maximum in spring (around April) (Cardoso et al. 2009a). In autumn (September-November) the amount of gonads is close to 0 (Cardoso et al. 2009a). Sexes are separate and fertilization occurs in the water column. In a field study in 2001-2003, spawning occurred in April/May and individuals with developed gonads were on their second year of life (Cardoso et al. 2009a). The youngest individual with developing gonads (i.e. that reached puberty) was 1+ years old (Cardoso, unpubl. data). Since it was collected on the 9th May 2002, age in days was estimated to be 492 days, considering that birth was on the 1st of January (in this case in 2001). Considering the observation in the field in 2001-2003, when spawning occurred in April/May, this value can be corrected to a spawning date of 15th April, meaning that age would then be around 388 days. This individual was 48.41 mm shell length and 86.22 mg ash-free dry weight (Cardoso, unpubl. data). In the German Bight, spawning occurred in the early eighties in March/April (Muhlenhardt-Siegel et al. 1983).

In the Wadden Sea, settlement of the larvae was seen to occur around May/June (Beukema and Dekker 1995), after a pelagic phase of about 1 month (Loosanoff and Davies 1963). Although in the northern German Wadden Sea Armonies (1996) registered several temporal pulses of spatfall, in the Dutch Wadden Sea two spawning periods were seen in 2002, a strong one in April/May and a weak one in August/September (Cardoso et al. 2009a). No other reports on *E. directus* spawning cycle in the Dutch Wadden Sea were found in literature. Studies on razor clams from other locations, describe patterns which vary between one spring event in *E. siliqua*, *Solen marginatus* and *E. directus* (Gaspar and Monteiro 1998, Kenchington et al. 1998, Fahy and Gaffney 2001, Remacha-Triviño and Anadón 2006), two spawning events in *E. macha* (Barón et al. 2004) and several spawning events over a long period in *E. arcuatus* (Darriba et al. 2004). Reproductive investment in terms of gonadal mass (for both males and females) in the Dutch Wadden Sea appeared to be only of 2.5% of gonads in relation to the

total body mass (Cardoso et al. 2009a). If only individuals with developed gonads are included this value slightly increases to 4 % (Cardoso et al. 2009a). Nevertheless, reproductive investment in *E. directus* in the Dutch Wadden Sea is much lower than the described for the razor clam *E. arcuatus* in Spain, in which about 1/3 of the body consisted of gonads during the reproductive period (Darriba et al. 2005). Growth and spatfall of most bivalve species living in the subtidal western Wadden Sea in 2002 were very low (e.g. *Macoma balthica* 8 ind.m⁻² in 2001 vs. 3 ind.m⁻² in 2002, *Cerastoderma edule* 704 ind.m⁻² in 2001 vs. 38 ind.m⁻² in 2002, *Mya arenaria* 87 ind.m⁻² in 2001 vs. 10 ind.m⁻² in 2002 and *E. directus* 10 ind.m⁻² in 2001 vs. 0 ind.m⁻² in 2002; Dekker et al. 2002, 2003), suggesting that the low reproductive investment of *E. directus* could have been the result of unfavourable environmental conditions in that year. If the weight of gonadal mass per volume shell is compared with data from other bivalve species of the Dutch Wadden Sea, values for *E. directus* are still very low. For the subtidal area, highest mean gonadal mass was around 0.02 mg ash-free dry mass (AFDM) per cm⁻³ shell in *E. directus* while in *Macoma balthica* this value was about 2.3 mg AFDM.cm⁻³ and in *Cerastoderma edule* and *Mya arenaria* around 0.8 mg AFDM.cm⁻³ (Cardoso et al. 2007; Cardoso et al. 2009a,b). As stated above, reproductive investment of *E. directus* was around 2.5% (gonadal ash-free dry mass relative to the total body ash-free dry mass) while in subtidal *M. arenaria* and *C. edule* it was around 12 and 10% respectively (Cardoso et al. 2009b)

Data on the reproductive cycle which are important for the DEB model are presented in Table 3.

