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Insight into shell growth dynamics of Mactromeris polynyma in the St. Lawrence Estuary using sclerochemistry Justine Doré^{1*}, Julien Thébault¹, Virginie Roy², Fabien Dewilde³, Gwénaëlle Chaillou^{4*} ¹ Univ Brest, CNRS, IRD, Ifremer, LEMAR, Plouzané, 29280, France ² Maurice Lamontagne Institute, Fisheries and Oceans Canada, 850 route de la Mer, C.P. 1000, Mont-Joli, QC, G5H 3Z4, Canada ³ Univ Brest, CNRS UMS3113, Plouzané, 29280, France ⁴ Canada Research Chair in Geochemistry of Coastal Hydrogeosystems, Québec-Océan, ISMER, UQAR, Rimouski, QC, G5L 3A1 Canada * Correspondence: Justine Doré, justine.dore91@gmail.com * Correspondence: Julien Thébault, julien.thebault@univ-brest.fr

24 **Keywords:** 25 Mactromeris polynyma; chemochronology; growth; Lower St. Lawrence Estuary 26 27 Abstract: 28 This study explores the potential of geochemical data to accurately date *Mactromeris polynyma* shells 29 collected in the Lower St. Lawrence Estuary, and to assess the species' utility as an environmental 30 archive. Previous growth studies of M. polynyma from the St. Lawrence ecosystem showed a possible 31 overestimation of annual growth lines using the classic sclerochronology methodology. Our study 32 proposes a new method based on chemochronology, coupling Ba/Ca and Sr/Ca ratio profiles to 33 distinguish between disturbance lines and actual annual growth lines. By aligning the Sr/Ca profiles 34 thanks to the peaks of Ba/Ca, drastic decreases in the Sr/Ca ratio have been highlighted. These drops, 35 which are detectable in all specimens, precisely locate the annual growth lines, making it possible to 36 study variations in the growth rate. Our chemochronology method is a powerful tool for precisely 37 aging M. polynyma, making this innovative method an alternative to sclerochronology techniques for 38 highlighting annual growth lines for species that are difficult to age. 39 40 **Highlights:** 41 Chemochronology as a new methodology to age specimens 42 Ba/Ca ratio as a tool to align Sr/Ca ratio profiles 43 Aligned Sr/Ca ratio profiles show common sharp decreases among specimens 44 Sharp Sr/Ca ratio decreases accurately locate annual growth lines 45 M. polynyma has great potential as an environmental archive

46 1. Introduction

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Biogeochemical archives such as sediment cores, coral skeletons, otoliths, or mollusk shells act as recorders of their surrounding medium and provide evidence of past environmental conditions when specific environmental proxies are developed. Freshwater and marine bivalves can furnish life-history information, such as a continuous record of their growth and environmental changes in the ambient water (Rhoads and Lutz, 1980; Rosenberg and Runcorn, 1975). Because many bivalve species are extremely long-lived, non-colonial animals (some living to >500 years; Butler et al., 2013; Strom et al., 2004; Thompson et al., 1980; Wanamaker et al., 2008), they are valuable in paleoclimate (e.g., Butler et al., 2010; Scourse et al., 2006), water quality (e.g., Black et al., 2017; Dunca et al., 2005; Gillikin et al., 2005), and age (e.g., Abele et al., 2009; Kilada et al., 2007, 2009) studies, Moreover, due to their vast biogeographic distribution and long geologic history (i.e., abundant fossils are present for the past ca. 500 million years), bivalves can provide environmental data from many different settings over millions of years (see Richardson, 2001, for a review). Bivalve shells grow by accretion of calcium carbonate crystals and form growth increments throughout ontogeny. Growth rate varies in response to changes in local and regional environmental conditions and/or biologic events, like spawning (Jones et al., 1983). Growth can stop at several periodicities, including ultradian, circatidal, circalunidian, fortnight, month, or annual time scales (Schöne, 2008), this cessation of growth form the so-called growth lines. Thus, bivalves act as a calendar, providing precise dates of high-resolution climate records and environment changes. The aim of sclerochronology is to date bivalves (and other calcium carbonate secreting organisms) by extracting these growth patterns (see Schöne and Surge, 2012, for a review) in order to reconstruct past environmental conditions. The St. Lawrence marine ecosystem (i.e., Estuary and Gulf) is one of the largest and most productive estuarine-marine ecosystems in the world (Gilbert and Dufour, 2008). It is also a complex coastal system (Lavoie et al., 2007; Savenkoff et al., 2007) that receives more than half of the freshwater inputs from eastern North America (Dufour and Ouellet, 2007). Due to human uses and pressures in

the Great Lakes Basin, freshwater as well as nutrient, organic matter, and contaminant inputs are leading to important changes in the Lower St. Lawrence Estuary and Gulf (EGSL), including hypoxia and acidification of deep bottom waters in the Laurentian Channel (Gilbert et al., 2005, 2007; Mucci et al., 2011; Jutras et al., 2020). Over the last 20 years, some studies have focussed on the sediment records along the hypoxic gradient, including both foraminifera (Genovesi et al., 2011; Thibodeau et al., 2006) and redox-sensitive trace metals (Lefort et al., 2012). Moreover, the Atlantic Zone Monitoring Program (AZMP), implemented since 1998 by Fisheries and Oceans Canada (DFO), has characterized the oceanic variability of the system at seasonal, inter-annual, and decadal scales (e.g., Blais et al., 2019; Galbraith et al., 2019). However, these studies mostly concentrate on the deep Laurentian Channel, while data from coastal areas along the St. Lawrence shores are still sparse except in some harbours (e.g., Degtlareva and Elektorowicz, 2001; Dutil et al., 2012). As far as we know, no studies have yet used mollusk sclerochronology to assess coastal environmental changes over time along the St. Lawrence shores, although the St. Lawrence coastal ecosystem has numerous bivalve species, such as the Arctic Surfelam Mactromeris polynyma, that could provide useful information on coastal environment changes (Bourdages et al., 2012). In the last three decades, DFO scientific missions have discovered numerous mollusk beds along eastern Canadian coasts (i.e., North Shore of the St. Lawrence Estuary and Gulf, the Îles-de-la-Madeleine in the southwestern Gulf of St. Lawrence, Scotian Shelf, Grand Banks) that revealed M. polynyma to be a commonly distributed and abundant coastal species (Brulotte, 1995; Cyr, 1994; DFO, 2006; Lambert and Giguère, 1994; Lambert and Goudreau, 1995a; Landry et al., 1992a). M. polynyma is known to be a sedentary endobenthic bivalve mollusk forming beds in sublittoral zones or under the low tide line. They are found buried in sandy sediments (Bourassa et al., 2008; Lambert and Goudreau, 1995b) at depths determined by the length of their siphons and consequently their size (e.g., adult surf clams can reach 150 mm in length). They feed by using their incurrent siphon to filter suspended materials. In the St. Lawrence Estuary and Gulf, Arctic Surfclams can be found at water depths ranging from 1 to 60 m, with a maximum density observed between 15 and 20 m in depth (Trottier and Goudreau, 2015). Due to its biological and ecological characteristics, M. polynyma could

