



“Age” determination of European flat oysters from the Voordelta reef

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Preface

This report reflects the results which have been obtained in a first attempt to "age" European flat oysters collected from a bed in the Voordelta area in the Dutch part of the North Sea. The practical work took place in November and December 2019. The timeframe within this work had to be accomplished was tight, there was no time to explore different methods of shell processing and growth line visualisation. This report therefore reflects a first estimate and confirmation of the ages of Dutch wild flat oysters and as such gives relevant information about the year in which the oyster bed in the Voordelta was established.

Texel, June 2020

Rob Witbaard, Pauline Kamermans, Oscar Bos.

Introduction

Natura 2000

The Natura 2000 site Voordelta in the Dutch part of the North Sea was established in 2008 (Ministry of LNV, 2008). Seven years later, in 2015, a natural (mixed) reef of European flat oyster (*Ostrea edulis*) and Pacific oyster (*Magallana gigas*) was discovered in this area (Christianen et al., 2018, Sas et al., 2017, 2018, Kamermans et al., 2015). The reef is situated near the north part of the Brouwersdam and is estimated to cover at least 40 ha.

Oysters are regarded as important bio-engineers and the beds (reefs) they can form are supposed to enhance biodiversity (Smyth & Roberts, 2010; Gregalis et al, 2009). In the past European flat oyster beds formed an important habitat of the North Sea ecosystem, but nowadays the species has virtually disappeared. Overfishing but also natural factors such as diseases have played a role (Smaal et al., 2017). Outside the Voordelta, there appears to be a perspective for the establishment of flat oyster (beds) in other parts of the Dutch North Sea (Smaal et al., 2015). Flat oysters (as a species) and oyster beds (as habitats) are listed on the OSPAR list of threatened and/or declining species and habitats (OSPAR Commission, 2008, 2009) and an OSPAR recommendation has been adopted on furthering their protection and conservation (OSPAR Commission, 2013). In addition, targets have been set in the Dutch Marine Strategy Part 1 (Min. IenW and Min. LNV, 2018) for the restoration of European flat oyster beds in the North Sea.

In the Netherlands, European flat oyster beds are however not yet explicitly subject to the Dutch Natura 2000 protection framework under the European Habitat Directive (EC, 1992), at least because they were not previously known to occur in the Dutch part of the North Sea. One option for such protection could be to consider European flat oyster beds as 'biogenic reefs' as part of habitat type H1170. In the Netherlands, the definition of reefs H1170 currently only includes geogenic reefs (Min. EZ, 2014). In a recent overview (Bos et al., 2019), it has become clear that also other reef builders such as Ross worm (*Sabellaria spinulosa*) are present in the Dutch part of the North Sea. Given the ambition for European flat oyster restoration, LNV considers to adapt the current definition of reefs to include biogenic reef builders.

The discovery of the reef in the Voordelta leads to the question whether the oyster bed was possibly already there at the moment of the site designation in 2008. Age estimation of oyster shells collected in the past could provide more insights. The ministry therefore commissioned the Royal NIOZ in collaboration with Wageningen Marine Research to perform an age estimation of oysters collected in 2015 and 2017 from the Voordelta area.

Age estimation

In our temperate marine climate there is a strong seasonal signal in temperature and food availability. In spring when daylight period is lengthening, primary production in the water column starts and water temperatures start increasing. This leads to strong seasonal cycles in the activity of marine invertebrates. In bivalves this leads to seasonal (shell) growth patterns which are recorded in their shells as growth lines or rings (Richardson, 2001). When seasonality is strong and well defined, such growth lines are

often externally visible. In environments which experience less seasonality or experience erratic disturbance events (hypoxia, storms etc.), the regular seasonal shell deposition might be interrupted by the production of disturbance lines, making the interpretation of growth lines difficult especially from the outside of the shells. For the shells used in this study the presence of disturbance lines is a likely option as the location from where they are collected is very shallow and extreme weather events (hot, cold, storms) are easily transferred to the bottom ecosystem.

Other difficulties for the recognition of growth lines can arise from the "complex" shell growth structure. In the European flat oyster (*Ostrea edulis*) external growth lines are obscured by the complex external shell structure and shape, locally changing growth directions, foliation and the presence of ornamentation. Hence for reliable age estimates, other methods, than externally reading of growth lines, are needed to estimate age. An approved method is the application of acetate peels on shell cross sections (Kummel & Raup, 1965). The externally visible growth lines result from structural and compositional differences in the shell carbonate. Externally visible growth lines are the external appearance of internal growth lines which are based on compositional and structural differences within the carbonate material. These structural differences can be visualized in cross-sections and transferred to sheet acetate to study these with a transmitted light microscope. For the age estimation from the oysters which are subject in this study, this technique has been applied.

In scientific literature there are not many studies which deal with the ageing and life span determination of "wild" European flat oysters (*Ostrea edulis*). In 1958 Walne studied oyster growth at the fisheries station in Conway (Wales, UK). He observed that flat oysters were growing between April and early November. Tissue growth appeared not always to run parallel with shell growth. On basis of his experiments he furthermore concluded that the variation in growth is so large that reliable estimates can only be achieved if very large number of specimens ($>>20$) are studied.

Richardson et al (1993) determined the age of *Ostrea edulis* from three locations around the UK. They used umbonal growth lines and found well-defined winter growth lines. Periodicity of the growth lines was established by studying 70 differently sized, cultivated, oysters of known age. This showed that the average number of counted lines matched the expected number when an annual forming periodicity is assumed.

Their detailed description of the appearance of the growth lines shows that the growth lines in the hinge portion of the shell are often associated with a cleft-like structure at the boundary between carbonate and conchiolin ligament (Figure 1).

