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Construction of long annually-resolved shell-based chronologies using *Glycymeris glycymeris* (Bivalvia; Glycymerididae) from the Bay of Brest, France

Amy Marie Featherstone

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Construction of long annually-
resolved shell-based chronologies
using *Glycymeris glycymeris*
(Bivalvia; Glycymerididae) from the
Bay of Brest, France

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Chapter 1: Introduction

Plus de 37% de la population mondiale vit actuellement à moins de 100 km des côtes de l'Océan Mondial (McGranahan et al. 2007) tandis que 70% des plus grandes villes du monde (de plus de 1,6 millions d'habitants) se trouvent en zone littorale (Small & Nicholls 2003). Cette forte présence humaine expose les écosystèmes côtiers de la planète à une série d'effets anthropiques directs et indirects (Stutz & Pilkey 2005). Le développement continu de ces zones se caractérise par l'intensification généralisée de l'urbanisation du littoral (McKinney 2002), du transport maritime (Hall & Jacobs 2012), de la pêche (Garcia & Grainger 2005) et de l'exploitation des ressources renouvelables et non renouvelables (Liu et al. 2011). Cette activité économique provoque une augmentation sensible des concentrations en métaux lourds et autres contaminants dans les eaux côtières, avec parfois pour conséquence l'eutrophisation des écosystèmes concernés (Tam & Wong 2000; Smith 2003; Tett et al. 2007). Les écosystèmes côtiers s'étant révélés les systèmes les plus sensibles aux effets anthropiques de tout l'environnement marin, nous devons étudier très attentivement leur comportement face aux événements et perturbations climatiques qu'ils subissent. Parmi les défis encore à relever figure celui qui consiste à établir une distinction précise entre les réponses d'origine naturelle et celles d'origine anthropique de ces systèmes vis à vis des différents systèmes climatiques qui les gouvernent.

1.1. Le changement climatique

Il a été maintes fois démontré que les récentes variations climatiques de notre planète, loin d'être uniquement l'œuvre de changements naturels, sont également dues à l'action de l'homme (Moritz et al. 2002; Smol et al. 2005; Behrenfeld et al. 2006; Hoegh-Guldberg & Bruno 2010; etc.). Certains des effets du changement climatique sur l'environnement marin sont identifiables et quantifiables : augmentation de la température de l'eau (Hughes et al. 2003), accélération de l'écoulement des glaces polaires causé par leur fonte (Zwally et al.

2002), élévation du niveau moyen des océans (Monioudi et al. 2017), diminution globale de la productivité primaire nette (Behrenfeld et al. 2006) et modifications de la circulation océanique (Clark et al. 2002). On observe également des changements dans la répartition des espèces marines, avec notamment des apparitions de plus en plus fréquentes d'espèces invasives et la diminution des captures par pêche. Ainsi, de nombreuses espèces de poissons ayant une grande importance commerciale ont migré vers le nord du fait du réchauffement climatique (Perry et al. 2005).

Les océans sont l'un des plus grands réservoirs de dioxyde de carbone (CO₂; Lampel et al. 2017). De nombreuses études ont montré que les océans ralentissent l'augmentation de la concentration atmosphérique en CO₂ liée aux activités humaines. On considère qu'environ un quart à un tiers des émissions anthropiques a été absorbé par les océans, même s'il est probable que le taux de séquestration du carbone puisse avoir évolué ces dernières décennies (Barnett et al. 2001; Sabine et al. 2004; Khatiwala et al. 2009; le Quéré et al. 2010). En dépit du rôle crucial des océans dans le système climatique mondial, notre connaissance de son fonctionnement est encore incertaine.

La plupart des prévisions de variabilité des océans repose sur la modélisation de leur fonctionnement. Ces modèles de prévisions sont donc alimentés, à l'origine, par des relevés instrumentaux historiques. Bien que ces modèles soient de puissants outils de prévision climatique, ils tendent à faire des erreurs sur les prévisions à l'échelle locale par manque de données rétrospectives cohérentes. Ce type de données facilite en effet la calibration des modèles (Braconnot et al. 2012). Reynolds et al. (2013) soulignent que cela est particulièrement le cas pour les données recueillies avant 1860 quand les méthodes de mesure étaient souvent rudimentaires et que les collectes des données n'étaient pas systématiques. Les études paléoclimatologiques basées sur des proxies environnementaux peuvent permettre

de surmonter ces difficultés (Wanamaker et al. 2011). La sclérochronologie des bivalves marins en est un bon exemple (Rahmstorf 2002).

Même s'il existe une grande variabilité interne naturelle des océans, en particulier des écosystèmes côtiers, Harley et al. (2006) suggèrent qu'un certain nombre de changements récents pourraient être causés par le réchauffement climatique. Ce pourrait ainsi être le cas pour la modification de la circulation des océans et de leur capacité de séquestration du CO₂.

1.1.1. La circulation océanique

La circulation océanique dépend de plusieurs processus dont, principalement, les tourbillons océaniques et la circulation méridienne de retournement (CMR) de l'Atlantique (*Atlantic Meridional Overturning Circulation* AMOC; Marshall & Speer 2012). La CMR est le résultat de l'application du principe d'Archimède, selon lequel les différences de densité de surface aux latitudes élevées permettent la convection océanique (McDougall et al. 2014). Ce phénomène, souvent appelé circulation thermohaline, préserve l'Europe du Nord du climat extrême que connaît, par exemple, le Canada situé à la même latitude. C'est pourquoi une modification éventuelle de cette circulation pourrait avoir des effets catastrophiques comme une brutale modification du climat (Dupont 2008). Certains modèles climatiques prévoient que la circulation thermohaline pourrait ralentir d'environ 30% sous l'effet de l'arrivée de grandes quantités d'eau douce provoquées par les anomalies climatiques et l'augmentation des précipitations dans les latitudes les plus septentrionales (Stouffer et al. 2006). Bien que très controversé dans la littérature spécialisée, ce refroidissement des eaux entraînerait l'apparition d'un climat plus froid en Europe du Nord. On trouvera de plus amples informations sur cette controverse au Chapitre 4.

1.1.2. Le CO₂

L'augmentation de la concentration atmosphérique en CO₂ a des effets directs et indirects sur les océans. En termes simples, le carbone se dissout dans les océans, puis se retrouve absorbé par les organismes photosynthétiques comme le phytoplancton (Wolf-Gladrow & Riebesell 1997). Cependant, des études ont montré que l'augmentation du CO₂ dans les océans diminue la capacité de ces derniers à dissoudre le carbone inorganique via le facteur Revelle [*Revelle factor*] (Sabine et al. 2004). L'absorption du carbone par les océans pourrait changer avec un réchauffement général de la planète, particulièrement dans les régions où les taux d'absorption du CO₂ sont les plus élevés, comme par exemple dans l'Atlantique Nord (Bousquet et al. 2000). Les taux les plus élevés de facteur Revelle sont observés dans les eaux froides des latitudes élevées car les eaux plus chaudes ont une capacité d'absorption du carbone moindre (Sabine et al. 2004). Néanmoins, les océans

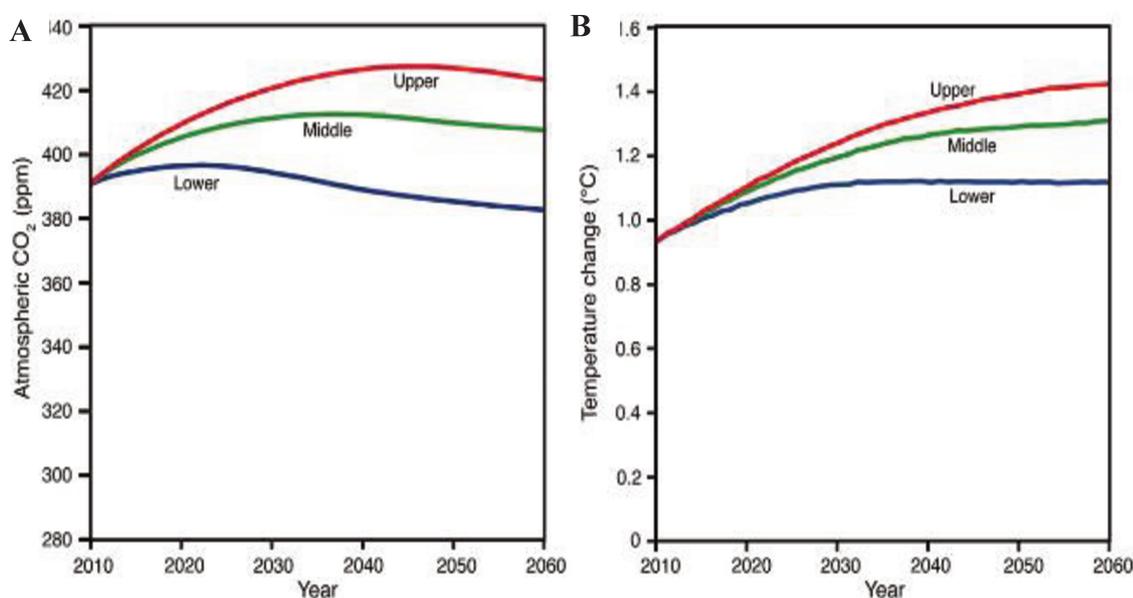


Fig. 1.1: Evolution future du CO₂ atmosphérique (A) et de la température moyenne mondiale (B). Chaque courbe indique des variations basées sur les émissions mondiales estimées du rapport spécial du GIEC (scénarios d'émissions A2). L'axe vertical indique les variations à partir de données de l'époque préindustrielle. Images adaptées de Davis et al. (2010).

continuent à absorber le carbone, ce qui provoque l'acidification des eaux, la baisse du pH des océans ainsi qu'un état de sous-saturation préjudiciable à la formation d'organismes marins dépendants du carbonate de calcium (Doney et al. 2009).

En résumé, la quantité de CO₂ pourrait changer radicalement dans le futur et cela va avoir un impact significatif sur le climat et l'environnement (Hoegh-Guldberg et al. 2007; Fig. 1.1). De ce fait, l'Atlantique Nord intéresse particulièrement les spécialistes du climat en raison de son influence dominante sur les systèmes météorologiques mondiaux et la variabilité de son environnement (Walther et al. 2002).

1.2. Les systèmes océaniques et atmosphériques influant sur l'Atlantique Nord

L'Océan Atlantique est le deuxième plus vaste après le Pacifique, couvrant environ 20% de la surface de la planète. Il est par ailleurs l'un des plus salés, avec une salinité de surface comprise entre 33 et 37 selon la latitude et la saison. Les valeurs maximales de salinité se retrouvent dans les régions subtropicales soumises à de faibles précipitations et à une forte évaporation. Les salinités les plus faibles sont quant à elles enregistrées au nord de l'équateur (du fait des fortes précipitations tropicales), aux hautes latitudes et le long des côtes à proximité des estuaires (Talley, 2002). La température de surface de l'Océan Atlantique varie selon la saison, la latitude et la courantologie. Les effets conjoints des variations de température et de salinité déterminent la densité de l'eau qui influence en retour les mouvements des masses d'eau (Lalli & Parsons 1997).

Une masse d'eau est identifiable par un certain nombre de propriétés physiques et chimiques (i.e., température, salinité, densité, traceurs chimiques) qui résultent de son

processus de formation. La redistribution des masses d'eau dans l'Atlantique Nord est liée au transport de chaleur, notamment entre les régions tropicales et les régions polaires (Scott & Marotzke, 2002). Par conséquent, il est important de comprendre et de quantifier les mécanismes qui induisent des variations de circulation océanique, aussi bien dans le passé que dans le futur. En particulier, si les conditions climatiques des derniers siècles furent relativement similaires à celles d'aujourd'hui (Thompson et al. 2002), elles ont aussi été influencées par des épisodes pluri-décennaux caractérisés par des écarts de températures notables par rapport à la moyenne à long-terme, probablement causés par des forçages climatiques externes anormaux. Cela a pu être mis en évidence par plusieurs reconstructions climatiques basées sur des données de type "proxy" (Overpeck et al. 1997; Jones et al. 2001; Mann & Jones, 2003).

La circulation de surface dans l'Atlantique Nord consiste en trois principaux courants inter-connectés entre eux : le Gulf Stream qui s'écoule vers le nord-est depuis les côtes d'Amérique du Nord; la dérive nord-atlantique (North Atlantic Current – NAC) qui est une branche du Gulf Stream se dirigeant vers le nord depuis les Grands Bancs de Terre-Neuve; et le front sub-polaire qui est l'extension nord-est de la dérive nord-atlantique (Marchal et al. 2016). Ce front sub-polaire est une région qui sépare le gyre sub-tropical du gyre sub-polaire. Ce système de courant transporte les eaux chaudes dans l'Atlantique Nord; sans lui, les températures dans l'Atlantique nord-est et en Europe chuteraient dramatiquement (Rossby 1996).

La circulation thermo-haline globale est induite par les effets conjoints du forçage thermo-halin et du mélange vertical (Rahmstorf 2003). Le gyre sub-polaire est une composante importante de la circulation thermo-haline et joue un rôle crucial dans la variabilité climatique (Tréguier et al. 2005; Moreno-Chamarro et al. 2016) de par l'existence d'un lien entre la force de ce gyre et la convection profonde en mer du Labrador (un site très

important pour la circulation thermo-haline). Il existe donc un lien fort entre la variabilité de la dynamique du gyre sub-polaire et l'évolution de la circulation thermo-haline dans l'Atlantique (Böning et al. 2006).

Les forçages atmosphériques ont également un impact sur la circulation thermo-haline dans l'Atlantique. Il est possible que les températures hivernales relativement douces dans l'Atlantique nord et en Europe occidentale puissent être reliées à des processus d'advection éoliens and non au transport de chaleur océanique (Seager et al. 2002). L'Oscillation Nord-Atlantique (North Atlantic Oscillation - NAO) est un facteur climatique ayant une influence sur la température de l'air, les précipitations, et la vitesse du vent à l'échelle du bassin nord-Atlantique (mais également sur les masses continentales d'Europe occidentale et d'Amérique nord-est), agissant à une échelle inter-annuelle (Lamb & Pepler 1987; Hurrell 1995; Trigo et al. 2008). Il existe des différences importantes dans les prédictions de différents modèles climatiques quant à l'évolution future de la NAO et quant aux réponses de cette oscillation au changement climatique (Woollings & Blackburn 2012). Si de récents travaux ont permis d'améliorer les capacités de prédiction des variations de la NAO à une échelle saisonnière (e.g. Cassou 2008; Scaife et al. 2014), la prédiction des tendances à long-terme reste à ce jour un challenge considérable.

D'autres forçages atmosphériques majeurs peuvent par ailleurs moduler la relation entre la NAO et le climat hivernal en Europe de l'est, par l'intermédiaire de processus non-stationnaires. La structure est-atlantique (East Atlantic pattern - EAP) est l'un de ces forçages. Initialement décrit par Wallace & Gutzler (1981), il est défini par un centre de basses pressions au-dessus de la région sub-tropicale de l'Atlantique nord, le long de la ligne nodale du dipole caractérisant la NAO. L'EAP est connu pour influencer le trajet et la position des tempêtes au-dessus de l'Atlantique nord (Woollings et al. 2010). De récents travaux ont montré que l'EAP, conjointement avec un autre forçage dénommé Scandinavian

Pattern (SCA), pouvait influencer la position des centres d'action du dipole de la NAO (Moore et al. 2013; Comas-Bru & McDermott 2014). Les phases de l'EAP et du SCA contrôleraient ainsi la relation entre la NAO et les conditions hivernales dans la région (température de l'air et précipitations notamment). Plus récemment, il a été démontré que la connection entre la NAO et l'EAP semble être à l'origine des anomalies de transport de chaleur et de vapeur d'eau au-dessus de l'Atlantique nord (Bastos et al. 2016). Toutefois, le rôle potentiel de l'EAP et du SCA dans la modulation des liens, étudiés par Brayshaw et al. (2011) et García-Bustamante et al. (2013), entre l'indice NAO et le régime des vents à la surface des océans reste encore à étudier.

Avec la récente prise de conscience du changement climatique à l'œuvre, il est plus important que jamais d'acquérir une vision globale du système climatique planétaire afin de mieux en prévoir ses futures évolutions (Moss et al. 2010). L'absence de données d'observation paléoclimatiques, aussi bien spatiales que temporelles, est un frein majeur à l'obtention d'estimations climatiques précises (Phipps et al. 2013). La sclérochronologie peut, aujourd'hui, contribuer à combler ce manque grâce à sa capacité à fournir quantité d'indicateurs (Wanamaker et al. 2011).

1.3. La sclérochronologie

Avec la perspective du changement climatique au premier rang des préoccupations de la politique environnementale, des études mettant en évidence les variations des systèmes climatiques passés sont indispensables (Reynolds et al. 2010; Tschakert & Dietrich 2010; Cameron 2012). Elles permettent, en effet, une meilleure compréhension des processus responsables de ces changements (Gröke & Gillikin 2008). L'océan affecte le système climatique de plusieurs façons, notamment en jouant un rôle dans les grands cycles

biogéochimiques et les échanges gazeux avec l'atmosphère (Rahmstorf 2002). Toutefois, les séries temporelles d'observation issues de relevés instrumentaux ne sont pas suffisamment longues pour fournir une image complète et précise de la variabilité climatique. Les changements de circulation océanique au cours des derniers millénaires peuvent potentiellement être enregistrés dans des archives biogéniques. Des reconstructions paléoclimatiques peuvent en effet être réalisées à partir de différents types de données proxy, à différentes échelles temporelles (sub-annuelle à séculaire). De nombreuses archives paléoclimatiques permettent déjà de réaliser des reconstructions haute-résolution. Le point crucial pour définir une archive haute-résolution est que son processus de formation permette une datation aussi précise que possible de toute partie de l'archive (e.g. possibilité de préciser au cours de quelle année s'est formée telle ou telle portion). La capacité des modèles à prévoir le futur des systèmes climatiques et océaniques se trouve renforcée par cet apport d'indicateurs de changements passés (Rahmstorf 2002; Harley et al. 2006; Brown et al. 2008; Collins et al. 2010). Ces indicateurs peuvent être des signaux géochimiques ou structurels, enregistrés de manière séquentielle dans des accrétions et des dépôts de structures biologiques ou géologiques représentatives de l'environnement dans lequel elles se sont formées (Gröke & Gillikin 2008).

La sclérochronologie est l'étude de la formation temporelle, sous forme d'incrément de croissance, de ces dépôts calcifiés sur le squelette en carbonate de calcium des organismes marins (Kirby 2000). En effet, ces indicateurs biologiques de la variabilité de l'environnement marin sont les équivalents, pour les océans, des indicateurs dendrochronologiques des écosystèmes continentaux (Jones 1983). Le terme de sclérochronologie est apparu pour la première fois en 1974 dans une publication de Buddemeier & Maragos (1974) qui portait sur l'étude radiographique de la croissance d'un corail. Ce terme est ensuite employé deux ans plus tard, pour la première fois, dans le titre

d'une publication de Hudson et al (1976). Depuis, et particulièrement au cours de ces quinze dernières années, la sclérochronologie a connu des avancées majeures et est actuellement en plein essor au sein de la communauté scientifique (Kowelski 2001). Schöne & Gillikin (2013) proposent d'utiliser ce terme uniquement pour l'étude de la structure physique des tissus durs, complétée ou non par une analyse géochimique, et de considérer la sclérochimie, discipline qui étudie uniquement la géochimie isotopique et élémentaire des tissus durs des organismes marins, comme une sous-discipline de la sclérochronologie. Cette différenciation est inspirée de celle que font déjà les dendrochronologistes qui distinguent l'étude des isotopes des cernes des arbres (dendrochimie) de celle de l'accroissement temporel de la largeur de ces cernes (dendrochronologie) (Bondi & Qeadan 2008). Si la dendrochronologie est de loin la discipline la plus connue, les cernes des arbres ne sont pas les seuls éléments à pouvoir fournir des informations sur l'environnement, et l'on peut aujourd'hui obtenir des données importantes à partir des coquilles de mollusques (Marali et al. 2017), des sclérosponges (Horta-Puga 2017), des carottes de glace (Stein et al. 2017), des algues coralliennes (Wanamaker et al. 2011), des sédiments (Allen et al. 1999), des coraux (Marschal et al. 2004), des otolithes de poisson (Alós et al. 2017), des spéléothèmes (MacDougal et al. 2017), des statolithes (Zumholz et al. 2007), des foraminifères (Ezat et al. 2017), des brachiopodes (Curry & Fallick 2002), ainsi que des dents de mammifères (Hamilton et al. 2017).

Les études de sclérochronologie reposent sur l'analyse de l'accroissement annuel des couches calcaires qui forment le squelette des animaux, notamment des mollusques bivalves, provenant en général du même milieu et de la même espèce. Les incréments correspondants à l'accroissement annuel de chaque coquille peuvent être comptés et on peut en déduire l'âge de l'animal. La mesure de la largeur de ces incréments permet également de quantifier la croissance annuelle de chaque individu. Les résultats obtenus peuvent être utilisés comme indicateur de la variabilité de l'environnement, notamment de la température de l'eau. Selon

Butler et al. (2013) la température peut en effet influencer de trois façons sur la croissance des coquilles : d'abord comme le résultat direct d'effets thermodynamiques, puis, indirectement, à travers la température des eaux de surface des océans qui modifie la disponibilité alimentaire (Gosling 2003), et enfin en modifiant la durée de la période de croissance des mollusques bivalves (Brocas et al. 2013; Schöne et al. 2004).

En effet, en fonction de l'environnement local, on observe une croissance synchronisée des animaux d'un même milieu (Helama et al. 2007; Black et al. 2008a; Coulson et al. 2014; Vihtakari et al. 2017). Si des incréments de croissance remarquables, synchronisés entre plusieurs individus d'une même population, peuvent être identifiés par des techniques dites de *cross-matching*, il devient alors possible de combiner de multiples séries individuelles afin de reconstituer une chronologie de référence (= chronologie maîtresse, *master chronology*) beaucoup plus longue et étoffée (Schöne & Gillikin 2013). Une fois que cette chronologie maîtresse est établie, il devient possible d'étudier les liens statistiques qui existent entre cet enregistrement de type proxy et le climat local, permettant alors de reconstruire la variabilité climatique dans le passé à des périodes où les enregistrements instrumentaux n'existaient pas. Ces reconstructions locales peuvent ensuite être utilisées pour réaliser des reconstructions à plus large échelle spatiale (CFRs, pour Climate Field Reconstructions) en les intégrant dans un réseau spatialement plus étendu d'enregistrements proxy.

Bien que ce champ d'étude n'en soit encore qu'à ses débuts, il a indéniablement commencé à prendre son essor, en particulier dans le domaine de la croissance des bivalves.

1.4. Le potentiel des bivalves comme archives marines

Les mollusques présentent de nombreux avantages par rapport aux carottes de sédiments ou aux coraux. Tout d'abord, la périodicité de formation des lignes de croissance de la coquille est parfois très élevée, pouvant aller chez certaines familles de bivalves jusqu'à indiquer la fréquence des marées (Richardson et al. 1980). Cette caractéristique confère à ces mollusques un potentiel considérable en matière de paléoreconstruction à haute résolution. On peut ainsi reconstituer des conditions environnementales à un niveau infra-annuel alors que les coraux et les sédiments n'offrent généralement qu'une information à l'échelle annuelle (Mann 2002). Un autre avantage des mollusques est leur couverture géographique très large par rapport à d'autres archives potentielles comme les coraux scléactiniaires qui se limitent aux latitudes inter-tropicales. Ils peuvent ainsi fournir des données d'observation du climat sur une échelle spatiale beaucoup plus large (Kearney & Porter 2005; Chauvaud et al. 2012).

Le nombre d'études consacrées aux coquilles des bivalves et, plus particulièrement, au lien entre la largeur de leurs incréments de croissance et leur environnement, est en constante augmentation. Les mesures de la taille de ces incréments ont déjà permis de reconstituer des oscillations climatiques (Holland et al. 2014) ainsi que des courbes de température de l'eau de mer (Jones et al. 1989). D'autres travaux ont permis de mettre en évidence des relations entre la croissance des bivalves et plusieurs autres variables environnementales. Ainsi, Helama et al. (2007) and Mette et al. (2016) ont pu démontrer que la croissance de la coquille d'*Arctica islandica* était étroitement liée à la NAO hivernale, tandis que Gutierrez-Mas (2011) a mis en évidence l'influence de l'élévation du niveau des mers sur des fossiles de *Glycymeris spp.* Enfin, Bušelić et al. (2015) ont mis en évidence, au moins en partie, l'existence d'une corrélation entre la croissance de *G. bimaculata* et la salinité des eaux environnantes.

Des rythmes de croissance endogènes ont pu être observés chez un certain nombre d'espèces de bivalves marins. Ainsi, des incréments de croissance des coquilles liés aux marées ont été identifiés chez *Chione fluctifraga* et *C. cortezi* et pourraient correspondre aux cycles d'émersion-immersion (Schöne et al. 2002). D'autres espèces, comme *Crassostrea gigas*, présentent une croissance réduite, voire même interrompue, pendant la gamétogenèse, toute l'énergie disponible de l'organisme étant alors mobilisée pour la production de gamètes (Dridi et al. 2007). Chez d'autres espèces encore, comme chez le bivalve antarctique *Yoldia eightsi*, on a pu observer une cyclicité de croissance endogène, qui demeure pour l'instant inexpliquée (Román-González et al. 2017).

Le seul inconvénient à l'étude de ces mollusques est une longévité le plus souvent moindre que celle des coraux. En effet, la plupart des bivalves vivent une quinzaine d'années environ, ce qui ne permet pas de disposer d'une fenêtre temporelle d'archivage très longue, même à très haute résolution (Ridgeway et al. 2011). Il y a toutefois des exceptions chez certaines espèces : *A. islandica*, par exemple, peut vivre plus de 500 ans (Butler et al. 2013) tandis que *Margaritifera margaritifera* (Geist 2010) et *G. glycymeris* (Reynolds et al. 2013) peuvent atteindre les 200 ans. Le prélèvement de différents spécimens vivants et morts sur un même site permet de reconstituer l'état du milieu jusqu'à plusieurs siècles en arrière (Weidman et al. 1994). De plus, les restes de coquilles de bivalves sont courants sur les sites archéologiques ou également à l'état de fossiles et sub-fossiles, ce qui permet d'accéder à des données sur les conditions environnementales, non seulement à une échelle historique, mais également géologique.

Pour toutes ces raisons, les propriétés des bivalves les rendent particulièrement appropriés pour nous aider dans la reconstitution des variations environnementales passées, pas uniquement à travers l'étude des incréments, mais également de la composition en

isotopes stables (Jolivet et al. 2015) et des concentrations en éléments traces (Thébault et al. 2009).

1.5. Formation de coquilles bivalves

L'un des principaux pré-requis pour utiliser les coquilles de bivalves comme archives environnementales est le fait de pouvoir dater très précisément chaque portion de coquille. Il est par conséquent essentiel de parvenir à une bonne compréhension des mécanismes de formation des structures de croissance formées périodiquement dans ces coquilles. De

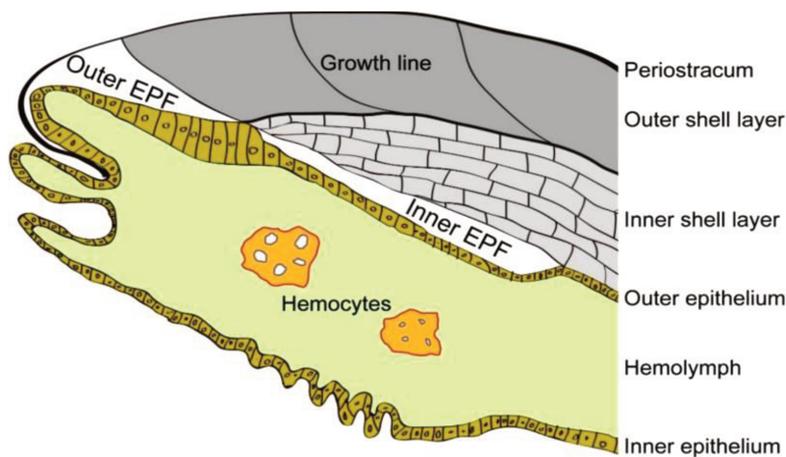


Fig 1.2: Dessin d'une coupe longitudinale d'une coquille de bivalve au niveau du bord ventral et du manteau. EPF désigne le fluide extra-palléal. Adapté de Wilbur & Saleuddin (1983).

nombreux travaux réalisés sur *Cerastoderma edule*

(Richardson et al. 1980),

Ruditapes philippinarum

(Kim et al. 1999) et

Saxidomus purpuratus (Kim

et al. 2003) ont démontré que

les rythmes de croissance

étaient contrôlés par des

mécanismes biologiques

(Rensing et al. 2001), eux-mêmes régulés par des facteurs environnementaux comme la température, la disponibilité en nourriture, la photopériode ou les cycles tidaux (Brockington & Clarke 2001). Les processus biologiques, en lien avec la saisonnalité de divers paramètres environnementaux, joueraient ainsi un rôle clé dans la définition de la longueur de la saison de croissance, mais aussi des dates de reprise et d'arrêt de croissance (Schöne 2008).

Le mécanisme par lequel les bivalves forment leur coquille est encore loin d'avoir été complètement élucidé. Il est notamment encore difficile de savoir si la formation de la coquille est un processus entièrement extracellulaire ou bien si une partie se déroule dans le milieu intracellulaire. Ainsi, deux modèles conceptuels ont été développés pour répondre à cette interrogation.

Le premier (*matrix model*) suggère que le processus de biominéralisation se déroule dans le fluide extra-palléal (EPF), un liquide biologique enfermé dans un compartiment situé entre l'épithélium externe du manteau et l'intérieur de la coquille (Fig. 1.2). Dans ce fluide, la précipitation du carbonate de calcium (CaCO_3) est facilitée par la sursaturation en ions calcium et bicarbonates. La cristallisation du CaCO_3 y est alors réalisée autour d'une trame de matrice organique sécrétée par l'épithélium externe du manteau (Bevelander & Nakahara 1969; Wheeler 1992). Ce modèle considère donc que la formation de la coquille prend place extracellulairement, au sein du fluide extra-palléal. A l'inverse, le modèle dit "cellulaire" considère la biominéralisation comme un processus partiellement intra-cellulaire : les cristaux de CaCO_3 seraient formés dans des hémocytes au sein de l'hémolymphe, puis transportés au niveau du front de calcification (Mount et al. 2004; Zhang et al. 2012). En dépit du fait que ces modèles proposent des mécanismes différents, tous deux s'accordent pour considérer que la biominéralisation de la coquille se déroule dans un micro-environnement isolé du milieu ambiant. Cela complique d'autant plus la mise en évidence d'éventuelles relations entre des variables environnementales et la composition isotopique et élémentaire ("impuretés") dans la coquille.

1.6. Les isotopes stables

L'analyse des isotopes stables de l'oxygène ($\delta^{18}\text{O}$) donne des résultats intéressants et plusieurs études ont montré que le ratio des ces isotopes dans les coquilles de mollusques est un bon indicateur des variations de température de l'eau de mer dans laquelle l'organisme, qui a fabriqué ce squelette calcaire, a vécu (Chauvaud et al. 2005). Cependant, les travaux d'Epstein et al. (1953) ont souligné l'influence de la composition isotopique du fluide ambiant au cours de la précipitation du carbonate. Schöne & Gillikin (2013) indiquent que $\delta^{18}\text{O}$ est un double indicateur car il enregistre simultanément les changements de température et la signature isotopique de l'oxygène de l'eau ambiante (Urey 1948). Il est donc indispensable de connaître la valeur du $\delta^{18}\text{O}$ de l'eau (présentant une relation linéaire à la salinité) afin de reconstituer précisément l'évolution de la température de l'eau à partir des données de $\delta^{18}\text{O}$ des coquilles.

Les isotopes de l'oxygène ont été utilisés avec succès dans le passé comme indicateurs de température de l'eau de mer, principalement grâce à l'équation mise au point par Grossman & Ku (1986) pour les bivalves aragonitiques. *Glycymeris glycymeris*, présent en rade de Brest, est présenté comme un cas particulier par Royer et al. (2013). En effet, la reconstitution précise de l'évolution des températures mesurées a nécessité la mise au point d'une nouvelle équation. L'équation de Grossman & Ku (1986) a toutefois été utilisée avec succès par Reynolds et al. (2013), qui ont mené leur étude dans le nord-ouest de l'Écosse. D'autres espèces du genre *Glycymeris* ont également été utilisés avec succès pour la reconstitution de paléotempératures (Bušelić et al. 2015; Kim et al. 2016). L'étude de Royer et al. (2013), citée précédemment, a servi de pierre angulaire aux travaux présentés ici et plus d'informations à ce sujet peuvent être trouvées au Chapitre 5.

Le $\delta^{13}\text{C}$ des coquilles de bivalves est supposé refléter la composition atmosphérique en CO_2 et les variations de la production primaire océanique (Gillikin et al.

2009). Il existe deux sources de carbone pour la fabrication des coquilles : le carbone inorganique dissous (CID) et le carbone rejeté par l'organisme, issu de son propre métabolisme. Ce dernier est le produit final du carbone consommé (McConnaughey et al. 1997). Dans leur étude, Lorrain et al. (2004) constatent que le rapport entre la quantité totale de carbone produite par le bivalve pendant la respiration et la quantité totale de carbone nécessaire à la fabrication de la coquille détermine la quantité de carbone métabolique disponible pour la formation de la coquille. De ce fait, tout résultat provenant de bivalves dont nous connaissons mal le métabolisme et le système respiratoire, doit être pris avec précaution. Chauvaud et al. (2011) complètent cette approche en arguant du fait que les variations à la fois ontogénétiques et saisonnières du $\delta^{13}\text{C}$ observées dans les coquilles montrent que celles-ci ne sauraient être utilisées comme indicateur des fluctuations passées du carbone inorganique dissous mais peuvent en revanche constituer un outil prometteur pour les études écophysiologicalues.

1.7. Les éléments traces

L'étude de la composition élémentaire des coquilles de bivalves est déjà considérée par la communauté scientifique internationale comme un outil prometteur de mesure des paramètres environnementaux, tels que la température de surface de la mer, la salinité, la dynamique du phytoplancton et la contamination par les métaux lourds (Liste 1.1). Les éléments trouvés dans les dépôts de carbonate peuvent être classés en deux catégories. La première regroupe les éléments, comme le calcium (Ca) et le magnésium (Mg), ayant une teneur dans la matrice carbonatée de plus de 100 ppm (Schöne et al. 2011). La seconde regroupe les éléments dont la teneur est inférieure à 100 ppm, comme le plomb (Pb) ou le cuivre (Cu) (Lifset et al. 2012). De nombreux auteurs ont démontré que les éléments mineurs

ou les éléments traces incorporés dans les carbonates biogènes peuvent être utilisés comme traceurs environnementaux (Reinfelder et al. 1997; Carré et al. 2006; Elliot et al 2009; Vihtakari et al. 2017).

Liste 1.1: exemples d'éléments mineurs, traces et ultra-traces utilisés dans les coquilles de bivalves comme proxies des conditions passées pour un certain nombre de variables environnementales (liste non-exhaustive).

Variable environnementale	Eléments	Références
Température	Sr/Ca, Mg/Ca, Sr/Li	Klein et al. (1996); Freitas et al. (2005); Kunioka et al. (2006); Carroll et al. (2009); Freitas et al. (2009); Yan et al. (2013); Füllenbach et al. (2015); Zhao et al. (2017a)
Salinité	Ba/Ca, Na/Ca, B/Ca	Roopnarine et al. (1998); Takesue et al. (2008); Poulain et al. (2015)
Production primaire	Ba/Ca, Mn/Ca, Li/Ca, Mo/Ca	Stecher et al. (1996); Vander Putten et al. (2000) ; Elliot et al. (2009); Thébault et al. (2009); Thébault & Chauvaud (2013); Lazareth et al. (2013)
pH	U/Ca, Na/Ca, terres rares (REE – rare earth elements)	Frieder et al. (2014); Ponnurangam et al. (2016); Zhao et al. (2017b)
Disponibilité en lumière	Sr/Ca	Hori et al. (2015)
Contamination par les métaux lourds	Pb/Ca, Zn/Ca, Cu/Ca, Cd/Ca, Fe/Ca, U/Ca,	Fang & Shen (1984); Price & Pearce (1997) ; Markich et al. (2002); Wallace et al. (2003); Dunca et al. (2009); Krause-Nehring et al. (2012); Holland et al. (2014)
Upwelling	Mn/Ca, Ba/Ca	Langlet et al. (2006); Langlet et al. (2007); Hatch et al. (2013)
Hypoxies et changements redox	Mn/Ca, Cu/Ca	Luoma & Bryan (1982); Barats et al. (2008)
Activités anthropiques	Na/Ca, U/Ca, Fe/Ca, REE	Holland et al. (2014) ; Ponnurangam et al. (2016)

1.7.1. Quelques exemples donnant lieu à des débats dans la littérature spécialisée

A cause de leur présence importante dans la matrice carbonatée, de nombreux travaux sont consacrés à l'étude du strontium et du magnésium dans le but de mieux comprendre leur processus d'incorporation (Shirai et al. 2014; Zhao et al. 2017b). Les analyses donnent des résultats différents et les interprétations divergent en fonction des espèces étudiées.