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be a valuable asset for sclerochronology and sclerochemistry studies (Mann et al., 2013) in the coastal EGSL and more generally in arctic and subarctic regions, where fisheries and economic interests are increasing (DFO, 2018). Several fisheries management reports describe *M. polynyma* as a slow-growing, long-living species (i.e., it would take 13 to 20 years to reach 80 mm in length, depending on the fishing bed) (Bourassa et al., 2008; Lambert and Goudreau, 1999; Landry et al., 1992b; Roddick et al., 2012).

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To reconstruct past environmental variations, associating a specific date of formation to a given shell portion is the first step in defining a timeline for proxies that are contained in growth rate (i.e., growth anomalies) and embedded in the carbonate shell material (i.e., geochemical variations) (e.g., Fenger et al., 2007; Hudson et al., 1976; Schöne et al., 2013; Strom et al., 2004). However, accurate age determination in long-living bivalve shells is not always straightforward because of uncertainties in locating growth lines on the external or internal shell. The determination of accurate annual growth lines depends on many interrelated variables. There are several sources of bias when using sclerochronology to identify growth lines: tools that emphasize growth lines (e.g., blue light excitation: Wanamaker et al., 2009; Mutvei's solution: Schöne et al., 2005), microscope type and magnification (e.g., digital microscope with 10x or 500x magnification), and the operator. In the case of M. polynyma, several technical reports (government of Canada publications) have revealed a bias in the growth estimates of specimens collected around the Gulf of St. Lawrence when counting lines on the external shell due to excessive number of lines (Lambert and Goudreau, 1997, 1999). Other reports on the growth rate of M. polynyma in the EGSL based their assess on the inside of a hinge plate cut out of the shell where excessive growth lines make visual counting difficult too (Bourassa et al., 2008; Roddick et al., 2012). In both case, the authors pointed out the difficulty to assess accurate ages with this species making the use of cross-dating method unenforceable despite its practice recommended in sclerochronology (e.g., Griffin, 2012). Thus, we highlight the necessity to use geochemical technique to counterbalance this issue.

An accurate localization of annual growth lines is essential to determine age and growth rate because individuals may form growth lines at different periodicities and even when they are stressed, making

localization much more difficult. On a time scale ranging from a few years to several decades, no study has shown any alteration in the geochemical composition of the shells after biomineralization (e.g., Schöne, 2013, Walliser et al., 2015). Thus, geochemical approaches could be used to determine the age of bivalve species with indistinct growth lines (e.g., Cardoso et al., 2013a; Gurney et al., 2005; Kilada et al., 2009; Richardson et al., 2004). Geochemical methods provide an additional tool to age shells while reducing reading errors with misidentifying growth lines. Such methods may be used on *M. polynyma* to determine annual growth lines and to test its use as an environmental archive.

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Several studies have highlighted the potential of using the oxygen stable isotopic ratio (δ^{18} O) and the strontium-to-calcium ratio (Sr/Ca) as tools to estimate and validate age by identifying annual growth lines of different bivalve species (e.g., Cardoso et al., 2013a, 2013b; Foster et al., 2009; Gurney et al., 2005; Witbaard et al., 1994). No consensus has yet been reached on the incorporation dependency of Sr into bivalve shells. The Sr/Ca ratio can be linked to water temperature (Richardson et al., 2004) and salinity (Dodd and Crisp, 1982) as well as metabolic activities like growth rate (Carré et al., 2006; Gillikin et al., 2005; Lorrain et al., 2005; Takesue and van Geen, 2004). In this study, the main objective is to propose a new geochemical approach as an alternative to standard sclerochronological techniques in order to build an age model for each M. polynyma shell, which is the first essential step for paleo-environmental studies. More specifically, we first develop a step-by-step methodology to locate annual growth lines combining synchronous patterns of trace element ratios. Secondly, we explore the growth pattern synchronicity between specimens of M. polynyma collected at our study site in the Lower St-Lawrence Estuary. According to our knowledge, the study is the first to combine geochemical profiles in order to age and evaluate growth rate of bivalve species with excessive disturbances lines, offering a new approach to assess the species' potential to record environmental variations.

2. Materials and sample site

The study site is located on the North Shore of the Lower St. Lawrence Estuary (LSLE, Quebec, Canada; Fig. 1), in DFO fishing area 1B, where three distinct beds of Arctic Surfclams have been identified (Fig. 1C). The sample site chosen for this study is the Baie-Comeau bed (called Baie-Comeau hereafter). *M. polynyma* shells were collected in late July 2018 at the northern edge of Baie-Comeau (49° 11′ 35.326″N – 68° 05′ 22.866″W) at 10 m depth during the DFO Maurice Lamontagne Institute's oceanographic campaign (IML-2018-029) on-board the CCGS *Leim* (Fig. 1C). The harvest was performed with a New England hydraulic dredge with jets of water at the base of the blade (pressure between 241 and 621 kPa). This technique has the advantage of softening the bottom sediment and reaching specimens buried up to 20 cm deep (DFO, 2018). Shells were collected alive and soft tissue were removed shortly after sampling. Seven shells were selected because of their size spectrum and good state of preservation (e.g., absence of holes or breaks in the shell). In this study, a restricted size range was chosen so that individuals were neither too large (i.e., potentially old, thus decreasing the analysis resolution of the last years of life) nor too small (i.e., to avoid having insufficient data) in order to facilitate geochemical analyses and data comparison between specimens.