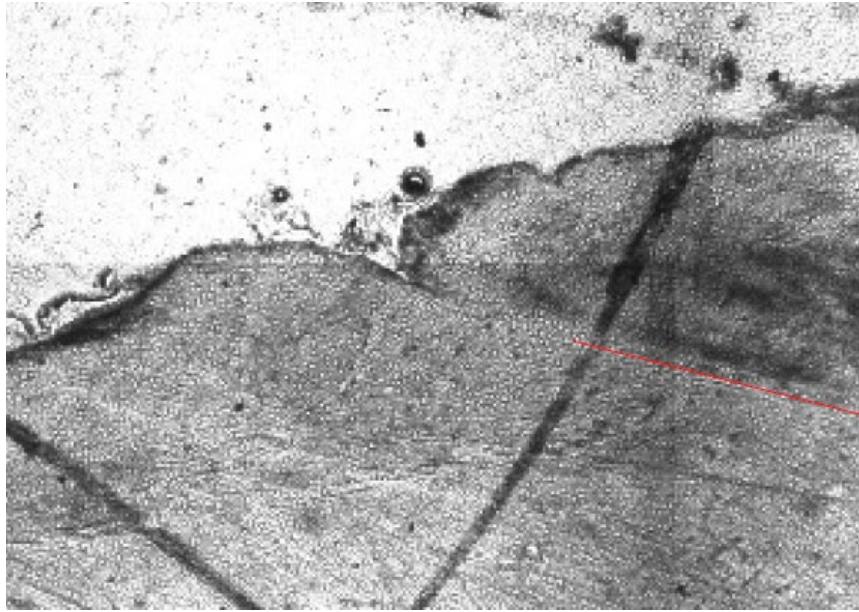


Figure 1. Photograph showing the cleft-like structure in an European flat oyster collected from the Voordelta. This feature was not always as well developed as described in Richardson et al (1993).

The definition of growth lines differs between populations, which is attributed to the differences in environmental forcing. Where Walne (1958) observed that European flat oyster growth ceased in early November, the results of Richardson et al (1993) suggest that, for this species, the growth line is formed between February and March when temperatures are at minimum. On basis of their analyses it is clear that there is a large variation in size at age, implying different growth rates between individuals thus supporting the observations of Walne (1958). Richardson et al (1993) furthermore observed that different populations differ in their age-length relationships. Such spatial differences thus stress the importance to establish the age-size relationship for clearly identifiable European flat oyster populations such as that at the "Voordelta" location.

Material and Methods

Shells of European flat oyster (*Ostrea edulis*) were collected in December 2015 and in April 2017 from the mixed oyster reef (*Ostrea-Magellana*) in the Voordelta (~51.7717°N, ~003.7979°E). Shells were kept frozen until analyses. On November 6th, the largest 12 doublets as subset of the collected specimens were supplied to the sclerochronology lab at NIOZ, with the aim to visualize and count the growth lines.

The soft tissue was already removed on November 4th by WMR. At NIOZ the shells were air dried for further processing. Habitus of all shells has been documented by photographing them externally and internally. From these photographs shell size was measured digitally with the aid of the software program Image-J (<https://imagej.nih.gov/ij/>). From the dried shells the carbonate weights of left and right hand valves have been determined separately.

Growth line visualisation

For growth line visualisation the acetate peel method has been used. This is standard method in bivalve sclerochronology. First a 3-5 mm thick section was extracted by cutting the flat valve on a Buehler isomet slow-speed diamond saw. Each of these sections was embedded in epoxi resin (THV500+harder 125) for reinforcement and to facilitate further processing (Figure 2) and trimmed so that the desired cross-section could be obtained (Figure 3).



Figure 2. The embedded cross-sections of the flat valve, ready for further processing.

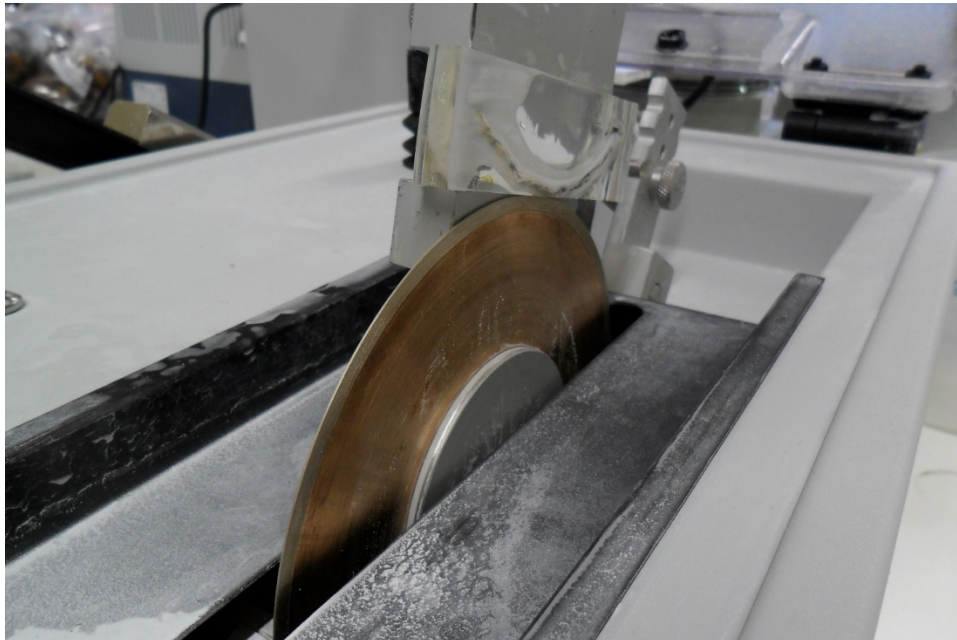


Figure 3. Trimming a cross-section to make it ready for grinding and polishing.

Sections were grinded down to the desired level, with high grade carborundum disks, starting at a coarse grit (320) up to extremely fine grit (4000). Then they were subsequently polished with polycrystalline diamond suspension (3 μ m) and aluminium oxide suspension (0.05 μ m). After polishing the cross-sections were etched in a 1 vol% solution HCl. Etching time varied between 60 seconds and 300 seconds. The etching process dissolves the carbonate rich parts while the organic rich parts (growth lines) remain untouched. This differential etching, creates a micro-topography on the shell cross section. This micro-topography is transferred to sheet acetate by applying a few drops of acetone to "melt" the acetate on the cross section. After evaporation of the acetone the sheet acetate with the transferred micro-topography can be peeled off the cross-sectional surface and clipped so that it fits in between microscopic object glasses. It can then be studied with transmitted light microscopy. Magnifications between 2.5 $\times$ and 10 $\times$ have been used to photograph the cross-section. Photographs were made with a microscope mounted Crc3 Zeiss camera in combination with the axiovision computer program. The Zeiss panorama application was used to stitch individual photographs into a composite photo of the entire shell cross section (Appendix A). From this photograph growth lines were identified and marked (Figure 4). This was always done in combination with the microscopic "live" view, as the resolution of the live view is slightly better than the resolution of the photographs.