2.3. Eggs and larval stage

During spawning, eggs (about 64-70 µm in diameter; Loosanoff and Davies 1963, Kenchington et al. 1998) and sperm are released into the water where fertilization occurs. In an experimental set up, D-stage (i.e. hatch, which is considered in the DEB model as moment of birth) occurred on average at a size of 136 µm after 5 days at 18 °C (Kenchington et al. 1998). Larva reached the pediveliger stage and were ready to settle after 15-16 days at a size of about 245 µm (Kenchington et al. 1998). However, Loosanoff and Davies (1963) describe a larval stage of 1 month (at 15°C). A compilation of data on larval stage, important for the DEB model, is presented in Table 3.

2.4. Growth

Maximum age reported in literature for *E. directus* was 7 years (i.e. life span was around 2555 days) (Armonies and Reise 1999), however, most individuals do not become older than 2-4 years (Armonies en Reise 1999, Wijsman et al. 2006). In the intertidal and subtidal western Wadden Sea, the maximum observed age was 6 years old, while in the coastal area the maximum observed age was 4 (Daan and Mulder 2006, Cardoso unpubl. data, Dekker unpubl. data). In other razor clams, especially *E. siliqua* is known to reach much higher ages of 19-25 years old (Fahy and Gaffney 2001, McKay 1992).

Maximum observed size was 17.1 cm in the intertidal of the western Wadden Sea (Dekker unpubl. data). The maximum size reported in Europe was 18.6 cm in German waters (corresponding to the 7 year-old individual, Armonies and Reise 1999) but the species can reach more than 20 cm in the western Atlantic coast (Kenchington et al. 1998). According to Lambert (1994), *E. directus* reaches a size of about 80 mm in two years and after 5 years it has around 150 mm. In the Dutch Wadden Sea, maximum observed weight was 1831 mg ash-free dry mass (Cardoso personal observation).

Unpublished data on growth of *E. directus* are shown in Figure 18 and Table 2. These data are from animals collected monthly in a subtidal area of the western Dutch Wadden Sea (53° 10' N, 5° 22' E), from November 2001 to January 2003. Note that the Wadden Sea is an area in which bivalves exhibit a reduced growth due to food limitation (Cardoso et al. 2006) and therefore growth of *E. directus* may be lower than described for other areas. Individuals were randomly collected with a 'Reineck' box corer (0.06 m²) in an area of 1–2 km² at a water depth of about 2.5 m. In the laboratory, shell length of each individual was measured to the nearest 0.01 mm total length (range: 8.5–141.0 mm) and age was determined by analysing the external shell year marks.

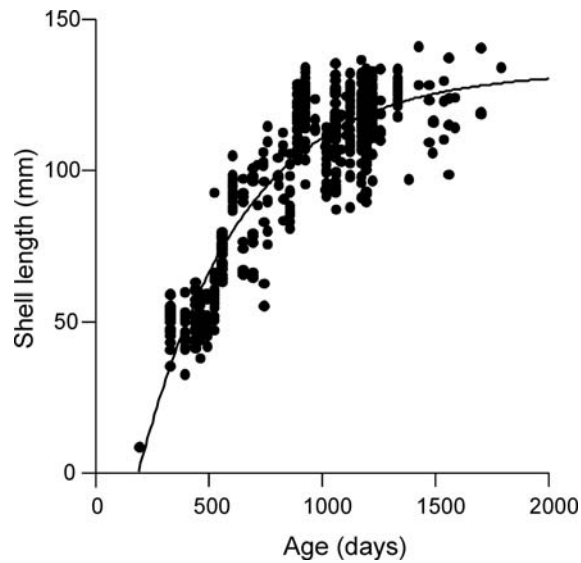


Fig. 18. Growth curve for *E. directus* in the subtidal Wadden Sea from 2001-2003 (Cardoso unpublished data).

A Von Bertalanffy growth (VBG) curve was fitted to this dataset, according to the expression

$$L_t = L_\infty * (1 - e^{-k*(t - t_0)})$$

where L_∞ is the estimated maximum length (mm), k is the growth rate constant (d^{-1}), t is age in days, t_0 is the age at settlement and L_t is the observed length at age t . VBG parameters L_∞ , k and t_0 were iteratively estimated using the software package SYSTAT (Wilkinson 1996). The

estimated VBG parameter L_{∞} (asymptotic length) resulted in a maximum length of 132.8 ± 2.7 mm, a growth rate constant of $0.002 \pm 0.0002 \text{ d}^{-1}$ and a t_0 of 189 days [corresponding to the beginning of June, which fits with the settlement period described by Beukema and Dekker (1995) and Cardoso et al. (2009)].