FIGURE 1 (2 columns)

3. Methodology for the validation of annual growth lines

To explore the potential of *M. polynyma* as an environmental archive, growth lines must first be determined to age and evaluate the growth rate of each specimen prior to reconstruction of past environmental variations. However, standard sclerochronological techniques as cross-dating were not conclusive to age specimens. Thus, geochemical analyses were performed on shells to identify growth lines having an annual periodicity (called annual growth lines hereafter). The step-by-step approach is presented in Fig. 1S.

3.1. Preparation of shells

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Because of external shell impurities and fragility, especially toward the ventral margin, hinge plates (HPs) were selected to study the growth of six specimens of M. polynyma (M07, M08, M10, M12, M14, and M15). In addition, the whole small and practically unbroken shell of one specimen (M17) was added to study the outer shell layer. Table 1 presents the morphometric features of the selected specimens. Two 1 mm thick "mirror" cross sections were cut from the six HPs and one entire shell along the axis of maximum growth, from the umbo to the ventral margin, using a low-speed precision saw (Struers, Secotom-10; rotation speed of 500 rpm; feed rate of 100 µm.s⁻¹) equipped with a 600 µm thick diamond-coated blade continuously cooled by water. Beforehand, shells had been embedded in a 1 cm wide thick layer of a quick-drying metal epoxy resin (Araldite Metal, Huntsman Advanced Materials) along the axis to protect shells from breaks during sectioning or manipulation. Each section was then glued on a glass side, ground with 1200 then 2500 grit wet table carborundum paper, and polished with 1 µm diamond liquid (ESCIL, Chassieu, France) on a polishing cloth fixed on a grinding turntable (Struers, TegraPol-25). To reduce scratches and contamination hazards, crosssections were cleaned between each grinding/polishing step with deionized water and dried to eliminate residual diamond liquid and/or abrasive material. For each specimen, a "mirror" HP cross section was used for sclerochronological analysis whereas trace element analyses (TEA) were performed on the other HP cross section (Fig. 2). Oxygen stable isotope analyses (SIA) was only performed on the entire shell of M17 (Fig. 2).

To visually locate growth lines, digital images of all "mirror" HP cross sections were taken with a stereomicroscope (20x magnification; Zeiss Lumar.V2) and a digital microscope (500x magnification; Keyence, VHX 600), both of which were equipped with a motorized stage. For the outer shell layer (OSL), only the stereomicroscope was used. To enhance growth line readability on HPs, cross sections were immersed in Mutvei's solution for 30 min at ambient temperature (Schöne et al., 2005) and the same imaging method was applied. Images were processed with ImageJ software to attribute a distance to each growth line.

FIGURE 2 (2 columns)

TABLE 1 (1 column)

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3.2. Stable isotope analysis (SIA)

The oxygen-isotope ratio ($\delta^{18}O_{\text{shell}}$) was measured in the OSL of specimen M17. This shell cross section was pre-rinsed ultrasonically with demineralized water. Discrete samples of powdered carbonate were then obtained using a computer-operated MicroMill® coupled with a 300 µm diameter drill. Short tracks about 900 µm long, 200 µm thick, and 10 µm deep (milling to 10 µm in depth allowed us to reduce the drill diameter to 200 µm) were drilled parallel to growth lines in the second half of the cross section (sampling started 7 mm from the ventral margin because of shell fragility). Consecutive tracks overlapped by at least 50 µm. These parameters allowed the sampling of ~15 µg of CaCO₃ with an average spatial resolution of ca. 150 µm (Fig. 2E). Isotopic analyses were performed at the Pôle Spectrométrie Océan (PSO, Plouzané, France) using a MAT-253 (Thermo Scientific) stable isotope ratio mass spectrometer (IRMS) coupled with a Kiel IV Carbonate Device (Thermo Scientific). Oxygen-isotope ratios were standardized with the VPDB scale using NBS-19 and NBS-18 international carbonate standards (Coplen, 1996). The assigned value was $\delta^{18}O_{VPDR} = -$ 2.20% for NBS-19 and $\delta^{18}O_{VPDB} = -23.20\%$ for NBS-18. External precision (1 σ), based on repeated measurements of an in-house standard, was $\pm 0.04\%$. Due to breaks, bioerosion, and the fragile OSL, $\delta^{18}O_{\text{shell}}$ was only measured on 22.4 mm within 7 mm of the ventral margin of the OSL, thus the date of death could not be used to determine date oscillations. In addition to the measured $\delta^{18}O_{shell}$, we calculated a monthly theoretical shell $\delta^{18}O$ ($\delta^{18}O_{theo}$) based on

In addition to the measured $\delta^{18}O_{shell}$, we calculated a monthly theoretical shell $\delta^{18}O$ ($\delta^{18}O_{theo}$) based on mean seawater temperature and salinity data from 2010 to 2018 measured close to the sample site. The $\delta^{18}O_{theo}$ was calculated using the paleothermometry equation developed by Grossman and Ku

- 227 (1986) for biogenic aragonite and re-evaluated using the most recent δ^{18} O correction of -0.27 (Hut,
- 228 1987) shown by the equation 1:

229 eq. (1)
$$T^{\circ}C = 20.6 - 4.34 \left(\delta^{18}O_{theo} - (\delta^{18}O_{sw} - 0.27)\right)$$

- where $\delta^{18}O_{sw}$ was deduced from the linear regression between salinities and $\delta^{18}O_{sw}$ of the seawater
- 231 measured by Tan and Strain between 1980 and 1996 (most complete and recent data) along the
- 232 Estuary and Gulf of St. Lawrence (Fig. 2S) and accessible on the NOAA website
- 233 (https://data.giss.nasa.gov/cgi-bin/o18data/geto18.cgi). We rewrote the paleothermometry relationship
- 234 to calculate $\delta^{18}O_{\text{theo}}$ values (eq. 2):

235 eq. (2)
$$\delta^{18}O_{theo} = \frac{19.4282 - T^{\circ}C + 4.34(\delta^{18}O_{sw})}{4.34}$$

- Salinities and water temperatures used to calculate $\delta^{18}O_{theo}$ are from the instrumented oceanographic
- buoy identified as PMZA-RIKI (48°40′01″N-68°35′11″W). This buoy is 70 km far from the sample
- site, but it provides the nearest oceanographic time series available. The $\delta^{18}O_{sw}$ was then written as
- 239 followed:

240 eq. (3)
$$\delta^{18}O_{SW} = 0.2859(\pm 0.015)^*S - 10.6997(\pm 0.35)$$

- According to equation 3, a value of zero for the $\delta^{18}O_{sw}$ corresponds to an ambient salinity of 37.42.
- Thus, a salinity variation of 1 reflects a fluctuation of 0.285% in the $\delta^{18}O_{sw}$ composition. $\delta^{18}O_{sw}$ was
- 243 then used in eq. (2) to accurately calculate $\delta^{18}O_{theo}$ at the location of buoy PMZA-RIKI. Then, a
- 244 macroscopic comparison was done between monthly $\delta^{18}O_{theo}$ and $\delta^{18}O_{shell}$ oscillations. Extreme
- positive values from $\delta^{18}O_{theo}$ and $\delta^{18}O_{shell}$ (e.g. winter temperatures) were matched and they were used
- 246 to validate the annual periodicity of the $\delta^{18}O_{shell}$ oscillations. The SIA points out the presence of
- numerous supplementary lines that are unlikely of seasonal or annual origin (Fig. 1S) and leads to the
- consideration of a new method for locating lines that are annual.

Because the shell is fragile and bioerosion mechanisms may remove the first years of life, the hinge plates were used for trace element analysis (TEA). Thus, trace elements of only one OSL (M17) and the other 6 HPs (Table 1, Fig. 2) were analyzed with a Nu Attom high-resolution inductively coupled plasma mass spectrometer (HR-ICP-MS) coupled with a Photon-Machines G2 laser ablation (LA-ICP-MS) system at the Research Centre on the Dynamics of the Earth System (GEOTOP), Université du Québec à Montréal (UQAM). Several trace elements were measured, but only ¹³⁵Ba, ⁸⁶Sr, and ⁴³Ca (internal standard) are presented here. Elemental ratios (E/Ca) were obtained using IOLITE software (https://iolite-software.com/) after instrumental drift correction and normalization. Operating conditions and processes are detailed in Doré et al. (2020). Raw data were smoothed using a 10- and 100-point running mean for HP and OSL profiles, respectively.

Element-to-calcium ratio profiles (Ba/Ca and Sr/Ca) were used to quickly and accurately detect annual growth lines in HPs. The Ba/Ca ratio profiles are often characterized by a flat background interrupted by sharp peaks detectable in conspecific and contemporaneous specimens from the same locality (Carré et al., 2006; Gillikin et al., 2006; Lazareth et al., 2003; Stecher et al., 1996; Thébault et al., 2009; Vander Putten et al., 2000) and they can be used as proxy of the phytoplankton dynamics (e.g., Doré et al., 2020; Stecher et al., 1996; Thébault et al., 2009) and as cross-dating tool by aligning profiles (Doré et al., 2020; Marali et al., 2017). However, even if Ba/Ca profiles recorded on *M. polynyma* hinge plates offer some inter-annual variabilities, information on the position of annual growth lines cannot be accurately inferred from these signals. Indeed, these lines are located somewhere within the flat portions of the Ba/Ca profile. Without additional clues, their exact position cannot be identified precisely. Conversely, Sr/Ca ratio profiles are usually formed of annual oscillations that can be very useful in highlighting a sharp decrease in growth (i.e., halt in growth) due to the positive correlation between Sr/Ca and shell growth rate (Carré et al., 2006; Gillikin et al., 2005; Lorrain et al., 2005; Takesue and van Green, 2004). Therefore, Sr/Ca signals could be useful to identify with a good accuracy the position of annual growth lines. Unfortunately, in some of our

specimens, Sr/Ca annual cycles could be interrupted with one or more sharp decreases that could be misinterpreted as annual lines. In addition, the relatively high inter-individual variability prevents accurate temporal alignment of Sr/Ca profiles. This issue could be fixed by aligning Sr/Ca profiles of different specimens using the likeness of their Ba/Ca signals to highlight a similar, simultaneous, Sr/Ca decrease, which can corresponds to an annual growth line. Thus, firstly, we used the maximum value of similar Ba/Ca peaks between signals to align Ba/Ca profiles, and, secondly, each profile was re-sampled as a function of the longest distance between two consecutive peaks using a homemade script built using R software (cran.r-project.org; available on request). Finally, these resampled and synchronized signals were then used to align inter-individual Sr/Ca profiles. This alignment visually highlights common inter-individual Sr/Ca patterns, which could identify annual growth lines. The positions of annual growth lines were set in the most likely locations (i.e., when most—but not always all—locations had a common Sr/Ca ratio pattern). To validate the position of annual growth lines, we compared positions of presumed annual growth lines determined by both SIA and TEA on the OSL of specimen M17.

3.4. Growth chronology from trace element profiles

Based on the age determined by TEA, a von Bertalanffy growth function (eq. 4) was applied and fitted to the shell length-at-age data:

294 eq. (4)
$$L_t = L_{\infty} (1 - e^{-K(t-t_0)})$$

where L_t is the shell length (in mm) at time t (in years), L_{∞} is the length reached after an infinite time of growth (in mm), K is the Brody growth constant defining the "speed" of growth (per year), and t_0 is the theoretical age at which the length would be zero (in years). Because only HPs were studied, the length referring to the external shell was calculated using an allometric relationship (eq. 5):

299 eq. (5)
$$L_t = a \left(\frac{L_{OSL}}{L_{HP}} \right)$$

300 where a is the length of each increment measured in the HP, and L_{OSL} and L_{HP} are the total length of the shell and the HP, respectively.

In addition, the ontogenetic trend (i.e., physiological effect on growth where the older a specimen the less it grows) was mathematically removed using a negative exponential curve then a cubic spline. Growth indices (GIs) were then obtained to examine the potential of *M. polynyma* to archive environmental variations that had equal influence on growth rates. Thus, GIs (eq. 6) were calculated for each year of each specimen by dividing measured and predicted increment widths (IW):

307 eq. (6)
$$GI_t = \frac{(IW_{measured})_t}{(IW_{predicted})_t}$$

where GI_t is the growth index at time t (in years), $IW_{measured}$ is the width of the measured annual increment at t, and $IW_{predicted}$ is the predicted increment width at t. The GIs for each time series were then standardized following eq. (7):

311 eq. (7)
$$SGI_t = \frac{GI_t - \mu}{\sigma}$$

where μ is the average and σ the standard deviation of all GI values at time t. SGIs were estimated using the dplR package in R (https://CRAN.R-project.org/package=dplR) (Bunn, 2008). A master chronology, representing the average of SGI chronologies, was then calculated using aged shells in order to maximise the accuracy of the method (i.e., hinge plates only).