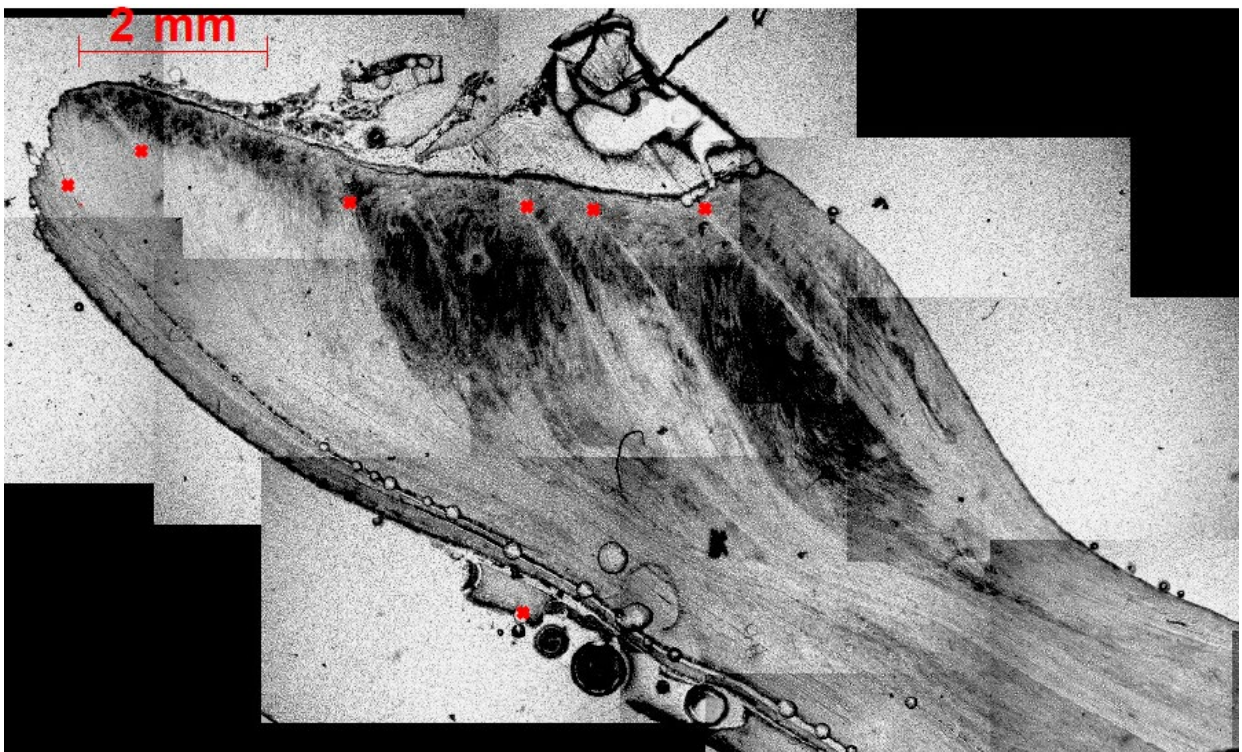


Figure 4. Composite photograph showing the appearance and marked growth lines in the hinge band of the flat valve of *Ostrea edulis* collected from the Voordelta.

Results

In total 12 shells with shell lengths ranging between 77 and 97 mm, were sectioned, photographed and studied (Appendix-A). Growth lines in the flat valve were sometimes poorly defined, hence, different etching times were used. Those hinge lines which could be traced back from hinge to valve were anyhow regarded to be the lines delimiting a growth season. For some lines there is uncertainty and for these, a judgement has been made on basis of expert knowledge.

The clarity of the growth lines differed greatly between specimens. In some shells, growth lines tended to "fan" at the outside of the shell (Figure 5). Such assemble of closely spaced lines are interpreted as the delineation of one growing season in view that the assemble of lines illustrates a intermittently ending and resumption of shell growth at the end of the growing season when the conditions sometimes favor and sometimes (temporally) interrupt shell growth.

Some of the specimens showed well-defined growth lines in the ligament. Close inspection at large magnification (up to 20×) suggests that in the ligament lines are visible which are formed with periodicities other than annual. For instance, in specimen 009 the ligament produced 31 very clear lines in the ligament, while in the valve only between 9-11 rings were counted. The fine lines in the ligament were also observed by Richardson et al (1993) and they regarded them as lines with a higher depositional frequency. Huyghe, (2019) was able to prove that such high frequency lines in Pacific oyster (*Magallana gigas*) were tidally in nature for non-juvenile specimens. Specimens of the same species, permanently immersed appear to have strong tidally-linked activity patterns (Tran et al 2011) and on basis of these observations, the very fine growth lines in the ligament might reflect this tidal component. In this study these thin, high frequency growth lines were not counted as being an annual growth line.

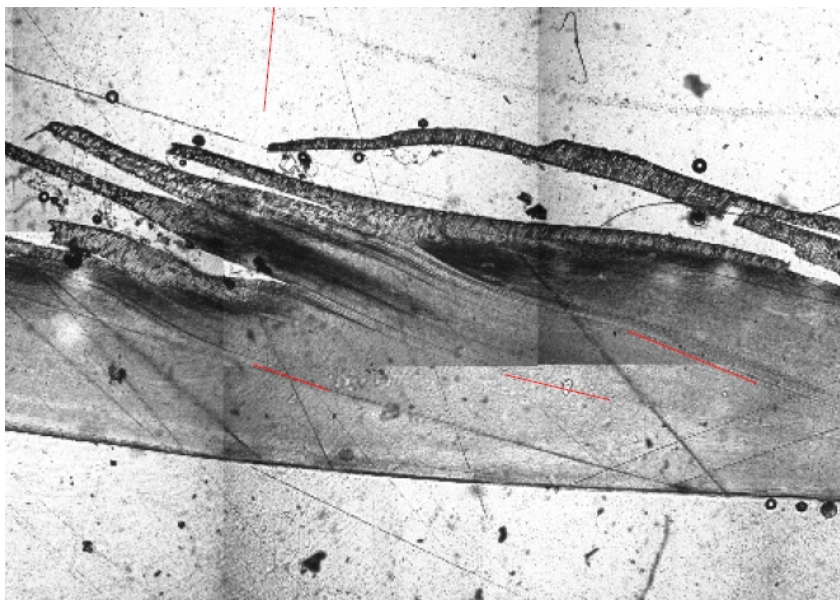


Figure 5. Example of growth lines in the valve. Very close to each other a high number of lines runs from the internal shell layers to the outside. Such clusters of lines are regarded as one growth interruption and possibly indicate an intermittent shell growth at the end of the season.