Le strontium a été longtemps étudié en tant qu'indicateur de température de surface. Cette hypothèse est étayée par l'existence d'une relation inverse entre la température et le ratio Sr/Ca chez d'autres organismes vivants comme le corail (de Villiers et al. 1995; Rüggeberg et al. 2008). Toutefois, de récentes études du ratio Sr/Ca des coquilles de mollusques laissent supposer que sa valeur pourrait être influencée par d'autres paramètres, même si certains travaux montrent que la température demeure l'élément moteur principal (Freitas et al. 2006). Parmi ces autres paramètres, on trouve le ratio Sr/Ca de l'eau, le taux de croissance (Stecher et al. 1996; Lorrain et al. 2005), les modifications de salinité de l'eau (Dodd & Crisp 1982) et l'activité métabolique du manteau chez certaines espèces comme *Mercenaria mercenaria* (Stecher et al. 1996) et *S. giganteus* (Gillikin et al. 2005b). De plus, l'interprétation de l'incorporation du strontium dans les coquilles peut différer d'un auteur à l'autre même lorsque la même espèce est étudiée.

L'étude de *Mytilus trossulus* (Klein et al. 1996), *M. edulis* (Wanamaker et al. 2007), *Isognomon ephippium* (Lazareth et al. 2003) and *Protothaca staminea* (Takesue & van Geen 2004) suggère que le magnésium pourrait servir de traceur pour la température de l'eau de mer. Malgré tout, comme pour le strontium, les mêmes incertitudes sont soulignées dans certaines publications, dont les conclusions sont parfois contradictoires. L'analyse du mollusque chilien, *Concholepas concholepas*, révèle que l'incorporation du magnésium dans

la coquille et le taux de croissance varient de façon cyclique avec le jour et la nuit (Lazareth et al. 2007). Pendant la journée, l'incorporation du magnésium semble corrélée à la température de l'eau mais, pendant la nuit, elle paraît contrôlée par le métabolisme de l'animal lui-même (activité trophique, formation d'incréments de croissance riche en matière organique). Les résultats obtenus par cette étude pourraient expliquer, en partie, les anomalies observées lors des études précédentes portant sur le même sujet.

Tous ces résultats mettent en évidence la nécessité d'un étalonnage rigoureux à l'échelle de chaque espèce. Il semble donc difficile, à l'heure actuelle, de tirer des conclusions sur le processus d'incorporation du strontium et du magnésium dans les coquilles de bivalves. Les nombreux débats autour de ces deux seuls éléments suffisent à prouver que l'analyse géochimique des coquilles est une discipline complexe. Il convient donc d'être prudent dans les interprétations que l'on peut en faire et surtout d'éviter de généraliser les résultats obtenus.

1.7.2. Indicateurs géochimiques de la dynamique de croissance du plancton

Les mesures instrumentales de la production primaire dans les océans sont rares. L'observation ininterrompue de la surface terrestre n'a, en effet, commencé qu'à la fin des années 70 lorsque les premiers satellites d'observation ont été lancés et reste actuellement beaucoup plus détaillée au-dessus des régions économiquement développées ou qui présentent un intérêt scientifique accru. Les paléocéanographes doivent donc s'appuyer sur les enregistrements de type proxy pour reconstituer la production primaire à des époques ou des lieux pour lesquels l'observation directe est impossible. De nombreuses études ont été consacrées à l'évaluation du potentiel des éléments trace présents dans les carbonates biogéniques marins et, plus particulièrement, dans celui des coquilles de mollusques, comme indicateur haute résolution de la dynamique de croissance passée du phytoplancton (Vander

Putten et al. 2000; Gillikin et al. 2006; Thébault et al. 2009). Le plus prometteur de cet indicateur potentiel est le baryum (Ba).

Un certain nombre d'études mettent en lumière l'existence d'un lien entre les efflorescences phytoplanctoniques et la présence de concentrations élevées en Ba dans les coquilles de plusieurs espèces de mollusques bivalves, telles que *M. californianus* (Spangenberg & Cherr 1996), *M. mercenaria* and *Spisula solidissima* (Stecher et al. 1996), *M. edulis* (Vander Putten et al. 2000), *Comptopallium radula* (Thébault et al. 2009) and *A. islandica* (Marali et al. 2017). Cependant, ce lien est réfuté par Gillikin et al. (2008) qui suggèrent que les taux élevés de Ba/Ca dans les coquilles de *P. maximus* and *S. giganteus* seraient dus à un mécanisme de forçage environnemental encore indéterminé. D'après Zhao et al (2017a), le processus d'incorporation de Ba/Ca dans les coquilles de *Corbicula fluminea* est complexe du fait de la forte influence exercée par la quantité de ressources alimentaires disponibles (et le taux de croissance des coquilles) sur l'enrichissement des coquilles en Ba. Zhao et al. (2017a) déclarent que si cela se vérifie pour toutes les espèces marines, alors la pertinence des études précédemment citées devra être réévaluée de façon critique.

Plus récemment, Thébault et al. (2009) ont découvert que le molybdène (Mo) pourrait devenir, grâce à *C. radula*, un nouvel indicateur de l'assimilation du nitrate par le phytoplancton dans les écosystèmes marins, et ainsi permettre la reconstitution de l'équilibre entre production nouvelle et production régénérée dans un paléoenvironnement. Toutefois, cette théorie se complique avec les travaux de Tabouret et al. (2012) qui montrent que le Mo dissous dans l'eau ne peut être à l'origine des taux élevés de Mo trouvés dans les coquilles de *Pecten maximus*.

1.7.3. Métaux lourds : des indicateurs de pollution

Outre les éléments déjà cités, de nombreuses études réalisées sur les coquilles de bivalves s'intéressent également à la teneur en éléments-traces métalliques (Cd, Cu, Mn, Ni, Pb, V, U), auparavant communément regroupés sous l'appellation "métaux lourds", afin de détecter la présence de polluants et de contamination anthropique (Boening 1999). Comme les sclérosponges, les bivalves ont la capacité d'accumuler des concentrations élevées de plomb dans leur coquille calcaire qui sert donc de système d'archivage des polluants. Toutefois, en raison d'une forte variabilité intra-annuelle et intra-individuelle, l'analyse de ces concentrations peut s'avérer difficile, en particulier chez *M. mercenaria* (Genest & Hatch 1981) et *C. radula* (Metian et al. 2008). Malgré cela, un registre chronologique des variations des concentrations environnementales en plomb entre 1949 et 2002 a pu être établi à partir de l'étude de onze coquilles de *M. merceneria* de Caroline du Nord (USA). Cet enregistrement révèle une augmentation du taux de plomb contenu dans les coquillages, qui coïncide avec la généralisation de l'utilisation de l'essence au plomb au début de l'ère industrielle (Gillikin et al. 2005a). Ces travaux confirment également qu'il est possible de retracer les fluctuations environnementales du plomb à long terme et de manière efficace à partir de l'étude de quelques spécimens seulement.

Ces résultats suggèrent aussi que les variations des autres éléments métalliques (Zn, Cu, Cd, Ni) contenus dans les coquilles des mollusques pourraient être liés aux apports pélagiques de métaux dans les océans (Carriker et al. 1996). Toutefois, des travaux menés sur *C. radula* montrent que ces concentrations (Ni, Cr, Cu, Mn, Co) résultent probablement davantage des flux benthiques des métaux dissous que des apports pélagiques (Metian et al. 2008). En effet, aucune relation significative entre les concentrations de métaux dans les coquilles et les métaux dissous dans la colonne d'eau n'a pu être clairement établie. De façon

générale, l'étude des éléments traces métalliques et de leur processus d'incorporation dans les coquilles de bivalves est un sujet complexe encore sujet à conjecture.

1.8. Objectifs

La présente étude a été financée par le Réseau de Formation Initiale Marie Curie (*Initial Training Network ITN*) dans le cadre du projet de collaboration internationale ARAMACC (Annually Resolved Archives of

Marine Climate Change;

<http://aramacc.com>). ARAMACC est un consortium qui regroupe les meilleures équipes européennes de recherche en sclérochronologie. Il a pour objectif la formation par la recherche de 10 doctorants (Early-Stage Researchers) et 1 post-

doctorant (Experienced Researcher). Le but premier de l'ensemble de ces travaux est d'utiliser les coquilles des mollusques ayant une grande

longévité comme archives des changements climatiques et environnementaux en Atlantique Nord. Cet objectif sera atteint en établissant un réseau d'enregistrements chronologiques pour l'Atlantique Nord, essentiellement fondé sur l'étude des coquilles d'*A. islandica* et *G. glycymeris* (Fig 1.3), tout en poursuivant l'application de la sclérochronologie des bivalves

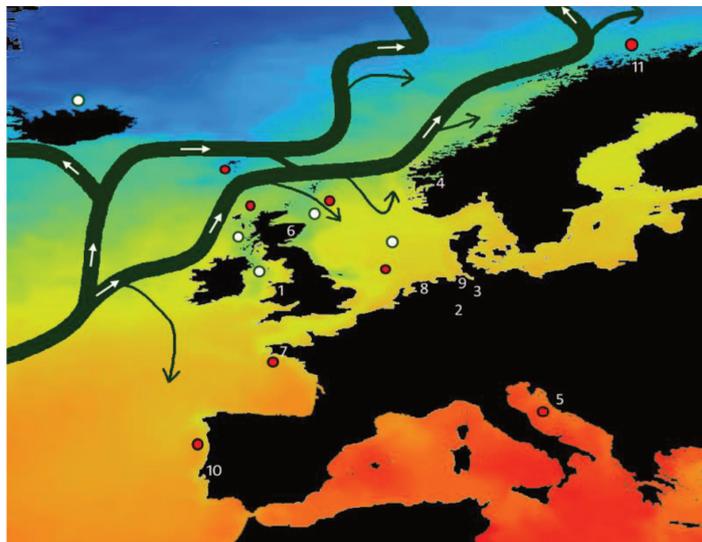


Fig 1.3: Couverture du projet ARAMACC en Atlantique Nord. Sites proposés pour la reconstitution de nouvelles chronologies à partir de bivalves (points rouges), sites des chronologies déjà disponibles (cercles blancs), et localisation des partenaires (chiffres blancs) du projet ARAMACC (source: <http://aramacc.com>)

dans les domaines de la biologie, de la modélisation climatique, des indicateurs proxy et du suivi environnemental.

Le présent travail de doctorat s'insère à part entière dans ARAMACC et consiste à reconstituer une chronologie pluri-décennale, à résolution annuelle à infra-annuelle, de la variabilité environnementale en rade de Brest (Bretagne, France) à l'aide des données extraites des coquilles de *G. glycymeris* (Chapitre 3).

Le **Chapitre 2** présente les méthodes utilisées et communes à tous les autres chapitres de ce manuscrit. Ces méthodes traitent principalement de la préparation des coquilles de *G. glycymeris* et de la façon dont elles sont mesurées et datées avec précision. Ce chapitre fournit également toutes les informations sur les espèces étudiées et leur situation géographique, ainsi que des renseignements sur la station de mesure SOMLIT située à l'entrée de la rade de Brest.

Le **Chapitre 3**, comme indiqué précédemment, porte sur la croissance des coquilles de *G. glycymeris* (construction d'une chronologie de référence). Une série d'objectifs ont été définis, notamment celui de déterminer s'il est possible d'effectuer une datation croisée (*cross-matching*) de coquilles d'individus de *G. glycymeris* prélevés vivants ou morts en rade de Brest. Il est également nécessaire de déterminer la pertinence de ces échantillons pour la reconstitution d'une chronologie qui soit concordante et fiable sur le plan statistique ainsi que pour l'identification des relations existantes entre les facteurs environnementaux et la croissance des coquilles.

Le **Chapitre 4** est consacré à la reconstitution des courbes de températures à l'aide des rapports des isotopes stables de l'oxygène de la coquille ($\delta^{18}\text{O}$) en remontant plus loin dans le temps qu'avec les mesures instrumentales. Ce chapitre présente également une tentative d'identification des relations existantes entre la courbe des températures reconstituées et les oscillations climatiques, dont on pensait jusqu'à présent qu'elles conditionnaient la

température de la rade de Brest.

Le **Chapitre 5** présente les résultats de l'analyse des éléments traces mesurés dans les coquilles de *G. glycymeris* entre 1969 et aujourd'hui. Cette analyse a pour but de mieux comprendre l'incidence des facteurs environnementaux et biologiques sur chaque élément. Au vu de la littérature disponible au moment de la rédaction de ce mémoire de thèse, cette étude est une première.

Enfin, le **Chapitre 6** est rédigé sous forme de conclusion et permet de mettre en évidence les facteurs climatiques les plus significatifs de la rade de Brest, de souligner les réflexions et les perspectives suscitées par les travaux présentés ici, ainsi que de proposer des idées pour de futures recherches.

Chapter 2: Common Methods

There were many aspects of this thesis which were repeated throughout each study and chapter. To avoid repetition and ease reading, they are addressed here

2.1. *Glycymeris glycymeris*

Several bivalve species can be used for sclerochronological analysis, the most common being *Arctica islandica*. The focus of this study is on *Glycymeris glycymeris* (Fig 2.1) which



Fig 2.1: Examples of *G. glycymeris* found in the Bay of Brest

is another species that has been used for reconstruction of past climatic and environmental variables (Reynolds et al. 2013; Royer et al. 2013; Brocas et al. 2013). *G. glycymeris* is a fairly large bivalve with a maximum

length of 80 mm (Royer et al. 2013) that inhabits the north eastern Atlantic continental shelf from Cape Verde to Norway in water depths of up to 100m and in areas with strong bottom currents (Savina & Pouvreau 2004). Previous research has shown (i) that the periodic growth increments in *G. glycymeris* are formed annually and are synchronous within populations (Reynolds et al. 2013; Royer et al. 2013; Brocas et al. 2013) and (ii) that individuals can live for nearly 200 years (Ramsay et al. 2000; Reynolds et al. 2013).

2.1.1. Biology and Ecology

G. glycymeris (Linnaeus 1758) is eurythermal (Raffi et al. 1985) and are stenohaline (Rombouts et al. 2012). Savina & Pouvreau (2004) regard the mollusc as an infaunal, subtidal, filter-feeding bivalve, which buries into sandy-gravelly sea floors.

Glycymerids are descendants of the ancient Arcoidea, which were established early in the radiation of the Bivalvia. Fossil specimens have been found to come from the Cretaceous (~ 130 MA) and appear to occupy similar ecological ranges to the present species in the North Atlantic. The shell of *G. glycymeris*, therefore, retains many primitive characteristics such as a basically ovate shape, a strong broad hinge plate with taxodont teeth, and roughly equal-sized adductor muscle scars. They are not as specialized anatomically as other bivalves; instead, they have adapted to physically harsh environments evidencing their role as functional generalists.

As with the shell, primitive features are visible in the anatomy, i.e. the adductor muscles are isomyarian and filibranch gills are present. The ciliary sorting mechanism of the gills carries material both dorsally and ventrally along the outer and inner demibranch, Food particles are carried anteriorly, to the labial palps and the mouth, along the dorsal margin of the lamellae and along the dorsal groove between the bases of the two demibranchs. This sorting mechanism is very efficient, reflected in the relatively small labial palps which are the main sorting region for most lamellibranches (Atkins, 1936; Morton, 1983). Rejected material is carried posteriorly along the ventral margin of the demibranchs, until it's finally discarded at the gill posterior, close to the exhalant stream.

A mucous food-string leaves the labial palps and enters the mouth and stomach, where particles may be sorted further. When comparing the structure and function of the stomach of several other bivalve species, Reid (1965) concluded that the stomach of *Glycymeris*

glycymeris more closely resembled a hypothetical primitive stomach, with a small but efficient area for sorting food particles. Such a simple anatomy of the stomach is usually found in bivalves where the major particle sorting area is located elsewhere, other than in the stomach (Reid, 1965). Brand and Morris (1984) state that particle sorting must take place on ctenidia only, evidenced by a low filtration rate of roughly $0.06 \text{ l h}^{-1} \text{ g}^{-1}$. However, Savina & Pouvreau (2004) found an average filtration rate 10 times higher. This considerable difference may be explained by *G. glycymeris* exhibiting a high variability in feeding activity. Brand and Morris (1984) found that the filtration time was around 60 %, suggesting that *G. glycymeris* could have a periodic feeding activity, with active periods (high filtration) alternating with “inactive” periods (no or low filtration, but digestion and defecation). The fact that faeces of *G. glycymeris* were very dense and compacted into small pieces tends to confirm this periodic activity.

Very low ventilation rates are recorded for the species, compared to other filter-feeding bivalves. The fact that most particle sorting takes place on the gills is thought to contribute to the observed low ventilation volume (Brand & Morris, 1984). However, although the ventilation rates were very low, the oxygen utilization proved to be unusually high for a bivalve without a respiratory pigment. This can be explained by an unusually long blood pathway through the gills (Atkins, 1936; Brand & Morris, 1984), or by oxygen uptake occurring by direct diffusion through the tissue surface. Due to this the species has considerable ability to withstand anaerobic conditions, although small individuals are better regulators of oxygen consumption than large individuals (Morris, 1978; Brand & Morris, 1984).

Only one study has been previously carried out on the reproduction of *G. glycymeris* in the Bay of Brest. In this study, Lucas (1965) assigned sexually mature animals to three gonad development stages. At stage A the individuals could not be sexed; at stage B gonad smears

showed that few gametes (oocytes or sperms) were present; while in stage C gametes were abundant. The population had ripe gametes all year round so that the proportion of stage C gametes was never lower than 70% (Lucas, 1965). The ability to have ripe gametes throughout the year has also been observed in British waters. Lebour (1937) obtained ripe eggs from *G. glycymeris* in Plymouth Bay in January, March and October, and Morris (1978) stated that the animals collected off the Isle of Man spawned year-round. Just less than three thousand individuals were examined by Lucas (1965) and only six individuals were found to be hermaphrodite. The sex ratio changed with size, from being mostly males at the onset of sexual maturity (~20 mm in shell height), to more females at a size of 40 mm (40% males). In order to explain the change in sex ratio with size, the possibility of higher mortality rates among the males was rejected and it was suggested instead that the change in ratio was caused by a sex change, supported by the occurrence of the hermaphrodite animals (Lucas 1965).

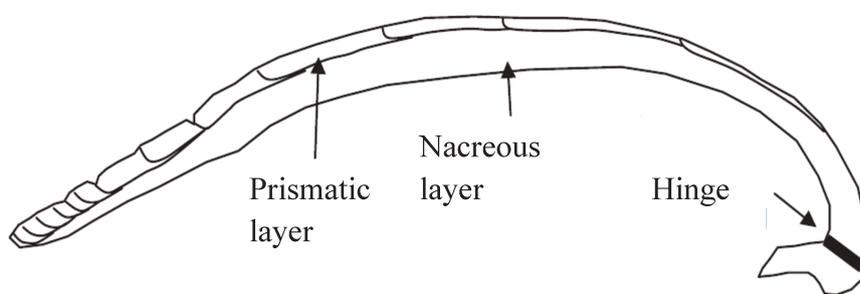
The foot of *G. glycymeris* is relatively large and consists of an upper visceropedal region (including the gonad) and a muscular lower part with two lateral flaps. When the shell is lying on the surface of the substratum, the foot extends into the sand with the two lateral flaps held together but at retraction the flaps separate, forming an anchor. When the animal has gained sufficient pedal anchorage, the shell is raised into an erect position and effective burrowing begins as a series of downward steps repeated. The digging cycle takes 0.5 to 3 minutes to complete, with smaller individuals undertaking a large number of short cycles rapidly to achieve the same depth as the larger individual.

During the digging cycle the ligament fails to give adequate force for reopening the shell valves against the surrounding substratum, indicating that it is relatively weak (Ansell & Trueman, 1967). Furthermore, Thomas (1976) has suggested that the weak ligament of arcoids, in general, has prevented infaunal species of this group becoming specialized for

deeper burrowing. Even though the ligament is thought to be weak, *G. glycymeris* has evolved very hard and robust shell.

2.2. Shell Layers

G. glycymeris shells are composed entirely of aragonite (Carter, 1980) and have a crossed-lamellar ultrastructural motif. It has a light coloured outer shell layer (prismatic layer



– Fig 2.2) separated from a darker inner layer by the pallial myostracum (nacreous layer – Fig 2.2). The prismatic layer is secreted by a marginal

Fig 2.2: Simplistic rendition of shell layers. Adapted from Gillikin et al. 2005)

band of cells, so that the shell grows at its outer edge, whereas the nacreous layer is derived from the main surface of the mantle. In order for shell secretion to take place, Wilbur and Saleuddin (1983) mention three major processes that must all be operative at the same time during crystal formation and shell growth: 1) active ion transport to the site of mineralization, 2) the synthesis of organic compounds of the shell matrix, which provides the framework for crystal formation and 3) the secretion of these organic compounds by the mantle epithelium.

Both the prismatic and nacreous layers contain two growth sub-layers (although it is much harder to observe in the nacreous layer): a relatively thin, dark, growth line and a wider, pale coloured, growth increment (Fig 2.3). The growth lines were farther apart in the early portions of the shell, formed during relatively rapid growth, compared to the margin of a large shell, formed when growth had slowed. The dark growth band is followed a light-

coloured growth increment, ending in an abrupt, thin, dark band before the next growth increment started.

The external lines of the relatively small *G. glycymeris* from the Bay of Brest are easy to interpret but the definition of the faint lines in the area between the umbo and the first clear

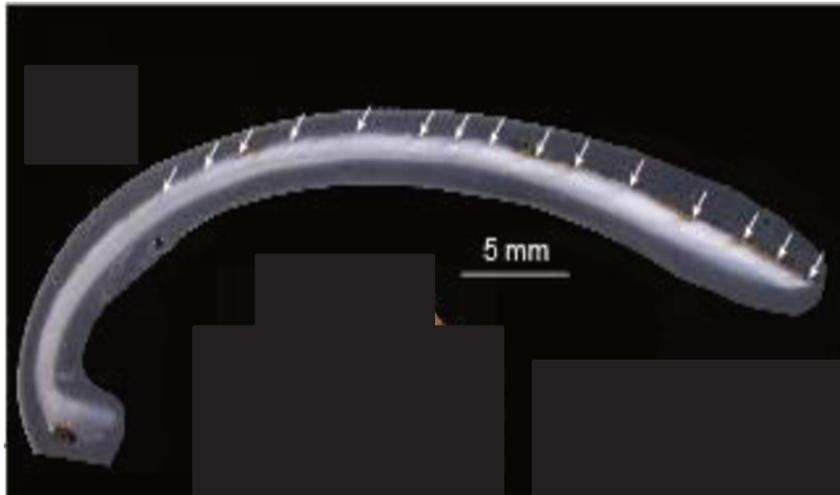


Fig 2.3: Image of *G. glycymeris* increments through the prismatic layer. Adapted from Zhao (2016).

growth increment is uncertain. Surface marks on larger shells have to be read subjectively as the substratum in which that *G. glycymeris* lives in is very coarse and abrasion of the shell surface, while the animal is burrowing,

can possibly result in shell erosion giving dull growth marks in old specimens. The use of external growth lines for ageing in this study was, therefore, ambiguous and considered to be too subjective. Due to this, acetate peels had to be created of the hinge portion of the shell.

2.2.1. Crossmatching

Crossmatching is the act of comparing growth increments of different individuals to identify common patterns through time. Samples within the same population, in this case shells, often exhibit the same pattern of variation through time as they experience the same factors limiting growth (such as temperature or food availability). This variation is what's used to crossdate many samples, finding the same wide and narrow increments in multiple samples. Once these wide and narrow bands are aligned and set to a particular date, more

samples can be added, potentially fossil specimens, to allow both a strengthening of the patterns found and the ability to have more incremental patterns going further back in time.

2.3. Shell Preparation

The morphometrics (shell length, height, width, and total dry shell mass) of all the collected shells were measured using an automatic vernier calliper at 0.01 cm precision and recorded. The shell mass was recorded on a balance to the nearest 0.1 g. The recorded biometrics were then used to select live- and dead-collected shells for sectioning. Shells which were damaged by the dredge or fouled were excluded.

The selected live- and dead-collected shells were sectioned using the methodology described by Ramsay et al (2001). A 3-4 cm section was

cut from the hinge through to the ventral margin along the axis of maximum growth using a diamond tipped blade mounted on a rotary grinding saw, making sure that the apex of the umbone was included in the section (Fig 2.4A). The cut section was then embedded into

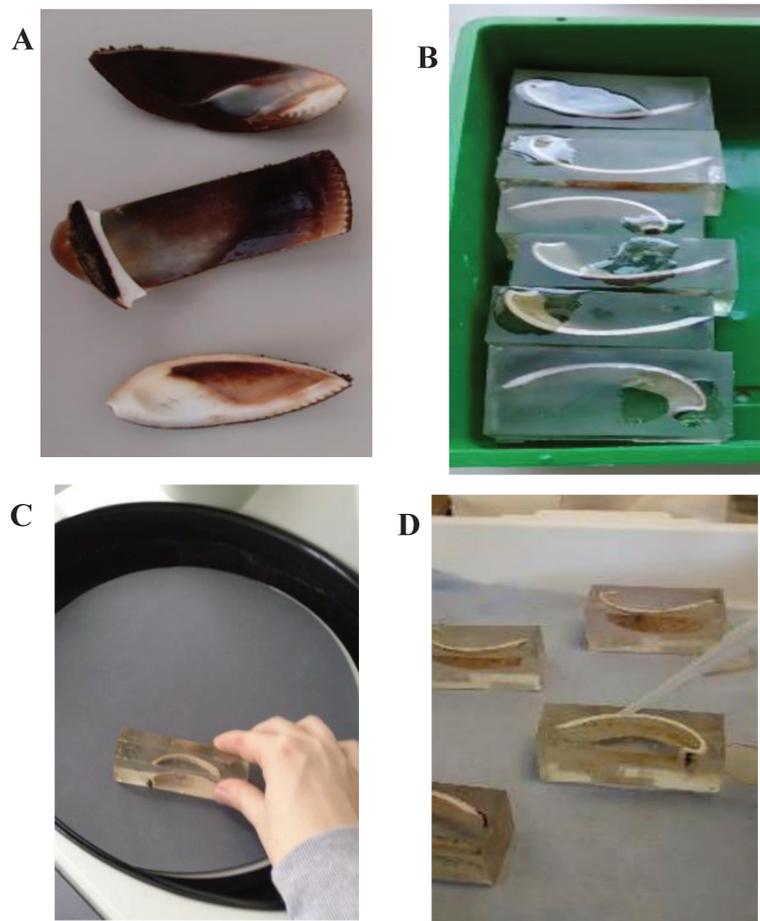


Fig 2.4: The process of shell preparation. A) *G. glycymeris* sectioned preserving the axis of maximum growth; **B)** Shells embedded in resin; **C)** Grinding and polishing; **D)** Adding ethyl acetate to create the acetate peel replicas.

polyester resin (Sody 33, Escil, France) and placed in an oven set at 30°C until dry before a final section was cut with a precision saw (Struers Secotom-10) along the axis of maximum growth (Fig 2.4B). The cut surface was ground using silicon carbon paper (grades 800–4000) fixed to a mechanical grinding machine (Struers TegraPol-35) before being polished using 3µm diamond paste (Fig 2.4C). The polished shell sections were then etched in 0.1M HCl for 90 seconds, soaked in a water bath and left to air dry (Ramsay et al. 2000; Ramsay et al. 2001; Reynolds et al. 2013). Acetate peel replicas of the etched surfaces were then produced using methods described by Richardson (2001) (Fig 2.4D).

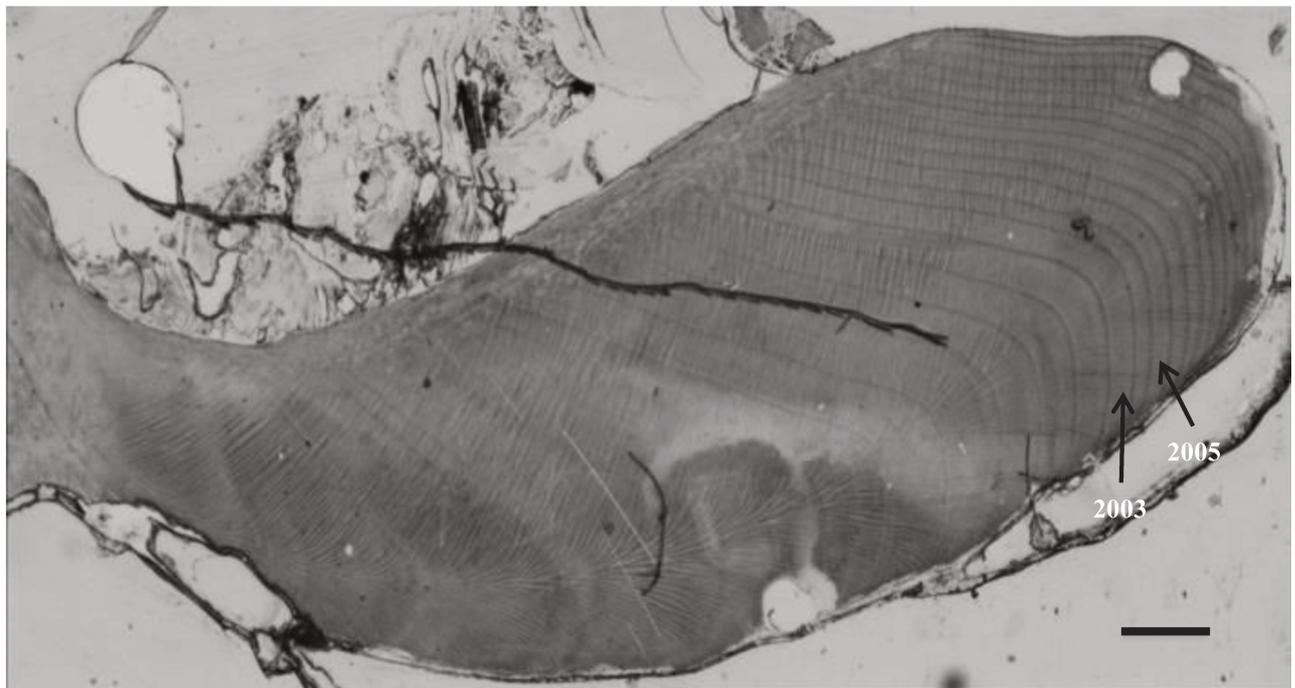


Fig 2.5: A digital photomosaic of an acetate peel replica of growth increments in the *G. glycymeris* hinge. Black scale bar is 500µm. Black arrows indicate marker years where there was increased growth. Shell ID: 14101004, collected in October 2014.

The prepared acetate peels were digitally photographed with a Zeiss AxioCam MRc5 digital camera mounted on a Zeiss Lumar.V12 light-transmitting microscope under 40 and 80× magnification (Fig 2.5). The software Axiovision V4.9.1 was used to create photo

mosaics from the individual photographs, and the growth increments seen in the images were crossdated visually using the list year method (Yamaguchi 1991). This technique is based on the assumption of synchronous growth in individuals sampled from the same area (Fritts 1976; Yamaguchi 1991). The growth increments were then digitally measured, using ImageJ software. Because the increment widths in *G. glycymeris* typically show little variability, a mean value was taken of three sets of measurements in order to minimize the effect of measurement error (Brocas et al. 2013). The growth measurements were taken in the hinge rather than the ventral margin as the hinge provides a more consistent orthogonal transect through the increments (Ramsay et al. 2000).

Stable isotope (Chapter 4) and trace element (Chapter 5) sampling was taken in the prismatic layer (within margin) and not the hinge. This is because, as the increments in the margin are wider, they can provide a more detailed interannual variability that is not available in the hinge. It has long been known that the increments found in the hinge (such as in Fig 2.3 and 2.5) are transposed to the margin (Jones 1980; Ramsay, 2000), due to the increments being formed during the growth period. To be sure the ages assigned to the margin increments were correct, the years counted in margin were compared to the overall age derived from the hinge. The shells which had clear increments, showed the marker years, and had the same number of years counted in both the hinge and the margin were chosen for further analysis.

For more information on the direct methods of stable isotope and trace element analysis, please see chapters 4 and 5 respectively.

2.4. Study Site

The Bay of Brest is a semi-enclosed marine ecosystem with an area of 180km² and an average depth of 8m. It's connected to the Atlantic shelf (Iroise Sea) by a strait to the west that is 2km width and 40m depth and is being fed by two rivers. The larger, the Aulne, has a catchment area of 1822km² and the smaller Elorn 280km² which provide up to 85% of the total freshwater input into the bay (Raimonet et al 2013).

Our study site, the Banc de la Cormorandière (48°20'26" N, 4°30'44" W), is a subtidal dune situated 20 m below the surface. It is located in the western part of the Bay of Brest, near its outlet and is subject to strong tidal currents (Fig 2.6). Living *G.*

glycymeris and dead shells were collected monthly between September 2014 and November 2015. In total 401 live specimens and 243 articulated paired valves from

dead *G. glycymeris* were collected in 20-25m water depth using a dredge deployed from the RV *Albert Lucas*. Of the 243 pairs of valves from dead specimens, 114 were excluded from further consideration because of extensive bioerosion. *G. glycymeris* live-collected from the same site in 2012 and archived at Université de Bretagne Occidentale were also used in the analyses.

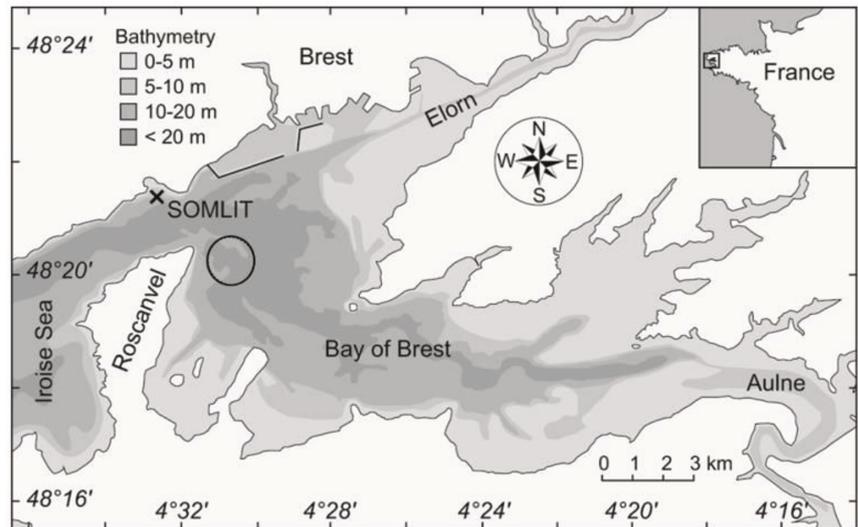


Fig 2.6: Location of Brittany in France and sample location within Brittany. Dotted circle indicates sample site and 'X' shows the position of SOMLIT-Brest research station.

Adapted from Thébault & Chavaud (2013).

2.4.1. Monitoring Stations

Service d'Observation en Milieu Littoral (SOMLIT) is a French marine monitoring programme, created by the Institut National des Sciences de l'Univers (INSU), which has a number of sub-surface monitoring stations around the French coast including the Mediterranean, the Atlantic Ocean and the English Channel (Fig 2.7). The sampling sites were chosen due to their variety, for example some stations are in marine waters, some estuarine and others (such as the Bay of Brest) are in coastal mixed systems. It was not just water mixing that was the focus of these decisions however, it was also taken into account whether the locations had historical monitoring and also how influenced the area is by tide,



Fig 2.7: Locations of SOMLIT stations

around France. Image created by Benoît Sautour. Accessed via: <http://somalit.epoc.u-bordeaux1.fr/fr/>

from the non-tidal (Marseille) to the mega-tidal (Luc/Mer and Wimereux).

Sampling at the majority of these stations has been taken at high tide, and at least twice a month since 1997.

Seawater temperature and salinity have been monitored weekly from 1998 to present at the SOMLIT-Brest station located at the outlet of the Bay of Brest (48°21'30" N, 4°33'06" W), using a Sea-Bird SBE 19 CTD profiler (Sea-

Bird Electronics, Inc.). The sampling station, which is open to oceanic influence, is located in front of Ste-Anne-du-Portzic at the boundary between the Iroise Sea and the Rade de Brest (Fig 2.7). Sampling is carried out weekly, at 2 m depth, and five physio-chemical factors are measured: temperature, conductivity, dissolved oxygen, pH and turbidity, as well as a biological parameter (fluorescence of chlorophyll α).

In addition, the buoy MAREL-Iroise provides automatic records of temperature, conductivity, dissolved oxygen, pH, and turbidity measured every 20 min at a location 50m from the SOMLIT-Brest station (48°21'28''N, 4°33'05'' E). These monitoring stations are located less than 3 km away from the shell sampling site.