3.5. Statistical analyses

Synchrony between aligned Sr/Ca signals was tested using a pairwise matrix correlation on a common growth period. Linear regression was used to calculate the coefficient of determination and Pearson's product-moment correlation was used to determine the coefficient of correlation; both were applied to smoothed HP signals. The associated *p*-value was also calculated. Robustness of the master chronology was assessed through calculation of the expressed population signal (EPS) using the dplR package as follows (eq. 8):

324 eq. (8)
$$EPS = \frac{n(R_{bar})}{(n(R_{bar}) + (1 - R_{bar}))}$$

where R_{bar} is the average of all correlations between pairs of SGI chronologies and n is the number of specimens used to construct the master chronology. If the EPS value is higher than the arbitrary threshold of 0.85 (Wigley et al., 1984), the variance of the estimated master chronology sufficiently expresses the variance of all SGI series.

4. Results and Discussion

4.1. A step-by-step chemochronology approach for dating specimens

Numerous growth lines are present on the OSLs and HPs of our seven *M. polynyma* specimens, and disrupt the identification of annual growth lines (Fig. 3S). If all lines were considered to be annual growth stops, our specimens would be centuries old, which is highly unlikely. Therefore, most of those excessive lines are probably so-called "disturbance" lines formed as a result of stress. The origin of this stress is unknown and could be related to predation or fishery (trawling) activity as observed for other bivalves as, for example, for *Ensis spp.* (Robinson and Richardson, 1998), *G glycymeris* (Ramsay et al., 2000) and *A islandica* (Klein and Witbaard, 1993; Witbaard and Klein, 1994). In this context, we could not visually estimate the age and growth rate of *M. polynyma* with a high level of confidence even using cross-dating method. To resolve this difficulty, we provide here an alternative step-by-step method based on geochemical ratio profiles. This chemochronology methodology allows to avoid the constraints of macroscopic observations and focuses on the geochemical content of the shells to age specimens.

4.1.1. Shell δ^{18} O and determination of annual growth lines

The oxygen-isotope ratio ($\delta^{18}O_{shell}$) was measured on 54 samples micromilled along the OSL of specimen M17. This isotopic profile showed four discernible oscillations (i.e., variation between two

accurate positive extreme values) along 22.4 mm (between 9.7 and 32.1 mm from the ventral margin; Fig. 3), ranging from -1.39 to +2.09% (mean = 0.30%; s.d. = 0.92%). These oscillations suggest a seasonality with alternatively high (corresponding to lower temperatures during winter) and low (corresponding to higher temperatures during summer) δ^{18} O values. The δ^{18} O_{theo} profile calculated based on *in situ* temperature and salinity also shows regular seasonal oscillations that range from around -1.82% to +3.22%, with an average of +0.73% $\pm 0.90\%$ (Fig. 3).

FIGURE 3 (2 columns)

Figure 3 shows the high overlap between $\delta^{18}O_{shell}$ and $\delta^{18}O_{theo}$ from 2013 to 2016, revealing the presence of disturbance/stress lines in addition to annual growth lines. These two oxygen-isotope ratio profiles provide a useful tool to visually eliminate non-annual growth lines and could inform on the growth cessation period. Indeed, even if global oscillation values are slightly different (because environmental data were recorded in the middle of the LSLE, approximately 70 km from Baie-Comeau), variations below 1%e for $\delta^{18}O_{shell}$ values (higher temperature), especially in 2014 and 2015, are similar to $\delta^{18}O_{theo}$ variations. However, variations are different for values above 1%e (lower temperature). It should be noted that $\delta^{18}O_{theo}$ values for some winter months (December to March) are lacking due to missing water temperature and salinity data during this ice-covered period. Moreover, when these values were available, we found no matches. Shells seem to stop recording variations of $\delta^{18}O$ with colder temperatures. However, as no annual growth line can be identified using only the oscillations of $\delta^{18}O$, no certainty can be advanced on the period of growth cessation at this stage of the study. Furthermore, some amount of time averaging during carbonate sampling cannot be exclude. Investigating the age and growth rate of M. polynyma shells using variability $\delta^{18}O_{shell}$ could have been applied on the condition that samples were taken up to the ventral margin (known date of death).

applied on the condition that samples were taken up to the ventral margin (known date of death). However, this study involves one major drawback: the slow shell growth rate and the relatively large diameter of the drill bit used for carbonate sampling preclude the accurate estimation of shell growth dynamics. In addition, accurate aging of shells using the OSL may be challenging due to the high risk of bioerosion, attacks of euendoliths, and potential breakage. Oxygen-isotope sampling and analysis

are also time consuming and expensive. Considering this, the use of trace elements archived in shell hinge plates may offer an efficient and low-cost alternative for determining the age of bivalve shells.

FIGURE 4 (2 columns)

4.1.2. Localization of annual growth lines using trace elements

Numerous studies have investigated variations in trace element concentrations related to biological and environmental changes in biogenic carbonate (Ballesta-Artero et al., 2018; Doré et al., 2020; Druffel, 1997; Freitas et al., 2006; Langlet et al., 2002; Lea et al., 1989; Poulain et al., 2015; Prendergast et al., 2017). Variations in Sr concentration have been related to an array of environmental or biological drivers, including water temperature, salinity, and metabolic activities such as growth rate (Dodd, 1965; Klein et al., 1996; Langlet et al., 2002; Lorrain et al., 2005; Richardson et al., 2004; Stecher et al., 1996; Wanamaker and Gillikin, 2019). Several studies related Sr/Ca ratio variations to the metabolic activity of the mantle epithelium (Carré et al., 2006; Langlet et al., 2002; Stecher et al., 1996) and/or to shell growth rate (Cardoso et al., 2013a; Carré et al., 2006; Pearce and Mann, 2006; Stecher et al., 1996).