An overview of the line counts of all shells is given in table 1. The number of observed lines varied between 5 and 14, suggesting that the oysters had ages between 5 and 14 years old. Figure 6 shows that oysters with more lines tend to be larger. Oyster #006 which was collected in December 2015 appeared

to have the largest number of lines (14) and the largest total carbonate weight. In comparison to the other shells collected in 2015 it indeed had a very thick shell. This supports the idea that it is a relative old individual as older shells tend to get relatively heavier shells (Sheldon, 1967). Carbonate deposition along the growing margin becomes less, but the amount of carbonate deposited at the inner shell surface remains the same. Thus, per mm length growth relatively more carbonate is deposited. Older shells become thicker and heavier, without significantly growing along their margin.

Table 1. Overview of the used oyster shells, their origin, shell height and the counted number of lines.

Specimen No.	Collection.Date	Area	Heigth.mm	N Lines
#001	15/12/2015	Voordelta	82.04	8-10
#002	15/12/2015	Voordelta	96.98	6-7
#003	15/12/2015	Voordelta	76.94	5
#004	15/12/2015	Voordelta	83.82	6-7
#005	15/12/2015	Voordelta	81.15	6+
#006	15/12/2015	Voordelta	95.74	14
#007	15/04/2017	Voordelta	89.62	7-8
#008	15/04/2017	Voordelta	86.03	10-11
#009	15/04/2017	Voordelta	87.3	10-(31)
#010	15/04/2017	Voordelta	96.08	8-9
#011	15/04/2017	Voordelta	88.78	9-11
#012	15/04/2017	Voordelta	77.98	6-7

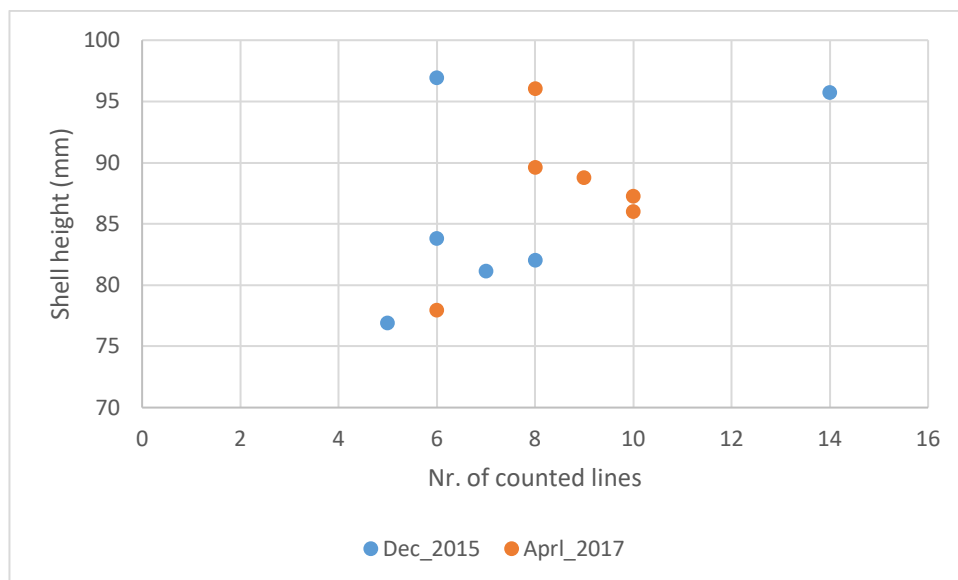


Figure 6. Plot of number of counted lines against shell height (largest distance from umbo to margin) in *Ostrea edulis* collected from the Voordelta.

Figure 6 summarizes the age-length relationship for oysters from the "Voordelta". The size range of the studied shells is limited between 75 and 100 mm. On basis of this it is impossible to fit a typical growth function such as a Von Bertalanffy curve. The graph however shows a clear relationship between the

counted number of lines and the valve height and the results match those presented by Richardson et al (1993). They found that an approximately 90 mm long shell had an age of between 10 and 15 years. Their published Figure 5 also shows a large variation in shell length at age. Specimens of 8 year old differed over 30mm in shell length. Similar differences can be observed in the data set presented here.

Discussion

The indicative size-age relationship which evolved from counts of the lines together with results published elsewhere suggests that the growth lines are annual. Although there is a huge variation of size with age, the relationship shows a convincing trend in which smaller specimens have lower number and bigger animals tend to have more growth lines. Assuming that these lines are indeed annual in nature, the estimated ages range between 5 and 14 years. The oldest found individual (#006) was collected in December 2015 and must have settled in the period 2001-2002. The results show that 5 out of 12 analyzed specimens had ages which suggest that the oyster bed was already present at the time when the Voordelta was designated as protected area.

For reliable age determinations of European flat oysters, validation of the forming periodicity of the growth lines is necessary. One of the most straightforward validation methods are mark-recapture experiments. Using specimens from such experiments in a growth line analyses can verify in how far the number of growth lines corresponds to the number of passed growth seasons since the marking operation. Clever sequential sampling within a year might also give insight on the timing of growth line formation. High end and quicker methods use temperature dependent chemical properties of the carbonate to verify the periodicity at which the growth lines are formed. One such method uses the stable isotope ratios of oxygen. This is nowadays a rather standard analytical method in sclerochronological studies to verify growth periodicities and check the interpretation of growth lines. (Witbaard et al, 1996; Cardoso et al 2013; Zimmet et al, 2019). It however not validates the age of the specimen. The stable isotope method is expensive and time consuming but also yield data on the temperature range at which the shells grow and thus gives insight about timing of growth and length of growing seasons and potentially the relation with other environmental characteristics. Other ways to link the shell growth patterns to seasonal variation in environmental conditions is the use of elemental ratios of the calcium carbonate. Durham et al (2017) tested laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) to determine seasonal profiles of Mg/Ca ratios. LA-ICP MS is a fast method when compared to stable isotope analyses, mainly because no time is needed for the physical sampling of the carbonate. Lartaud et al (2010) showed that $[Mn^{++}]$ in the oyster carbonate is linked to seawater temperature and shell growth rate. Manganese profiles in shell cross sections therefore can be used as well and can be measured by using cathodoluminescence (CL). This method was used by Huyghe et al (2019) to visualise the complete range of annual and tidally induced growth lines in Pacific oysters (*Magallana gigas*). Sometimes one method is not conclusive in the determination and are combinations of methods needed as Doldan et al (2018) applied to arrive at an age estimate of Argentine flat oysters *Ostrea puelchana*.