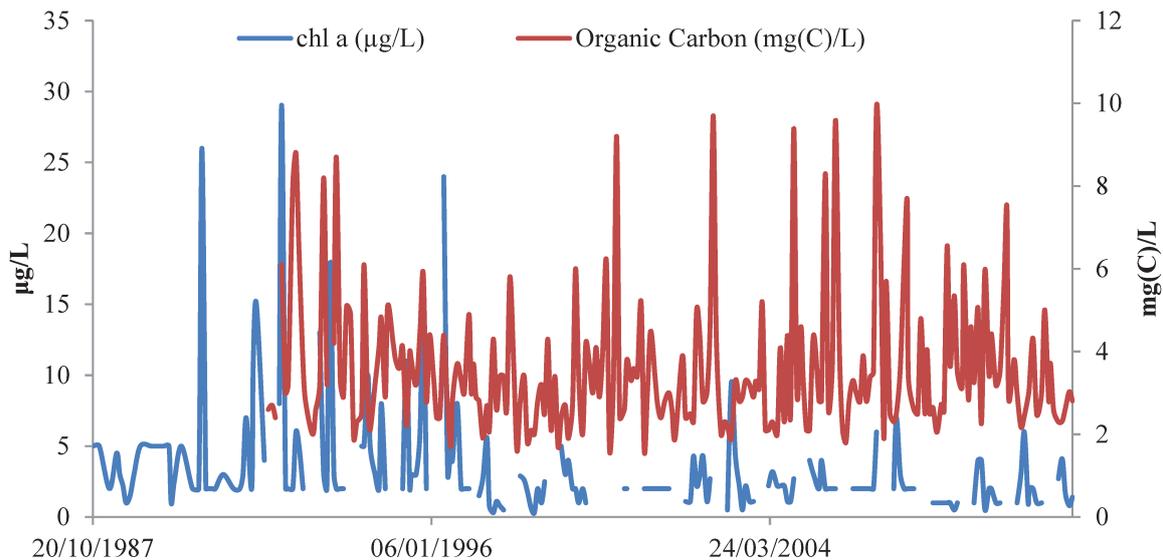


Fig 2.8: Organic carbon and chlorophyll a from the River Elorn.

Data for environmental analysis was also taken from the River Elorn (48°26'34" N, 4°05'4" W) and the River Aulne (48°11'39" N, 3°57'11" W). This was accessed through HydroBank (<http://www.hydro.eaufrance.fr>), using station codes 4178000 (Elorn) and 4179500 (Aulne). Water temperature, atmospheric pressure and flow rate were measured at these stations monthly from 1981 to 2011 (1988 to 2011 in the case of the River Elorn). Monthly physiochemical data of the river water is also accessible through this database, however, the temporal availability differs depending upon the factor, for example organic carbon has been measured from 1992 and chlorophyll a from 1987 (Fig 2.8).

Rainfall was also deemed to be an interesting factor for analysis as the Bay of Brest is semi-enclosed (Fig. 2.9). This data was obtained daily by Klein Tank et al. (2002) at 94 m above sea level at 48°26'39" N, 4°24'42" W. This particular dataset can be accessed using

station identifying code 434 (Brest-Guipavas) on the European Climate Assessment and Dataset website (<http://www.ecad.eu/>).

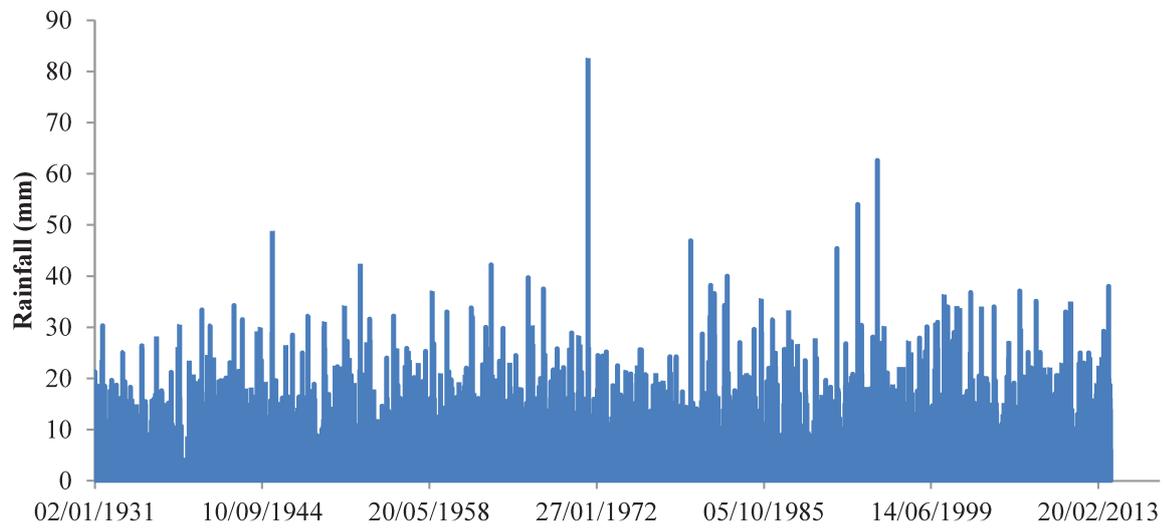


Fig 2.9: Rainfall measured at Brest-Guipavas

**Chapter 3: Influence of riverine input on the
growth of *Glycymeris glycymeris* in the Bay of
Brest, North-West France**

3.1. Abstract

A crossdated, replicated, chronology of 114 years (1901-2014) was developed from internal growth increments in the shells of *Glycymeris glycymeris* samples collected monthly from the Bay of Brest, France. Bivalve sampling was undertaken between 2014 and 2015 using a dredge. In total 401 live specimens and 243 articulated paired valves from dead specimens were collected, of which 38 individuals were used to build the chronology. Chronology strength, assessed as the Expressed Population Signal, was above 0.7 throughout, falling below the generally accepted threshold of 0.85 before 1975 because of reduced sample depth. Significant positive correlations were identified between the shell growth and the annual averages of rainfall (1975-2008; $r = 0.34$) and inflow from the river Elorn (1989-2009; $r = 0.60$). A significant negative correlation was identified between shell growth and the annual average salinity (1998-2014; $r = -0.62$). Analysis of the monthly averages indicates that these correlations are associated with the winter months (November – February) preceding the *G. glycymeris* growth season suggesting that winter conditions predispose the benthic environment for later shell growth. Concentration of suspended particulate matter within the river in February is also positively correlated with shell growth, leading to the conclusion that food availability is also important to the growth of *G. glycymeris* in the Bay of Brest. With the addition of principle components analysis, we were able to determine that inflow from the River Elorn, nitrite levels and salinity were the fundamental drivers of *G. glycymeris* growth and that these environmental parameters were all linked.

3.2. Introduction

Annually-resolved paleoenvironmental archives such as tree-rings (Bond-Lanberty et al. 2014; Silva et al. 2016; Seiler et al. 2017) ice cores (Melott et al. 2016; Steiger et al. 2017; Stoker et al; 2017) and corals (Caragnano et al. 2017; Kubota et al. 2017) have provided valuable insights into the terrestrial and tropical marine environments of the past. Up until the last decade, however, no proxy archive with a similarly high resolution had been developed for the marine environment of the mid and high latitudes (Wanamaker et al. 2011). During the past decade, with the use of the shells of long-lived bivalve molluscs, in particular *Arctica islandica* (Butler et al. 2010; Wanamaker et al. 2012; Butler et al. 2013), *Panopea abrupta* (Black et al. 2008b; Black 2009; Black et al. 2009), and *Glycymeris spp.* (Reynolds et al. 2013; Peharda et al. 2016; Reynolds et al. 2017), this gap in knowledge has begun to close. These species, as well as others, can be used to build extended decadal to multicentennial annual-resolved chronologies using techniques established within the field of dendrochronology (Butler et al. 2013). These chronologies, especially when they can be precisely dated by being anchored in time with shells of a known date of death, provide a stratigraphy for geochemical and increment width proxies which, when successfully calibrated against instrumental series, can be used for paleoenvironmental reconstructions at an annual resolution (Schöne & Gillikin 2013).

Growth increments found in the shells can provide useful information about the biology, ontogeny and environment of the individual and its population (Richardson 2001; Zhao et al. 2016; Milano et al. 2017). These increments can be formed regularly, for example as annual or tidal increments, or intermittently as a result of disturbance events that cause metabolic stress to the animal (Richardson 2001). Endogenous growth rhythms have been found in a number of marine bivalve species. For example, tidal growth increments in shell formation have been identified in *Chione fluctifraga* and *C. cortezi* and are characterized as

corresponding to emersion–immersion cycles (Schöne et al. 2002). Other species exhibit reduced or even interrupted shell growth during gametogenesis due to the allocation of energetic resources towards the production of gametes, such as *Crassostrea gigas* (Dridi et al. 2007). In other examples, such as the Antarctic bivalve *Yoldia eightsi*, endogenous cyclicality in growth has been observed for which there is no clear explanation (Román-González et al. 2017).

Growth increment widths have also been successfully used to reconstruct seawater temperatures (Black 2009; Royer 2013) and climate oscillations (Holland et al. 2014). More intermittent links with seawater temperatures were described by Butler et al (2010) and Marali and Schöne (2016). In addition, other studies have found relationships between bivalve growth and various other environmental variables. For example, Helama et al (2007) and Mette et al (2016) found that *A. islandica* shell growth was related to the winter NAO, Gutierrez-Mas (2011) found *Glycymeris spp.* fossils to be responding to sea-level rise and Bušelić et al (2015) correlated the growth of *G. bimaculata*, in part, with the salinity of the surrounding waters.

The focus of this study is on *Glycymeris glycymeris* which is another species that has been used for reconstruction of past climatic and environmental variables (Reynolds et al. 2013; Royer et al. 2013; Brocas et al. 2013). *G. glycymeris* is a fairly large bivalve with a maximum length of 80 mm (Royer et al. 2013) that inhabits the north eastern Atlantic continental shelf from Cape Verde to Norway in water depths of up to 100m in areas with strong bottom currents (Savina & Pouvreau 2004). Previous research has shown (i) that the periodic growth increments in *G. glycymeris* are formed annually and are synchronous within populations (Reynolds et al. 2013; Royer et al. 2013; Brocas et al. 2013) and (ii) that individuals can live for nearly 200 years (Ramsay et al. 2000; Reynolds et al. 2013). This species is therefore an ideal proxy archive for marine paleoenvironmental studies as it can

provide replicated and crossdated chronologies of shell material, with annual resolution and absolute dating. If sufficient quantities of suitable fossil material are available it will be possible to extend these chronologies back through several centuries.

The study site is in the Bay of Brest, a semi-enclosed mixed marine/estuarine ecosystem with an area of 180 km² and an average depth of 8 m. The Bay of Brest is connected to the Atlantic shelf (Iroise Sea) by a strait to the west that is 2 km wide and 40 m deep, and is fed by two rivers. The larger river, the Aulne, has a catchment area of 1822 km² and the smaller Elorn has a catchment of 280 km². These two rivers provide up to 85% of the total freshwater input into the bay (Raimonet et al. 2013). It is therefore a suitable region for an investigation into the influence of river inflow upon the hydrography, biological and environmental dynamics within the bay.

A major difference between the open ocean and semi-enclosed bays has to do with the dynamics of seasonal phytoplankton blooms (Watanabe et al. 2017). Increasing nutrient loading during the last decade, resulting from intensifying agricultural practice, has focused attention on the possible trend towards coastal eutrophication. Estuarine ecosystems, particularly those in enclosed or semi-enclosed bays, are subject to high nutrient loading, but each bay responds differently to such inputs (Sugimoto et al. 2016). While the emergence of eutrophic conditions is characteristic of some coastal areas, others, including the Bay of Brest, have not yet exhibited such a critical evolution (le Pape et al. 1996).

A typical configuration of factors drives biogeochemical dynamics in such semi-enclosed bays: density stratification is potentially induced by aperiodic river discharges, but stirring by tidal currents and wind prevents sustained vertical stratification in these partially mixed waters (le Pape et al. 1996). At the other end of the system, hydrodynamic exchanges with the open ocean limits the accumulation of organic matter (Jennerjahn & Ittekkot 2002);

however, intermittent inputs by river flow can maintain nutrient availability even after periods of high consumption (Mallin et al. 2003).

The objective of this study is to create an extended and annually resolved *G. glycymeris* chronology and to evaluate its potential as an environmental indicator and archive of proxy records for past climatic and hydrographic variability. In order to achieve this objective, the following goals were set; (i) to determine the feasibility of crossdating live- and dead-collected shell material using *G. glycymeris* samples from the Bay of Brest, north-west France, (ii) to establish that these samples can be used to build a well-replicated and statistically robust chronology, (iii) to identify relationships between environmental factors and the growth of *G. glycymeris*.

3.3. Methods

3.3.1. Chronology Construction

The morphometrics (shell length, height, width, and total dry shell mass) of all the collected shells were measured using an automatic vernier calliper at 0.01 cm precision and recorded. The shell mass was recorded on a balance to the nearest 0.1 g. The recorded biometrics were then used to select 44 live- and 30 dead-collected shells for sectioning. The selected shells were those which were the largest by height, and the heaviest by total shell mass, chosen on the basis that these were likely to be the longest-lived (see Butler et al. 2010). Shells which were damaged by the dredge or fouled were excluded.

Standard statistical techniques derived from dendrochronology were used to crossdate the growth increment series (Black et al. 2008; Scourse et al. 2012; Butler et al. 2013; Brocas et al. 2013; Reynolds et al. 2013 Peharda et al. 2016). Only those samples within which the

growth increments were well defined and which were taken from individuals that were over 20 years old were used for chronology development. In total 20 live-collected and 18 dead-collected individuals satisfied these criteria. The year of collection (2012, 2014 or 2015) was assigned to the most recent partial growth increment of the live-collected specimens; this is the incomplete increment found on the outer edge of the hinge.

The dendrochronological crossdating application COFECHA V6.06P (Holmes 1983; Grissino-Mayer 2001) was used to crossdate the growth increments between different individuals. For the dead collected specimens, where the date of death was unknown, 2004 was initially assigned to the most recent growth increment so that the series could be input to COFECHA. COFECHA was configured to fit a 20-year cubic smoothing spline with 50% wavelength cut-off to the measured time series. This 20-year smoothing was chosen due to the young average age of the specimens included in the analysis along with the standard 50% wavelength cut-off. Each time series was then divided by the values predicted by the spline, isolating high-frequency variability and standardizing each series to a mean of one (Grissino-Mayer 2001). The overall average of the correlations between each individual and the average of all others was reported as the series intercorrelation. The dead collected individuals were reassigned new arbitrary ages at ten-year intervals until COFECHA could give a potential fit. Once a fit was found the list year method was used to check that the match was also visually correct. The successfully crossdated shell series were used to construct a master chronology using the dendrochronology application ARSTAN for Windows (version 41d, Cook & Krusic 2006).

Detrending methods that have been previously applied in sclerochronological studies to produce *G. glycymeris* (Reynolds et al. 2013) and *G. pilosa* chronologies (Peharda et al. 2016) were then used. We first applied an adaptive power transformation to each series to stabilise variance throughout the growth series (Cook & Peters 1997). Once this was

completed, a negative exponential function was fit to the transformed series to remove the ontogenetic growth curve. In 6 cases, however, ARSTAN indicated that a 15-year cubic spline with 50% frequency cut off provided the best fit (Cook et al. 1990) by announcing that the previous function would lead to a negative detrended growth curve. ARSTAN creates three versions of the master chronology (standardized (STD), residual (RES) and ARSTAN (ARS)), which model autoregression in different ways. In this study, there were no significant differences between the three versions, so only the STD chronology will be used in the remainder of this analysis.

3.3.2. Chronology Validation

Accelerator mass spectrometry (AMS) radiocarbon (^{14}C) dating was used to validate the crossdating between four dead-collected *G. glycymeris* that cross matched with each other but could not be visually incorporated into the master chronology. Prior to sampling, the valves were cleaned and the periostracum removed. A single CaCO_3 subsample from each shell, drilled close to the ventral margin, was taken by Beta Analytic, Miami, USA, for analysis. Conventional $\Delta^{14}\text{C}$ determinations were corrected for a regional marine radiocarbon reservoir age effect (MRRE) using ΔR of -48 ± 45 years (Struiver & Braziunas 1993) and, as all samples were post-bomb (post-1950), they were calibrated using regional bomb-pulse calibration curves created by Scourse et al (2012), on the basis of the marine box model used by Reimer et al (2009). Shallow, well-mixed, locations like the Bay of Brest have a response similar to the German Bight or Oyster Ground which approximate the atmospheric bomb-pulse because carbon is readily exchanged and mixed in such settings. As such the pMC (% modern carbon) values were compared with the German Bight and Oyster Ground curves found in Scourse et al (2012).

3.3.3. Environmental Analysis

The STD chronology standardized growth index (SGI) was compared to available variables from the local SOMLIT-Brest monitoring station, as well as the North Atlantic Oscillation (NAO). The NAO is an index of fluctuations in atmospheric pressure at sea level between the subpolar and subtropical regions (Hurrell et al. 2001). It is an indicator of weather patterns (wind, temperature, moisture, etc.) in the North Atlantic, especially the strength and direction of westerly winds and storm tracks during the winter months (Dawson et al. 2002). The winter NAO index used here is defined as the normalized pressure difference between the Azores (high-pressure) and Iceland (low-pressure) (Hurrell et al. 2001), averaged over the months December-February.

The same winter months were averaged for the East Atlantic Pattern (EAP) and compared to the SGI. The EAP has a strong impact in Western Europe by influencing sea surface temperature or modulating mean precipitation rates and hydrological processes (Tréguer et al. 2014).

The SGI was compared with temperature, salinity and chlorophyll α data acquired by the SOMLIT-Brest monitoring station from 1998 to 2014. Further comparisons were made between the SGI and rainfall using a dataset from Brest-Guipavas first published by Klein Tank et al (2002).

Data about the river flow rates of both the Elorn and the Aulne, nitrite and suspended particulate matter (SPM) was provided by Hydro Bank, which is administered by the Service Central d'Hydrométéorologie et d'Appui à la Prévision des Inondations (service du Ministère de l'Ecologie, du Développement Durable et de l'Energie). This data can be found at <http://www.hydro.eaufrance.fr/>.

The correlating environmental data were standardised ($\mu = 0$, $\sigma^2 = 1$) and analysed using principal components analysis (PCA). Missing values were corrected using a mean value imputation. The scores for the principal components that accounted for the majority of the variance (PC1, PC2 and PC3) were tested for significant correlations (using Pearson's correlation) with the SGI.

3.4. Results

3.4.1. Biometrics and Growth

In total 401 live- and 243 dead-collected *G. glycymeris* with paired valves were collected between September 2014 and November 2015. The mean shell length of all specimens collected was 59.2 mm ($\sigma = 9.3$ mm). The mean length of the dead-collected valves was 61.8mm ($\sigma = 6.9$ mm; range 34.6 mm to 77.1 mm), and that of the live-collected valves was 52.5 mm ($\sigma = 8.2$ mm; range between 24.6 mm and 69.4 mm). There was also a difference between the shell mass of the live- and dead-collected shells, the overall average being 42.4 g ($\sigma = 19.4$ g). The dead-collected paired valves weighed an average 55.3 g ($\sigma = 14.7$ g; range 8.6 g to 114.8 g), while the live-collected shells weighed much less with an average of only 34.62 g ($\sigma = 14.1$ g; range 3.1 g to 95.9 g).

The age range of the live-collected shells was 5 to 43 years, with an average of 19 years ($\sigma = 9$ years). This is low compared to the ages of the shells live-collected in 2012, whose average age was 27 years ($\sigma = 9$ years; range 25 years to 44 years). The dead collected shells had a greater longevity with an average of 44 years ($\sigma = 17$ years; range 24 years to 70 years). The maximum age of live-collected specimens from all of the 2015, 2014 and 2012

collections was 50 years, whereas the longest lived dead-collected valve analysed was 70 years. The mean longevity of the shells used to construct the chronology was 32 years.

3.4.2. Chronology

G. glycymeris has strong synchronous growth that allows it to be crossdated.

Signature years of increased growth, such as 2005, 2003, 2001, 1995 and 1983, were found in

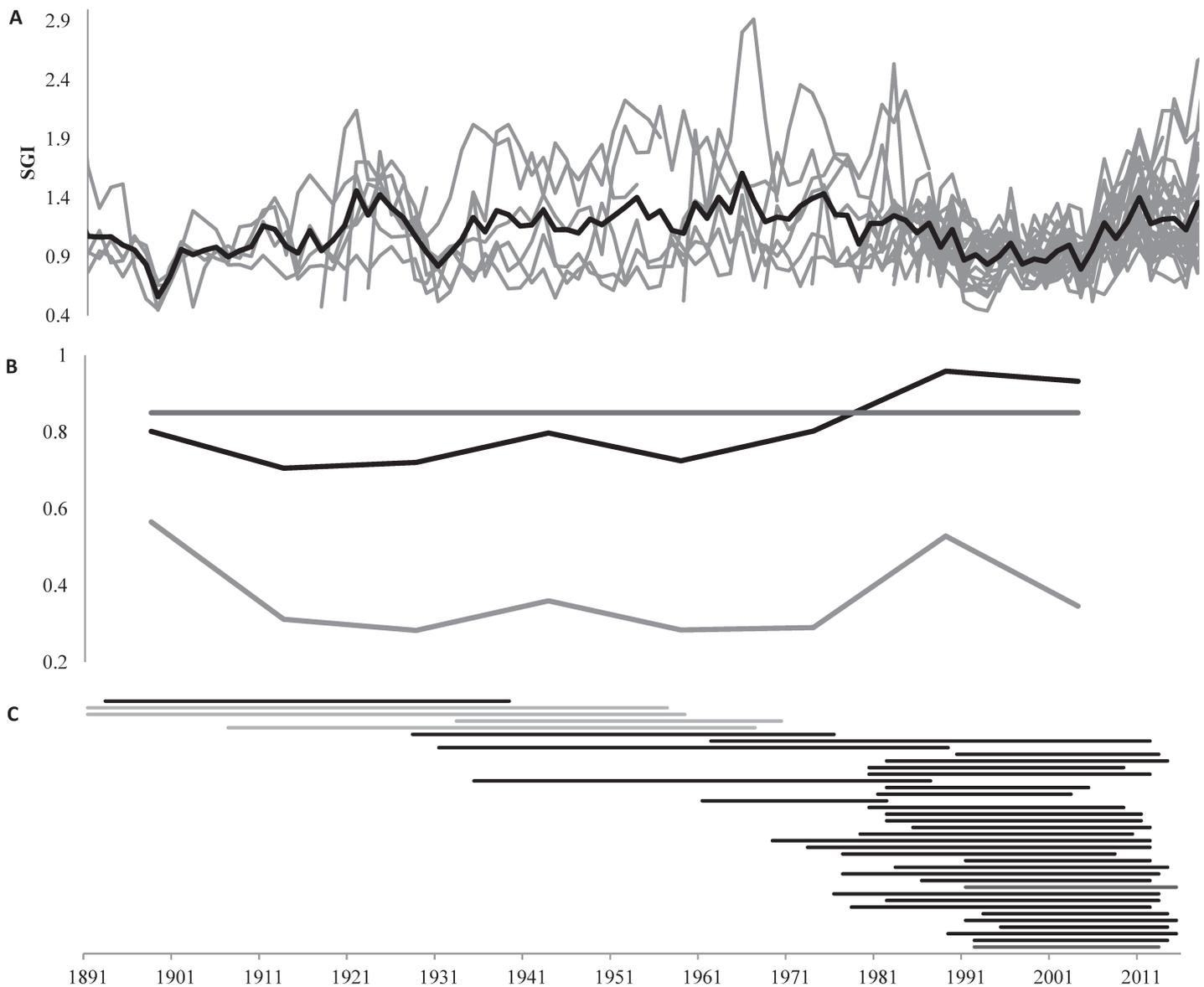


Fig 3.1: Chronology Information **A)** Detrended increment measurements (grey lines) and ARSTAN created standardized (STD) chronology (black line); **B)** EPS and RBar values calculated in a 20-year window with a seven-year overlap. The horizontal line shows the 0.85 threshold (see text); **C)** The position of each of the shells in the chronology. The grey lines indicate shells sent for radiocarbon analysis.

almost all samples. No missing increments were observed in any sample. Non-annual growth lines were present in all sampled individuals, but these were easily distinguished from the annual increments as they were always lighter in colour than the annual banding.

The chronology created with only live-collected specimens has a mean sensitivity of 0.189, with an interseries correlation of 0.497. When the dead collected shells are added to extend the chronology further back in time, the mean sensitivity remains almost identical at 0.190, and the interseries correlation rises slightly to 0.502. Using only live-collected individuals, the replicated chronology extends from 1975 to 2015, with five individuals in the chronology at 1975 (Fig 3.1C). When series from the dead collected shells are added, the replicated chronology extends back to 1901 with at least 3 individuals. One specimen reaches back to 1891. The Expressed Population Signal (EPS), a measure of chronology strength, falls below the conventional threshold of 0.85 (Wigley et al. 1984); see Discussion for further explanation) prior to 1975 because of the rapid fall in sample depth as live-collected specimens drop out of the chronology, but remains above 0.7 throughout. The chronology was truncated at 1901 because the running EPS (calculated over a 20-year window with a 7-year overlap) could not be calculated before that point (Fig 3.1C).

3.4.3. Chronology Validation

The four AMS ^{14}C determinations derived from the ventral margins of shells were dated as post-bomb (post 1950) and were therefore calibrated using the curves for German Bight and Oyster Ground described by Scourse et al (2012). The calibration indicated that the individuals had died c.1960 or post 2005 (Appendix 4), confirming their placement in the master chronology between 1955 and 1970.

3.4.4. Environmental Drivers

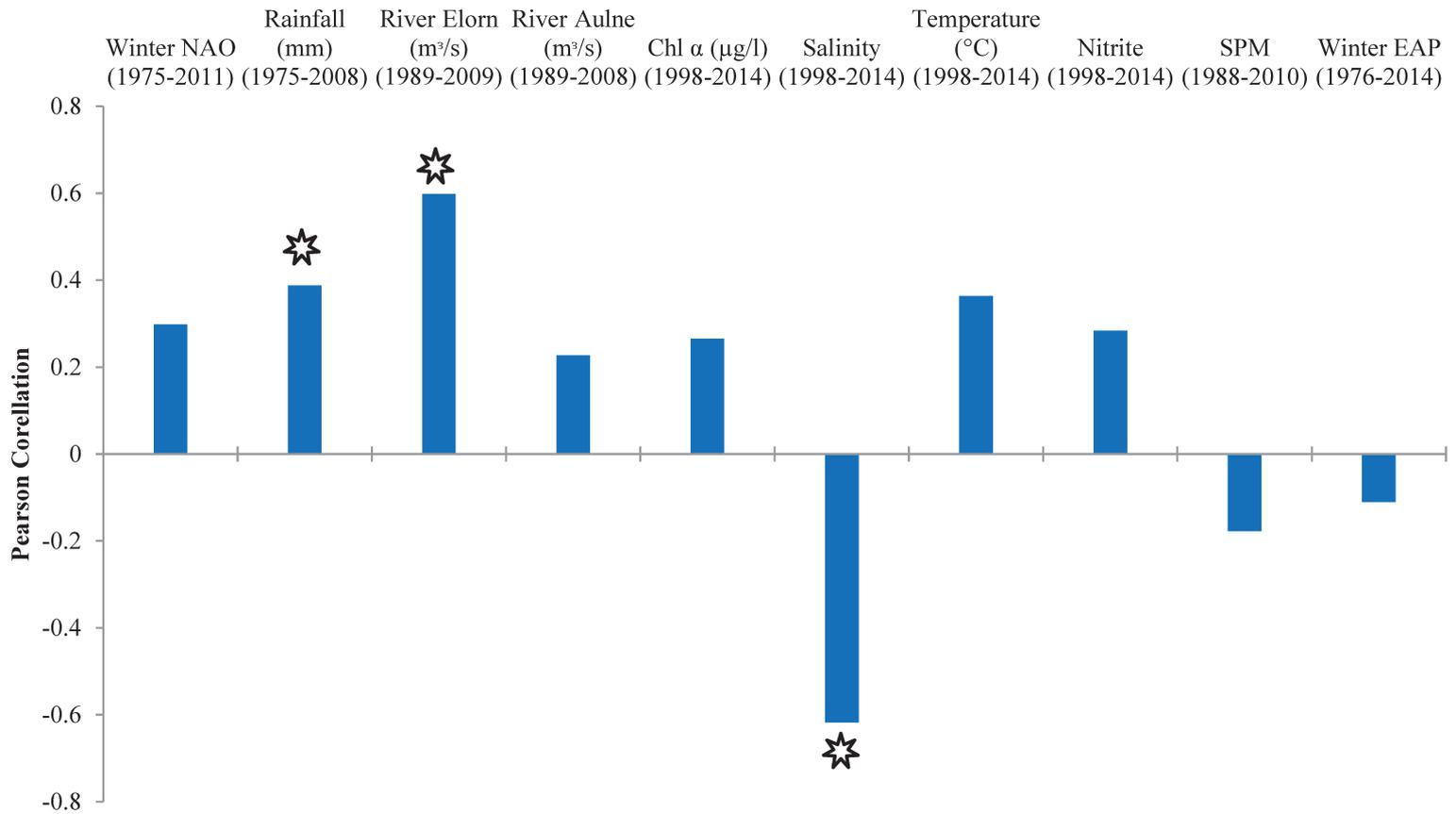


Fig 3.2: Pearson correlation coefficients of annually averaged environmental data with SGI. Significant correlations ($p < 0.05$) are marked with a star.

In the Bay of Brest, growth of *G. glycymeris* was negatively correlated to annually averaged salinity ($r = -0.62$, $p = 0.006$) over the period 1998-2014. Growth was positively correlated with the annually averaged flow rate of the River Elorn ($r = 0.60$, $p = 0.005$) over the period, 1989-2009 and with rainfall ($r = 0.34$, $p = 0.03$) over the period 1975-2008 (Fig 3.2). No significant correlations were found with any other annually averaged variable.

When the monthly averages of each environmental variable were correlated with the chronology, the strongest correlations with salinity were for the months of January ($r = -0.62$, $p = 0.008$) and February ($r = -0.54$, $p = 0.020$), and also for November ($r = -0.61$, $p = 0.01$)

and December ($r = -0.71$, $p = 0.001$) of the previous year (Fig 3.4). The months for which SGIs correlated most strongly with inflow of the River Elorn were January ($r = 0.52$, $p = 0.022$) and February ($r = 0.53$, $p = 0.019$) as well as December of the previous year ($r = 0.49$, $p = 0.033$) (Fig 3.3 & 3.4). In February alone, there were significant correlations with nitrite concentration (NO_2) ($r = -0.464$, $p = 0.022$) (Fig 3.3) and suspended particulate matter (SPM) ($r = 0.500$, $p = 0.01$) (Fig 3.3 & 3.4).

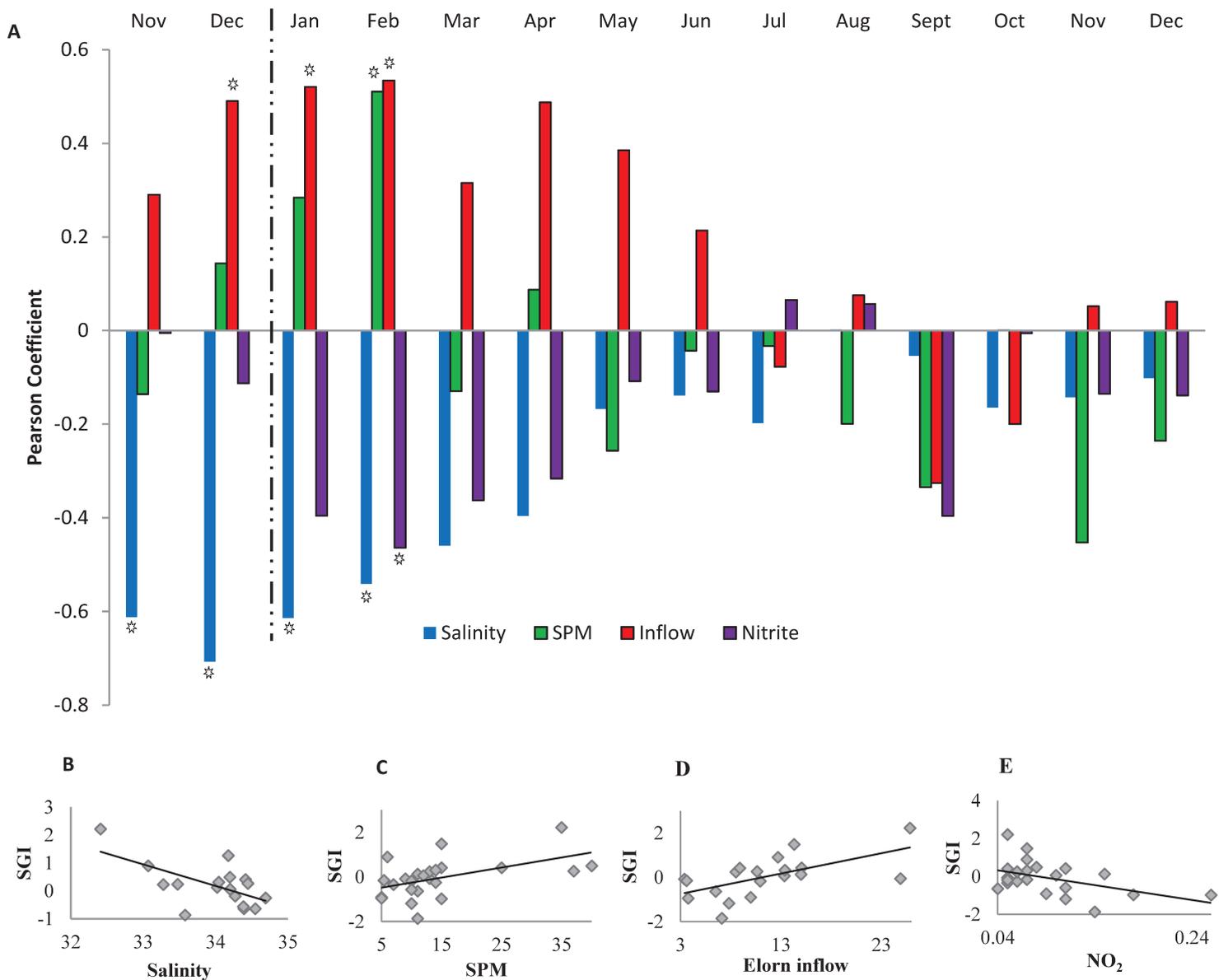


Fig 3.3: Comparison of chronology with environmental data. **A)** Pearson correlation coefficients of monthly environmental data with the SGI. Significant correlations ($p < 0.05$) are marked with a star. Dotted line indicates the change between years (e.g. previous and current year). **B-E)** biplots of significant environmental data (averaged monthly) and the chronology



Fig 3.4: Correlated monthly environmental data compared and annual growth index.
 Line representation of biplots in Fig 3.3B, C and D

3.4.5. Principal Components Analysis

The standardised environmental parameters of nitrite, River Elorn inflow, salinity and rainfall were analysed using a PCA. The first principle component (PC1) accounted for 51.9% of the variance, PC2 accounted for 21.2% and PC3 of 17.7%. PC4 and PC5 accounted for less than 15% of the variance and were subsequently disregarded from further analysis (Appendix 5). Two of the environmental parameters had similar strength loadings on PC1 (River Elorn Inflow; 0.51; nitrite, 0.55). Another strong loading in PC1 was salinity (-0.52) although it was a negative loading. SPM was the strongest loading on PC2 (0.93) and rainfall was strongest in PC3, showing strong synchrony between these environmental factors and their respective principle components (Fig 3.5 (see Appendix 6 for all loadings)). PC1 was positively correlated with the SGI ($r = 0.35$, $p = 0.02$) whereas neither PC2 ($r = -0.12$, $p = 0.44$) or PC3 ($r = 0.17$, $p = 0.29$) were found to have significant correlations.