In our study, the similarity (size and occurrence) of Ba/Ca peaks between specimens (Fig. 4) was used to align inter-specimen Sr/Ca profiles, since this signal does not give information on the position of annual growth lines. In this way, a comparison between different Sr/Ca ratio profiles (i.e., from several *M. polynyma* specimens) aligned using Ba/Ca peaks would help to locate sharp and

to align inter-specimen Sr/Ca profiles, since this signal does not give information on the position of annual growth lines. In this way, a comparison between different Sr/Ca ratio profiles (i.e., from several *M. polynyma* specimens) aligned using Ba/Ca peaks would help to locate sharp and synchronous decreases in Sr/Ca profiles, similar to how cross-dating techniques are applied to reveal growth lines.. After alignment, profiles show highly synchronized sharp Sr/Ca ratio decreases (Fig. 5) among the six HPs, even though all profiles were only slightly, but significantly, correlated (average r = 0.38; *p*-value < 0.001; Table 2). Previous studies considered that lower Sr incorporation was linked to a decrease of growth rate (or cessation, forming a growth line) (e.g., Carré et al., 2006 for *Mesodesma donacium* and *Chione subrugosa*; Stecher et al., 1996 for *Spisula solidissima*). Therefore,

the common Sr/Ca decreases that we observed here indicate, with a high degree of confidence, the positions of annual growth lines.

FIGURE 5 (2 columns)

406 TABLE 2 (1 column)

4.1.3. Validation of the chemochronology methodology with determination of age and growth patterns

Figure 6 shows decreases in the Sr/Ca ratios, for M10 specimen as an example. The decreases coincide with the growth line positions on the "mirror" HP and, therefore, locate accurate annual growth lines. The annual portions of Ba/Ca ratio profiles delimited by these annual growth lines are also similar to portions of the Ba/Ca profile measured in the OSL as highlight by oxygen-isotope ratios (Fig. 7). This similarity enables annual growth lines to be accurately located on the OSL which coincide with sharp decreases in the Sr/Ca ratio and δ18O_{shell} values corresponding to colder temperature. In this case, growth cessation of *M. polynyma* could be related to winter temperatures, as already observed for many other high-latitude bivalves (Killam and Clapham, 2018), such as *Arctica islandica* (Weidman et al. 1994), *Hiatella arctica* (Sejr et al., 2002) and *Serripes groenlandicus* (Khim et al., 2003), and cold water temperatures mostly coincide with ice-covered periods. The annual growth lines localization also provides an interesting observation of the inter-annual growth rate variability. Overall, 8 to 10 carbonate samples were micromilled each year except in 2013 (where there were 15 samples; Fig. 3). Since all carbonate samples were equidistant, this suggests a higher growth rate in 2013 than the following years. This could be due to a natural ontogenetic decrease or to environmental changes.

The step-by-step chemochronology methodology allows the location of annual growth lines in a specific portion of the shell that can be matched to a precise calendar year. Once the specimen's age is determined, associated environmental effects can then be explored. Even though the mechanisms causing annual growth line formation for *M. polynyma* are still poorly understood, our results are in agreement with other studies on marine bivalves that have suggested a common control between shell

Sr incorporation and bivalve growth rate (Gillikin et al., 2005; Stecher et al., 1996; Takesue and van Geen, 2004; Vander Putten et al., 2000).

FIGURE 6 (2 columns)

FIGURE 7 (2 columns)

Using the geochemical approach and the date of death (i.e., date of field sampling), we estimated the ages of specimens M07, M08, M10, M14, M15, and M12 to be approximately 11, 12, 21, 21, 21, and 22 years, respectively (Fig. 5). This approach can be applied even if one specimen has an erratic growth pattern compared to the others. For example, despite Ba/Ca ratio variations between specimen M14 and the others are similar (Fig. 4), a delay in two concomitant Sr/Ca ratio drops was noted, indicating punctual slowing in growth for M14 (Fig. 5). This specific anomaly, only observed on M14, was likely caused by a local and probably discrete unknown disturbance, which could reflect a predator attack or any other type of individual disruption.

4.2. Exploration of the growth rates: Regional comparison and intra-site synchrony

In Figure 8, the growth curve of *M. polynyma* from Baie-Comeau was constructed using length-at-age data (i.e., distance from the umbo to each growth line revealed by the chemochronology) (Fig. 8A) that was compared to growth curves calculated either on the external shell (Lambert and Goudreau, 1999; Landry et al., 1992a) or the hinge plate (Bourassa et al., 2008; Roddick et al., 2012; Trottier and Goudreau, 2015) of specimens collected at six other sites. In each case, the von Bertalanffy growth function was used (Fig. 8B; see section 3.4 for details). Shells collected at Baie-Comeau showed a slow growth rate with a maximum size of 146.35 mm (Table 3). Variability in specimen growth rate and longevity can be observed between our sampling (Baie-Comeau) and those from previous studies in the Gulf of St. Lawrence (Table 3; Fig. 8B). Growth estimated with the von Bertalanffy growth

function for specimens from the Îles-de-la-Madeleine ($L_{\infty} = 122.37$; K = 0.049) was highly different, not only from those in Baie-Comeau ($L_{\infty} = 146.35$; K = 0.058) but also from all other sites. Environmental conditions, such as the water depth, the bottom water temperature and salinity as well as the productivity on the sampling site are probably key factors explaining the observed growth differences. Further studies are needed to explore the role of these later parameters on the local growth rates of M. polynyma such as multi-annual environmental monitoring.

FIGURE 8 (2 columns)

TABLE 3 (2 columns)

Testing the robustness of our growth rate estimation method is difficult because specimens from Baie-Comeau have not previously been the subject of growth studies, so no comparison can be made between the classical sclerochronological method and our geochemical approach. Globally, the growth rate estimated for Baie-Comeau specimens shows a similar trend with North Shore GSL sites and Banquereau Bank, for the first 15 years at least. This corresponds to a specimen length of ~90 mm. However, after 15 years, our model predicts a higher growth rate than previously reported. For example, for a length of 130 mm, our model gives an age of 38 years. In Longue-Pointe-de-Mingan (fishing zone 4B, ca. 400 km from Baie-Comeau; Fig. 1b), a length of 130 mm can be reached only for specimens older than 50 years (Bourassa et al., 2008). That length is never reached with the other models, which overestimate the age of *M. polynyma* relative to the model that we calculated. It is currently impossible to separate the environmental factors from the methodological approach to explain these differences, even though Lambert and Goudreau (1999) still noted an overestimation risk due to excess growth lines. Further geochemical investigations on specimens dated in previous studies should be planned to validate the different methodologies. Moreover, a better understanding of growth rate drivers would probably improve bivalve fishery management in the EGSL.