For absolute age validation of the individuals more work is needed and the most straight forward and cheap method could be a mark and recapture experiment performed over a long time span. The age - size relationship could be improved by using a larger size-range of animals for age determination.

Conclusion

Based on the earlier studies and the appearance of the growth lines, the indicative size-age relationship which evolved from counts of the lines, it is likely that the counted lines are annual in nature. Age at size matches to relationships found for a UK population (Essex-Black River). If this assumption is true, it means that the 14 year old shell (#006) which was collected in December 2015 must have settled in the period 2001-2002, depending on the exact timing of line formation.

In this study 12 shells were analyzed. Assuming annual growth lines, any shell collected in 2015 older than 7 years (i.e., 2 shells), or collected in 2017 and older than 9 years (i.e., 3 shells) would have been present before 2008, that is, before designation of the Natura 2000 site Voordelta in 2008. The results show that this is the case for 5 out of 12 of the analyzed shells. This leads to the conclusion that at the moment of Natura 2000 site designation, the oyster bed probably was already present to some extent.

Cited Literature

- Bos, O.G, J.W.P. Coolen, J. T. van der Wal, 2019. Biogene riffen in de Noordzee. Actuele en potentiële verspreiding van rifvormende schelpdieren en wormen Wageningen University & Research rapport C058/19, 48pp.
- Cardoso, J.F.M.F, S.Santos, J.IJ.Witte, R. Witbaard, H. W. van der Veer, J.P. Machado. 2013. Validation of the seasonality in growth lines in the shell of *Macoma balthica* using stable isotopes and trace elements. J. Sea Res. 82: 93-102.
- Christianen MJA, Lengkeek W, Bergsma JH, Coolen JWP, Didderen K, Dorenbosch M, Driessen FMF, Kamermans P, Reuchlin-Hugenholtz E, Sas H, Smaal A, van den Wijngaard KA, van der Have TM (2018) Return of the native facilitated by the invasive? Population composition, substrate preferences and epibenthic species richness of a recently discovered shellfish reef with native European flat oysters (*Ostrea edulis*) in the North Sea. Mar Biol Res 14:590–597.
- Doldan, M.S., M. de Rafelis, M.A. Kroeck, M.S. Pascual and E.M. Morsan, 2018. Age estimation of the oyster *Ostrea puelchana* determined from the hinge internal growth pattern. Mar. Biol. 165: 119. DOI 10.1007/s00227-018-3375-2.
- Durham, S.R. D.P. Gilikin, D.H. Goodwin and G.P. Dietl, 2017. Rapid determination of oyster lifespans and growth rates using LA-ICP-MS line scans of shell Mg/Ca ratios. Paleogeography, Palaeoclimatology, Palaeoecology 485: 201-209.
- Gregalis, K.C., M.W. Johnson and S.P. Powers, 2009. Restored oyster reef location and design affect responses of resident and transient fish, crab, and shellfish species in Mobile Bay, Alabama. Transactions of the American Fisheries Society 138: 314–327.
- Huyghe, D., M. de Rafelis, M. Ropert, V. Mouchi, L. Emmanuel, M. Renard and F. Lartaud, 2019. New insights into oyster high-resolution hinge growth patterns. Mar. Biol. 166:48. DOI/ 10.1007/s0027-019-3496-2.
- Kamermans, P., Lengkeek, W., Van der Have, T. & Smaal, A. (2015). Herstel platte oester op de Noordzee: vooronderzoek schelpdierlocaties Voordelta. IMARES, Sascon, Bureau Waardenburg b.v. (<http://library.wur.nl/WebQuery/wurpubs/fulltext/380164>).
- Kummel, B., Raup, D., 1965. Handbook of paleontological techniques. —W.H. Freeman and Company, San Francisco and London.
- Lartaud, F., M. de Rafelis, M. Ropert, L. Emmanuel, PGEairon, M. Renard, 2010. Mn labeling of living oysters: artificial and natural cathodoluminescence analyses as a tool for age and growth rate determination of *C. gigas* (Thunberg, 1793) shells Aquaculture 300(1); 206-217.
- Ministerie van Economische Zaken [Min. EZ] (2014) Profieldocument Riffen (H1170) (http://www.synbiosys.alterra.nl/natura2000/documenten/profielen/habitattypen/Profiel_habitatype_1170_2014.pdf).

Ministerie van Infrastructuur en Waterstaat & Ministerie van Landbouw, Natuur en Voedselkwaliteit [Min. IenW & Min. LNV] (2018) Mariene Strategie (deel 1). Actualisatie van huidige milieutoestand, goede milieutoestand, milieudoelen en indicatoren. 2018-2024. Den Haag, the Netherlands.

Ministerie van Landbouw, Natuur en Voedselkwaliteit [Min. LNV] (2008) Aanwijzingsbesluit Voordelta (<https://www.synbiosys.alterra.nl/natura2000/documenten/gebieden/113/Besluit%20Voordelta.pdf>)

OSPAR Commission (2008) List of Threatened and/or Declining Species and Habitats (OSPAR Agreement 2008-06). (<https://www.ospar.org/documents?d=32794>).

OSPAR Commission (2009) REVISED OSPAR Background Document for *Ostrea edulis* ad *Ostrea edulis* beds. OSPAR Comm Biodivers Ser:17.

OSPAR Commission (2013c). OSPAR Recommendation 2013/4 on furthering the protection and conservation of *Ostrea edulis* in Region II of the OSPAR maritime area and *Ostrea edulis* beds in Regions II, III and IV of the OSPAR maritime area. OSPAR(2) 13/4/1, Annex 7. < <https://www.ospar.org/documents?d=32968> >.

Richardson, C.A. , A. Collis, K. Ekaratne, P. Dare and D. Key, 1993. The age determination and growth rate of the European flat oyster, *Ostrea edulis*, in British waters determined from acetate peels of umbo growth lines. ICES J. Mar. Sci. 50: 493-500.

Richardson, CA (2001) Molluscs as archives of environmental change. Oceanography and Marine Biology: Annual Review 39: 103–164.

Sas H, Kamermans P, Van der Have TM, Christianen MJA, Coolen JWP, Lengkeek W, Didderen K, Driessen F, Bergsma J, Van Dalen P, Van Gool A, Van der Pool J, Van der Weide BE (2018) Shellfish bed restoration pilots Voordelta The Netherlands (https://www.ark.eu/sites/default/files/media/Schelpdierbanken/ARK_Annual_Report_Voordelta_2017_LR.pdf).