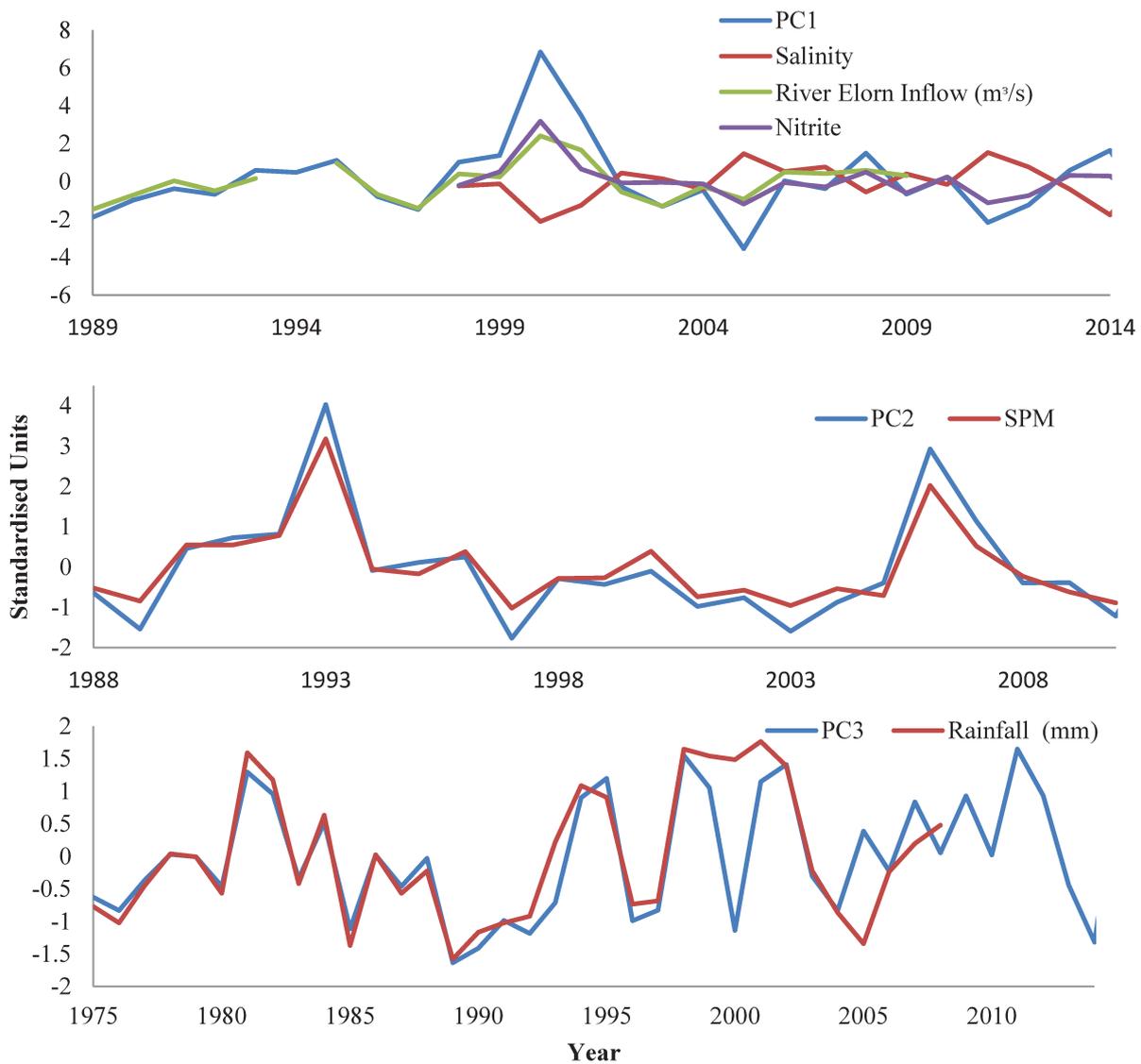


Fig 3.5: Environmental data with the respective leading principle component (PC) scores. Environmental parameters included in analysis are: salinity, River Elorn inflow rate, nitrite levels and rainfall. Each environmental parameter was normalised (mean = 0, variance = 1)

3.5. Discussion

In this study we examined the internal growth increment series in shells of the marine bivalve *G. glycymeris* collected from the Bay of Brest, France. Series from 20 live- and 18 dead-collected shells were successfully crossdated to construct a replicated chronology

extending from 1901 to 2014. The longest-lived shell analyzed was a dead collected individual whose longevity was estimated at 70 years, making it the longest-lived individual of this species so far found in north-west France (Royer et al. (2013) have previously reported a maximum longevity of 46 years). It is interesting to note that other studies, conducted at higher latitudes, found significantly greater longevity in *G. glycymeris* (Ramsay et al. 2000; Brocas et al. 2013; Reynolds et al. 2013), with Reynolds et al. (2013) identifying a specimen that had lived for 192 years. Reynolds et al. (2013) collected their samples from north-west Scotland, whereas Ramsay et al (2000) and Brocas et al (2013) sampled the waters surrounding the Isle of Man. This supports the hypothesis that there is a latitudinal trend, with longevity increasing at higher latitudes. Moss et al (2016) found this effect in many different bivalves including species from the commercially important genera *Veneridae*, *Pectinidae*, and *Mytilidae* and attributed it to differences in temperature and the limited and highly seasonal food availability that affects populations at higher latitudes. They theorise that the longer lifespan may be a consequence of limited metabolism, and that long life could be the key to reproduction because with limited food availability individuals could not always rely on the energy resources to allow annual spawning.

Wigley et al (1984) determined that an EPS of 0.85 indicates that the signal in the chronology is a sufficiently good representation of the signal in the whole population. This chronology achieves that threshold after 1975, but because of the reduced sample depth, EPS drops below 0.85 before that date, sometimes falling as low as 0.7. Since all the comparisons with environmental data here relate to the period after 1975, the chronology signal strength can be considered adequate for these analyses. The use of this chronology as a robust reconstruction tool is contingent on its being strengthened prior to 1975 with the addition of growth increment series from more shells.

The significant correlation observed between *G. glycymeris* growth and the inflow of the River Elorn is to be expected. The rivers feeding into the Bay of Brest are a substantial source of nutrients that sustain a large amount of primary productivity in the bay (Tréguer et al. 2014; Salt et al. 2016). Although the River Elorn is the smaller of the two rivers, it is closer to the sample site than the larger River Aulne, and it is therefore reasonable to conclude that it might have a more direct influence on the growth of this *G. glycymeris* population. Although a significant negative correlation was found between growth and salinity, it is unlikely that there is a direct relationship between the change in salinity and growth. Colonese et al (2017) found a similar relationship between growth, freshwater circulation and salinity using intra-shell $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ values of the freshwater mollusc *Anomalocardia flexuosa* from southern Brazil. However, *G. glycymeris* is a marine stenohaline species with an optimum salinity at 34 (Rombouts et al. 2012), so it is unlikely that an increase in salinity would cause growth to decrease, especially as average salinity in the Bay of Brest is below the upper tolerance limit for the species and very close to the optimum. It can therefore be assumed that another variable, also related to river inflow, is controlling growth. Del Amo et al (1997) reported correlations (Spearman's rank correlation) between concentrations of silicic acid and phosphate and river inflows during 1993-94 that were higher for the Elorn than for the Aulne. The long-term trend to lower Si:N molar ratios (le Pape et al 1996) has resulted in silicates and phosphates, rather than nitrates, being the main limiting nutrients in the Bay of Brest (del Amo et al. 1997; Salt et al. 2016). It is therefore feasible that the Elorn is more significant than the Aulne in the delivery of limiting nutrients to the Bay of Brest, so that shell growth is more sensitive to changes in inflow from the Elorn. This hypothesis should, however, be treated with caution as it is based on measurements for a single annual cycle.

The strong positive correlation between growth and suspended particulate matter (SPM) in February supports the hypothesis that food availability is an important driver of shell

growth in the Bay of Brest. As *G. glycymeris* is part of the endofauna, living below the surface of the sediment, the predominant effect of nutrients on shell growth must occur after the particles settle. Active pumping and biodeposition by benthic suspension feeders have been found to increase the rate of settlement of suspended matter on the sediment (Ellis et al. 2002). For example, De Vries & Hopstaken (1984) have previously estimated, for Grevelingen (The Netherlands), that biodeposition by benthic suspension feeders increases particulate matter settlement by at least three times compared to passive sedimentation (Chauvaud et al. 2000). In the Bay of Brest, Barnes et al (1973) found that biodeposition by *Crepidula fornicata* led to siltation of sediment as well as significantly reduced particle resuspension. This activity by *C. fornicata* appears to be a crucial factor in the development of a silicate pump in the Bay of Brest, with the biologically limiting silicates retained in the bay as a result of such biodeposition and contributing to diatom dominated phytoplankton blooms during the spring and summer (Chauvaud et al. 2000; Rageneau et al. 2002). *C. fornicata* is found in great numbers (500-1300 individuals/m²) close to the sample site of this study (Chauvaud et al. 2000; Dawson et al 2002), and it is likely that the retention of nutrients in the area through biodeposition is directly related to the availability of nutrients to *G. glycymeris* at this site.

High levels of chlorophyll α are not necessarily correlated with high growth rates in bivalves (Chauvaud et al. 1998). Lorrain et al (200) observed that large bottom concentrations of chlorophyll α , particularly after diatom blooms, could have a negative effect on the ingestion or respiration of *P. maximus* juveniles, either by gill clogging or by oxygen depletion at the water-sediment interface associated with the degradation of organic matter. *G. glycymeris* has a ciliated gill structure which allows for potential food to be sorted upon the gills themselves, rather than through the digestion process and unwanted material is passed to the edges of the gills demibranchs (Atkins 1936). This structure of constant sorting

and movement of particles along the gills means that gill clogging is more likely than in species with a lophophore or siphon (Steel- Petrović 1975).

Overall, phytoplankton is thought to be only a small part of the diet of *G. glycymeris* (Savina & Pouvreau 2004) and therefore SPM is likely a much better representation of food availability for this species within the normal seasonal cycle. This is not the first time such a conclusion has been made. Galap et al (1999) states that bacteria enriched detritus, collected from the sediment, constitutes as the principle nutrient source for *G. glycymeris* in the Douarnenez Bay, France. Also, this is not restricted to *G. glycymeris* in France, the same has been observed in Mali Ston Bay, Croatia, where the main food source of *G. nummaria* is detritus, particularly in the autumn/winter months (Najdek et al 2016).

The negative correlation observed with nitrite (NO_2) is likely due to the high toxicity of the compound. Widman et al (2008) found that, after ionised ammonia, NO_2 was the most toxic nitrogen based compound for *Argopecten irradians irradians*. Argumugan et al (2000) observed that *Mytilus galloprovincialis* and *Crassostrea gigas* produce NO_2 as a by-product of their reactive oxygen intermediates, although these are the only species found to do so. While *Mercenaria mercenaria* and *C. virginica* have been shown to have a strong tolerance for nitrite (Epifanio & Srna 1975), the same has not been observed in *G. glycymeris*. More research needs to be carried out on the *Glycymeris* genera as up until now there have been no studies of their response to environmental NO_2 . This is particularly important in areas such as the Bay of Brest, where inflows of nitrogen compounds have increased ten times over the course of the 20th century (Tréguer & Queguiner 1989).

The addition of principle components analysis (PCA) related nitrite levels to the inflow from the River Elorn as well as finding a negative relationship to salinity (Appendix 6) grouping them together for analysis within PC1. This leads to the conclusion that the nitrite

levels in the Bay of Brest are being fed by the River Elorn. As this grouping correlated strongly with the SGI, it can be concluded that all of these factors are driven together and that they influence the growth of *G. glycymeris*. More information is needed to pull apart exactly which of these environmental factors is the most important. Although correlations were found with SPM levels in February (Fig 3.4), the lack of correlation between the SGI and PC2 (Fig 3.5) implies that this correlation may be coincidental.

In contrast with other bivalve growth studies (Schöne et al 2003b; Brocas et al 2013), no significant correlation was found between the growth of *G. glycymeris* and the winter NAO (wNAO). The wNAO reflects winter conditions in Northern Europe, as it indicates the direction of storms caused by winds blowing across the Atlantic (Trigo et al 2002). Royer et al (2013) found that the growing season for *G. glycymeris* in the Bay of Brest is May-October, and is therefore lagging the period of maximum pressure gradients in the North Atlantic (upon which the wNAO index is based) by several months. The same authors also found no correlation between the wNAO and growth in their population from the Chausey Islands, and concluded that this was because of the time lag. However, it has been suggested by Schöne et al (2003) that the state of the NAO in winter could predispose the environment to favour shell growth later. Correlations between the wNAO and shell growth, even if they are only intermittent (Mette et al 2016), seem to occur at higher latitudes than the populations in NW French waters investigated by Royer et al (2013) and in this study. This suggests that the effect of the positive phase of the wNAO is to divert the Atlantic storm tracks into UK and Norwegian waters. The result is that the lagged signal of the wNAO is more strongly expressed in bivalve populations living directly under the path of the stronger storms that occur when the wNAO is in its positive phase. For the *G. glycymeris* population studied here, it is likely that local factors are more important for growth than major climate oscillations. For example, Grall & Glémarec (1997) describe the river Elorn as being heavily polluted by

agricultural runoff and this may lead to larger scale environmental signals such as the NAO being masked in the shell growth records (Dunca et al. 2005).

Tréguer et al (2014) found a positive correlation between sea surface salinity, rainfall and the East Atlantic Pattern (EAP) in the Bay of Brest. It could be assumed that, because the growth rate of *G. glycymeris* in this location is controlled by rainfall and river runoff (indirect correlation with salinity), it would be linked with the EAP as well. However, our study shows that this is not the case. Tréguer et al (2014) only used data spanning fifteen years, on the other hand this study utilised almost forty years by comparing the EAP with the created SGI, going back further in time than salinity observations within the bay allow. The difference between the results in this paper and those by Tréguer et al (2014) lends weight to the requirement of longer proxies in order to establish significant climatic trends (Mann et al. 2008).

3.5.1. Assessment of reproducibility

The ability to use *G. glycymeris* as a sclerochronological record is completely dependent on the amount of shell material available. This is particularly important with fossil shell material as availability must be reasonably extensive along a temporal distribution. This study shows that growth of *G. glycymeris* in the Bay of Brest is synchronous at Banc de la Cormorandière and that 20 live-collected shells are able to crossmatch to create a statistically robust growth index; however, 18 dead-collected shells are not statistically viable (as the EPS drops below 0.85 when dead-collected shells are added), despite successful cross-matches being identified (Fig 3.1B). This is because the dead-collected samples cross a temporal distribution (Fig 3.1C) and do not achieve the typical minimum of c. 8–10 shells (Brocas et al., 2013; Butler et al., 2009; Reynolds et al., 2013) required over any given period to create a robust master chronology.

The addition of AMS¹⁴C dating provides independent support for the interspecies cross dating, as well as an indication of the temporal distribution of the population. There is close agreement between the ¹⁴C determinations from all four shells and the cross matched dates, supporting the hypothesis that cross matching is a viable tool for aging.

3.5.2. Conclusions

This study indicates that *Glycymeris glycymeris* in the Bay of Brest is highly sensitive to the fresh water inflow from the River Elorn, as well as to food availability mediated by increased SPM in the late winter. As the length of the chronology extends to periods before measured environmental data was available, it will be possible to reconstruct such variables using the chronology SGIs, subject to improvement of the chronology signal (EPS) by adding more shells to the chronology before the 1970s. It will also be possible to integrate other biological data (such as fish otolith chronologies, changes in benthic species composition, and phytoplankton observations) into this research, using mixed effects models to test the relationships between local environmental variables and different combinations of ecological and biological responses. Given the large quantities of fossil material available in the relatively small area sampled for this study, we are confident that a robust chronology extending further back in time can be constructed. In addition, with the use of radiocarbon dating, we will be able to construct floating chronologies for earlier periods of climatic and environmental interest. This study highlights the importance of location as a factor in the degree to which individual growth in a population responds to climatic and environmental change. Localised records such as these have great potential for the calibration of regional climate models as they provide unique sources of annually-resolved and locality-specific paleoclimate information that is often not available from instrumental measurements.

**Chapter 4: A 45-year sub-annual
reconstruction of seawater temperature in the
Bay of Brest using shell oxygen isotope
composition of the bivalve *Glycymeris*
*glycymeris***

4.1. Abstract

A reconstruction of sea surface temperature (SST) spanning 45 years (1966-2011) was developed from $\delta^{18}\text{O}$ obtained from the aragonite shells of *Glycymeris glycymeris*, collected monthly from the Bay of Brest, France. Bivalve sampling was undertaken between 2014 and 2015 using a dredge. In total 401 live specimens and 243 articulated paired valves from dead specimens were collected, of which 24 individuals were used to reconstruct SST.

Temperatures computed with the equation by Royer et al. (2013) compared well with observed SST during the growing season between 1998 and 2010 (Pearson Correlation: $p = 0.002$, $r = 0.760$). Furthermore, a significant negative correlation existed between SST and the North Atlantic Subpolar Gyre (SPG) index ($p = 0.001$, $r = -0.50$) and a positive correlation with the East Atlantic Pattern (EAP) index when lagged by one year ($p = 0.002$, $r = 0.46$). This led to the conclusion that EAP and SPG are the possible driving forces behind SST in the Bay of Brest. As the SPG controls air temperature in Northern Europe and the EAP controls water temperature in Southern Europe, this leads us to believe that the Bay of Brest is an interaction area between these two climate systems. As such, this locality is interesting as the $\delta^{18}\text{O}$ of the shells can be used a proxy for both the SPG and EAP, and temperature reconstructions can provide a unique insight in how these climate systems interact prior to the instrumental era.

4.2. Introduction

High-resolution records of climate change in the North Atlantic demonstrate frequent and rapid fluctuations in the ocean-atmosphere system (Bond et al., 1993; Dansgaard et al., 1993, McManus et al., 1994; Thouveny et al., 1994; Stuiver et al., 1995; Fuller et al. 1998). It is important to study past climates in order to give long-term context to modern climate warming. Numerous studies have focussed on climate change of the past few centuries to millennium, using both modelling experiments based on estimated climate forcings (Murphy et al. 2004) and empirical reconstructions using proxy data (Mann et al. 1998). From the late 19th century, meteorological stations have been providing records of temperature, precipitation, and other climate indicators over both the northern and southern hemispheres (Jones et al. 1998). This has been predominantly land-based stations, supplemented by ship-based logs of sea-surface temperatures (SST), until more recent decades (García-Herrera et al. 2005).

In order to study climate before the development of the robust instrument record, evidence derived from climate proxies must be used; indirect observations that serve as substitutes (Pollack & Huang 2000). The requirement for these reconstructions is exact dated, high-resolution data that can be calibrated with instrumental data (Mann et al. 1999). It is also important to note the seasonality of the proxy data when forming a climate reconstruction (Mann et al. 1998).

The focus of this study is to use stable oxygen isotope data from the shell of *Glycymeris glycymeris* to reconstruct past seawater temperatures for periods before the availability of local instrumental data (1998-present). According to an oxygen isotope study by Berthou et al. (1986), *G. glycymeris* growth bands are formed annually with their boundaries corresponding to growth slowing down. Using these growth lines, it has

previously been shown that *G. glycymeris* can live up to two centuries (Ramsay et al. 2000; Reynolds et al. 2010). The distribution and longevity of *G. glycymeris* make this organism a suitable subject for paleoenvironmental studies.

The objective of this study was specifically to use $\delta^{18}\text{O}_{\text{shell}}$ from *G. glycymeris* to investigate the climatic drivers of temperature in the Bay of Brest. In order to achieve this objective, the following goals were set: (i) successfully reconstruct seawater temperatures further back in time than instrumental measurements; (ii) identify relationships between the reconstructed temperature and the climate oscillations that are thought to influence the temperature in this study area (such as Tréguer et al. 2014).

G. glycymeris in the Bay of Brest is thought to have a growth season of May to October (Royer et al. 2013). Although they don't grow over the months covered by winter North Atlantic Oscillation (NAO_{winter} (December, January, February)), studies have shown that the European climate over the summer is significantly linked to the NAO_{winter}, such as Saunders et al. (2002) which looked in detail at summer snow extent. The majority of studies, however, state that NAO_{winter} only indicates winter climates (Hurrell 1995; Rodwell et al. 1999; Osborn 2006). As such, any relationships found between SST and NAO_{winter} will likely be lagged by 1 year. The same can be said for the East Atlantic Pattern (Wibig 1999; Conrad et al. 2003).

4.3. Methods

Twenty-four specimens of different sizes (and ontogenetic ages) were collected alive. As samples were taken from the juvenile years, shells were chosen for their wide increments in order multiple drill points to be taken. In order to assign calendar years to each increment found in the prismatic layer of the shell, acetate peel replicas were prepared using methods described in Chapter 2.

The section was then cut again using the precision saw to 3-6mm thick sections, and polished again with 3 μ m diamond paste. The goal of this was to obtain a thick cross-section to sample shell powder (here aragonite) for $\delta^{18}\text{O}$ analysis. The polished slab was rinsed several times ultrasonically with demineralized water prior to sampling for isotopic composition. Calendar dates were marked by each increment in the margin by scratching a mark in the resin.

4.3.1. Oxygen isotopic analysis

Due to ontogenetic growth trends, shells were sampled only between the second and eighth year of growth to maximise the number of samples taken from each annual increment and thus increase the temporal resolution. The period of growth assigned to *G. glycymeris* sampled from the Bay of Brest was from early May to the end of October, as highlighted by Royer et al. (2013) for the same sample region.

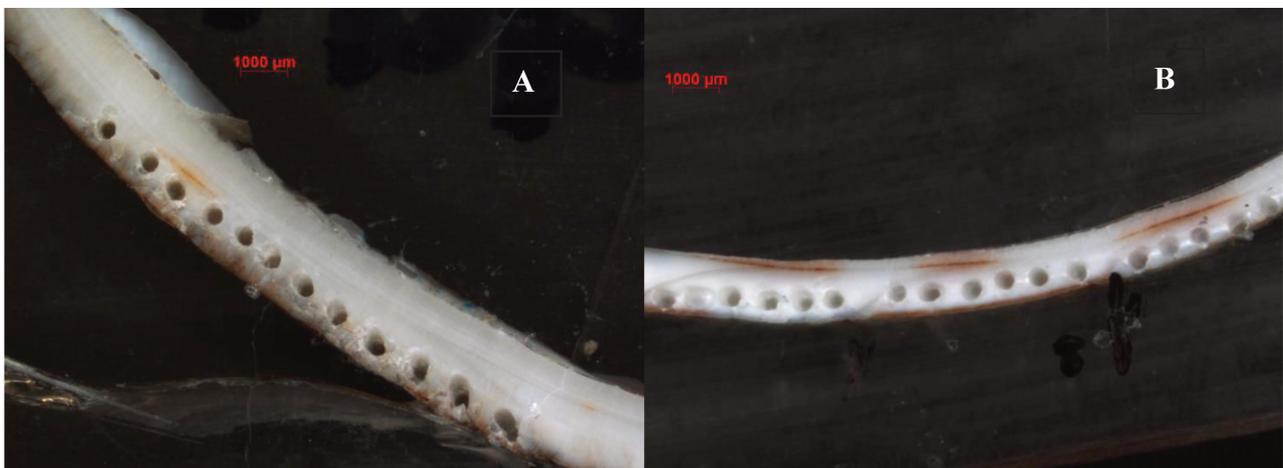


Fig 4.1: Examples of drill sampling on the prismatic shell layer for $\delta^{18}\text{O}$ analysis. Specimens 14111007 (A) and 14121022 (B)

Shell aragonite powder (n= 636) was sampled in the direction of growth using an automated high-resolution microsampling device (MicroMill, New Wave Research) equipped with a 300- μ m tungsten carbide drill bit (model H71.104.003, Gebr. Brasseler GmbH & Co. KG, Germany). Between 8 and 33 (on average, 12) carbonate samples were collected from the

prismatic layer of the 24 shells. Drill holes were ca. 350 μm (Fig 4.1) in diameter and provided aragonite fractions of 50 to 120 μg (on average, 94 μg). Samples were processed in a Thermo Finnigan MAT 253 continuous flow-isotope ratio mass spectrometer coupled to Gas Bench II at the Institute of Geosciences, University of Mainz. Samples were dissolved in concentrated phosphoric acid in helium-flushed borosilicate exetainers at 72°C (two hours reaction time). Stable oxygen isotope ratios are reported relative to the Vienna Pee-Dee Belemnite (VPDB) standard based on a NBS-19 calibrated Carrara marble ($\delta^{18}\text{O} = -1.91\text{‰}$). The average internal precision and accuracy – based on eight individual measurements per sample – was 0.06‰ and the long-term accuracy (1σ), based on 421 blind measurements of NBS-19, was ± 0.04 ‰.

In order to determine past temperatures using $\delta^{18}\text{O}_{\text{shell}}$, we used a fractionation equation developed by Royer et al. (2013), calibrated for aragonitic *G. glycymeris* shells from the Bay of Brest:

$$(1) T(^{\circ}\text{C}) = 18.11(\pm 0.13) - 2.66(\pm 0.15) \times (\delta^{18}\text{O}_{\text{aragonite VPD}} - \delta^{18}\text{O}_{\text{water VSMOW}}).$$

Using this equation requires shell oxygen isotope data that are not adjusted for differences in acid fractionation factors of aragonite (shell) and calcite (standards). For more detailed explanation of this correction see Füllenbach et al. (2015).

The water oxygen isotope composition in the Bay of Brest was estimated using the linear relationship between $\delta^{18}\text{O}_{\text{water}}$ and salinity (S) (measured at SOMLIT) as reported by Chauvaud et al (2005):

$$(2) \delta^{18}\text{O}_{\text{water VSMOW}} = 0.164(\pm 0.001) \times S - 5.38(\pm 0.02).$$

The salinity term used in equation 2 represents the average from May-October between 1998 and 2011, and ranges of 34.69 to 35.24 ($1\sigma = 0.16$).

4.3.2. Reconstruction of growth rates

A long-term SST reconstruction was developed based on $\delta^{18}\text{O}_{\text{shell}}$ taken from increments formed between 1966 and 2011. The isotope data was aligned to the SOMLIT SST curve by assigning the SST derived using the $\delta^{18}\text{O}_{\text{shell}}$ to the instrumental temperature curve, assuming average $\delta^{18}\text{O}_{\text{water}}$, so the best fit was obtained. It was then realigned based on the actual $\delta^{18}\text{O}_{\text{water}}$ calculated using Chauvaud et al. (2005) until the r values no longer increased. This was undertaken using sinusoidal temperature variation, a model developed by Judd et al. (2018).

4.3.3. Data Analysis

The average reconstructed SST was calculated for the growth intervals of all annual increments from 1966 to 2010 and linearly regressed against the North Atlantic Oscillation (NAO), the East Atlantic Pattern (EAP) and the Atlantic Subpolar Gyre (SPG) indices as reconstructed with Miami Isopycnic Coordinate Ocean Model (MICOM) by Hátún et al. (2005).

The NAO index is used to describe fluctuations in atmospheric pressure at sea level between the subpolar and subtropical regions (Hurrell et al. 2001). It is an indicator of weather patterns (wind, temperature, moisture, etc.) in the North Atlantic, especially the strength and direction of westerly winds and storm tracks during the winter months (Dawson et al. 2002). The NAO index used here is defined as the normalized pressure level difference between the Azores (high-pressure) and Iceland (low-pressure) (Hurrell et al. 2001). The EAP has a strong impact in Western Europe by influencing SST or modulating hydrological processes and mean precipitation rates (Tréguer et al. 2014). The SPG in the North Atlantic is formed by the North Atlantic Current in the south and the east and part of it propagates into the Norwegian Current in the north (Herbaut & Houssais 2009). The heat released from the

Norwegian Current into the atmosphere maintains a moderate climate in northern Europe (Bonan 2008). More details on how these climate events affect the Bay of Brest can be found in Table 4.1.

Table 4.1: Influences of the NAO and EAP on Western European climate in both their positive and negative phases.

	NAO	EAP
Positive Phase	High air temperature Lower precipitation Strengthening of the subpolar gyre – lower surface salinity	Above average SST Increased precipitation
Negative Phase	Lower air temperature Higher precipitation and more frequent storm events Weakening of the subpolar gyre – higher surface salinity	Below average SST Lower precipitation.

4.4. Results

4.4.1. Determination of the seasonal timing and rate of shell growth

Reconstructed temperatures were compared to the temperatures recorded by the

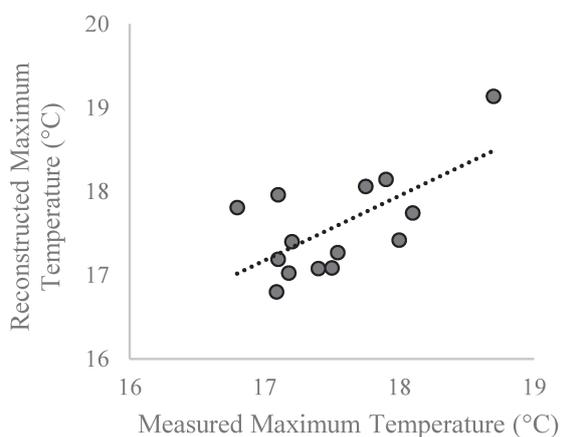


Fig 4.2: Crossplot of measured and reconstructed maximum annual temperature.

SOMLIT monitoring station between 1998 and 2010, highlighting that annual maxima are recorded in the shells with a good fidelity (Pearson Correlation: $p = 0.002$, $r = 0.760$ (Fig 4.2)), whereas annual minima are not.

The full range of reconstructed temperatures was 13.9°C to 19.1°C (Fig 4.4B). As with

Royer et al. (2013), it was found that the period of growth for *G. glycymeris* in the Bay

of Brest lasts from May to October (Fig 4.3), though it should be taken into account that a sampling distance of 350µm was left between the growth lines and the first and last sample in each increment.

From the model developed by Judd et al. (2018), more accurate dates were placed upon the start and end of the growth cycle for all years (1966-2010). The start of growth ranged from 10th May to 6th July, with most start dates in June, and growth finished between the 10th of August and the 28th of October.

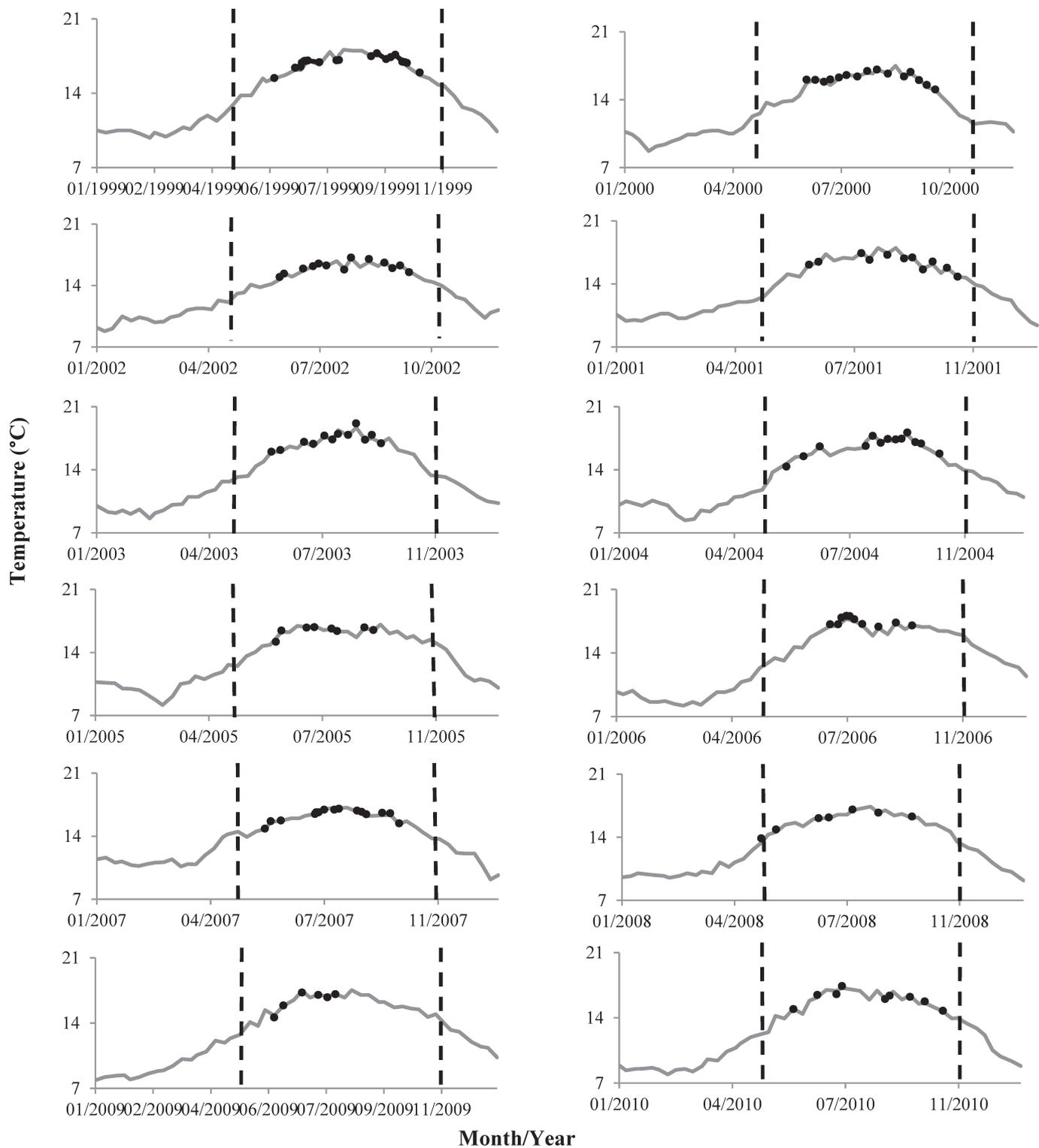


Fig 4.3: Time series of oxygen isotope-derived temperatures calculated from the paleotemperature equation (black dots) and measured temperatures in the Bay of Brest (grey line) separated by each sampling year (1999-2010). Vertical hashed lines represent the first week of May and the last week of October, respectively, the growing season determined by Royer et al. (2013).

The reconstructed temperatures were aligned annually using the sinusoidal curves generated by using the model created by Judd et al. (2018). This can be seen visually in Fig 4.4A. The modelled first and last day of growth per annum can be found in more detail in appendices 8 and 9.

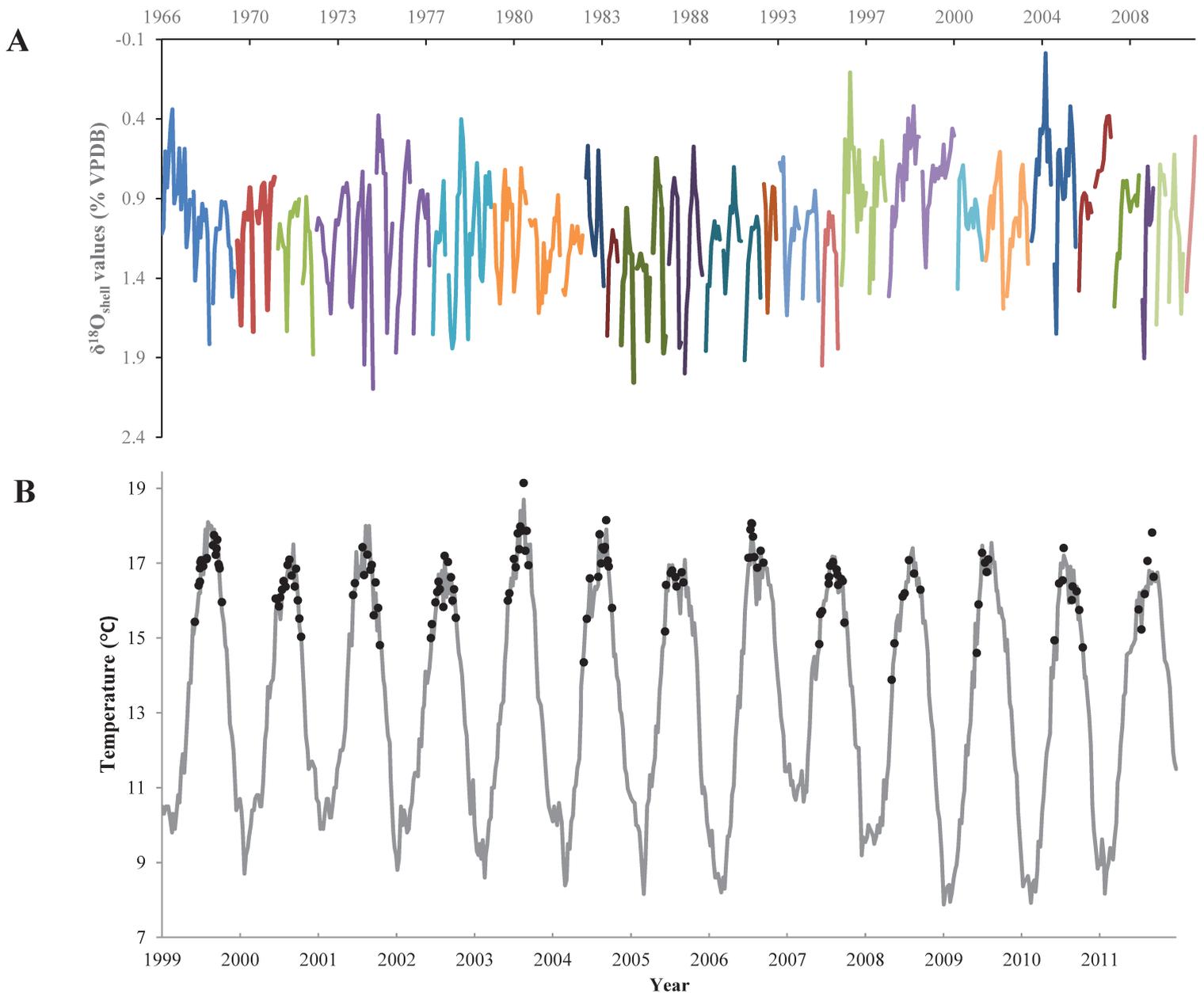


Fig 4.4: $\delta^{18}\text{O}_{\text{shell}}$ values and reconstructed temperature. (A) Inverted $\delta^{18}\text{O}_{\text{shell}}$ values taken from juvenile growth portions of 24 shells spanning 1966-2011. Each colour indicates a different shell. (B) Time-series of oxygen isotope-derived temperature from the paleotemperature equation (black dots) and measured temperatures in the Bay of Brest (grey line) from 1999-2011.