FIGURE 9 (1 columns)

The chemochronology method provided a reliable method to study growth rate anomalies (SGIs) of $M.\ polynyma$ in Baie-Comeau (Fig. 9). SGIs of the different specimens exhibited chaotic variations before 2010 (with specimen M14; Fig. 9A), highlighted with an EPS value dropping below the threshold of accuracy (e.g., 0.85). By removing the specimen M14, the master chronology showed a higher level of confidence, even if EPS values were still under the arbitrary threshold of 0.85 before 2008 (Fig. 9B). This difference in EPS values before and after 2008 was probably exacerbated by the low number of studied specimens. Indeed, it is rare to obtain a robust master chronology with only three specimens. To date, according to our knowledge, the minimum sample depth leading to a robust master chronology was found to be four shells of *Arctica islandica* (Estrella-Martinez et al., 2019). Nevertheless, both master chronologies obtained in our study showed a strong positive growth anomaly in 2013. This is consistent with the number of δ^{18} O_{shell} samples—higher than in other years—characterizing 2013 in the OSL of specimen M17. These results underlined a common response among the individuals studied to variations in their environments, demonstrating great potential as environmental archives especially considering the high EPS values after 2008. This technique is promising but more shells are needed to confirm the environmental response.

5. Summary and perspectives

This pioneer work offers an alternative to age bivalve species with misidentified annual growth lines, using shell geochemistry to accurately build an age model. Previous methods using standard sclerochronological techniques may have under- or over-estimated the age of M. polynyma shells. Here, we proposed a new methodology based on chemochronology (i.e., using geochemical data) to precisely locate annual growth lines (Fig. 2). The present study showed (i) the utility of the δ^{18} O profile to reveal disturbance lines among non-identified annual growth lines in the outer shell layer, and (ii) the strong relationship between Ba/Ca and Sr/Ca ratios that allowed the alignment and localization, respectively, of annual growth lines with a high level of confidence. The presence of

common sharp Sr/Ca ratio decreases between aligned profiles provided information on the position of single annual growth lines.

This chemochronological approach made it possible to reconstruct the temporal variations of standardized growth indices (i.e., master chronology) between 1998 and 2017. Our results highlighted that SGI variations are highly synchronous between specimens after 2008. Therefore, *M. polynyma* shells likely have a great potential for reconstructing past environment conditions before the instrumental era if enough specimens are available and fulfil specific criteria (i.e., over a size spectrum and state of preservation). Furthermore, synchronous trace element profiles between contemporaneous specimens could inform on past environmental variability. Indeed, numerous study linked peaks of Ba/Ca ratios to phytoplankton dynamics (e.g., Doré et al., 2020; Stecher et al., 1996; Thébault et al., 2009; Vander Putten et al., 2000). Further investigation should be done to compare variations of SGI and trace element time series with local and regional environmental variability (e.g., fluctuations in water temperature, primary production) in order to calibrate these tools as environmental proxies. All these findings support the idea that *M. polynyma* is a promising biogenic archive that could be integrated into future environmental studies of the coastal zone in sub-arctic ecosystems.

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Supplementary figures: 551 552 553 Fig. 1S. Diagram of the step-by-step approach used to identify annual growth lines on hinge plates 554 and to determine growth rate of *Mactromeris polynyma* specimens 555 Fig. 2S. Locations of salinity and δ¹⁸O_{sw} measurements made by Tan and Strain between 1980 and 556 557 1996 that were used to calculate the linear regression used in eq. (2). 558 559 Fig. 3S. Photographs of the hinge plate of specimen M07 using different optic tools and treatments. 560 (A) Shell was polished and then photographed with a stereo-microscope at 20x magnification. (B) 561 Shell after treatment with Mutvei's solution and photographed with a digital microscope at 500x 562 magnification. (C) Mosaic of images (500x magnification) showing the whole hinge plate. 563 564 References 565 566 Abele, D., Brey, T., Philipp, E., 2009. Bivalve models of aging and the determination of molluscan lifespans. 567 Exp. Gerontol. 44, 307-315. https://doi.org/10.1016/j.exger.2009.02.012 568 Ballesta-Artero, I., Zhao, L., Milano, S., Mertz-Kraus, R., Schöne, B.R., van der Meer, J., Witbaard, R., 2018. 569 Environmental and biological factors influencing trace elemental and microstructural properties of 570 islandica shells. Sci. Total Environ. 645, 913-923. 571 https://doi.org/10.1016/j.scitotenv.2018.07.116 572 Black, H.D., Andrus, C.F.T., Lambert, W.J., Rick, T.C., Gillikin, D.P., 2017. δ¹⁵N values in *Crassostrea* 573 virginica shells provides early direct evidence for nitrogen loading to Chesapeake Bay. Sci. Rep. 7, 574 44241. https://doi.org/10.1038/srep44241 575 Blais, M., Galbraith, P.S., Plourde, S., Scarratt, M., Devine, L., Lehoux, C., 2019. Chemical and biological 576 oceanographic conditions in the Estuary and Gulf of St. Lawrence during 2018. Can. Sci. Advis. Sec. 577 Res. Doc. 2019/059, iv + 64 pp.