Sas H., Kamermans P., Van der Have T.M., Christianen M.J.A., Coolen J.W.P., Lengkeek W., Didderen K., Driessen F., Bergsma J., Van Dalen P., Van Gool A., Van der Pool J., Van der Weide B. (2017). Shellfish bed restoration pilots Voordelta, The Netherlands. Annual report 2017, 30-03-2018 (https://www.ark.eu/sites/default/files/media/Schelpdierbanken/ARK_Annual_Report_Voordelta_2017_LR.pdf). Sascon, Wageningen Marine Research, Bureau Waardenburg.

Sheldon, R.W. (1967). Relationship between shell-weight and age in certain molluscs. Journal of the Fisheries Research Board of Canada. 24. 1165-1171. 10.1139/f67-096.

Smaal, A, Kamermans, P, Kleissen, F, van Duren, L & van der Have, T. (2017). Platte oesters in offshore windparken (POP). Wageningen Marine Research rapport, no. C035/17, Wageningen Marine Research, Den Helder. DOI: 10.18174/412950

Smyth, D and D. Roberts, 2010. The European oyster (*Ostrea edulis*) and its epibiotic succession. Hydrobiologia (2010) 655:25–36

Smaal, A.C. , Kamermans, P. , Have, T.M. van der , Engelsma, M.Y. , Sas, H. (2015) Feasibility of Flat Oyster (*Ostrea edulis* L.) restoration in the Dutch part of the North Sea. Yerseke : IMARES (Report / IMARES Wageningen UR C028/15).

Tran,D., A. Nadau,, G. Durrieu, P. Ciret, J.-P Parisot, and J.-C Massabuau, (2011). Field chronobiology of a molluscan bivalve: how the moon and sun cycles interact to drive oyster activity rhythms. *Chronobiol.Int.* 28,307–317. doi:10.3109/07420528.2011.565897

Walne, P.R., 1958. Growth of Oysters (*Ostrea edulis* L.). *J. mar. biol. Ass U.K.* 37: 591-602.

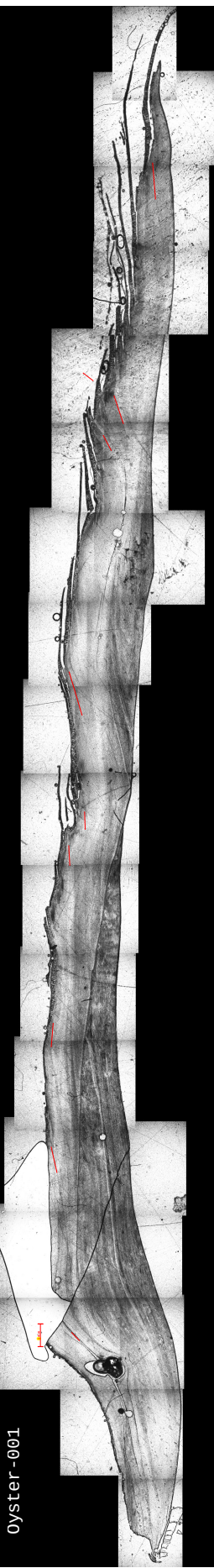
Witbaard, R., M.I. Jenness, K. van der Borg, G. Ganssen, 1994. Verification of annual growth increments in *Arctica islandica* L. from the North Sea by means of oxygen and carbon isotopes. *Neth. J. Sea Res.* 33(1): 91-101.

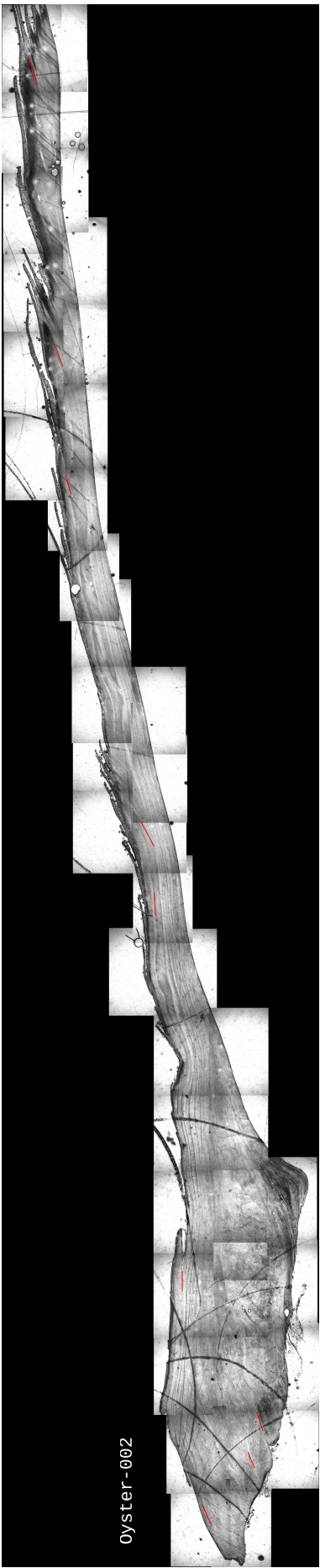
Zimmit, J.B. R. Lockwood, C.F.T. Andrus and G.S. Herbert, 2019. Sclerochronological basis for growth band counting: A reliable technique for life-span determination of *Crassostrea virginica* from the mid-Atlantic United States. *Paleogeography, Paleoclimatology, Palaeoecology* 516:54-63.

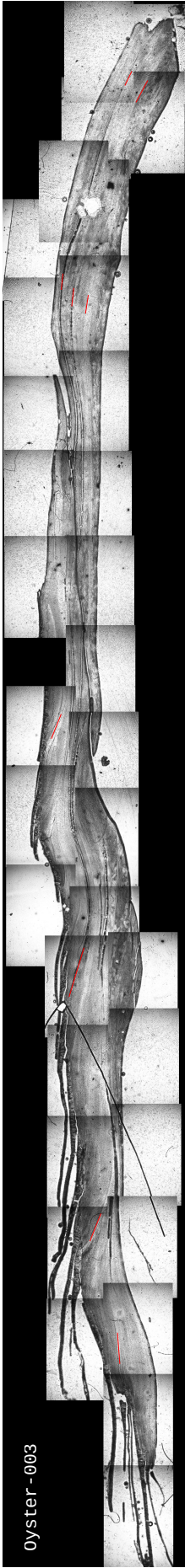
Annex 1

Foto's van doorsnedes van alle schelpen.