4.4.2. Expanding the temperature reconstruction

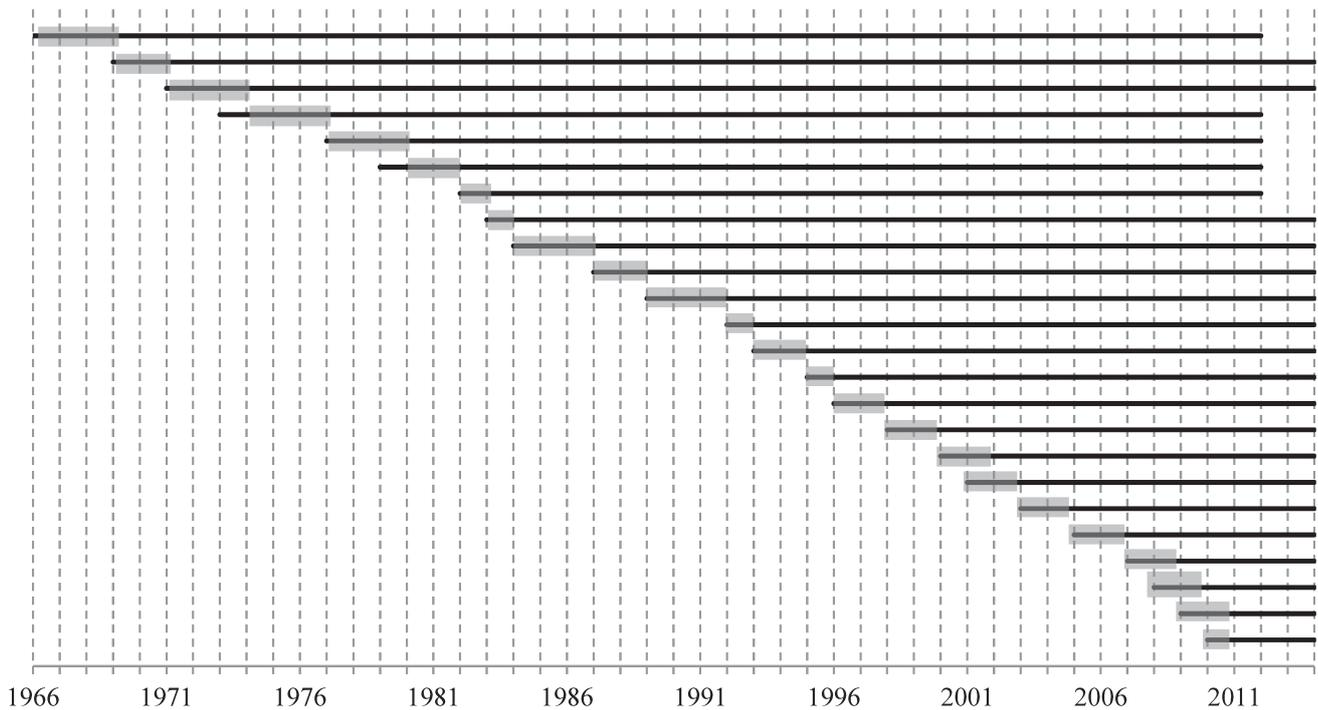


Fig 4.5: Lifespans of the 24 shells analysed for oxygen isotope composition. Grey sections indicate growth increments drilled for stable isotope analysis

A $SST_{(May-October)}$ reconstruction for the Bay of Brest was developed to cover the time interval 1966-1994. Equation 1 was applied to oxygen isotope ratios taken from the juvenile growth increments of 24 specimens with overlapping lifespans (Fig 4.5). Due to salinity varying by 2.39 between May and October, there was an uncertainty of $\pm 0.44^{\circ}C$ (calculated using equation 2). The reconstructed temperatures show that maximum seawater temperatures through the growth season greatly varied over time, with a “cool” phase until 1995 ($16.0 \pm 0.1^{\circ}C$) and a “warmer” phase afterwards ($16.5 \pm 0.2^{\circ}C$) (t-test; $p < 0.001$, $t = -4.267$). The maximum temperature reached before 1995 was $17.9^{\circ}C$ in 1966 whereas, after 1995, the maximum temperature was $19.1^{\circ}C$ (Fig 4.6A). This shift was also apparent for the reconstructed temperature that prevailed between May and October (henceforth referred to as reconstructed $SST_{May-Oct}$). The maximum $SST_{May-Oct}$ was $16.7^{\circ}C$ before 1995 and $17.5^{\circ}C$ afterwards (Fig 4.6B). The years that had the warmest days, according to the reconstructed

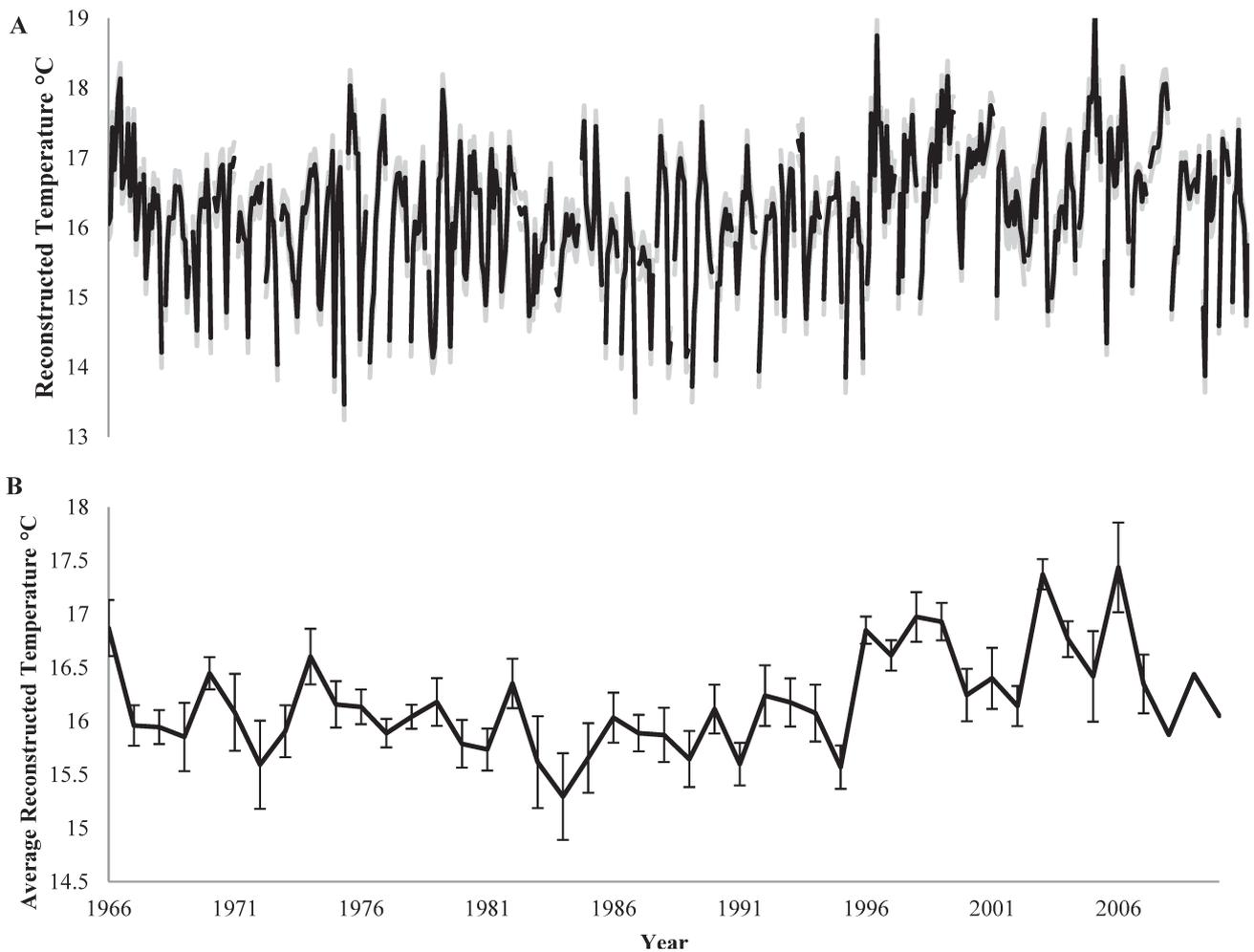


Fig 4.6: Oxygen isotope reconstructions A) Time series of oxygen isotope-derived temperature at the Bay of Brest from 1966 to 2010 (black line) during the growing season of May-October. Grey shading represents the uncertainty related to the use of an average $\delta^{18}\text{O}_{\text{water}}$ value; B) Average oxygen isotope-derived temperatures during the annual growing season. Error bars represent error present in the equations (1 & 2) as well as machine error.

SST_{May-Oct} were 1996 and 2003, while the years with the coldest days during the growth period were 1973 and 1984 (Fig 4.6A). This is reflected in the reconstructed SST_{May-Oct} when averaged which show that the overall warmest summers were in 2003 and 2006, while the coldest summer was in 1984 (Fig 4.6B). As there was no temperature recorded below 13.2°C (Fig 4.6A), we can infer that this is the lowest temperature at which shell growth can occur in *G. glycymeris* in the Bay of Brest. Over the period of 1966–2010 the reconstructed temperatures

between May and October (Fig. 4.6B) show a significant linear warming trend of $0.07\text{ }^{\circ}\text{C}/\text{decade}$ (95% CI).

4.4.3. Relationship with climate indices

$\text{SST}_{\text{May-Oct}}$ were compared to the SPG. A significant negative correlation was found between the reconstructed $\text{SST}_{\text{May-Oct}}$ and the SPG index ($r = -0.500$, $p = 0.001$). As the subpolar gyre circulation increases in 1981, temperature decreases and the opposite occurred in 1995 (Fig 4.7A). Overall the two plots trend in opposite directions, with a change in 1995. Before then, the SPG has a weak increasing trend (while the reconstructed temperature is slightly decreasing). After 1996, the SPG weakens rapidly and temperatures in the Bay of Brest increase.

A positive correlation was found between the $\text{SST}_{\text{May-Oct}}$ and the EAP index when lagged by one year ($r = 0.460$, $p = 0.002$; Fig 4.7B). This means that the EAP strength in 2000, for example, would have an effect on temperature in the Bay of Brest in 2001. No correlation was found between reconstructed temperature and the NAO index ($r = -0.261$, $p = 0.114$; Fig 4.7C).

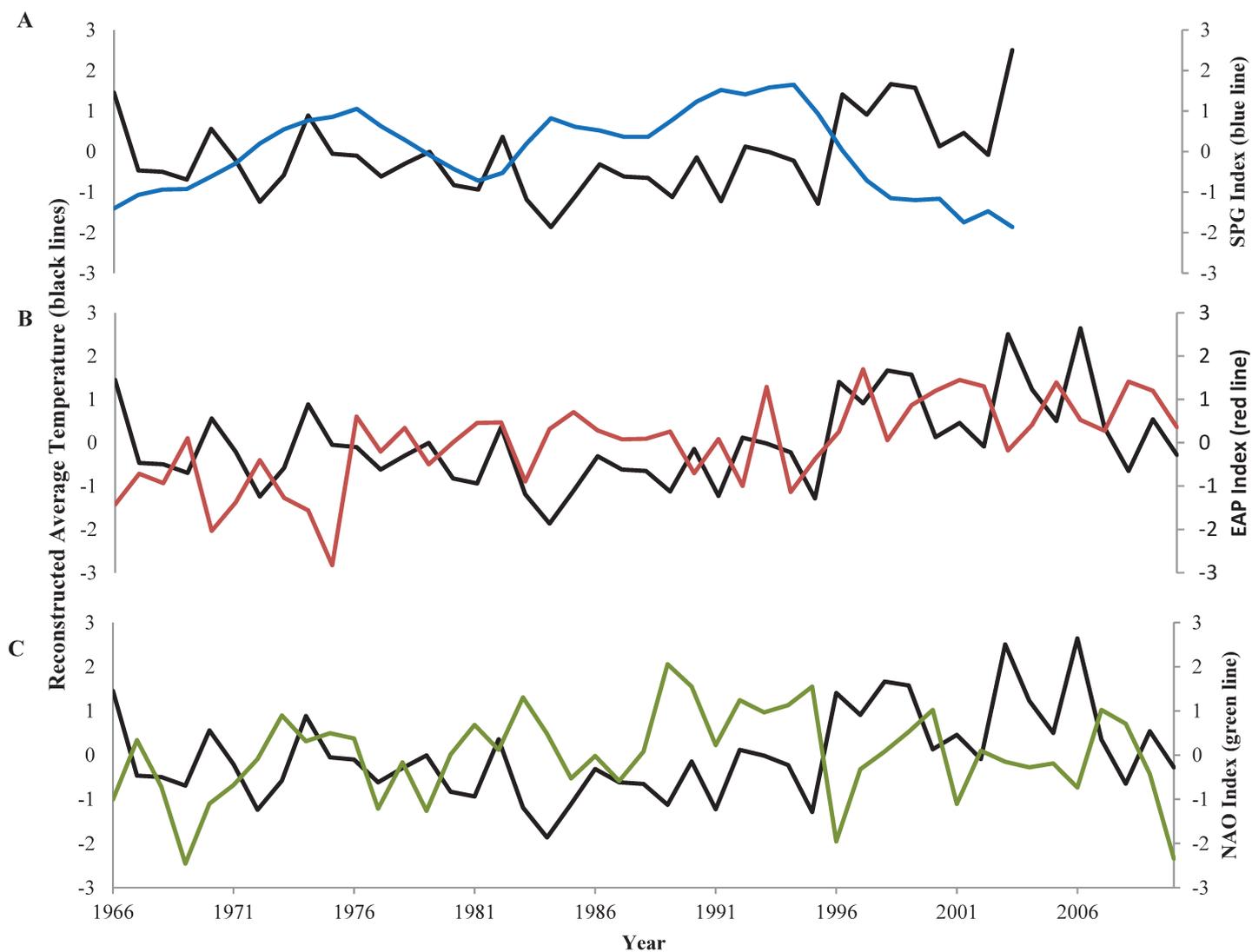


Fig 4.7: Comparison of the standardised indices of annual average reconstructed temperature and climate systems

4.5. Discussion

The data presented in this study demonstrate that $\delta^{18}\text{O}$ in the shell of *G. glycymeris* is a good proxy for SST variability and reliable paleoclimate reconstructions can be carried out using this archive. By comparing and relating *G. glycymeris* temperature reconstructions with similarly sensitive climate indices, it is possible to reconstruct palaeoceanographic and atmospheric variability over the late Holocene or even as far back as the early Oligocene where *Glycymeris* species have been found in the fossil record (Walliser et al. 2015). These temperature reconstructions could be invaluable for the calibration of predictive numerical climate models (Mann 2002).

Our study showed a negative correlation between the SPG index and the reconstructed SST_{May-Oct} in the Bay of Brest (Fig 4.7A), with an increase in strength between the late 1960s and early 1990s, before a sharp decline in 1995 to the early 2000s. Other studies (for example Sgubin et al. 2017) demonstrate little change in the overall strength of the SPG since the mid-1960s and others rapid warming (weakening) since the mid-1990s (Corbière et al. 2007; Metzl et al. 2010; Yang et al. 2016). Hermanson et al. (2014) attributed this to the weakening of the AMOC, leading to the amount of heat transported into the SPG being reduced and its strength depleted. As the AMOC is expected to decrease due to climate change (Rahmstorf et al. 2015), climate modellers (e.g Sgubin et al. 2017) have stressed the need to consider the potential risks associated with an SPG reduction when developing future strategies of adaptation to climate change as well as when searching for possible early warning signals (Lenton et al. 2012).

Previous studies suggest that the recent strengthening SPG has increased the likelihood of several climate impacts including more frequent hurricanes (Msadek et al. 2014) as well as a large number of wet and warm summers over much of Europe (MacLeod et al. 2016).

Hermanson et al. (2014) predicted a cooling of the SPG after this warm spike in 2003 (as is shown in our reconstructed temperatures (Fig 4.6B)) but not a complete reversal to previous cold conditions. Due to this, they expect the climate impacts previously mentioned that relate to a strong SPG to persist but become less frequent. Although there are only 7 years present in our reconstruction after 2003, in Fig 4.6B, we can see temperatures fluctuate and peak again in 2006 before somewhat settling for another six years. Between 2006 and 2010 has a higher average temperature than before the warm spike in 2003, just as Hermanson et al. (2014) predicted.

The year 2003 (the warmest summer (Fig 4.4B)) is interesting from a meteorological perspective. Reichstein et al. (2007) conclude that the perceived heatwave was rather a drought spell for most of the biosphere, although our data shows that the temperature was also abnormally high in North West France. Schär et al. (2004) suggest, from a modelling point of view, that the 2003 heatwave can be regarded as a proxy of future climate that will be warmer and with more extremes.

Tréguer et al. (2014) attributed the changes of $SST_{(winter)}$ in the Bay of Brest to fluctuations of the NAO. The paper by Tréguer et al. (2014) used data collected by SOMLIT-Brest and compared temperature anomalies to the NAO index from the period 1998-2015. The dissimilarity in results between our study and Tréguer et al. (2014) is most likely due to the difference in the time intervals of the study. *G. glycymeris* grow only between May and October, completely missing the winter months studied by Tréguer et al. (2014). Any potential relationship found between reconstructed temperatures and the NAO would have been lagged by a year, which is not the case (Fig 4.7C).

Tréguer et al. (2014) admitted in their study that the time studied was not enough to provide a complete picture of the climate processes influencing the Bay of Brest, as such we

put forward another theory. After 1997, the NAO and the SPG started acting, overall, in phase with each other, with important turning points in 1996 and 1994 respectively, before the Tréguer et al. (2014) study period. Lohmann et al. (2009a) states that the asymmetric SPG-response to the sign of persistent NAO-like forcing as well as the different time scales involved demonstrates a strong non-linearity in the North Atlantic Ocean circulation response to atmospheric forcing. Under persistent NAO⁺ forcing the initial strengthening of the SPG is, after about 10 years, replaced by a weakening of the gyre (Lohmann et al. 2009a), potentially leading to an increase in the salinity of the Bay of Brest. This change in the gyre is caused by a strong AMOC response to the formation of intermediate and deep waters in the SPG region under NAO⁺ forcing (Lohmann et al. 2009b).

Precipitation is thought to be the driving factor of sea surface salinity in areas influenced by the SPG (Grist et al. 2015). As such salinity in the Bay of Brest will be expected to change through these aforementioned NAO phases. In this study, salinity was only recorded from 1998 to present, which means that a change in the NAO⁺ forcing may not have been completely observed. Also, an average salinity from 1998-2011 was used to reconstruct temperatures further back in time, using Equation 1. This means that, although the recreated temperatures found from 1998-2011 matched the recorded temperature, this may not be the case for further in the past. SST from the Bay of Brest is not available from 1966-1997 so we cannot be completely certain of the reconstruction, however the reconstructed temperatures follow the same climate signals both before and after this time (Fig. 4.7A & 4.7B) allowing us to extrapolate that salinity in the Bay of Brest did not change exponentially through the reconstructed time-frame. This may be due to the enclosed nature of the study area.

The EAP index has also been shown to correlate with the reconstructed SST_{May-Oct} of the Bay of Brest. The EAP describes climate patterns at lower latitude than the NAO and SPG and contains a strong subtropical link (Santana-Casiano et al. 2007). The positive phase of the

EAP is usually associated with high SST throughout Europe in all months of the year and was suggested by Cannaby & Hüsrevoğlu (2009) as the cause of a recent warm anomaly in Irish waters. As the EAP appears in this study as a driving factor of SST, we suggest that the effect of the EAP on ocean–atmosphere heat and freshwater fluxes and current structures requires further investigation. Cannaby & Hüsrevoğlu (2009) state that within the central North Atlantic basin, including the SPG and Subpolar Front, the Atlantic Multidecadal Oscillation and EAP patterns are out of phase. Due to this, the current warming of the SPG associated with the current positive phase of the Atlantic Multidecadal Oscillation is inhibited by the concurrent positive phase EAP.

4.5.1. Conclusions

Temperatures in the Bay of Brest, between May and October, can be reconstructed using the $\delta^{18}\text{O}$ values of *G. glycymeris* shells, when the equation provided by Royer et al. (2013) is used. This shows that there is potential to constructing a long-term temperature chronology for this area by determining $\delta^{18}\text{O}$ of longer-lived, or fossil shells.

The climate systems exerting the greatest influence on SST of the Bay of Brest are the East Atlantic Pattern (EAP) and the North Atlantic subpolar gyre (SPG). The SPG has been described as controlling air temperature in Northern Europe and the EAP as controlling water temperature in Southern Europe, leading to the conclusion that the Bay of Brest is an interaction area between these two climate systems. As such, this locality is interesting as the temperatures recorded by the shells can be used a proxy for both the SPG and EAP, and temperature reconstructions can provide a unique insight in how these climate systems interact prior to observations. However, this link between the SPG and EAP will make interpretation difficult as it must be known which pattern was more dominant at the time in question.

**Chapter 5: Sr/Ca and Mg/Ca ratios as
paleotemperature proxies using *Glycymeris
glycymeris* in the Bay of Brest, North-West
France**

5.1. Abstract

The elemental composition of bivalve shells is regarded by the international scientific community as a promising tool to record environment parameters such as sea surface temperature, salinity and primary productivity. However, some studies have shown that the variation of trace elements within shells may be species dependant and/or change with conspecifics in different locations due to changes in complex networks of environmental interactions. Before now, the elemental composition of *G. glycymeris* was unstudied.

Five specimens were chosen for analysis; three shells aged less than 10 years and two shells aged 45 years. Data from a ThermoElement2 HR-ICP-MS coupled with a 193-nm laser ablation unit (CompexPro 102 Coherent) (LA-ICP-MS) were calibrated using NIST 612, NIST 614 and BIR-1G. All five shells were analysed with ~120 µm diameter transects, through the axis of growth, the laser firing directly onto the shell as a movement speed of 5 µm per second. Analysis was made in the prismatic layer, from the ventral margin to the umbo.

As this was the first time the trace element composition of *G. glycymeris* species was studied, a wide arrange of elements were first analysed to test which would be viable for study. This was later narrowed to ²⁵Mg and ⁸⁶Sr. Magnesium (Mg) and strontium (Sr) showed the same signal in all samples ($p < 0.0001$, $r = 0.527$), implying that their incorporation into the shell is being driven by the same environmental or biological factor. One shell was removed from further analysis with regards to Mg/Ca ratios as it was found to be statistically different from other samples. When the Sr/Ca and Mg/Ca ratios are averaged over the years 2007-2012, they correlated negatively with temperature (Sr/Ca: $p = 0.0130$, $r = -0.605$; Mg/Ca: $p = 0.0176$, $r = -0.584$), and chlorophyll α (Sr/Ca: $p = 0.0013$, $r = -0.732$; Mg/Ca: $p = 0.0267$, $r = -0.551$) and positively with phosphate present in the water column (Sr/Ca: $p =$

0.026, $r = 0.553$; Mg/Ca: $p = 0.0384$, $r = 0.52126$). When the overall laser transects were averaged and compared with weekly temperature between May and October (2007-2012) for both Sr/Ca ($p = 0.0732$, $r = 0.281$) and Mg/Ca ($p < 0.0001$, $r = 0.661$), there was a positive correlation. As there were only two shells spanning the time period from 1998-2007, the correlations from the annual average should be taken with caution.

5.2. Introduction

Geochemical analysis of biogenic minerals has become one of the most powerful tools available for paleoecological reconstructions, especially with regards to sclerochemistry (Warter & Müller, 2017). An increasing number of approaches and organisms are being utilized for the estimation of such variables as marine paleotemperatures, pH, productivity, and salinity. Biogenic carbonates with recognizable growth banding, such as, coral skeletons (Lewis et al. 2007; Montagna et al. 2007), fish otoliths (Ruttenberg et al. 2005; Schaffler & Winkelman, 2007) and mollusc shells (Carré et al. 2006; Thébault et al. 2013) are of special interest for paleoecologically researchers as these bands can present a record of environmental conditions (Schöne et al. 2005). Due to these growth bands, spatially-resolved geochemical analysis of secreted carbonate via: laser-ablation inductively-coupled-plasma mass spectrometry (LA-ICPMS), secondary ion mass spectrometry ((nano)SIMS) or electron micro probe analyser (EMPA), enables retrieval of the trace elemental and isotopic inventory in chronological order, resulting in time-series records (Warter & Müller, 2017).

As LA-ICP MS has a potential for rapid and accurate high-resolution trace element analysis at relatively low costs, as well as minimal sample preparation requirements, it has become a routine analytical tool in a wide area of research applications (Kamber 2009). Except from the common analysis by independent adjacent spots, compositional trace elemental variability can be measured using two other methodologies: lateral profiling, i.e. laser ablation along a defined transect on the sample's surface (as is the case in this study) and depth profiling, which involves static removal of layers of material at low laser repetition rates (1–2 Hz) to establish a depth-composition relationship (Warter & Müller, 2017). It has been shown that it is possible to resolve daily compositional variability in planktonic foraminifera (Sadekov et al. 2009) using LA-ICPMS depth profiling. This indicates that LA-

ICPMS compares well with other high-resolution methods, such as (nano)SIMS (Warter & Müller, 2017).

The use of Mg/Ca and Sr/Ca ratios in biogenic carbonates as geochemical proxies has grown rapidly in recent times and complements the more traditional $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$ proxies (Freitas et al. 2005). Mg/Ca and Sr/Ca ratios in both calcite and aragonite deposits, have been used as proxies of seawater temperature in many studies (e.g. Elderfield & Ganssen 2000; Marshall & McCulloch 2002; Wanamaker et al. 2008). As these studies have gained ground in the literature, there has been active research into the evaluation of additional factors (both biological and environmental) that may influence non-equilibrium incorporation of magnesium (Mg) and strontium (Sr) into biogenic carbonates (Mavromatis et al. 2013). These series of works have been undertaken on corals (Wei et al. 2000; Reynaud et al. 2007), foraminifera (Russell et al. 2004; Cleroux et al. 2008), brachiopods (Brand et al. 2003; Pérez-Huerta et al. 2008), ostracods (Wansard et al. 1998; Jin et al. 2006), echinoids (Dickson 2004; Ries 2004), belemnites (McArthur et al. 2007; Bodin et al. 2009), bivalves (Freitas et al. 2006; Freitas et al. 2009), gastropods (Ingram et al. 1998), and otoliths (Arai et al. 1996; Martin & Thorrold 2005). One very important outcome of all these studies is that they have highlighted species specific differences in how elements are incorporated into calcium carbonate deposits.

Shell Mg/Ca and Sr/Ca ratios vary significantly between different bivalve species and even among conspecific and contemporaneous specimens from one locality (Schöne et al. 2010). Dodd (1965) reported a large negative correlation between temperature and Sr/Ca ratios in *Mytilus edulis* and so did Surge & Walker (2006) in *Mercenaria campechiensis*. Gillikin et al. (2005c) observed the opposite in *Saxidomus gigantea* and Freitas et al. (2006) found an inverse correlation during autumn to early spring (October to March–April) and a positive correlation from late spring through summer (May–June to September) in *Pecten*

maximus. Lorrain et al. (2005) and Schöne et al. (2011) are two studies of many that have also observed a correlation of the Mg and Sr content and the growth rate or ontogenetic age of bivalves.

The present study is the first to measure skeletal Mg/Ca and Sr/Ca ratios in *G. glycymeris* and attempts add *G. glycymeris* into the literature surrounding Mg and Sr and their relationship to temperature.

5.3. Methods

For analysis, three shells aged less than 10 years and two shells aged 45 years were chosen. The younger shells were intended to provide calibration and indicate potential correlations by comparing their element signal intensities with environmental data collected by the SOMLIT monitoring station. The older shells would have expanded upon any relationships found (see chapter 2 for more details on chronology building and shell aging).

Data from a ThermoElement2 HR-ICP-MS coupled with a 193-nm laser ablation unit (CompexPro 102 Coherent) (LA-ICP-MS) were calibrated using NIST 612, NIST 614 and BIR-1G using the preferred values defined by GeoReM (<http://georem.mpch-mainz.gwdg.de/>). The laser was shot (~120 µm spots) directly into the standards to allow alignment of isotopic profiles. This was done with each standard three times before and after each session with the laser to account for drifting during the day, as well as once with each standard between transects. All five shells were analysed with ~120 µm diameter continuous transects, through the axis of growth, the laser firing directly onto the shell at a movement speed of 5 µm per second. Analysis was made in the prismatic layer, from the ventral margin to the umbo. During acquisition, signal intensities were recorded for ^{43}Ca , ^{98}Mo , ^7Li , ^{11}B , ^{25}Mg , ^{55}Mn , ^{86}Sr , ^{208}Pb , ^{238}U , ^{23}Na , ^{109}Ag , ^{67}Zn , ^{68}Zn , ^{75}As , ^{31}P , and ^{138}Ba . ^{43}Ca was used as

an internal standard to correct for laser beam energy drift, focus variation at the sample surface, and ICP-MS drift. For each transect, the mean signal intensity of the argon carrier gas was considered as background. Thus, all data points were corrected before further treatment. ^{31}P had to be instantly omitted from further analysis as the standards did not provide sufficient data for correction.

The isotope/Ca ratio of the shell underwent manipulation in the form of omitting obvious anomalous peaks. These anomalies were defined at points which were 10x greater than the data points surrounding them (e.g. three points before and after). ^7Li , ^{109}Ag , ^{67}Zn , ^{68}Zn , and ^{75}As were removed from further analysis after this stage as no obvious patterns were found in the data (Fig 5.1). This could be due to low intake levels by *G. glycymeris* or the detection limits of the equipment used. A large amount of ^{98}Mn was found in the ventral margin of the older shells, leading to the high averages in (Fig 5.2) however levels became too low to detect as the laser ventured towards the hinge (Appendix 10). Due to this it was removed from further study. ^{208}Pb , ^{138}Ba , and ^{238}U also showed great difference between individual samples (Fig 5.2), and, due to this variation, it is possible that incorporation of these two elements may be controlled by other drivers than environment (such as ^{208}Pb in *M. edulis* (Vander Putten et al. 2000)). It is beyond the scope of this study to investigate such things. As there is already a lot of literature surrounding ^{25}Mg and ^{86}Sr incorporation, these elements were chosen to be studied in more detail.

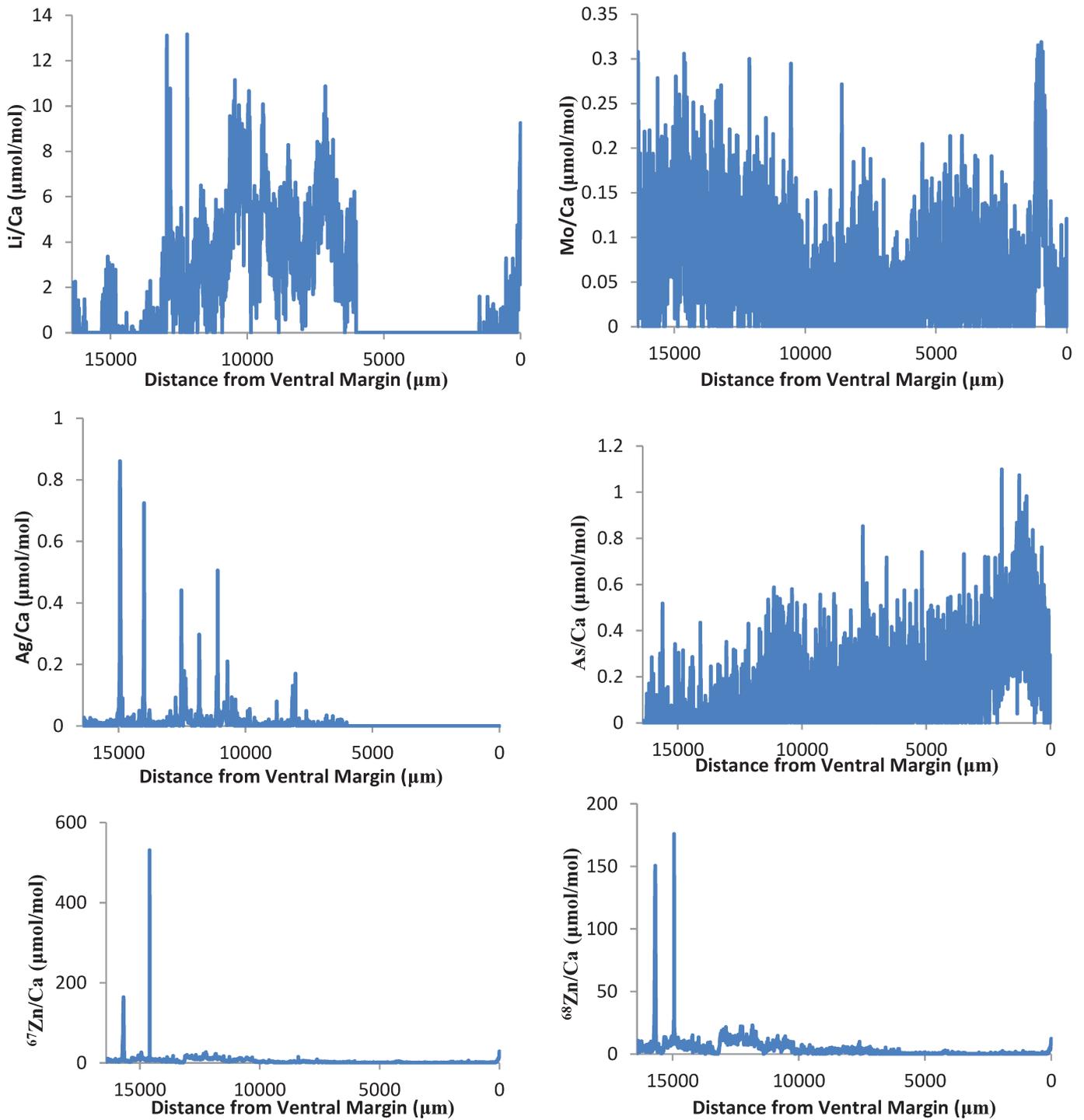


Fig 5.1: Examples of omitted measured trace elements. Measurements obtained from sample CoA071. Direction of growth is from left to right.

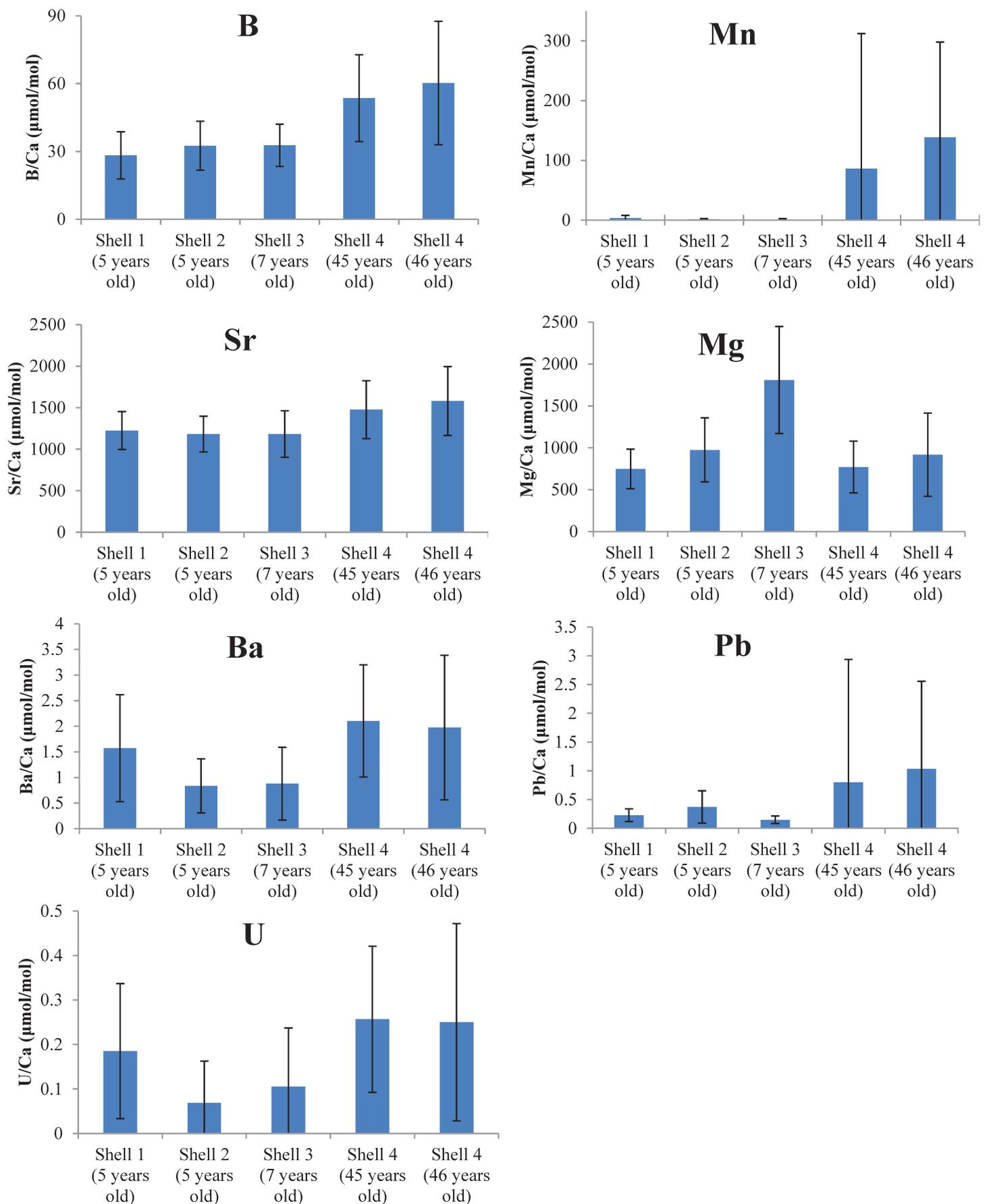


Fig 5.2: Comparisons of average signal intensity of different elements within each studied shell. The error bars represent standard deviation, cut at a minimum of 0.