Table 2. Mean shell length with age for the subtidal Wadden Sea (Cardoso unpubl. data). Time (days) was derived assuming spawning (birth) occurred on the 1st of January.

Time (d)	Shell length (cm)	Time (d)	Shell length (cm)
195	0.86	1017	10.07
329	5.05	1018	10.30
330	4.36	1059	10.65
394	4.73	1060	11.42
441	5.07	1061	9.51
462	4.99	1081	11.77
492	5.27	1082	10.93
493	4.36	1108	11.81
525	6.03	1109	11.45
560	7.21	1124	9.46
604	9.30	1125	11.79
651	9.37	1172	12.03
652	7.31	1174	10.05
693	9.62	1193	11.75
694	7.34	1195	10.36
695	10.08	1223	12.16
696	9.11	1224	10.62
715	9.51	1256	11.78
742	10.35	1335	12.62
743	7.42	1383	9.69
759	8.38	1425	13.47
760	11.20	1473	12.02
807	10.01	1489	10.57
809	9.51	1490	11.60
828	10.89	1537	12.09
830	8.68	1558	12.54
858	10.19	1560	9.87
859	8.70	1588	11.90
891	11.74	1700	12.61
926	11.98	1789	13.39
970	11.59		

Data on maximum life span (maximum observed age), ultimate length (maximum observed length), ultimate weight (maximum observed weight) and Von Bertalanffy growth rate, which are important for DEB modelling are compiled in Table 3. We use European values

because DEB modelling within this project is focusing in a European population of *E. directus*, and therefore life history characteristics of European populations are more useful for comparing DEB model predictions with field data.

2.5. Physiology

There is hardly any information on physiology of *E. directus* or other razor clams. Witbaard and Kamermans (2009) measured the filtration rate of *E. directus* under different algal concentrations and silt content in the water. Filtration rate varied between 0.4 and 5.3 litres per hour (L/h), corresponding to 0.2 to 3 L/h per gram ash-free dry weight (g AFDW). Algal concentration did not have an effect on the filtration rate but higher silt content in the water column significantly decreased filtration rate. These results are similar to the ones found by Shumway and co-authors (1985), which found a filtration rate of 0.93 L/h/g AFW for *E. directus*.

No data on respiration were found in literature for either *E. directus* or any other razor clam species.

Table 3. Life-history parameters for *E. directus* .

Type	Value	Unit	Remarks
Age at birth	5	d	at 273+18 K, Kenchington et al. , 1998
Age at puberty	492	d	at 273+12 K, Dutch Wadden Sea, Cardoso unpubl. data
Length at birth	0.0136	cm	at 273+18 K, Kenchington et al. , 1998
Length at puberty	4.841	cm	at 273+12 K, Dutch Wadden Sea, Cardoso unpubl.
Ultimate length	18.6	cm	at 273+9.8 K, German Wadden Sea, Armonies and Reise 1999
Dry weight at puberty	0.086	g	at 273+12 K, ash-free dry weight , Dutch Wadden Sea, Cardoso unpubl. data
Ultimate dry weight	1.831	g	at 273+12 K, ash-free dry weight , Dutch Wadden Sea, Cardoso unpubl. data
V. Bertalanffy growth rate	0.002	1/d	at 273+12 K, for an Lmax of 133 mm, Dutch Wadden Sea, Cardoso unpubl. data
Life span	2555	d	at 273+9.8 K, Sylt, German Wadden Sea, Armonies and Reise 1999

2.6. Length-weight relationship of *Ensis directus*

The relation between shell length and weight of shellfish is often described as an allometric relationship

$$W = aL^b$$

where, W is the weight, L is the shell length and a and b are constants.

In this study the length-weight relationship was determined from data of *E. directus* that has been retrieved from the IMARES database. A total of 780 individuals collected in various cruises along the Dutch coast, were selected for analysis. From each individual, shell length (mm) and fresh weight (g) was measured. A power function was fitted to the data using the function `nls()` within the software package R. The results of the parameter estimations for a and b are presented in Table 4.