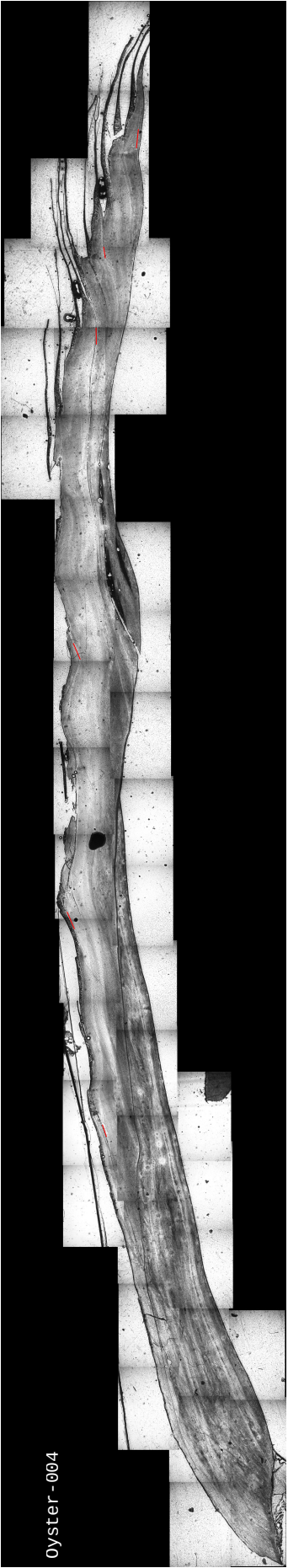
Oyster -001



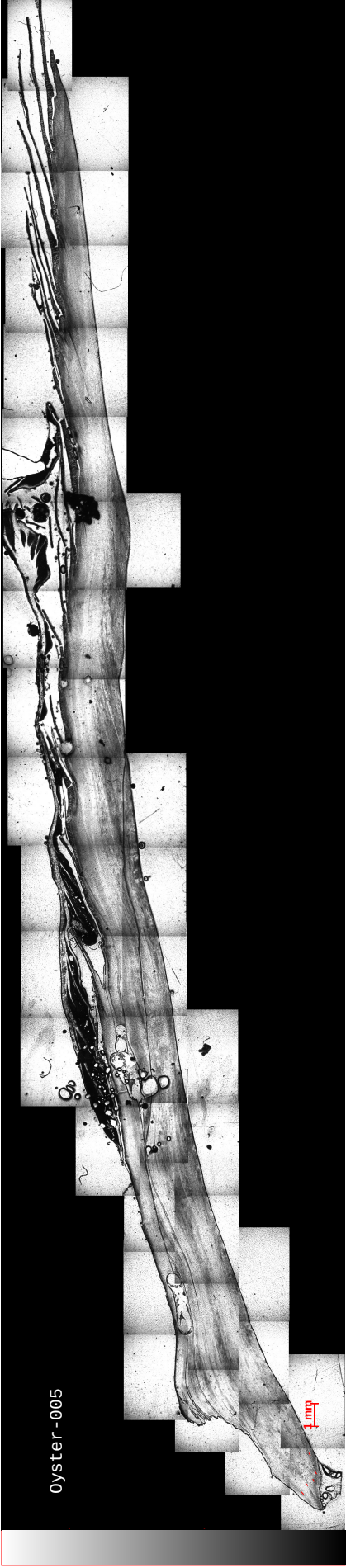




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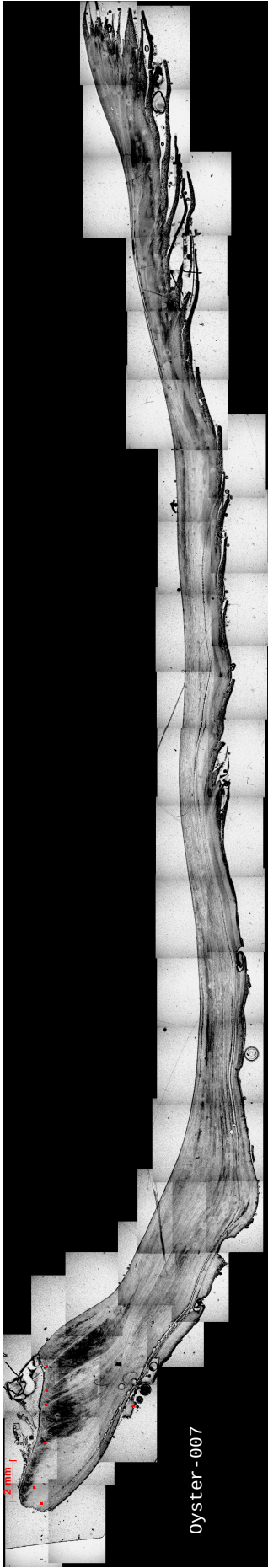