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Figure captions

848 Fig. 1. Map of DFO fishing areas for *Mactromeris polynyma* in the Estuary and Gulf of St. Lawrence. 849 (A) Location of the Estuary and Gulf of St. Lawrence, (B) DFO fishing areas with specific beds, and 850 (C) beds of *M. polynyma* with the location of the sample site (purple triangle). 851 852 Fig. 2. (A) Schematic of the different cut axes made on M. polynyma shells to obtain cross sections 853 used for geochemical analyses. Red dashed lines indicate the maximum growth axis on which 854 geochemical analyses were performed. (B) Image of the hinge plate cross section where trace element 855 analyses (TEA) were performed using LA-ICP-MS. (C) Image of the whole shell cross section (from 856 the umbo to the ventral margin). Two kinds of geochemical analyses were performed: (D) trace 857 element analyses (TEA) and (E) oxygen stable isotope analyses (SIA) in the outer shell layer. Note 858 that SIA was only performed on specimen M17. 859 860 Fig. 3. Temporal variations in the theoretical monthly δ^{18} O (red line) on a 22.4 mm section calculated 861 with eq. (2) and δ^{18} O measured in the OSL of the specimen M17 (black line). Each coloured dot 862 863 represents the theoretical value of $\delta^{18}O_{theo}$ calculated using average monthly temperatures and salinities measured between 2010 and 2018. The grey area represents the $\delta^{18}O_{theo}$ standard deviation. 864 The vertical dashed grey lines show the location of growth lines visually identified on the OSL. The 865 dashed black lines are presumed annual growth lines based on $\delta^{18}O_{shell}$ (see section 4.1.2). Note that 866 867 the y-axis is reversed. DOG: Direction of growth. 868 869 Fig. 4. Ba/Ca profiles measured in HPs of six specimens (M15, M07, M08, M10, M12, and M14) 870 from the end of the HP toward the umbo. Grey lines represent the raw data and coloured lines the 10-871 point running mean. Triangles mark the peaks used to align the profiles. DOG: Direction of growth. 872 873 Fig. 5. Aligned profiles of Sr/Ca based on Ba/Ca peaks for the six studied specimens (HPs analyzed). 874 Dashed vertical lines indicate drastic Sr/Ca decreases detectable in all specimens. Triangles mark

minimum values of Sr/Ca near or at the dotted lines. DOG: Direction of growth.

Fig. 6. Profile of the Sr/Ca ratio of specimen M10 and photograph of its hinge plate. Vertical dashed grey lines represent minimum Sr/Ca ratio values that are common among specimen profiles; their respective distances from the end of the ventral margin are displayed on the hinge plate photograph (blue arrows). The dashed blue lines show the growth lines associated with arrows.

Fig. 7. Variations in the $\delta^{18}O_{shell}$ and trace element ratios measured from the ventral margin toward the umbo in the OSL are shown. Profiles were smoothed with a 100-point running mean. The red curve presents the Ba/Ca profiles recorded on the M17 OSL and the grey curves show Ba/Ca profiles recorded on the HPs of the 6 other specimens (M07, M08, M12, M10, M15 and M14). Triangles show the average position of annual growth lines among the 6 HPs (see Figure 5); vertical dashed grey lines represent the excessive number of growth lines visually identifiable on the OSL while vertical dashed black lines represent the location of annual growth lines determined by both coupled Ba/Ca and Sr/Ca ratios and oxygen stable isotope ratio ($\delta^{18}O$). Note that the y-axis of $\delta^{18}O_{shell}$ is reversed. DOG: Direction of growth.

Fig. 8. (A) Von Bertalanffy growth curves estimated from the length-at-age of the six studied specimens (i.e., six analyzed HPs) from age 1 to 22 collected in Baie-Comeau. Shell M17 was not added to the curve because its age was unknown. (B) Growth curves calculated to age 70 and estimated for specimens originated from five different beds studied previously in the Gulf of St. Lawrence.

Fig. 9. Standardized annual growth rates (SGIs) of *M. polynyma* shells determined by localization annual growth lines *via* comparisons of inter-individual Sr/Ca ratio patterns. Coloured dots and lines represent the SGIs for the six specimens (A) and without specimen M14 (B) with the averaged chronologies (black). The number of shells in the chronology through time (sample depth) is represented by the grey shadow. EPS values (green curve) from the master chronologies were computed with a five-year running window with an overlap of three years.

Tables

Table 1. Morphometry of collected specimens. The length (anterior–posterior axis), height (dorso–ventral axis) and hinge length for the seven shells are in mm. HP: hinge plate (first 6 shells); OSL: outer shell layer.

-	Shell ID	Length	Height	Hinge length
•	M07	77.48	57.47	9.26
НР	M08	87.75	65.97	10.77
	M10	104.06	78.94	13.28
	M12	103.55	78.08	13.29
	M14	99.25	73.5	12
	M15	109.14	83.06	14.14
OSL	M17	86.28	65.47	10.66

Table 2. Pairwise correlation matrix between the six hinge plate profiles (M07, M08, M10, M12, M14, and M15) of Sr/Ca (10-pt running mean). Coefficients of correlation (r) were calculated after Sr/Ca profiles were aligned using Ba/Ca peaks (see Fig. 7). Stars indicate the p-value: *** <0.001, ** <0.01, * <0.05.

		MU7	MU8	MIO	MIZ	M14	M15
_	M07						
	M08	0.52 ***					
Sr/Ca	M10	0.30 ***	0.39 ***				
Sr/	M12	0.39 ***	0.28 ***	0.57 ***			
•	M14	0.12 ***	0.28 ***	0.49 ***	0.42 ***		
	M15	0.08 *	0.29 ***	0.61 ***	0.61 ***	0.31 ***	

Table 3. Parameters estimated from the von Bertalanffy growth function for six different beds located in the Gulf of St. Lawrence (see Fig. 1B for details). The length reached after an infinite time of growth (L_{∞}), the Brody growth constant per year (K), and the theoretical age at which the length would be zero, in years (t_0). The associated standard deviation (sd) and 95% confidence interval (CI) is shown only for the Baie-Comeau bed. Sampling years and references for other studies are also given.

Beds	Year	\mathbf{L}_{∞}	K	t_0	References
Baie-Comeau	2018	142.46	0.06464	0.9126	This study
		sd = 10.29	sd = 0.0082	sd = 0.2103	
		CI ± 20.41	CI ± 0.01634	CI ± 0.4169	
Longue-Pointe-de-Mingan	2004	134.00	0.0686	0.2120	Bourassa et al., 2008
Moisie River	1993	119.47	0.0825	0.1926	Lambert and Goudreau, 1999
Rivière-au-Tonnerre	1994	110.63	0.0980	0.4870	Lambert and Goudreau, 1999
Iles de la Madelaine	1992	122.37	0.0490	-0.8990	Landry et al., 1992
Banquereau Bank	2010	119.56	0.0830	0.0980	Roddick et al., 2012
Natashquan	2010	125.52	0.0777	0.5899	Trottier and Goudreau, 2015

