5.3.1 Statistical Methods

A linear regression (Pearson coefficient) was undertaken on all collected strontium and magnesium ratios to see if parallels can be made between their shell incorporation. Because both Sr/Ca and Mg/Ca ratios function as paleotemperature proxies in many organisms collected data from the shells was compared to instrumental sea surface temperature obtained from SOMLIT monitoring station between 2007 and 2012. The ratios were also compared to phosphate levels, salinity, chlorophyll α counts, nitrate levels, and dissolved oxygen, all from SOMLIT, as well as river flow rates of both the Elorn and the Aulne, provided by Hydro Bank.

Mg/Ca and Sr/Ca ratios, averaged between all shells, were also compared with temperature gathered by SOMLIT between May and October of years 2007-2012. This was to determine incorporation levels over the year, and establish any relationships using a Pearson coefficient. In order to be confident that this average was representative of the samples, a Kruskal-Wallis test was undertaken to look for significant differences in Mg/Ca ratios in all specimens per year. If any were identified, a Mann-Whitney pairwise was used to identify exactly which shells differed from the others.

5.4. Results

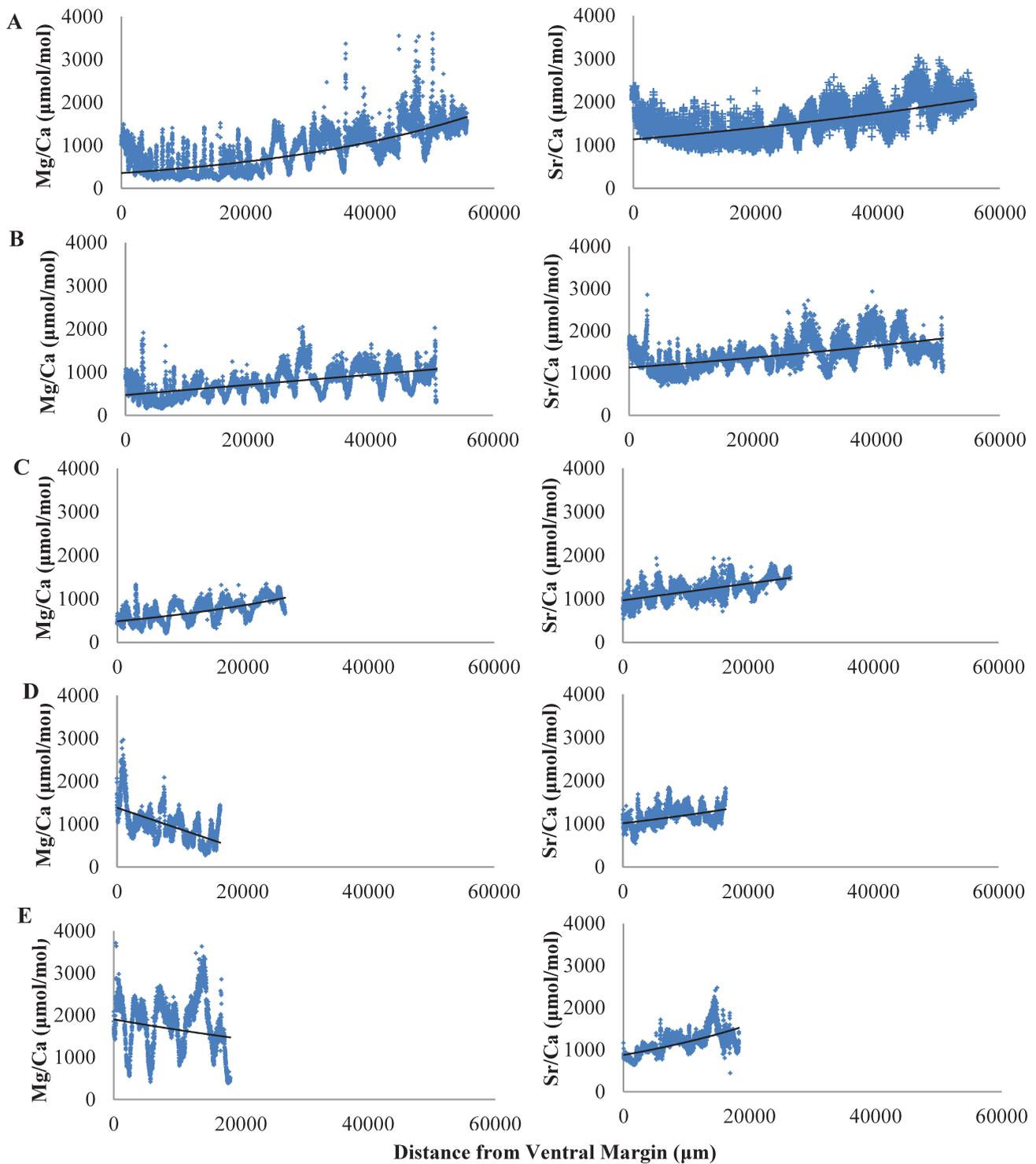


Fig 5.3: Mg/Ca and Sr/Ca ratios in shells of *G. glycymeris* from the Bay of Brest. Each letter (A-E) indicates a different analysed shell. Shells A and B are the oldest (Specimens A14122011 and 14101011), C-E are juveniles (14101029, CoA071 and CoA163). Lines represent an exponential trend curve. Growth is from left to right.

5.4.1. Shell Mg/Ca ratio profiles

Shell Mg/Ca ratios range from 156.93 to 3709.45 $\mu\text{mol/mol}$ (Fig. 5.3) across all analysed shells. Interannually, Mg/Ca ratios follow a common pattern, with lower values in the Autumn/Winter months and peaks in the summer. Overall, shells A14122011, A14101011 and 14101029 showed a similar trend in that they contained more Mg as the shells got older (increase in the exponential curve (Fig 5.3; A, B & C)). Shells CoA071 and CoA163 showed a negative trend, containing less Mg as they got older (Fig 5.3; D & E). There is a statistical difference in the annual variation of samples from the years 2007 to 2012 (Kruskal-Wallis: $p=0.008$, $H(\chi^2) = 13.74$). Further investigation with a Mann-Whitney pairwise identified CoA017 as significantly different from most of the other samples (Table 5.1). As such, this shell was removed from further Mg/Ca ratio analysis.

Table 5.1: Mann-Whitney p values of annual averages of Mg/Ca levels between 2007 and 2012. Blue fields identify statistical significance

	14101011	14101029	10122011	CoA017	CoA163
14101011		0.095	0.2101	0.012	0.095
14101029	0.095		1	0.012	0.144
14122011	0.210	1		0.022	0.531
CoA017	0.012	0.012	0.022		0.060
CoA163	0.095	0.143	0.531	0.060	

5.4.2. Shell Sr/Ca ratio profiles

Shell Sr/Ca ratios range from 443.34 to 3024.35 $\mu\text{mol/mol}$ (Fig. 5.3) across all analysed shells. Interannually, Sr/Ca ratios follow a common pattern, with lower values in the Autumn/Winter months and peaks in the summer. Overall, all shells showed a similar trend in that they contained more Sr as the shells aged, there is an increase in the exponential curve in Fig 5.3. There is no a statistical difference between the average annual Sr/Ca levels in samples between 2007 and 2012 (the years used for further analysis) (Kruskal-Wallis: $p=0.056$, $H(\text{chi}^2) = 9.696$).

A significant positive correlation between strontium and magnesium levels in shells was highlighted during the linear regression ($p < 0.0001$, $r = 0.527$).

5.4.4. Environmental Drivers

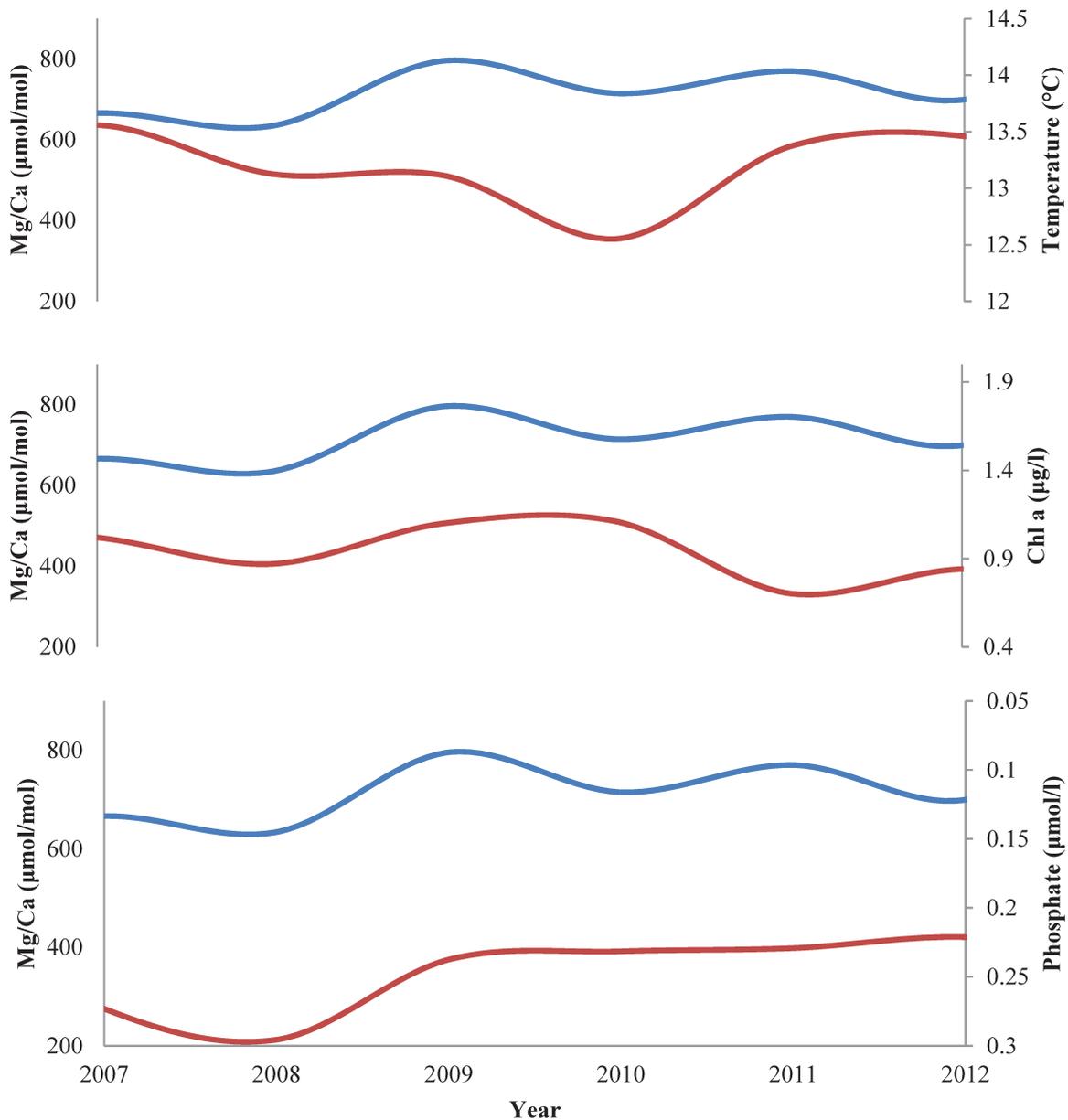


Fig 5.4: Comparison of Mg/Ca ratios (blue line) with significantly correlated environmental data (red line). Significant values were found with inverted temperature (A), inverted Chl a measurements (B) and phosphate amount (C), all measured by SOMLIT.

Yearly averages of magnesium in 4 shells from 2007 - 2012 negatively correlate with temperature ($p = 0.0176$, $r = -0.584$), levels of chlorophyll a ($p = 0.0267$, $r = -0.551$) and phosphate ($p = 0.0384$, $r = -0.52126$) (Fig 5.4). There were no correlations found with the other measured parameters.

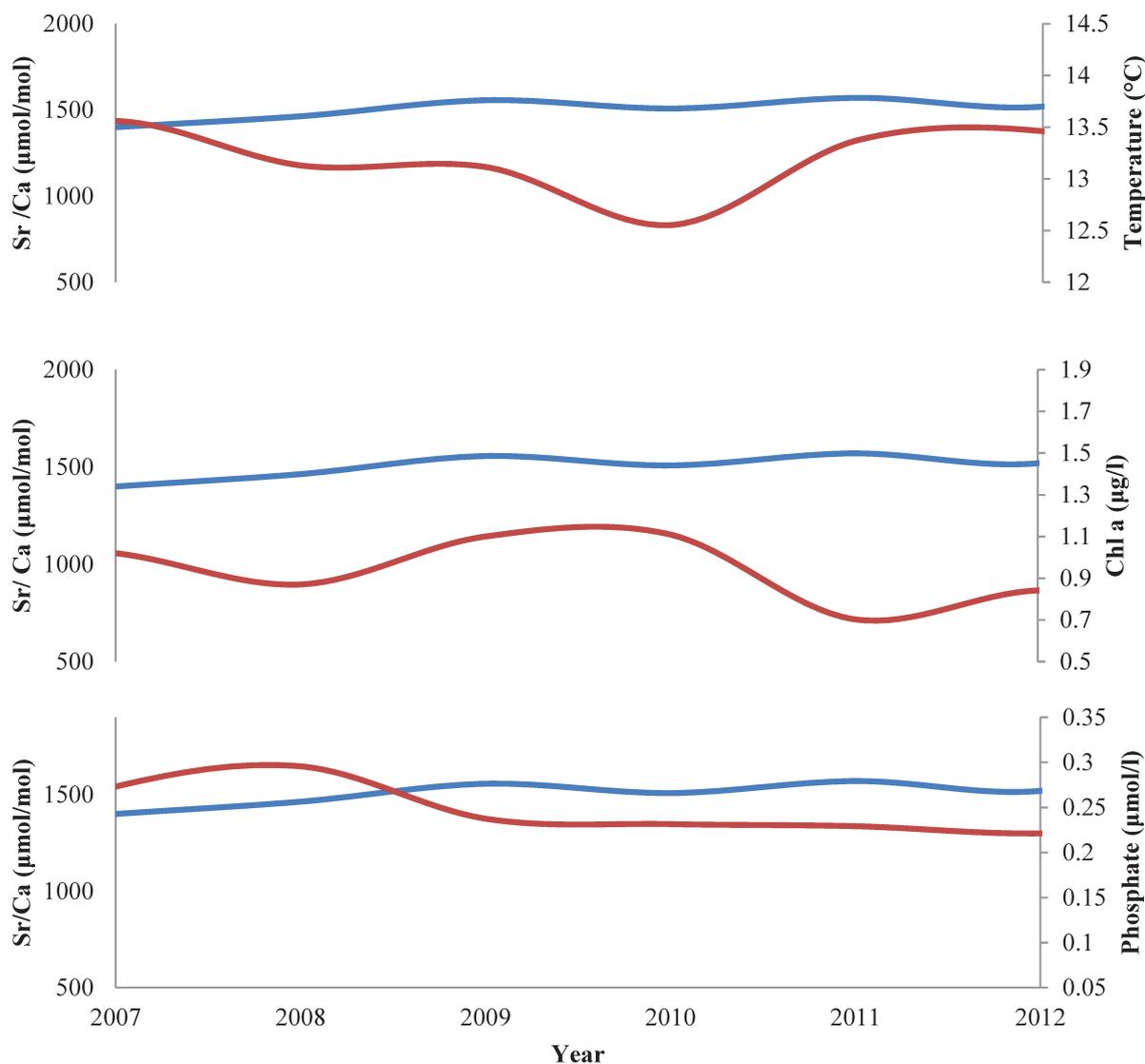


Fig 5.5: Comparison Sr/Ca ratios (blue line) with significantly correlated environmental data (red line). Significant values were found with inverted temperature (A), inverted Chl a measurements (B) and positive with phosphate amount (C), all measured by SOMLIT.

Yearly averages of strontium in all shells from 2007 – 2012 also negatively correlate with temperature ($p = 0.0130$, $r = -0.605$), levels of chlorophyll a ($p = 0.0013$, $r = -0.732$) and positively correlate with levels phosphate in the water ($p = 0.026$, $r = 0.553$) (Fig 5.5). No other correlations could be made with the other measured parameters. Seasonally, temperature correlates positively with both Sr/Ca ($p = 0.0732$, $r = 0.81$) and Mg/Ca ($p <$

0.0001, $r = 0.661$) in all analysed shells (Fig 5.6). This is the opposite for what has been found on an annual scale.

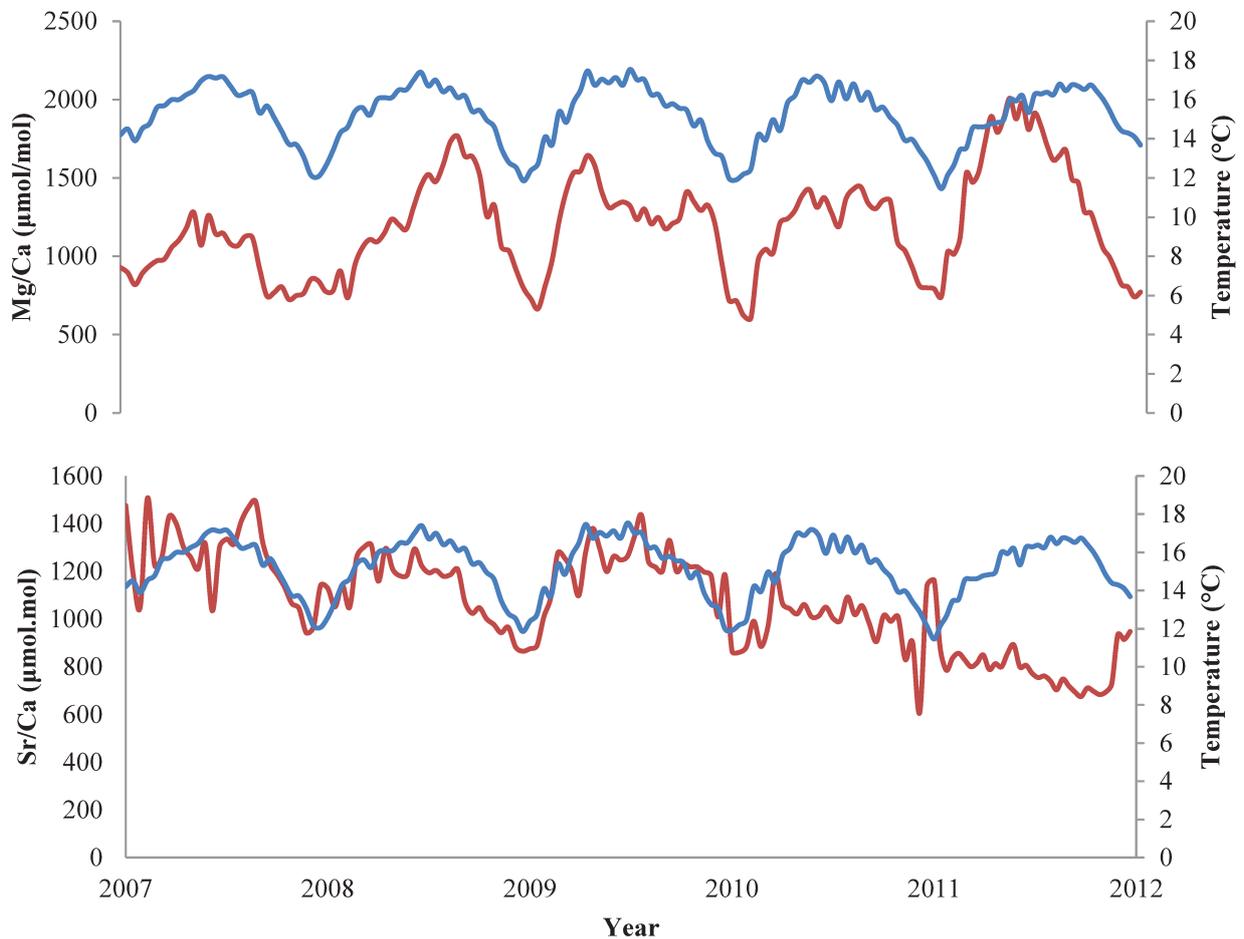


Fig 5.6: Comparison of temperature from May to October (blue line) with element ratios (red line). A) Shows magnesium and B) shows strontium, both averaged over all shells

5.5. Discussion

In this study we examined the Mg/Ca and Sr/Ca ratios found in the prismatic layer of *G. glycymeris* collected in the Bay of Brest, France. Three juvenile and two aged shells were successfully analysed for their trace element content. The annual ratio of Mg/Ca and Sr/Ca correlated positively with phosphate and was shown to have an inverse relationship with temperature and chlorophyll α (Fig 5.4 & 5.5 respectively). Due to these results, we can

assume that as temperature in the Bay of Brest decreases annually, the amount of chlorophyll α also decreases and phosphate levels increase. When this happens, *G. glycymeris* incorporate more magnesium and strontium into their shell. Seasonally, both Sr/Ca and Mg/Ca have a positive relationship with sea surface temperature. This is the first time these elements have been studied in this species.

The results of this study clearly indicate that the local environment (temperature, chlorophyll α , and phosphate availability) exert a major control over the Sr/Ca and Mg/Ca ratios of *G. glycymeris* shells on an annual scale. The literature surrounding the subject states that temperature is the predominant driving factor in this case and that chlorophyll α and phosphates are also correlating with temperature. It is well known that when temperature increases, there is increased energy for photosynthesis, leading to greater amounts of chlorophyll α in the water column (Stal & Walsby 2000; Ting et al. 2002; Fu et al. 2007). Fu et al. (2007) found that when temperature increases, cyanobacteria absorb more phosphates, reducing the amount in the marine environment.

There has previously been a relationship found with phosphates and magnesium/strontium through the phosphoproteins (Marsh & Sass 1985), which bind materials within the shell of heterodont bivalves (Marsh 1990). In their native state, phosphoprotein particles contain large amounts of calcium, magnesium, and inorganic phosphate ions (Marsh & Sass 1985) as well as trace amounts of transition elements, which bind calcium, forming the shell. It has even been theorised that magnesium represents part of the substrate for the phosphorylation reaction (Kolassa et al. 1979). As stated before, *G. glycymeris* is a primitive taxodont bivalve and therefore does not contain phosphoproteins to aid in the binding of the shell structure. As far as we are aware it is unknown what process binds the shell of *G. glycymeris* together, although it is most likely to be chitinase (Steinhardt et al. 2016), and more research is needed.

Other studies have shown that $\text{Sr}/\text{Ca}_{\text{shell}}$ is directly related to $\text{Sr}/\text{Ca}_{\text{water}}$ in marine bivalves, (Lorens & Bender 1980) as well as freshwater bivalves (Faure et al. 1967; Bailey & Lear 2006) and gastropods (Buchardt & Fritz 1978). If specific pumps were present and Sr^{2+} was actively transported to the EPF, one would expect a positive relationship between metabolic rate (growth rate) and $\text{Sr}/\text{Ca}_{\text{shell}}$ (Mucci & Morse 1983) such as seen in Fig5.6 (there is more strontium present within the shell, the closer it is to the juvenile years where growth is at its highest). It is assumed that the great majority of Sr^{2+} comes from the ambient water and can directly pass through ion channels of the mantle epithelia to reach the EPF (Stewart, 1984) or is first absorbed by the gills (Simkiss 1981) where it then enters the haemolymph and finally reaches the EPF through ion channels of the inner mantle epithelium (Bayne et al. 1979; Simkiss and Taylor 1995). As there is a relationship between growth rate and Sr/Ca content, it can be assumed that biological processes are dominant in the regulation of strontium in *G. glycymeris* shells.

In agreement with this the majority of previous studies on bivalves have concluded that the Sr/Ca and Mg/Ca ratios do not reflect water temperatures, but merely growth rates or other physiological parameters (e.g. Purton et al. 1999; Lorrain et al. 2005; Carré et al. 2006). Some authors have assumed that there is an indirect correlation between shell Sr/Ca and Mg/Ca values and calcification temperatures because metabolism is influenced by the ambient environment (e.g., Gillikin et al. 2005c; Freitas et al. 2006). However, no previous approach has tested whether environmental variables can be reconstructed from trace elemental and calcium ratios if the vital effects are mathematically eliminated (Schöne et al. 2011).

In order for elements to be incorporated into a shell, they must first reach the site of calcification. To do this, they must first pass through biological membranes in the extrapallial fluid (EPF) in the extra-pallial space, an enclosed environment situated between the mantle, a

thin organ that completely encloses the other soft-parts of the animal, and the inner shell surface (Allam & Paillard 1998). Passing through these membranes can alter the original chemistry obtained from the seawater (Wedderburn et al. 2000). Ca^{2+} -ATPase and carbonic anhydrase (CA) are two enzymes that have been identified as being important to calcification (Tanbutté et al. 1996). Ca^{2+} -ATPase achieves this by pumping Ca^{2+} to the EPF, while removing 2H^+ , and catalyses the reaction of bicarbonate to CO_2 , which can then easily diffuse through membranes (McConnaughey 1998). Due to its smaller size and differing electrochemical characteristics, Mg is excluded from the site of calcification (Schöne et al. 2013). However, Sr^{2+} and Ca^{2+} are similar in size and atomic properties (Jan & Jan 1976). Therefore, the Ca^{2+} -ATPase allows Sr^{2+} to enter the calcification fluid along with Ca^{2+} , so that the Sr/Ca ratios of the bivalve shell should be identical to that of the ambient water (Gaetani & Cohen 2006). Whether the Mg/Ca and Sr/Ca ratios are due to their effect on biological systems or not is beyond the scope of this study.

The variability between individuals (in particular magnesium (Fig 5.3) also points to Sr and Mg incorporation being controlled by physiological factors. Individual bivalves may employ differential ion-regulatory mechanisms to maintain homeostasis for calcification (Sokolova et al. 2012). This may have profound implications for biomineralisation processes, because shell formation is a complex process requiring considerations from aspects, calcification and mineralisation. It has been stated that the rate of local adaption of an organism to a changing environment depends on the degree of genetic variability in a population (Lande and Shannon 1996) and that differing environmental conditions can stimulate the expression of genetic differences among individuals (Kurihara 2008), although this is mostly observed in harsh environments (unlike the Bay of Brest).

Another reason for variability between individuals could be the energy cost of depositing strontium, magnesium and calcium. The active removal of protons from the EPF is

energetically expensive. Under low temperature conditions or reduced food availability, there is decreased metabolism, and energy must be budgeted to more important processes (such as reproduction) (Hughes 1970). Increased rates of ATP synthesis and ion transport require a high energy supply, so that bivalves will inevitably reallocate energy away. Metabolism in bivalves differs due to many factors, including sediment grain size and burrowing (de la Huz et al. 2002), and individuals respond to differently to environmental stress and pollution (Bayne et al. 1979). This means that energy budgeting differs between individuals, including calcification rate and trace element incorporation.

5.5.1. Conclusions

In this study, the element chemistry in the shells of *G. glycymeris* in the Bay of Brest, France, was analysed. It was found that Sr/Ca and Mg/Ca ratios are strongly regulated by the same factors. In this case, on an annual scale, those environmental effects are seawater temperature, chlorophyll α counts and the amount of phosphate present in the marine environment. In agreement with inorganic, coral, and sclerosponge aragonite, Mg/Ca and Sr/Ca ratios of *G. glycymeris* are inversely proportional to temperature, as well as (in this case) chlorophyll α and are positively correlated with phosphate. Both chlorophyll α and phosphate levels have been shown to have relationships with temperature in the literature. Seasonally, the temperature of the water positively correlated with Mg/Ca and Sr/Ca ratios.

A large portion of literature around Mg/Ca and Sr/Ca is determining whether they can be used as proxies for paleotemperature recreations. This study has shown that the ratios taken from the prismatic layer of *G. glycymeris* can be used as a proxy, however we can only specify this is the case with this species. Further studies are required in order to analyse how the amount of trace elements present in the calcite is controlled by physiological processes.

Chapter 6: Conclusions and Future Studies

6.1. General Conclusions

Sclerochronology is a rapidly growing area of study particularly in the high and low latitudes. The mid-latitudes are starting to become more studied however with the increase in interest in the shells of bivalves, Bivalves (along with other sclero-species such as corals) have been shown to aid in developing proxies for physical signals sequentially recorded within accretions or deposits of biological or geological structures that reflect the environment in which they were formed. This study highlights the ability of *G. glycymeris* to provide proxy records by accurately recording environmental variability in both the shell growth and shell geochemical archives.

Given considerable discussion of results within each chapter, only main conclusions are reiterated here along with a brief synopsis of the study limitations and future research perspectives

6.1.1. Chronology

As demonstrated by the findings in chapter 3, *Glycymeris glycymeris* in the Bay of Brest is highly sensitive to the fresh water inflow from the River Elorn, as well as to food availability mediated by increased suspended particulate matter (SPM) in the late winter. It was concluded that the riverine inflow rates were secondary in importance to growth in the wake of food availability (SPM). This highlights that the growth rates of *G. glycymeris* in this location are a potential proxy for SPM levels for future studies. As the length of the chronology extends to periods before measured environmental data was available, it will be possible to reconstruct such variables using the chronology SGIs. This study highlights the importance of location as a factor in the degree to which individual growth in a population responds to climatic and environmental change. Localised records such as these have great potential for the calibration of regional climate models as they provide unique sources of

annually-resolved and locality-specific paleoclimate information that is often not available from instrumental measurements.

6.1.2. Temperature Reconstruction

Chapter 4 demonstrated that the Bay of Brest is an interesting location with regards to climate. As temperatures reconstructed from $\delta^{18}\text{O}$ obtained from within *G. glycymeris* shells correlated with high fidelity to observed sea surface temperatures, it solidified the species as a potential proxy for paleoclimate temperature reconstructions. This is particularly exciting because *Glycymeris spp.* have been found in the fossil record all the way back in time to the Oligocene.

This chapter also revealed that the Bay of Brest is very interesting location with regards to its climate. Reconstructed sea surface temperatures (SST) correlated with the East Atlantic Pattern (EAP) and the North Atlantic subpolar gyre (SPG), which are important climate systems impacting temperature in both southern and northern Europe respectively. That SSTs correlated with both of these environmental circulations indicates that the Bay of Brest is a point of overlap and interaction area between the two. As such, this location is interesting as the temperatures recorded can be used a proxy for both the SPG and EAP and temperature reconstructions can provide a unique insight in how these climate systems interact prior to observations.

6.1.3. Trace Elements

This study in chapter 5 disclosed for the first time the environmental factors governing magnesium (Mg) and strontium (Sr) incorporation into *G. glycymeris*. Both elements were controlled by temperature. Mg and Sr were inversely correlated with temperature when annual averages were compared, but were positively correlated during the year (between May and October). It is hard to come to a conclusion about why this is

happening as there is no literature on trace element incorporation *G. glycymeris* and the general consensus is that this process is species specific. As far as we are aware, this has not been observed before.

6.2. Study Limitations

1. As the samples were collected from inside the Bay of Brest, there was little in the way of climate signals observed. This is because, within the semi-enclosed environment of the area, the rivers were the driving force for growth. On the other hand, by only working in a semi-enclosed coastal environment, we have observed important parallels that are interesting to future management of coastal areas. Some climate signals were observed, especially in the case of reconstructing sea surface temperatures.
2. Multiple species would have also provided interesting insights into the driving forces behind environmental conditions in the Bay of Brest, however due to time constraints this was not possible to achieve.

6.3. Future research perspectives

1. Future studies should focus on expanding outside the Bay of Brest, into the open ocean such as Île-Molene or Pointe Saint-Mathieu. *G. glycymeris* from these locations will, most likely, not be as affected by the freshwater input as those within the Bay of Brest. It is possible that the growth, $\delta^{18}\text{O}$, and trace elements in these ‘open water’ populations would be influenced by completely different environmental or biological factors. This could lead to increased knowledge about

G. glycymeris itself, the differences between semi-enclosed and open water locations, as well as how bivalves in general are affected by the environments they are living in. This would be of particular interest with regards to trace elements because there have been suggestions that species will incorporate elements into their shells differently depending on locations.

2. Expanding the chronology further back in time would also be a potential future project. Not only would this lead to more information about growth rates and how river inflow and SPM have changed since before records began, but it would also provide a platform to create a very long temperature reconstruction using $\delta^{18}\text{O}$ or long running trace element archives. Given the large quantities of fossil material available in the relatively small area sampled for this study, it is almost certain that a robust chronology extending further back in time can be constructed. In addition, with the use of radiocarbon dating, future projects would be able to construct floating chronologies for earlier periods of climatic and environmental interest and eventually anchor them with live collected data from this study.
3. More information must be gathered on trace element incorporation into *G. glycymeris*. Controlled laboratory experiments, such as those undertaken by Zhao et al. (2016; 2017), specifically on *G. glycymeris* could make great leaps in understanding not only how the animal biologically assimilates trace elements into its shell, but also how these biological controls are affected by the environment (if element incorporation is biologically controlled at all). An insight into biomineralisation in *G. glycymeris* would also be advantageous to start answering questions such as why different paleotemperature equations have been successfully used for the same species in different locations (Grossman & Ku

(1986) in Scotland by Reynolds et al. (2013) and Royer et al. (2013) in the Bay of Brest by this study).

4. The sclero team in the University of Brest is very lucky as it has samples and shell data from a variety of species over a large temporal scale (in particular *Pecten maximumus*). Another interesting study could be to gather all of these data and samples to try and observe localised biological, chemical or environmental patterns which may influence cross-species. In this way, biological factors could be ruled out as drivers of different parameters.

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Appendices

Appendix 1: Shell Biometrics and Location of Collection

Available upon request sent to Dr. Julien Thébault (julien.thebault@univ-brest.fr)

Appendix 2: Measured growth increments in the hinge of all analysed shells

Available upon request sent to Dr. Julien Thébault (julien.thebault@univ-brest.fr)

Appendix 3: Measured growth increments in the hinge of shells used to create EPS in Chapter 3

Available upon request sent to Dr. Julien Thébault (julien.thebault@univ-brest.fr)

Appendix 4: pMC, Fraction Modern and $\Delta^{14}\text{C}$ of four *G. glycymeris* shells sent for radiocarbon dating.