Oyster-004



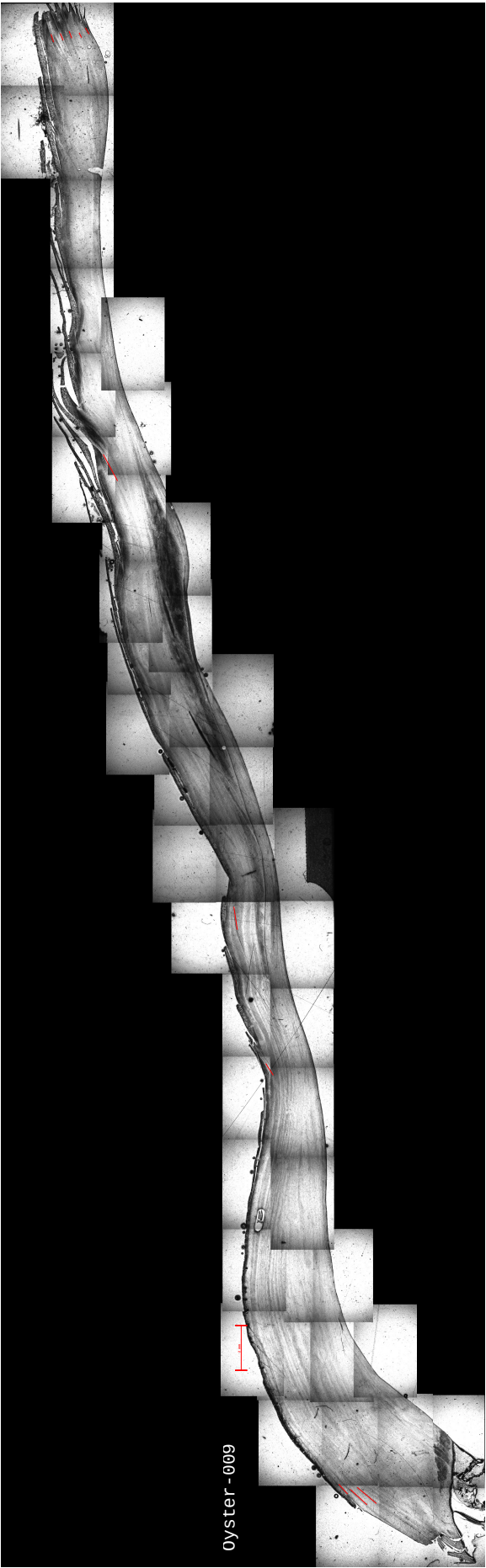


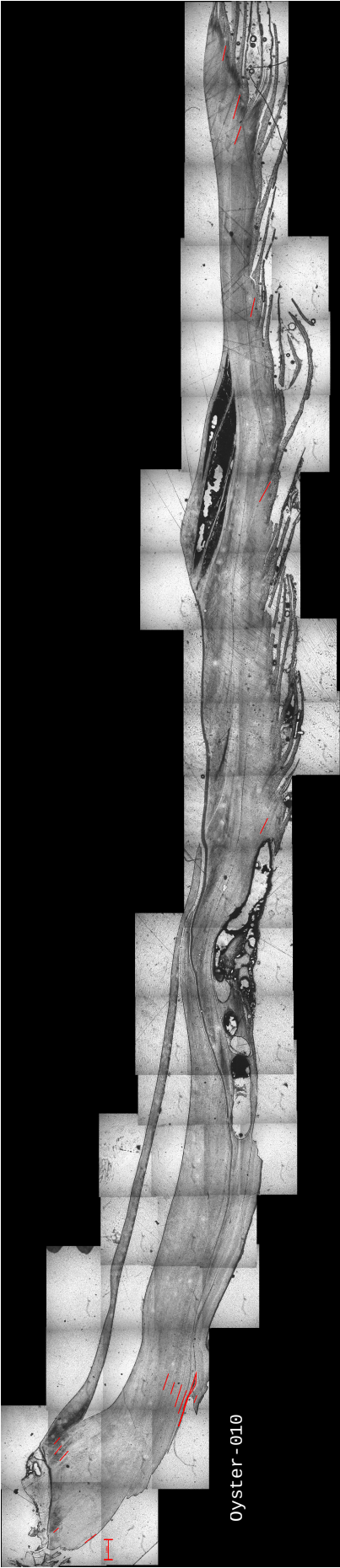
Oyster-006



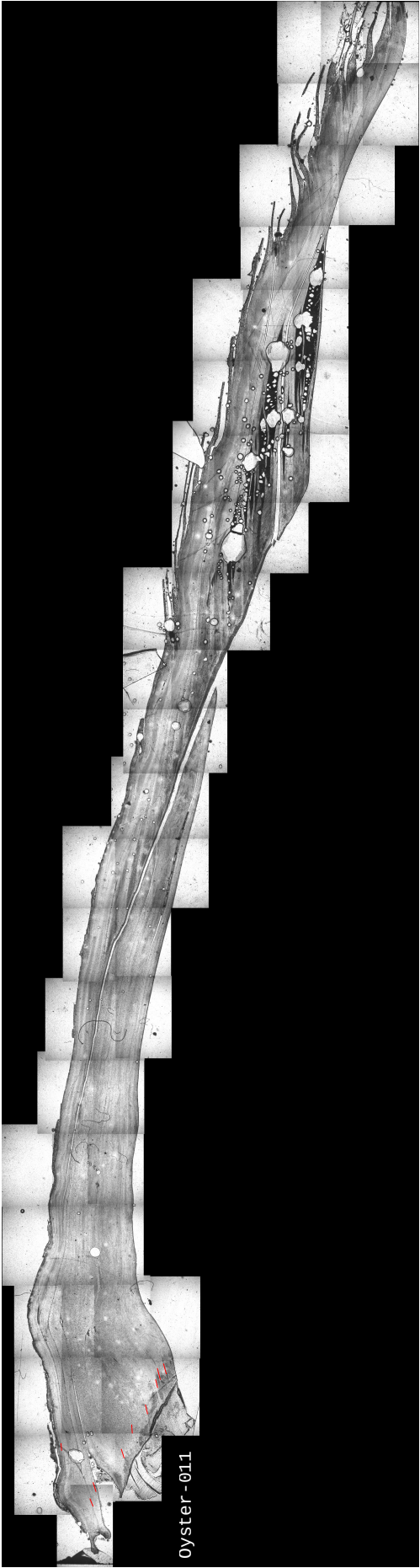


Oyster-008

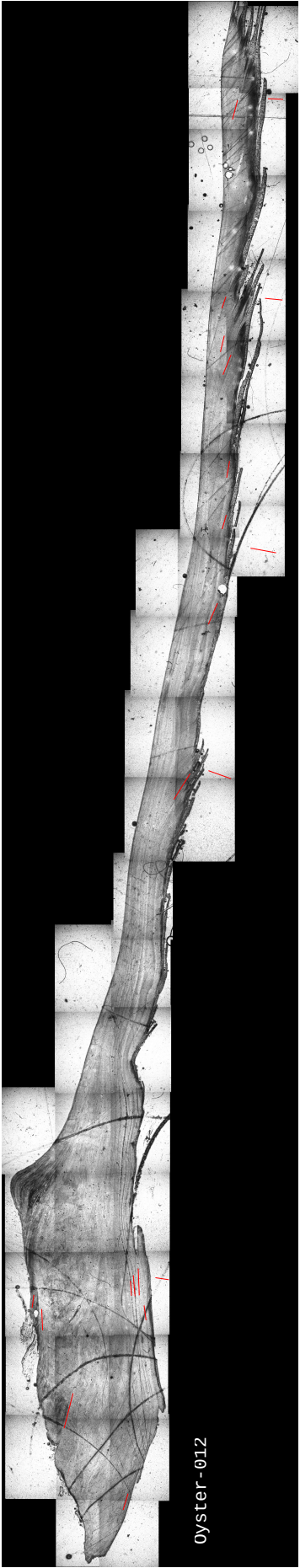




Oyster-010



Oyster-011



Oyster-012

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