Table 4. Parameter estimation of the length-weight relationship of *E. directus*. Parameters are estimated by non-linear regression.

	Estimate	Std. Error	t value	Pr (> t)
a	9.010e-06	1.532e-06	5.882	6.06e-09
b	3.099e+00	3.615e-02	85.727	< 2e-16

This resulted in the following power function for the wet weight (g) of *E. directus* as a function of shell length (mm):

$$W = 9 \times 10^{-6} \times L^{3.1}$$

Additionally, we have used a quantile regression in order to fit the power function to the lower part of the data, i.e. the individuals with the lowest amount of gonads and reserves. It can be assumed that the body mass of these individuals consists mainly of structural tissue. The quantile regression has been performed with the function `nlrq()` within R. The value of tau was set to 0.05 indicating that 5 percent of the datapoints were below the resulting curve. The results of the parameter estimations for a and b are presented in 5.

Table 5. Parameter estimation of the length-weight relationship for individuals with structural mass only. Parameters are estimated by non-linear quantile regression.

	Estimate	Std. Error	t value	Pr (> t)
a	2.0e-5	1.0e-5	2.09079	0.03687
b	2.84868	0.11418	24.94816	0.00000

This resulted in the following power function for the fresh weight (g) of *E. directus* as a function of shell length (mm):

$$W = 2.0 \times 10^{-5} \times L^{2.85}$$

According to the DEB theory the relation between the physical volume of an animal and its length is given by:

$$V_w = (\delta_M L_w)^3$$

where V_w is the volume of structural mass, L_w is the shell length and δ_M is the species-specific shape factor.

Besides structural mass (muscle and shell), the total body mass is also composed of reserves (glycogen) and gonads. The latter components are influenced by condition and reproductive stage. Kooijman (2010) has estimated a shape factor of 0.187 for *E. directus*, based on data from Swennen *et al.* (1985). In Figure 19, the length-weight relationship is plotted based on a shape factor of 0.187, assuming a density of 1 g cm^{-3} .

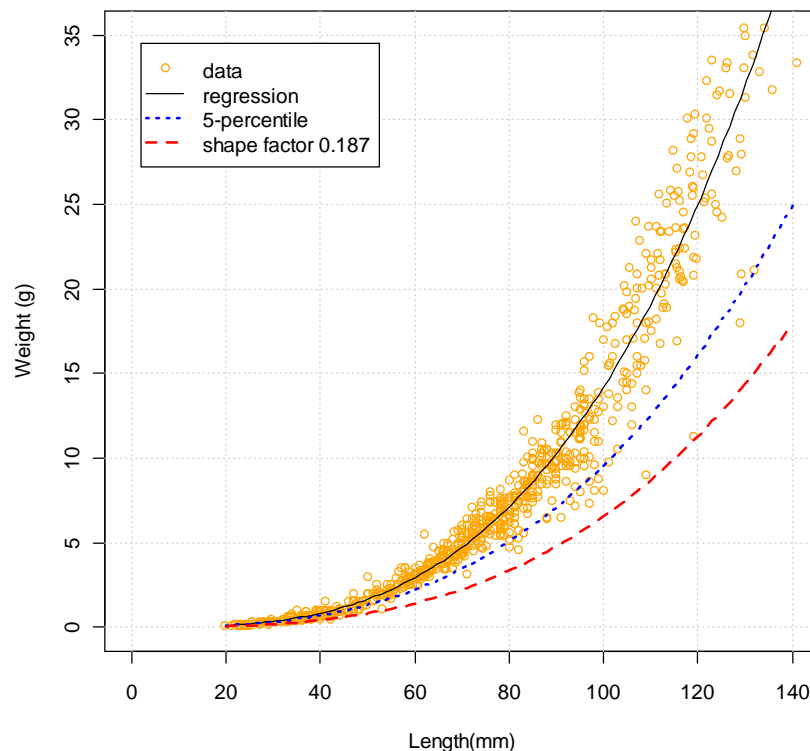


Fig. 19. Length-weight relationship *E. directus*. Dots indicate measured data of 781 individuals. Black solid line represents the fitted power function through the data. The broken blue line indicates the results of the quantile regression. The broken red line is the curve based on a shape factor of 0.187.