Percent Modern Carbon (pMC)	Fraction Modern	$\Delta^{14}\text{C}$
105.2 +/- 0.3 pMC	1.0524 +/- 0.0026	52.4 +/- 2.6 o/oo
96.9 +/- 0.4 pMC	0.9694 +/- 0.0036	-30.6 +/- 3.6 o/oo
99.6 +/- 0.4 pMC	0.9963 +/- 0.0037	-3.7 +/- 3.7 o/oo
102.6 +/- 0.3 pMC	1.0265 +/- 0.0026	26.5 +/- 2.6 o/oo

Appendix 5: Correlation matrix of annual averaged environmental data

PC	Eigenvalue	% variance
1	2.59279	51.856
2	1.05997	21.199
3	0.884272	17.685
4	0.310491	6.2098
5	0.15248	3.0496

Appendix 6: Loadings of the environmental data on principal components

	PC 1	PC 2	PC 3	PC 4	PC 5
Rainfall	-0.0011988	0.11132	0.12235	0.98621	-0.0058181
River Elorn	0.057075	0.67754	0.71444	-1.65E-01	0.0050399
Nitrite	0.019785	0.72249	-6.86E-01	4.10E-03	0.080518
Salinity	0.00027532	-0.061256	0.05249	6.28E-03	0.99672
SPM	0.99817	-0.052912	-0.027114	0.010537	-0.002166

Appendix 7: Measured $\delta^{18}\text{O}$ and $\delta^{13}\text{C}$

Available upon request sent to Dr. Julien Thébault (julien.thebault@univ-brest.fr)

Appendix 8: Modelled and measured first and last days of growth (1999-2010)

	Measured First Day of Growth	Measured Last Day of Growth	Modled First Day of Growth	Modled Last Day of Growth
1999	10 June	14 October	8 June	25 October
2000	21 June	18 October	19 June	20 October
2001	19 June	22 October	18 June	22 October
2002	17 June	11 October	17 June	10 October
2003	11 June	16 September	10 June	18 September
2004	31 May	11 October	31 May	11 October
2005	15 June	9 September	13 June	10 September
2006	10 July	19 September	6 July	20 September
2007	6 June	2 October	6 June	5 October
2008	10 May	21 September	10 May	26 September
2009	12 June	4 August	13 June	10 August
2010	6 June	20 October	5 June	20 October
2011	8 July	16 September	30 June	20 September

Appendix 9: Modelled first and last Days of growth (1966-1998)

	Modelled First Day of Growth	Modelled Last Day of Growth
1966	16 May	25 October
1967	2 June	13 October
1968	14 June	19 October
1969	23 June	6 October
1970	26 May	15 September
1970	18 June	31 August
1972	20 June	23 October
1973	19 May	27 September
1974	18 June	19 October
1975	5 June	6 October
1976	19 June	17 October
1977	6 July	26 October
1978	28 May	27 September
1979	4 July	15 September
1980	29 June	20 October
1981	17 June	26 September
1982	5 June	6 October
1983	1 July	27 October
1984	3 July	26 August
1985	9 June	19 October
1986	19 May	30 September
1987	26 May	22 October
1988	30 June	14 October
1990	31 May	1 September
1991	21 June	26 September
1992	23 June	17 October
1993	17 June	4 October
1994	11 June	19 October
1995	6 June	22 October
1996	14 June	28 October
1997	27 May	28 September
1998	19 May	20 October

Appendix 10: Measured trace elements ($\mu\text{mol/mol}$)

Available upon request sent to Dr. Julien Thébault (julien.thebault@univ-brest.fr)

40-year reconstruction of seawater temperature in the Bay of Brest using shells of dog cockles, *Glycymeris glycymeris*

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Introduction
 Global climate change is a major focus of politicians and scientists worldwide, the consensus being that anthropogenic activities are influencing environmental changes, thus affecting the Earth's ecosystems. In order to add credence to this, we must build a record of past long-term natural variability in the environment. In recent years bivalve mollusc shells have been successfully used as paleoenvironmental and paleoclimatic archives through the analysis of their geochemical composition. *Glycymeris glycymeris* is a bivalve found in coarse-grained subtidal sediments, with a large distribution area in the NE Atlantic. Thanks to its remarkable longevity of up to 200 years in the northern part of its geographical distribution area, it has a strong potential for building long-term climate reconstructions in temperate settings. The purpose of this study was to create a $\delta^{18}\text{O}$ temperature reconstruction for as long a time as possible, and compare that with climate dynamics.

Methods
 Specimens dredged in the Bay of Brest (Brittany, France; Fig. 1) were investigated for their stable isotope composition. 636 aragonite samples were micromilled in early ontogeny growth increments (average of 12 samples per increment) of 24 individuals with overlapping lifespans, and subsequently analysed for their oxygen isotope composition on a Finnigan MAT 253 CF-IRMS coupled to a GasBench II at the University of Mainz, Germany. The continuous time-series of oxygen isotope ratios was then converted into a series of reconstructed seawater temperatures using (i) in situ temperature and salinity data obtained from a coastal monitoring station, and (ii) the paleotemperature equation developed by Royer et al. (2013):
 $T(^{\circ}\text{C}) = 18.11 - 2.66 \times (\delta^{18}\text{O}_{\text{Aragonite VPDB}} - \delta^{18}\text{O}_{\text{Water VSMOW}})$

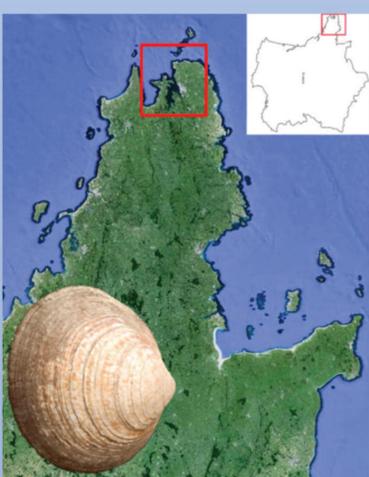


Fig. 1: Map of the study area

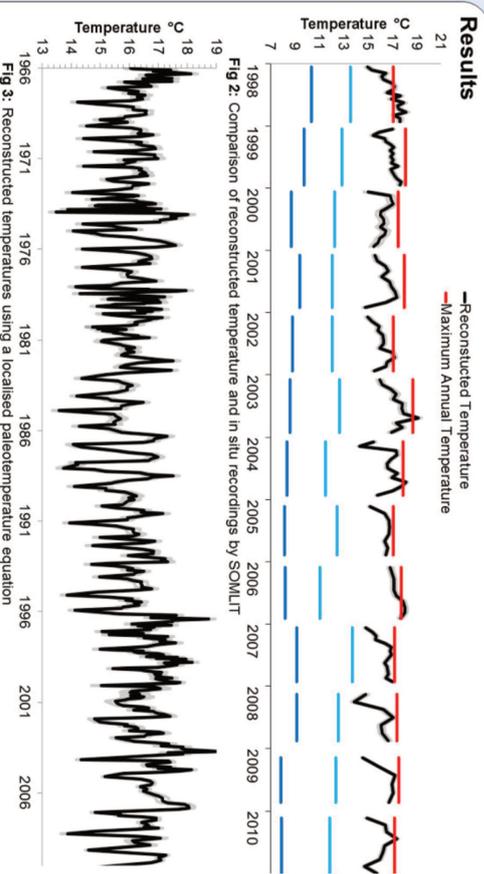


Fig. 2: Comparison of reconstructed temperature and in situ recordings by SCMLIT

- Reconstructed temperatures were compared to the temperatures recorded sub-surface by the SOMLIT-BREST monitoring station between 1998 and 2010 (Fig. 2), highlighting that annual maxima are recorded in the shells with a good fidelity.
- Temperature reconstruction was extended back to 1966 (Fig. 3). It shows that annual seawater temperature maxima greatly varied over time, with a "cool" phase until 1995 and a "warmer" phase afterwards.
- This shift also appears when reconstructed temperatures are averaged annually over the period of growth (Fig. 4). Before 1995, the average temperature was 16.01°C , over half a degree less than the average of 16.6°C after 1995.

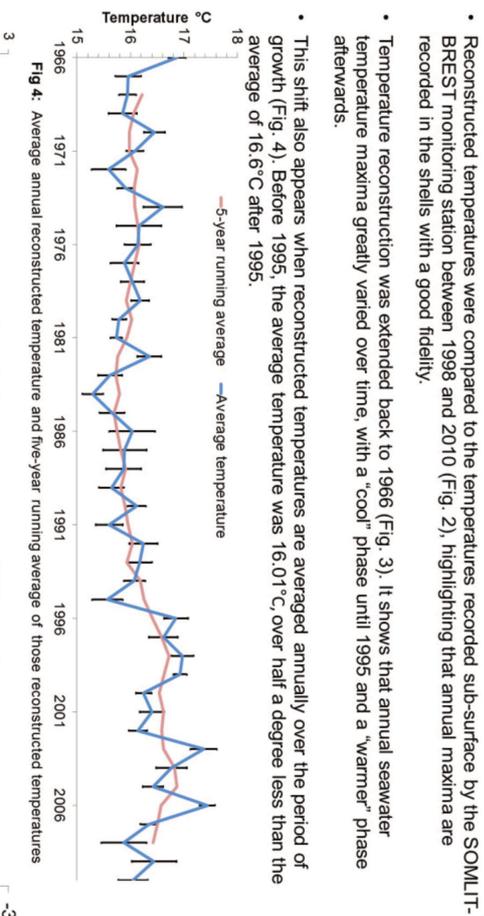


Fig. 3: Reconstructed temperatures using a localised paleotemperature equation

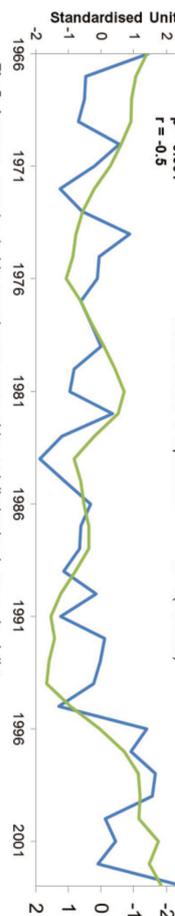


Fig. 4: Average annual reconstructed temperature and five-year running average of those reconstructed temperatures

Conclusions

- *G. glycymeris* may be used for decadal, high-resolution, reconstructions of past sea surface temperatures in areas where instrumental data is not available.
- Temperature reconstructions from $\delta^{18}\text{O}$ in *G. glycymeris* are accurate to local observed data and can be used to develop approximate temperatures further back in time.
- The temperature in the Bay of Brest, France may be directly related to the strength of the subpolar gyre circulation

ARCTIC FRONTIERS

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RESEARCH ARTICLE

Combined Use of Morphological and Molecular Tools to Resolve Species Mis-Identifications in the Bivalvia The Case of *Glycymeris glycymeris* and *G. pilosa*

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Abstract

Morphological and molecular tools were combined to resolve the misidentification between *Glycymeris glycymeris* and *Glycymeris pilosa* from Atlantic and Mediterranean populations. The ambiguous literature on the taxonomic status of these species requires this confirmation as a baseline to studies on their ecology and sclerochronology. We used classical and landmark-based morphometric approaches and performed bivariate and multivariate analyses to test for shell character interactions at the individual and population level. Both approaches generated complementary information. The former showed the shell width to length ratio and the valve asymmetry to be the main discriminant characters between Atlantic and Mediterranean populations. Additionally, the external microsculpture of additional and finer secondary ribs in *G. glycymeris* discriminates it from *G. pilosa*. Likewise, landmark-based geometric morphometrics revealed a stronger opisthogyrate beak and prosodic ligament in *G. pilosa* than *G. glycymeris*. Our Bayesian and maximum likelihood phylogenetic analyses based on COI and ITS2 genes identified that *G. glycymeris* and *G. pilosa* form two separate monophyletic clades with mean interspecific divergence of 11% and 0.9% for COI and ITS2, respectively. The congruent patterns of morphometric analysis together with mitochondrial and nuclear phylogenetic reconstructions indicated the separation of the two coexisting species. The intraspecific divergence occurred during the Eocene and accelerated during the late Pliocene and Pleistocene. *Glycymeris pilosa* showed a high level of genetic diversity, appearing as a more robust species whose tolerance of environmental conditions allowed its expansion throughout the Mediterranean.

and analysis, decision to publish, or preparation of the manuscript.

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Introduction

Mollusc shells have been recognized as useful archives of environmental data spanning in time from several years to millennia [1–3]. Based on their widespread distribution, from polar to tropical regions, and from freshwater to saltwater ecosystems, bivalve shells can provide valuable information pertinent to the reconstruction of environmental variations [4]. Sclerochronological analysis of bivalve shells includes the investigation of interannual variations in growth increment widths as well as variations in geochemical composition and these studies have developed rapidly over the past decade [5]. The longest-lived bivalve, *Arctica islandica* (Linnaeus, 1767), has been extensively used for sclerochronology in the North Atlantic and the maximum longevity of this species has been estimated at 507 years [1–2,6–7]. The potential for another species to act as a paleoenvironmental archive, *Glycymeris glycymeris* (Linnaeus, 1758), has been recently tested in the Irish Sea [8], NW France [9] and NW Scotland [3]. The expansion of sclerochronological studies into the Mediterranean requires identification of bivalve species that could be used as paleoenvironmental archives. Studies on species of *Glycymeris* are especially important since there are no comparable long-lived bivalves in the Mediterranean. In addition, species of glycymerids are locally abundant both alive as well as in the fossil record [10–13]. To date, studies on *Glycymeris* in this region are few and only one growth chronology, that for *G. bimaculata* (Poli, 1795), has been published [14].

The coexistence of two other species, *Glycymeris glycymeris* and *Glycymeris pilosa* (Linnaeus, 1767) hamper their use as proxies because they have been historically misidentified. An uncertain distribution and frequency of *G. glycymeris* in the Mediterranean has been recognized due to confusions with another species of the same genus—*G. pilosa* [15]. Other studies considered them as two forms of the same species because of their heavy and globose shapes (see description in [16]). There is no doubt about the presence of *G. glycymeris* in the North Atlantic, however, its presence in the Mediterranean has been poorly documented and there is a lack of studies on living individuals. Likewise, there are discrepancies whether or not *G. pilosa* (but treated as *G. glycymeris*) has been historically collected from the Eastern Atlantic. Some systematic papers have classified *G. pilosa* as a variety of *G. glycymeris* [17–18]. According to the Check List of European Marine Mollusca [19], both species are still considered as synonyms. Abundant populations of *G. pilosa* can be found in the Adriatic Sea. They have, however, been mistakenly identified as *G. glycymeris* in earlier studies [12]. Only during the last decade, have taxonomists started to identify them as two different species based on morphological characters [16,20]. The World Register of Marine Species [21], aiming to resolve critical issues of nomenclature, also considers them to be distinct species. For this reason, clarifying the erroneous consideration of *G. glycymeris* and *G. pilosa* as synonymous is essential for any further studies on sclerochronology and ecology.

According to the literature, *Glycymeris glycymeris* is distributed in the Northeast Atlantic (the Hebrides, Faroe Islands, Norway, North Sea, English Channel and Bay of Biscay), the Azores, Canaries and Madeira Archipelago's and the Mediterranean. Likewise, *Glycymeris pilosa* has been recorded from Western Sahara, Mauritania, Madeira and the Canaries Archipelago's and the Mediterranean Sea (up to Israel) [15–16,18,20,22].

Glycymerids are descendants of the ancient Arcoidea, a distinct lineage that was established early in the radiation of the Bivalvia [23–24]. Since its appearance during the Cretaceous (~130 Ma), fossil individuals seem to occupy similar ecological ranges to the present species in the North Atlantic, which could be explained by generic conservatism [23]. They are not as specialized anatomically as other bivalves; instead, they have adapted to physically harsh environments evidencing their role as functional generalists [23]. The high degree of conservatism expressed by the genus *Glycymeris*, not only in terms of their morphology [23] but also

reflected in shell ultrastructure [11], and a slow adaptation to different environments have added difficulties to the process of distinguishing species. Often, the variation between species is determined by differences in size and external sculpture. Such changes may have been triggered by adaptations to different environments and can be detected using both morphological and molecular phylogenetic tools (i.e. molecular clock) [25]. Species of *Glycymeris* have a very robust shell and prefer gravel to hard substrata under strong current regimes rather than in fine sediments and calm waters [26]. They are shallow burrowers with mainly nocturnal behaviours [23]. Earlier studies quantitatively measured and interrelated a wide range of glycymerid shell characters [23]. Aiming to classify species of *Glycymeris*, Goud & Gulden [20] selected certain distinctive morphological traits to distinguish between *G. glycymeris* from *G. pilosa*. Some of these traits coincided with those espoused in Thomas [23] but they also introduced others including shell microsculpture measurements of primary and secondary rib counts, which, it transpired, were species-uniform and distinct. The importance in modern taxonomy of DNA analyses contrasts with morphological studies in biodiversity research [27]. The exclusive reliance on one or other method may, however, fail to detect variations. Comprehensive studies including morphometrics and molecular analyses may, therefore, provide a more accurate approach to species discrimination. The genetic analyses of bivalve species identifications have been used recently to correct mislabeling [28–29]. The identification of molecular markers (i.e. mitochondrial and nuclear genes) has also been used to track genetic diversity and population structure [24,30–31]. Other methods such as the molecular clock have been used to describe phylogenetic relationships and evolutionary histories [32–34].

The aim of this study was to resolve the historical misidentification of *G. pilosa* in the Mediterranean and corroborate its separation from *G. glycymeris* as distinct species by combining morphometric and genetic analyses. This has been achieved by measuring several characters, following classical and landmark-based approaches, and through mitochondrial (COI) and nuclear (ITS2) markers.

Materials and Methods

Study area

Atlantic specimens came from the Isle of Man (54° 26' 54.49" N, 4° 20' 21.73" W) issued by the Department of Environment, Food and Agriculture (authorization n°SF204.011/2015), and from the Bay of Brest (48°20' 29.88" N, 4°30' 45.66" W) issued by the Directorate of Maritime Affairs (authorization n°101/2014), hereafter referred to as UK and France groups, respectively. Mediterranean samples were collected in two locations along the eastern Adriatic coast in Croatia: Pag Bay (44° 27' 42" N, 15°01' 36" E) and Pašman Channel (43°56' 49" N, 15°23' 18" E) issued by the Ministry of Science, Education and Sport (authorization n°533-19-14-0008/2014 and 533-19-14-0006/2015), hereafter referred to as Pag and Pašman sample groups, respectively. Unfortunately we were unable to collect specimens of both species neither in the Atlantic nor the Mediterranean.

A total of 107 shells classified as either *G. glycymeris* or *G. pilosa* were live collected. Samples from UK and France represented two groups of *G. glycymeris* (17 and 30 specimens, respectively), whereas two groups of *G. pilosa* came from Pag and Pašman (30 specimens from each location) (Fig 1). Due to logistical constraints (limited to one sampling cruise) and physical damage during handling, sample size of the UK population was smaller. Monthly sampling was performed at other sites ensuring enough samples in good condition. All morphometric measurements were conducted on the right valve (identified according to the position of the beaks, turned towards the posterior end of the valves; [16]). Samples from collections in the National Museum of Wales, Cardiff were also used for corroboration of measurements. All

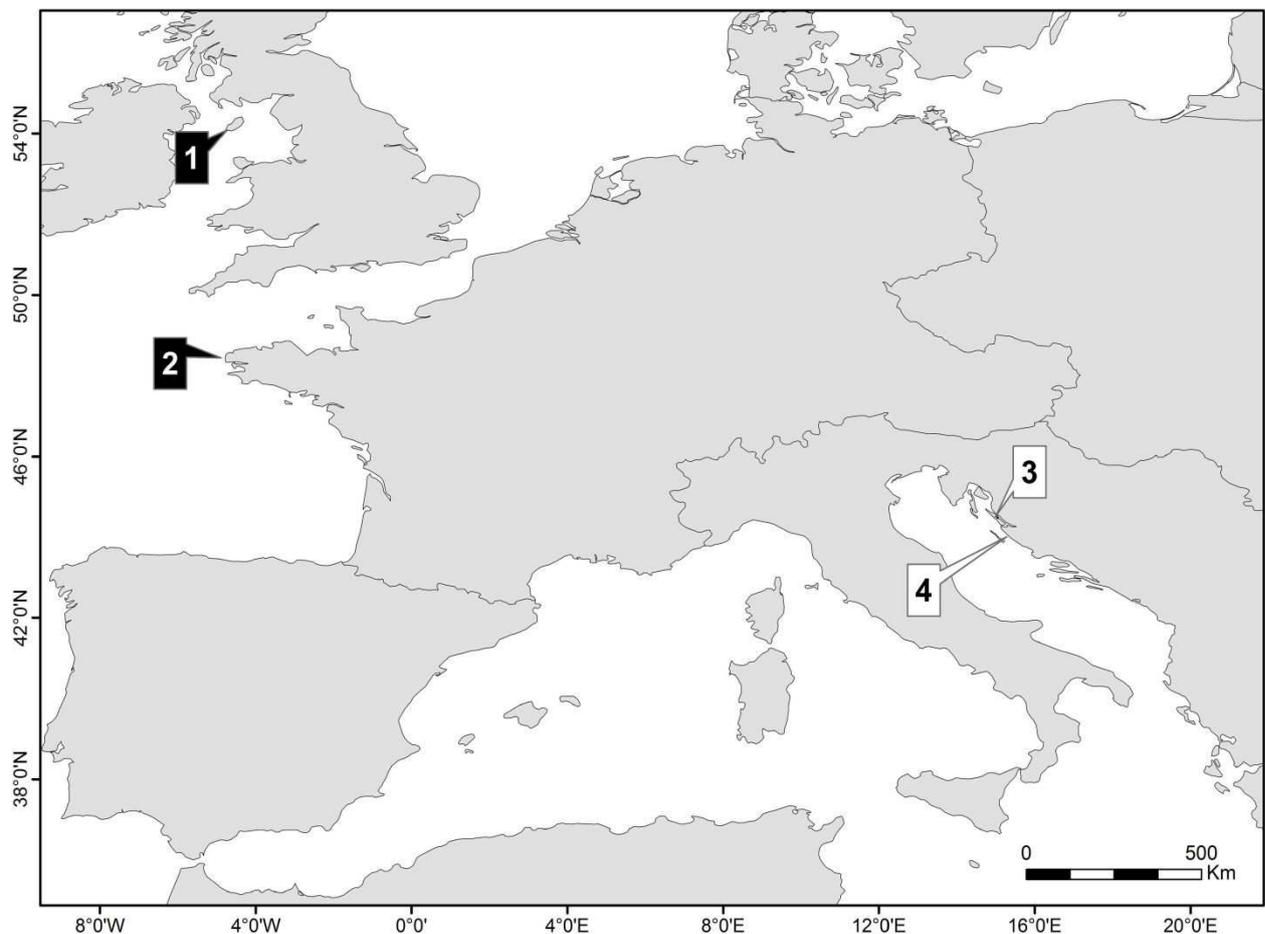


Fig 1. Collection sites along the Northeast Atlantic and Mediterranean Sea. Specimens identified as *Glycymeris glycymeris* (black fill) and *Glycymeris pilosa* (white fill) came from: (1) Isle of Man (UK), (2) Bay of Brest (France), (3) Pag Bay (Croatia), (4) Pašman Channel (Croatia).

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material examined with accession number beginning NMW.Z is held in the collections at the NMW and is available for institutional loan on request. The remaining material is held at the Institute of Oceanography and Fisheries (IOF), Split ([S1 Table](#)).

Morphometric analysis

Classic and novel morphometric analyses were chosen based on the bidimensional and tridimensional approaches of each method, to explore the range of morphological variation. Due to the absence of sexual dimorphism in the shell, males and females were treated indistinguishably [23]. To identify shell morphometric relationships among populations we conducted bivariate and multivariate analysis.

Classical morphometric analysis. The basis for the selected morphological data came from Thomas [23]. This study described the evolutionary conservatism of the Glycymerididae family by looking at the interrelationships of shell characters ([Fig 2](#)). The characters were measured with a digital caliper (accuracy of ± 0.02 mm). Additional morphological characters were included based on sculptural traits (e.g. rib counts). This step was introduced in our analyses because Goud & Gulden [20] counted the number of secondary ribs within a primary rib to

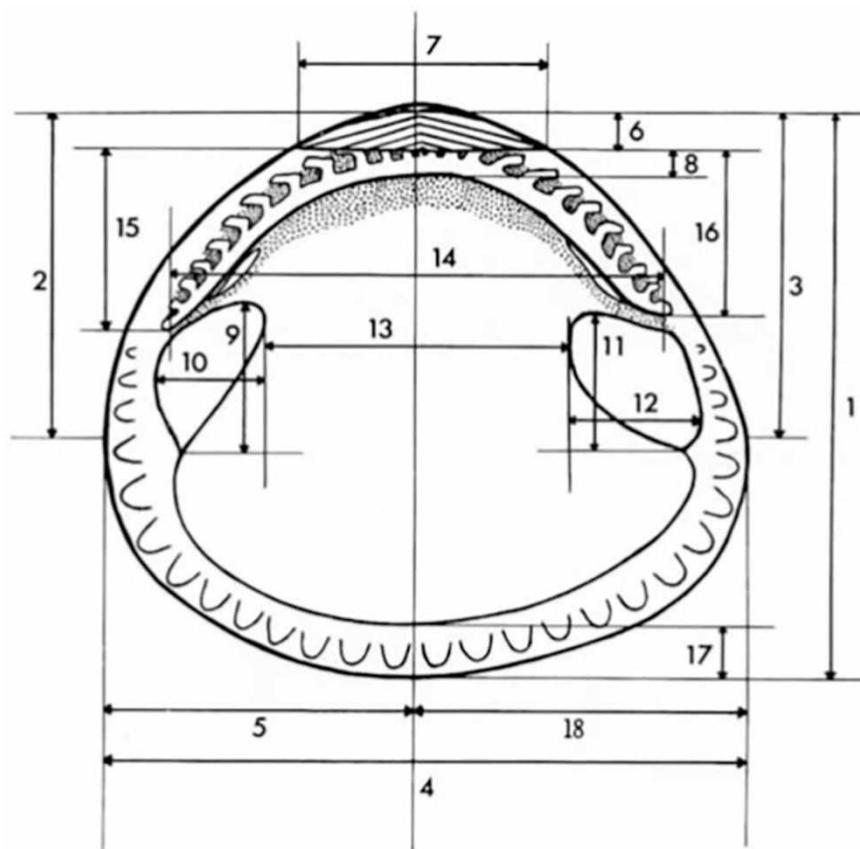


Fig 2. Classical morphometrics measurements. Height of shell (measured from umbo)(1), height of anterior extremity (2), height of posterior extremity (3), length of shell (4), anterior length (5), posterior length (18), asymmetry (5/18), height of ligamental area (6), length of ligamental area (7), median height of hinge plate (directly below umbo)(8), height of ligamental area / height of hinge plate (6/8), height of anterior tooth row (15), height of posterior tooth row (16), distance between last anterior and posterior teeth (14), height of anterior adductor scar (9), length of anterior adductor scar (10), area of anterior adductor scar ($9 \times 10 / 2$), height of posterior adductor scar (11), length of posterior adductor scar (12), area of posterior adductor scar ($11 \times 12 / 2$), distance between inner margins of adductor scars (13), adductor moment = sum of adductor scar areas * mean distance from hinge axis, height of crenulated extra-pallial margin (17) and the width (W). Measurements taken for each morphometric character adopted from Thomas (1975) with permission from the Palaeontological Association. Right valve of a *Glycymeris*.

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separate *Glycymeris* species; however, we were unable to consistently see the primary ribs clearly enough to be able to use this as a unit of measurement for the secondary ribs. Instead, a fixed length of shell (5 mm) was counted for secondary ribs. Externally, measuring 30 mm from the beak to the middle external of the valve, a marker measuring 5 mm was temporarily adhered at a right angle to the secondary ribs. In this way a consistent distance of shell was counted for secondary ribs. A tiny sticker marked with a 5 mm ruler curved with the shell to get an accurate count for each specimen. This method also ensured that if secondary ribs increased with age and hence closer to the ventral part of the shell, then shells of different sizes/ages could still be used in the count, provided they were not smaller than 30 mm in height.

To reduce redundancy in the Principal Component Analysis (PCA), we used Pearson correlation coefficients (through linear regression analyses) for data exploration. Those characters from the morphometric dataset that expressed more significant linear relationships among the

populations were computed as ratios for a size free shape multivariate analyses. A PCA was performed after data standardization on: shell width to shell length (W/L), shell length to shell height (L/H), asymmetry to shell height (A/H), height of ligament to shell height (HL/H), height of ligamental area to shell height (LA/H), length of ligamental area to shell length (LL/L), area to shell size ratio of both the anterior and posterior adductor muscles (AM/S & PM/S), height of the crenulated extra-pallial margin to shell height (M/H) and the log of the adductor moment (logAM) to find those variables contributing the most to the individual variability. Due to the wide range of variables at different scales a correlation matrix was used in the PCA. A Linear Discriminant Analysis was sought to discriminate differences among populations. Statistical analyses were carried out using the open software PAST v3.0 [35].

Landmark-based geometric morphometrics (GM). The GM analyses implemented in software MorphoJ [36] were used to quantify shape variation from 60 images of *Glycymeris* sp. from different localities (15 samples from each group). For all specimens, the inner side of the right valve was photographed and digitized with the software TPS dig2 [37]. From these images 12 landmarks and 2 semi-landmarks were defined (Fig 3). Landmarks were subjected to a

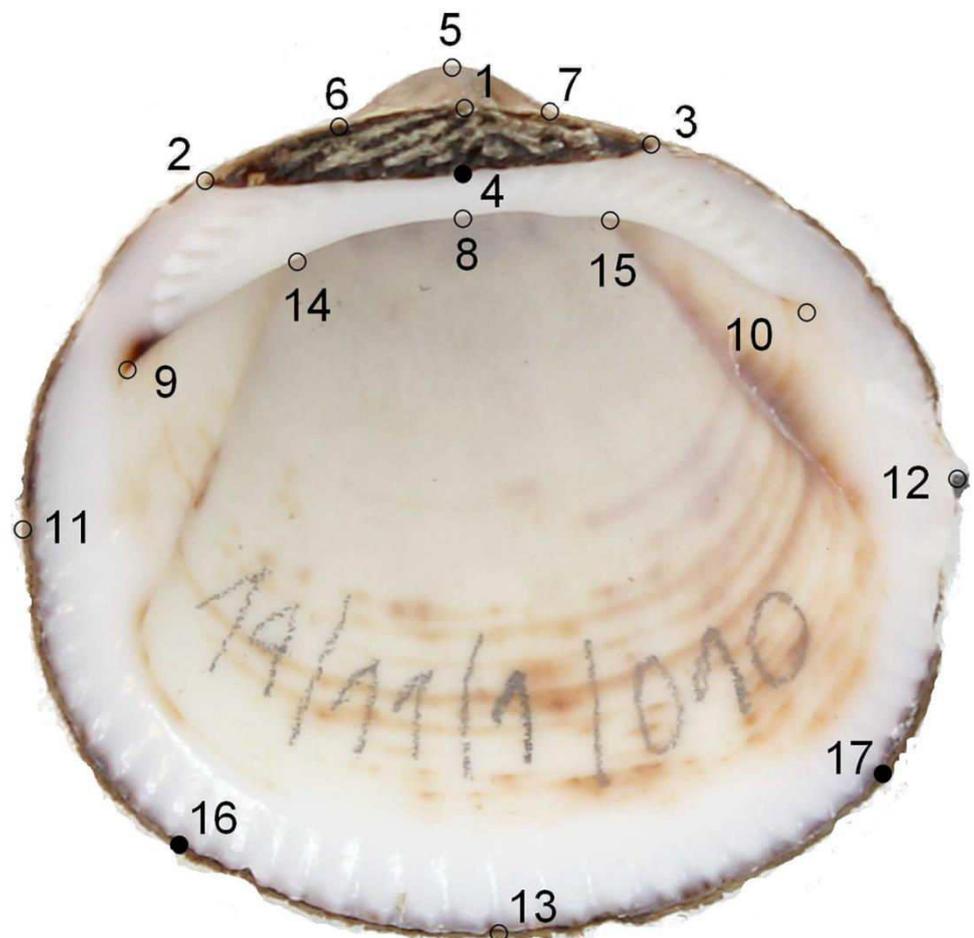


Fig 3. Geometric morphometrics landmarks. This image represents a right valve of *Glycymeris glycymeris* with the position of 12 landmarks (open dots) and 3 semi-landmarks (fill dots).

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standard generalized Procrustes alignment to remove differences between specimens based on scale, rotation, and location. A PCA was also applied to the Procrustes coordinates to explore variation in the shape components. Multivariate regression of shape with centroid size as the independent variable was computed to assess intrapopulation allometry. Permutation test with 10,000 rounds was used to evaluate the independence between the shape and size variables. Finally, a canonical variate analysis (CVA) was performed to find out which shell shape features best discriminated between species.

Sampling and DNA extraction

Based on the preliminary morphological determination, 15 specimens from each location were allocated for DNA sequencing. In addition, two specimens of *G. nummaria* (Adriatic origin) and two of *G. bimaculata* (from Balearic and Adriatic Seas) were sampled for phylogenetic analysis. Adductor muscle samples were stored in 96% ethanol. Genomic DNA was extracted from 30–50 mg of tissue using the DNeasy Tissue Kit (Qiagen Inc.) following the manufacturer's protocol. Two molecular markers were analyzed; internal transcribed spacer 2 (ITS2) rDNA was amplified according to Oliverio & Mariottini [38] while a partial fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI) was amplified following Folmer et al. [39]. Products sequencing were performed by Macrogen Inc. (Seoul, Korea) on an ABI 3730 automatic sequencer.

From GenBank database, 5 species of Arcida (*Arca ventricosa*, Accession number AB076935.1; *Tegillarca granosa*, HQ896817.1; *Cucullaea labiata*, AB050892.1; *Cosa waikikia*, AB084107.1; *G. glycymeris*, KC429093.1) were additionally used as ingroup samples for COI reconstruction while two pteriomorph species from different orders (*Isognomon legumen*, AB076950.1; *Spondylus gaederopus*, JF496776.1) and the protobranch *Nucula ataccellana* (KJ950273.1) were chosen as outgroups (see [34]). To fulfill ITS2 data set, *G. glycymeris* (FN667988.1) originating from Tuscan Archipelago, Mediterranean Sea [32] were used to accommodate intra family analysis.

Sequences were screened using BLAST searches (ncbi.nlm.nih.gov), trimmed and aligned in ClustalW 2.1 [40]. All new haplotypes have been deposited in GenBank database under Accession numbers: KX785175–KX785221.

Genetic diversity and phylogenetic analyses

Molecular diversity was measured using Dnasp 5.10 [41] calculating the number of haplotypes (H), polymorphic sites (S), haplotype and nucleotide diversity. Intra- and interspecific distance was calculated in MEGA6 [42]. The demographic histories for *G. glycymeris* and *G. pilosa* were tested using Tajima's D [43] and Fu's F_{ST} [44], where negative values indicated population expansion or historical bottleneck [45]. The significance of tests were assessed using 10,000 samples simulated under a model of constant population size in Arlequin 3.5.1.2 [46].

Phylogenetic reconstructions were estimated using maximum likelihood (ML) and Bayesian (BI) analysis on COI and ITS2 datasets. jModelTest [47] was used to determine the most suitable model of sequence evolution under the Akaike information criterion (AIC). The general time reversible with the gamma distribution shape parameter (GTR+G) model was chosen for COI gene and Jukes–Cantor [48] for ITS2 gene. A BI tree was constructed using MrBayes 2.0.6 [49] as implemented in Geneious (v. 2.0.3) running at least two independent Monte Carlo Markov Chain (MCMC) analyses with 2,200,000 generations sampled every 400 generations, with a 200,000 tree burn-in. Maximum likelihood trees were constructed using the PhyML [50] plug-in of Geneious with the BEST topology search option and 1000 bootstrap replicates.

Divergence time estimates

Divergence times of *G. glycymeris* and *G. pilosa* were estimated using BEAST 1.8.1 [51] as implemented on the CIPRES web portal [52], on the COI dataset. In respect to Combosch & Giribet [34], three fossil-based calibration points were used: (i) the root age of Bivalvia between 520.5 and 530 Ma, (ii) the age of Arcida at 478.6 (± 5) Ma based on *Glyptarca serrata* [53], and (iii) the age of Glycymerididae at 167.7 (± 5) Ma based on *Trigona tumida* [54]. Analyses were performed under relaxed uncorrelated lognormal clocks and a constant size coalescent population model. These priors were selected according to the Bayes factors from TRACER 1.6 [55] calculated to compare models, although all tested models including strict or relaxed molecular clock combined to exponential growth coalescent population model or Yule process speciation model produced similar divergence time estimates. Four independent runs with MCMC chain length of 5×10^7 were conducted, sampling every 5×10^3 generations. Convergence diagnostics were checked using [55] and phylogenetic trees were summarized in a target tree by the Tree Annotator program included in the BEAST package by choosing the tree with the maximum clade credibility after a 50% burn-in.

DNA-based species delimitation

For single marker species delimitation (COI), coalescent tree-based methods as the generalized mixed Yule-coalescent model (GMYC; see [56]) and the Poisson tree process model (bPTP; see [57]) were applied in order to identify the number of phylotypes. The GMYC analysis was conducted using R Statistical Platform [58], with the use of splits package. The input for the GMYC was an ultrametric single locus gene tree obtained with BEAST [51]. The coalescent tree-based bPTP method was performed using the web interface available at <http://species.h-its.org/ptp/> on the Bayesian majority-rule consensus non-ultrametric tree as input.