As can be seen from the figure, the measured weight of all the organisms is higher than calculated with a shape factor of 0.187. The curve of the shape factor indicates the weight of the structural volume. The rest of the flesh weight is composed of reserves and, to a minor extent of gonads. The shape factor of 0.187 suggests that on average more than 50% of *E. directus* biomass is composed of gonads and reserves.

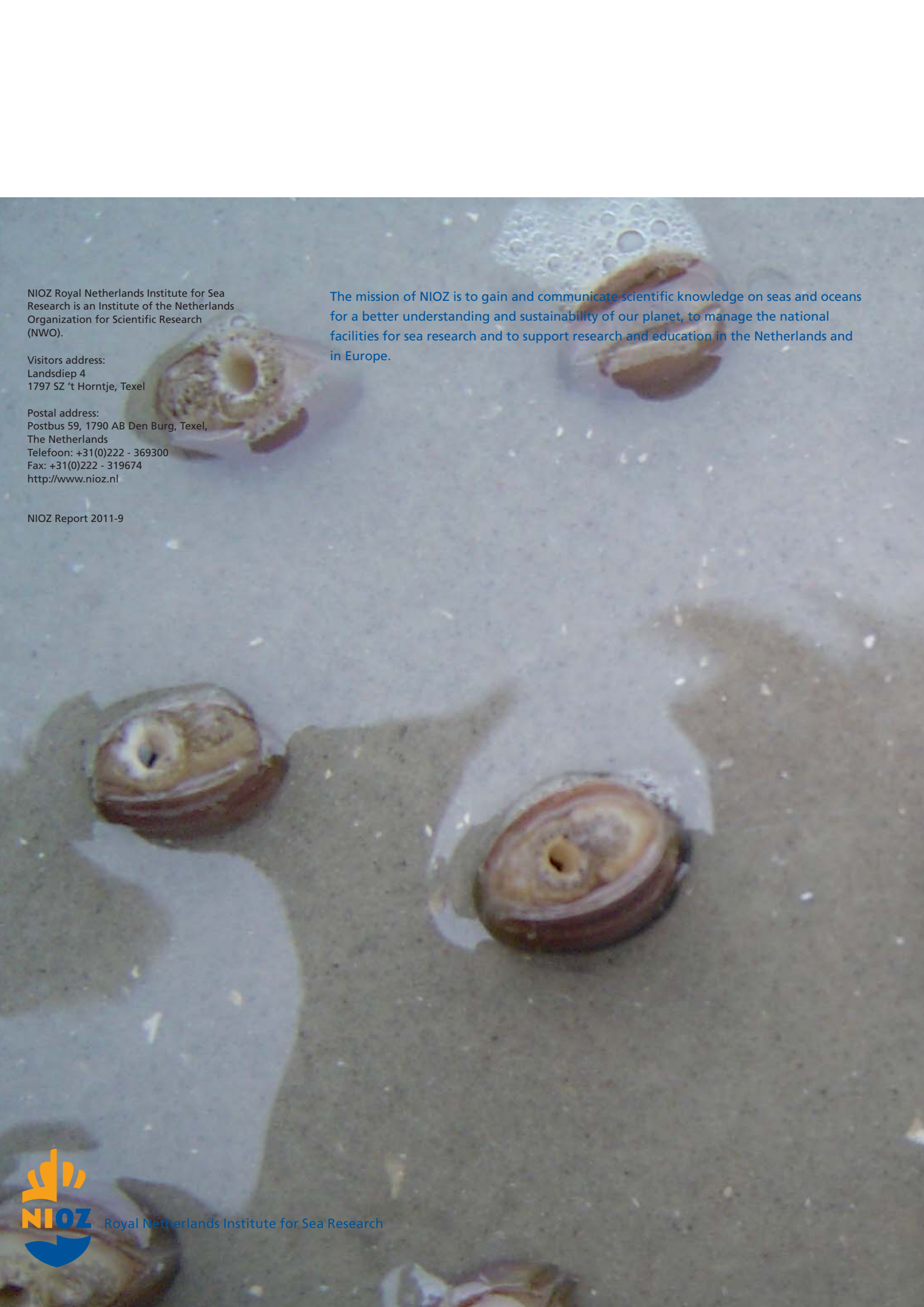
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