Results

Morphometric analysis

Classical morphometric variables showed significant differences among populations (MANOVA, Wilk's Lambda test, $F = 17.43$, $P < 0.001$). As a result of bivariate analysis among all the 24 measured characters, the dataset to carry out PCA was reduced to those areas representing the umbo region, adductor muscles and shell dimensions contributing in a total of 10 ratios (W/L, L/H, A/H, HL/H, LA/H, LL/L, AM/S, PM/S, M/H, logAM). Linear regression analyses indicated the shell width to be highly correlated with the shell length in populations of *G. pilosa* ($r > 0.94$, $p < 0.001$, with a $R^2 > 0.89$) and somewhat less for *G. glycymeris* ($r > 0.77$, $p < 0.001$, with a $R^2 = 0.57$). Geographically, Pag presented the most dispersed data. The height of the ligament to shell height showed a high correlation in all populations except Pašman ($R^2 > 0.66$, $p < 0.001$ and $R^2 = 0.32$, $p < 0.05$, respectively). Contrary, the shell length to shell height ($R^2 > 0.85$, $p < 0.001$), the length of the ligamental area to shell height ($R^2 > 0.59$, $p < 0.001$), anterior adductor muscle area to shell height ($R^2 > 0.61$, $p < 0.001$) and the log of the adductor muscle ($R^2 > 0.82$, $p < 0.001$) did not distinguish among populations. Asymmetry, marked major differences between Atlantic ($R^2 > 0.81$, $p < 0.001$), and Mediterranean ($R^2 < 0.08$, $p > 0.05$) populations, where the later was the most asymmetric. UK appeared as the most symmetric population ($R^2 = 0.25$, $p > 0.05$) and Pašman as the most asymmetric ($R^2 = 0.06$, $p > 0.05$). The length of the ligament indicated a greater correlation to shell length for shells from Brest, UK and Pag ($R^2 > 0.86$, $p < 0.001$), while data were a bit more dispersed for Pašman ($R^2 < 0.64$, $p < 0.001$). The margin height ($R^2 > 0.51$, $p < 0.001$) and the posterior adductor muscle area ($R^2 > 0.63$, $p < 0.001$) showed a high correlation to shell height for Brest, UK

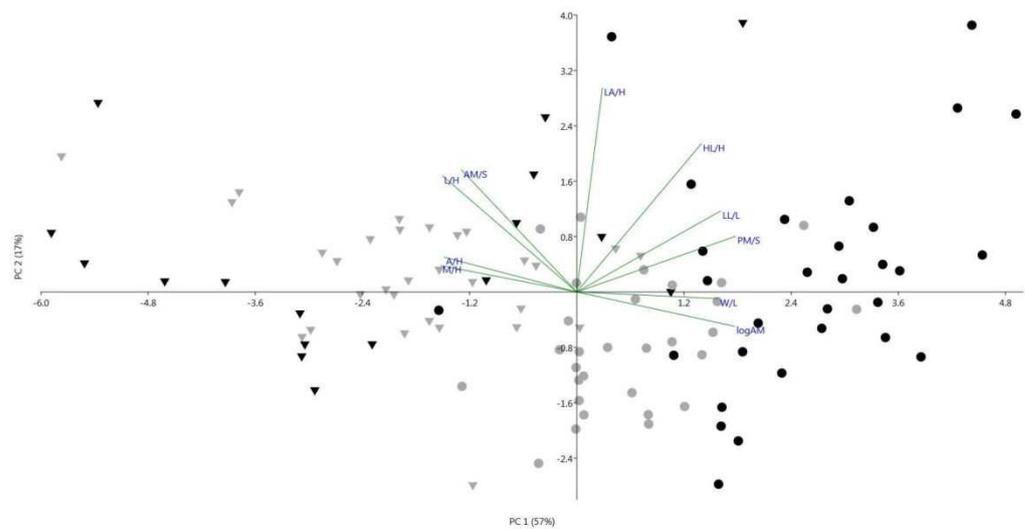


Fig 4. Biplot of the mean scores on the first two principal components based on 10 morphological character ratios. Symbols were assigned to each population: *Glycymeris glycymeris* (black inverted triangle, UK; grey inverted triangle, France) and *Glycymeris pilosa* (black circle, Pag; grey circle, Pašman).

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and Pašman populations. Population in Pag appeared less correlated for these features ($R^2 = 0.16$, $p < 0.05$ and $R^2 = 0.37$, $p < 0.001$, respectively). The first Principal Component (PC 1) explained 57.4% of the variation (eigenvalue 5.74), weights on W/L, A/H, L/H, LL/L, PM/S, M/H and the logAM were highly positively. The weightings for PC 2 (eigenvalue 1.67) indicated LA/H and HL/L to be the most important, explaining a 16.7% of the variation (Fig 4).

The first linear discriminant (LD 1) explained the majority of the total variance (90.96%) (S1 Fig). The Confusion matrix in LDA (Table 1) showed that misplacements of individuals in populations of the same species represented 6.6%, 17.6%, 3.3% and 26.6% for Brest, UK, Pag and Pašman populations, respectively. The intraspecies variability was higher for *G. pilosa*.

Concerning the microsculpture, *G. pilosa* had a more robust, rugged sculpture compared to the finer reticulate sculpture of *G. glycymeris* (Fig 5). A total of five specimens each of Pašman, Pag, Isle of Man and Brest samples were used to determine if the secondary ribs differed between shells collected at different locations (Table 2).

Pag Bay and Pašman Channel, the two populations of *G. pilosa*, fall between 10–15 secondary ribs per 5 mm. Isle of Man and Brest, the two populations of *G. glycymeris*, fall between 22–28 per 5 mm. This is consistent with other specimens of *Glycymeris* from other locations from the collections of the National Museum of Wales and other populations of *G. pilosa* collected from Istria and Cetina River. Additional material from the museum collection was included in the measurements (S1 Table).

Table 1. LDA Confusion matrix on the four populations of *Glycymeris*.

	Brest	UK	Pag	Pašman	Total
Brest	28	2	0	0	30
UK	3	14	0	0	17
Pag	0	0	29	1	30
Pašman	0	0	8	22	30
Total	31	16	37	23	107

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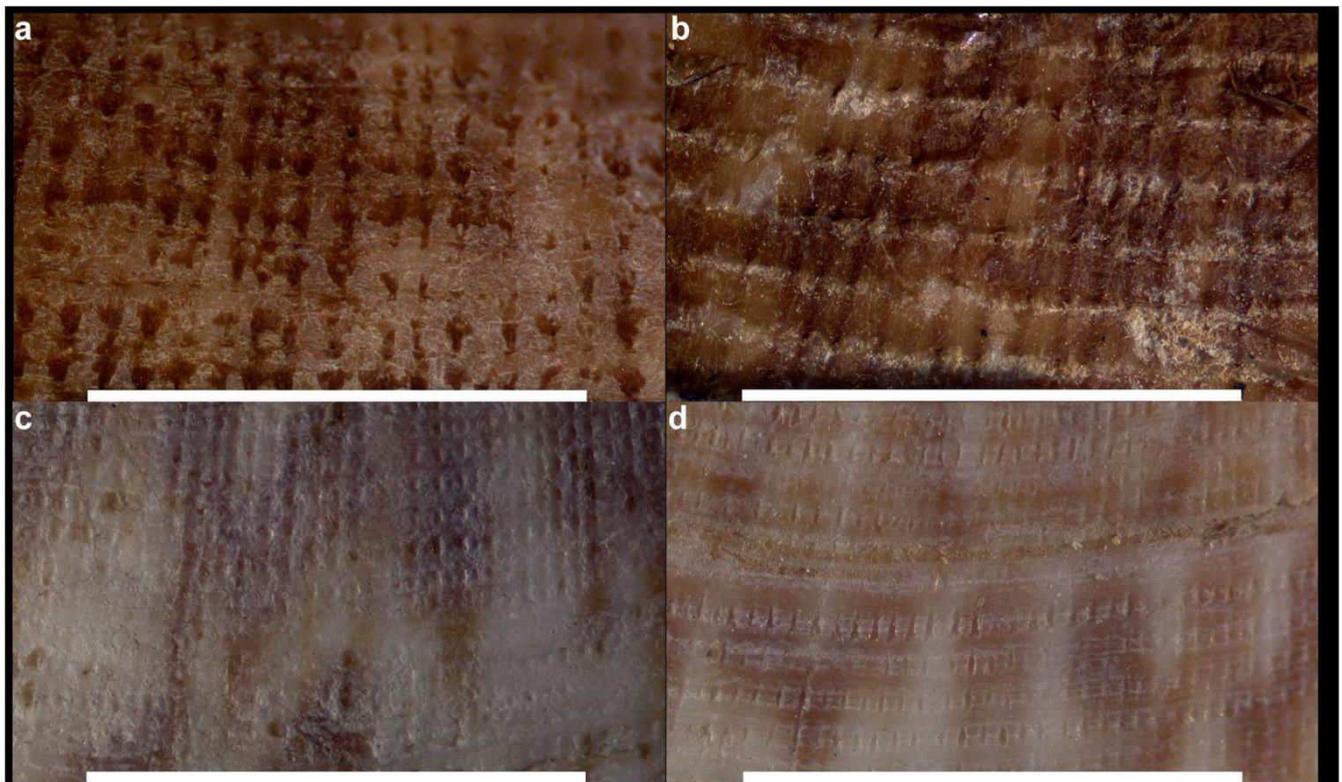


Fig 5. Detail of the microsculpture and rib count measurements of *Glycymeris pilosa* and *Glycymeris glycymeris*. The white line is used as a scale of 5mm. Measured populations correspond to (a) Pašman, (b) Pag, (c) UK and (d) Brest.

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Following the landmark-based geometric morphometrics, growth allometry of the *Glycymeris* sp. was observed and accounted for 14% of the total amount of shape variation. The resulting PCA revealed that the first two components PC 1 and PC 2 (eigenvalues 0.0015 and 0.0010) explained 29.5% and 19.3% of the total variability among the landmarks, on all analyzed specimens (Fig 6). At the primary axis of variation (PC 1), segregation by the species (*G. glycymeris* vs. *G. pilosa*) was observed, and it mainly described changes in the umbo region and enlargement of the posterior-ventral axis of the shell. The second PC axis was related to the development of the ligament area and elongation of the anterior region. The shell shape variations among species from different localities were successfully discriminated using CVA (Fig 6). All groups differed significantly between each other as revealed by permutation testing of Procrustes distances. The morphological differentiation measured by Procrustes distance was similarly large between the *G. glycymeris* from both sampling locations and *G. pilosa* from Pašman (0.09) or Pag (0.06), respectively. The population distances within species (*G. glycymeris*

Table 2. Secondary rib counts counted on the microsculpture in all four *Glycymeris* populations.

Location	Specimen 1	Specimen 2	Specimen 3	Specimen 4	Specimen 5
Pag Bay	12	12	15	12	15
Pašman Channel	14	14	10	11	12
Isle of Man	26	22	25	23	25
Bay of Brest	28	25	26	25	26

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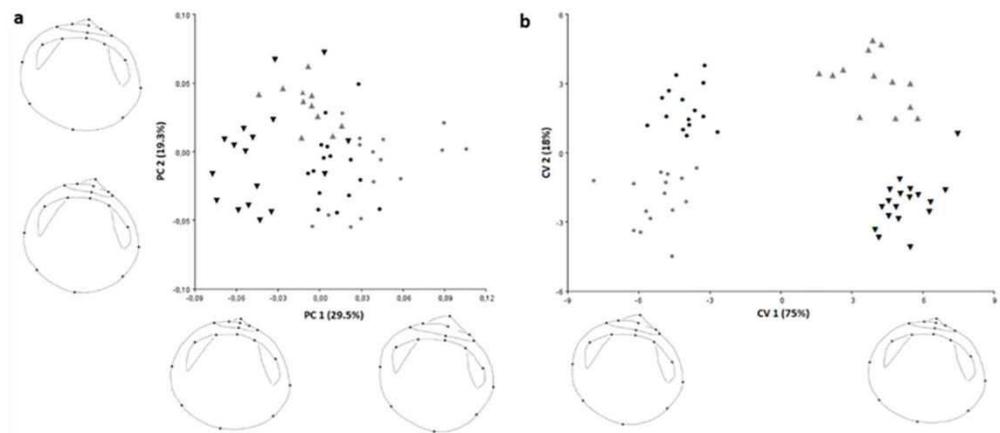


Fig 6. Plot of the principal components (PCs) based on Procrustes distances (a). Shape changes associated with the PCs are shown as extreme shell shapes representing the positive and negative end of each axis. Percentages of explained variance for each axis are in parentheses. Plot of the canonical variate analysis (CVs) of overall shell shape variation along the first 2 canonical axes (b). Wrapped outline drawings show shape changes associated with variation along first axis. *Glycymeris glycymeris* (black inverted triangle, UK; grey inverted triangle, France) and *Glycymeris pilosa* (black circle, Pag; grey circle, Pašman). Percentages of explained variance are in parentheses.

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from UK vs. France, *G. pilosa* from Pag vs. Pašman) were similar and considerably smaller (0.05–0.04). The first canonical axis (CV 1) explained the majority of the total variance (75%). Depicting the between species changes along discriminant functions by warped outline drawings revealed that shape was altered most in umbo and upper posterior-anterior region (Fig 6).

Complementary, images of both shell valves are shown in Fig 7, represented by one specimen from each population.

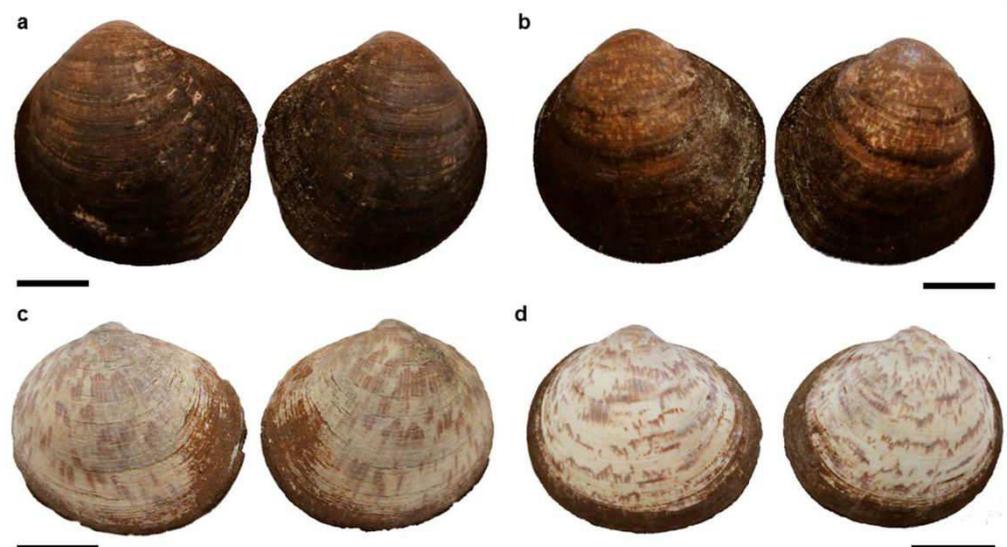


Fig 7. Left and right valves of *Glycymeris pilosa* from Pašman (a) and Pag (b) and *Glycymeris glycymeris* from UK (c) and Brest (d) populations. Scale bar 2 cm.

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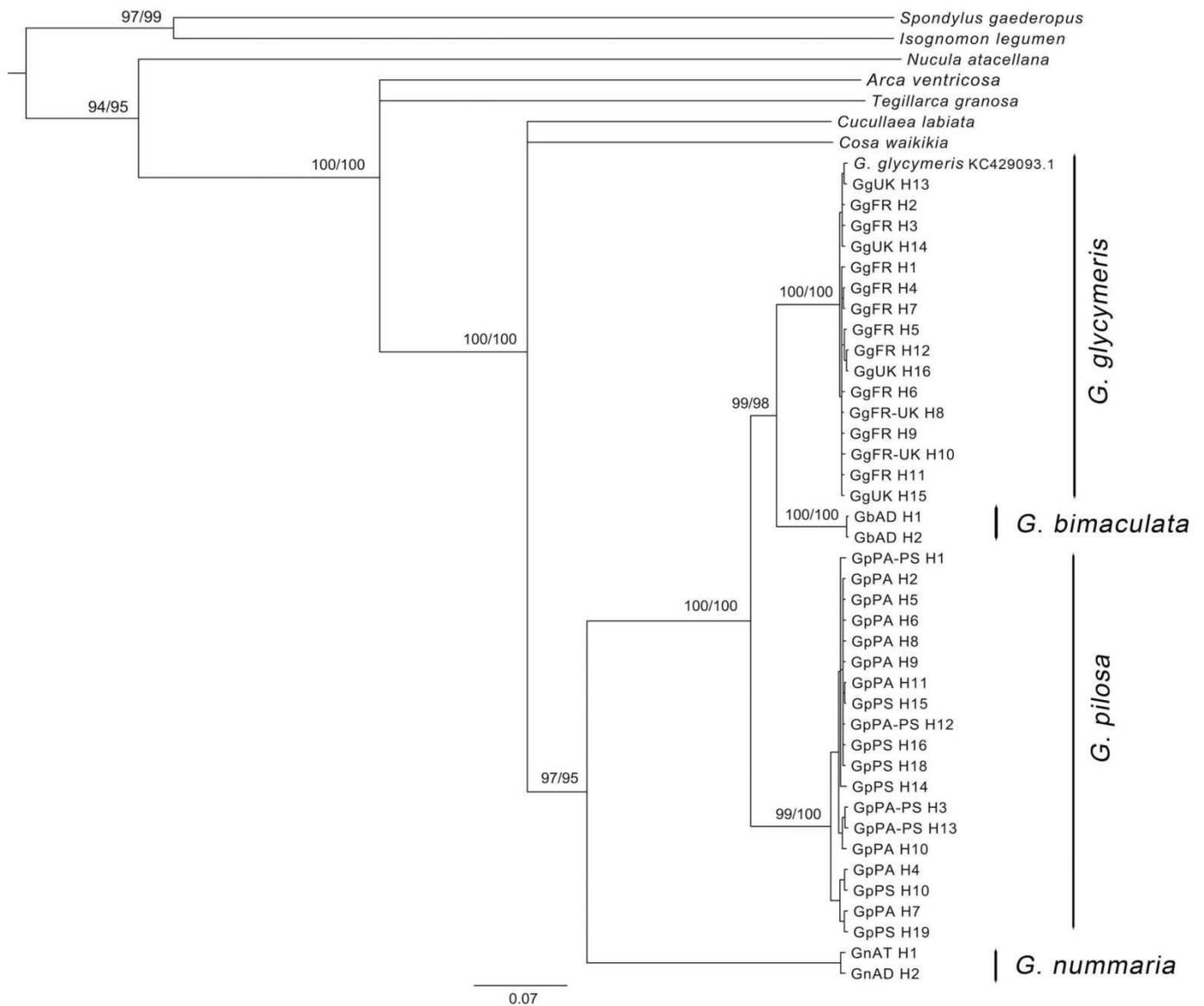


Fig 8. Bayesian posterior probabilities and bootstrap support for MrBayes and Maximum Likelihood analyses. Illustration of tree topology based on COI haplotypes of *G. glycymeris* (Gg), *G. pilosa* (Gp), *G. nummaria* (Gn), *G. bimaculata* (Gb) and outgroups. Posterior probabilities followed by bootstrap values are included at the nodes. The origin of the haplotypes (H) is indicated as follow: UK, United Kingdom; FR, France; AD, Adriatic Sea; AT, Atlantic Sea; PA, Pag; PS, Pašman.

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Molecular analysis

Fragments of 669 and 299 bp were obtained for COI and ITS2 genes, respectively. A total of 16 COI haplotypes and three ITS2 haplotypes were identified among *G. glycymeris* dataset. The COI gene contained 15 variable sites, whereas the ITS2 gene contained only two variable sites. For the *G. pilosa* dataset, a total of 32 variable sites and 19 haplotypes were identified for COI gene while the ITS2 gene contained only one variable site and two haplotypes. Genetic diversity indices for each gene and species are summarized in [S2 Table](#), indicating that COI displayed

quite higher values of both haplotype and nucleotide diversities ($h = 0.96$; $\pi = 0.056$) in comparison to the nuclear gene ($h = 0.62$; $\pi = 0.005$). For both genes, the most common haplotypes of *G. glycymeris* and *G. pilosa* were presented in both sampling locations (S3 Table). COI interspecific divergence accounted for 11% and for ITS2 was 0.9%, respectively. Mean intraspecific divergence in the *G. glycymeris* dataset were 0.4% (COI) and 0.04% (ITS2), while in the *G. pilosa* were 1.1% (COI) and 0.1% (ITS2), respectively.

Neutrality tests applied to search demographic species pattern revealed significant negative values for Tajima's D and Fu's F_{ST} for both *G. glycymeris* and *G. pilosa*, indicating a recent expansion of mtDNA haplotypes (S3 Table).

For COI gene, the dated topology (BEAST), Bayesian and ML analysis gave congruent results. Phylogenetic reconstructions distinguished four strongly supported species of Glycymerididae (bootstrap support 97% to 99%, posterior probabilities 0.95–0.99) in the ingroup (Fig 8). Also, the analyses of COI data supported close relationship between *G. glycymeris* and *G. bimaculata*, having *G. pilosa* species as a sister clade. By contrast, deep genealogical divergence among *G. nummaria* and other *Glycymeris* species were recorded. The ITS2 tree (S2 Fig) was much less resolved, with three well-supported groups recognized, among which *G. glycymeris* and *G. bimaculata* were grouped into one clade. The *G. glycymeris* specimen (FN667988.1) originating from Tuscan Archipelago, Mediterranean Sea was assigned in *G. pilosa* clade, suggesting species mislabeling in the GenBank database.

The divergence time for *G. nummaria* clade was estimated to occur at around 142 Ma (95% highest posterior density interval [HPD] 132.5–151.5), while divergence of *G. pilosa* clade at one side and *G. glycymeris* and *G. bimaculata* clades on the other, started to occur at around 46 Ma (95% HPD 20.9–80.1). The divergence time of *G. glycymeris* and *G. bimaculata* clades was dated around 25.3 Ma (95% HPD 9.5–45.3). Intraspecific haplotype divergence of *G. pilosa* started to occur 7 Ma (95% HPD 2.7–11.8) with the major haplotype diversification observed during late Pliocene and Pleistocene (0.4–3.0 Ma). Intraspecific divergence in the *G. glycymeris* clade also predates the Pleistocene (0.4–2.5 Ma) (S3 Fig).

Both species delimitation analyses performed by implementing the coalescent tree-based approach (GMYC and bPTP) produced similar results and provided strong support for the *a priori* defined morphospecies. The GMYC model delimited 11 species (4 *Glycymeris* and 7 out-group species) with a confidence interval of 9 to 13, and exhibited a significantly better likelihood than the null model (ML GMYC = 648.6, lnL NULL = 656.5; p -value < 0.001), pointing that a boundary between and within species was identified. The bPTP method identified on average 12.5 species (estimated number of species between 9 and 17) by simple heuristic search, resulting in Bayesian support values above 0.8.

Discussion

The combination of two morphometric analyses (classical and GM approaches) and the use of molecular tools allowed the identification of *G. pilosa*, avoiding confusion with *G. glycymeris* and selecting the main features that distinguish them. The tridimensional nature of using the classical method alongside the landmark-based approach allowed measurement of width to length ratio (W/L), which became one of the main discriminant traits, while at the same time pointing out the variations in the umbo region and the ligamental area, which otherwise would have been hidden. Based on our results a set of 7 characters explained nearly 50% of the individual variation that distinguished *G. glycymeris* from *G. pilosa* (Fig 4). The bivariate analyses allowed identifying the morphological meaning of these characters indicating that Mediterranean populations presented a more globose and posterior-ventral elongated shell. UK specimens were the most symmetric population, whereas Pašman was the most asymmetric.

Pašman specimens also showed an allometric growth in other characters such as the height of the ligament to shell height ratio (HL/H) or the length of the ligament to shell length (LL/L). Allometry has a greater effect in larger individuals, usually together with an elongation of the posterior-ventral margin [23]. Accordingly, our results were consistent with these observations since individuals from Pašman, were also the largest specimens. However, glycymerids present a very high individual variation in asymmetry, which might be site or size specific [23]; thus, this character (A/H) should be considered along with others before assuming it distinguishes between species. In the PCA analyses, the ligamental length to shell length ratio (LL/L), the length to height ratio (L/H) and the logAM strongly separated the Mediterranean and the Atlantic populations; however, this separation contrasted with the high correlation of these three variables observed in all populations; thus, they should not be used alone for species distinction. The trend for the length to height ratio was also observed in other studies [20]. The dimyarian condition of *Glycymeris*, characterized by the presence of two adductor muscles, was not a distinctive character for most populations neither in the anterior and posterior adductor muscle dimensions nor the log of the adductor moment. Our results indicated muscle sizes to be constant through ontogeny based on the differences in size as described in other studies (see [23]). LDA confirmed differences among populations and species and also showed the magnitude of the intraspecific variation.

The GM analyses showed that the beaks appeared strongly opisthogyrate, that is, pointing towards the posterior margin and this feature was more evident in *G. pilosa* than in *G. glycymeris*, as previously observed [20,16]. A prosodetic condition of the ligament (ligament lying in front of the beak) was more marked in *G. pilosa* rather than *G. glycymeris* (amphidetic to slightly prosodetic), a feature that wasn't contemplated in the classical approach but it appeared as an outstanding variable in PC 2 (Fig 6). This secondary component of variation is explained by the measurement of adult individuals; prosodetic ligament is a character mostly manifested in juveniles of *G. pilosa*, thus, it could be a weak distinctive character [16]. Our results corroborated this observation and do not support the statement that the ligament condition is a clear feature to distinguish between species, at least, if age is not taken into consideration [18,23].

The use of secondary ribs has been previously used to differentiate between *Glycymeris* sp. [20,59]. These differences were corroborated in our detailed observation on microsculpture, which allowed distinguishing between species; *G. glycymeris* presented a finer sculpture and thus, a higher number of ribs, than *G. pilosa*. Additionally, information from live collected specimens can be useful for a reliable identification of species. Associated with the microsculpture is the distribution of the periostracum, a very distinctive trait from live collected specimens, with a greater length and density of periostracal hairs and a velvety appearance in *G. pilosa* and finer and less hairy in *G. glycymeris*. Of course, periostracum is not preserved in fossils. A complementary morphological feature in living specimens is their coloration. Whereas *G. glycymeris* tend to present reddish zigzag bands over a light yellow to dark brown bottom on the outer shell surface, *G. pilosa* varies from light to dark brown background where zigzag bands are hard to identify [18] (A.Purroy pers. obs.). The interior of the valves is normally paler in *G. glycymeris* whereas *G. pilosa* presents a partial or complete dark brown to violet coloration blotch, covering at least the posterior ventral area. This coloration studied in *G. nummaria* and *G. bimaculata* was reported by Crnčević [60] and Eterović [61] to be caused by endolith activity.

Aiming to enable the application of our morphometric results, the following summarized statements can be of assistance when discriminating between both species. Globosity (W/L) and posterior-ventral elongation—measured as asymmetry—(A/H) were more evident in *G. pilosa* although they should not be stand-alone characters for species discrimination since

allometry and individual variation may have a great effect. Measurements on the length and height of the ligamental area with respect to shell length (LL/L) and height (LA/H) on the first, and to shell height (HL/H) on the latter were not very indicative of differences, except in the population with largest specimens (Pašman) where HL/H and LL/L were less correlated. Further, observations on the ligamental condition showed to be slightly prosodetic in *G. pilosa*, although it is a character mostly manifested in juveniles thus a weak distinctive trait by itself. The strongly opisthogyrate beaks were more evident in *G. pilosa*. In contrast, both adductor muscle dimensions were quite constant through ontogeny and they would not be distinctive. Complementary, when considering both living and well-preserved fossils, looking at the secondary ribs present on the microstructure appeared to be a very reliable one, showing a higher number and a finer sculpture in *G. glycymeris*. In addition, on the periostracum, *G. pilosa* presents a greater length and density of periostracal hairs, quite distinguishable in live-collected specimens. Also, on the outer shell surface, the characteristic reddish zigzag bands over yellow to dark brown bottom from glycymerids, which was observed in *G. glycymeris*, were much lighter or absent in *G. pilosa*. Further, the inner valve presents a brown to violet coloration blotch in most specimens whereas is paler in *G. glycymeris*. Altogether, the main discriminant traits when measured along were the globosity, and the umbo and ligamental areas. Clearly, a well-preserved shell increases the confidence in discriminating between species.

The congruent patterns of mitochondrial and nuclear phylogenetic reconstructions indicated the separation of the two co-existing species, *G. glycymeris* and *G. pilosa*, with a 100% bootstrap support and 99% posterior probability. Such a conclusion is accompanied with the high divergence rate (11%) observed between two mtDNA lineages. Also, it has been seen that the COI marker is more informative for the phylogeny of both higher-level and closely related *Glycymeris* species, whereas the ITS2 showed less resolving reconstruction for closely related species such as *G. glycymeris* and *G. bimaculata*. Due to the slower evolution rate of nuclear genes, a considerable structure can appear in mitochondrial genes before concurrent changes occur in nDNA [62]. Interestingly, the COI phylogenetic reconstruction revealed that *G. bimaculata* from Mediterranean Sea clustered together with *G. glycymeris* from Atlantic Sea, with a later divergence in comparison to *G. pilosa*. This interesting evolutionary scenario, not supported by morphospecies characteristics in our study, should be investigated in detail to confirm *G. bimaculata* species-specific distinctness.

Both *G. glycymeris* and *G. pilosa* showed absence of phylogenetic structure, i.e. the most frequently observed haplotypes occupied all sampling locations. With regards to *G. glycymeris*, the most common haplotypes (H8 and H10) that were found both in UK and France populations may represent the ancient haplotypes that probably evolved when the English Channel was just a river (Channel River). At that time (ca. 21 000 BP), Britain and Ireland were part of continental Europe, partly covered by the Fennoscandian ice sheet [63]. According to the divergence time estimation applied on mtDNA, the speciation process of *G. glycymeris* and *G. pilosa* started to occur in the Eocene, during a time of warm climate, while the accelerated intraspecies divergence occurred during the late Pliocene and Pleistocene. During the late Miocene and early Pliocene, the Mediterranean experienced dramatic palaeoceanographic events, a more restricted system with warmer temperatures was delimited with higher salinity and nutrient impoverishment of the marine environment [64–65]. The desiccation during the Messinian Salinity Crisis (MSC) ~5.6–5.3 million years ago caused a major extinction of the Mediterranean marine fauna. A new environmental setting with open-marine conditions (temperate waters and high productivity), with the opening of the Strait of Gibraltar, favored the introduction of species of Atlantic origin, known as Boreal Guests [64,66–67]. Fossil *Glycymeris* sp. shells coming from the Early Pleistocene deposits cropping out in the Arda River section (Castell'Arquato Formation, Italy), have been found to resemble recent specimens of *G. glycymeris* from Brittany (France) in

shell ultrastructure comparisons. This finding confirms their introduction during this period and suggest that it didn't change much during the last 2 million years [11], supporting the high conservatism of glycymerids [23]. Other fossil malacofauna confirmed the existence of *G. glycymeris* in Pliocene sediments [45,68–69] and during the Pleistocene [10].

The diversity of *Glycymeris* species in the Mediterranean includes *G. glycymeris*, *G. pilosa*, *G. bimaculata* and *G. nummaria* (known in the past as *G. violacescens* (Lamarck, 1819) and as *G. insubrica* (Brocchi, 1814), the latter as a Pliocene fossil). Whereas both *G. glycymeris* and *G. pilosa* coexist in the western Mediterranean, there is still uncertainty regarding the eastern basin [15] indicating Sicily as a possible geographical barrier for *G. glycymeris* [16]. The presence of this or both species in the eastern Mediterranean has led to misleading citations of *G. pilosa* as *G. glycymeris* throughout time (e.g., [12,70]) making it unclear whether *G. glycymeris* can be found in the eastern Mediterranean Sea. Although the collection of living specimens of *G. glycymeris* in the western Mediterranean has not been possible for this study, there is evidence of its presence (recorded captures, collections and personal communications) on the coasts of Spain, France and Italy [16] (Natural History Museum Collection in Milan, R. Chello pers. comm., S. Giacobbe pers. comm.). The only citation of *G. glycymeris* in the eastern Mediterranean corresponds to the Hellenic seas [71]; however, it could not be confirmed. Traditionally, the distribution of shallow marine organisms has been affected by abiotic factors such as salinity and temperature. The field settings of the present study represent two different environments. The Atlantic populations of *G. glycymeris* live in deep waters (approx. 20 m) under strong current regimes within sand and occasionally muddy areas [26]. Mediterranean populations of *Glycymeris pilosa*, come from shallower, intermittently calm waters (between 3–6 m), within a sediment layer of coarse skeletal sand with occasionally shell gravel on a limestone platform, characteristic of the Adriatic coast. Their presence in these kinds of habitats may be explained as an advantage to colonize nutrient-limited habitats due to their free-burrowing mobility and it is this ability that makes Glycymerid species opportunistic [23,72]. Alike, these opportunistic animals may appear or disappear from a specific area due to undetermined factors [10], such as fluctuations in food supply [65].

The expansion of *G. pilosa* across the entire Mediterranean more progressively than *G. glycymeris*, could be explained by a higher adaptive capacity to environmental changes of the first, and a lack of adaptation to unfavorable ecological changes on the latter, which can ultimately cause the extinction or near extinction of a species [63]. This evidence is supported by the potential absence of *G. glycymeris* in the Eastern Basin.

The presence of *G. pilosa* as the only living species of the Glycymerididae in Israel, surrounded by fossil shells of *G. nummaria* [73], supports the evidence that *G. pilosa* might be more robust to environmental changes, persisting along the Eastern Mediterranean. This robust nature is reflected in the higher level of intraspecific genetic divergence in *G. pilosa* Mediterranean immigrants (COI, 1.1%) compared to the *G. glycymeris* from Atlantic (COI, 0.4%). Such a feature has already been recognized in mammals and birds where environmental harshness (lower primary productivity, decreased rainfall and more variable and unpredictable temperatures) is positively correlated with intraspecific divergence [74].

This assumption cannot be confirmed despite our efforts in collecting living specimens of *G. glycymeris* in the Western basin, mainly because the scarce presence of individuals (based on previously cited pers. comm.) and its non-commercial nature that hampers its access at fish markets.

To gain a better understanding in the introduction and expansion of *Glycymeris* in the Mediterranean or even to compile all European glycymerids, further studies on fossil shells to build hypothetical divergent pathways would be very interesting. Additionally, revising existing records in Museum collections is encouraged to identify potential misleading identifications.

The Mediterranean Sea has been assessed for potential impacts on marine biodiversity, indicating the central Adriatic as one of the areas with major cumulative threats to invertebrate species [75]. Since Marine Protected Areas (MPAs) are still scarce in the Mediterranean, delimiting species with functional or evolutionary traits is critical to identify priority areas for marine biodiversity protection [76]. The present study provides the basis for the correct identification of an invertebrate species with high potential for future ecological and sclerochronological studies in an area under high anthropogenic threat.

Conclusions

The present study resolves the misleading identification of *G. pilosa* as *G. glycymeris* through the combined use of morphological (classic and landmark-based) and genetic tools. It also sets a guideline for the correct identification of these species. *Glycymeris pilosa* presents a more globose shape and tends to be more asymmetric than *G. glycymeris*. Microsculpture provides robust information for the identification of the studied species. The mitochondrial and nuclear genes proved the genetic distance between species; the COI marker appeared to be more informative for the phylogeny of both higher-level and closely related *Glycymeris* species than the ITS2. The molecular clock showed that the ancient clades leading to *G. glycymeris* and *G. pilosa* diverged during the Eocene and based on our results, their coexistence in the Mediterranean could have been driven by geological events such as the Messinian Salinity Crisis. Climatic and biotic changes influenced the expansion of both species throughout the Mediterranean favouring *G. pilosa*—a more tolerant species and better adapted to unfavorable changes. The outcome of this study sets the baseline for future studies on palaeoenvironmental archives in the Mediterranean.

Supporting Information

S1 Fig. LDA biplot of the previous PCA. Symbols are assigned to each population: *Glycymeris glycymeris* (black inverted triangle, UK; grey inverted triangle, France) and *Glycymeris pilosa* (black circle, Pag; grey circle, Pašman).
(PNG)

S2 Fig. Bayesian posterior probabilities and bootstrap support for MrBayes and Maximum Likelihood analyses. Illustration of tree topology based on ITS haplotypes of *G. glycymeris* (Gg), *G. pilosa* (Gp), *G. nummaria* (Gn) and *G. bimaculata* (Gb). Posterior probabilities followed by bootstrap values are included at the nodes. The origin of the haplotypes (H) is indicated as follow: UK, United Kingdom; FR, France; AD, Adriatic Sea; AT, Atlantic Sea; PA, Pag; PS, Pašman.
(TIF)

S3 Fig. Evolutionary time tree of Glycymeridae relationships inferred from Bayesian inference analyses with BEAST of COI gene. Text adjacent to selected nodes indicates median ages. Blue bars indicate 95% highest posterior density intervals for nodes of interest. Text below selected nodes indicates posterior probabilities.
(TIF)

S1 Table. Other material of *Glycymeris pilosa* and *Glycymeris glycymeris* from National Museum of Wales collection used for rib count measurements.
(DOC)

S2 Table. Number of COI and ITS2 haplotypes obtained in each population of *G. glycymeris* and *G. pilosa*. Pop 1 in *G. glycymeris* stands for population sampled in United Kingdom,

while Pop 2 stands for population sampled in France. For *G. pilosa* Pop 1 was sampled near the Island of Pag and Pop 2 in vicinity of the Island of Pašman, both in the Adriatic Sea. (DOC)

S3 Table. Descriptive statistics of genetic diversity and demographic history of *Glycymeris glycymeris* and *Glycymeris pilosa*, based on COI and ITS2 sequence data. N is number of analysed sequences, h is haplotype diversity (\pm SD), π is nucleotide diversity (\pm SD) and S is number of segregating sites. (DOC)

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**CONSTRUCTION OF LONG ANNUALLY-RESOLVED SHELL-BASED CHRONOLOGIES
USING *GLYCYMERIS GLYCYMERIS* (BIVALVIA; GLYCYMERIDIDAE) FROM THE BAY OF BREST,
FRANCE**

ABSTRACT

This PhD thesis uses *Glycymeris glycymeris*, a temperate bivalve in the Glycymerididae family, to reconstruct environmental variability in the Bay of Brest, France. This work comprises of geochemical data archived within shells, compared with several biological and ecological variables. The aims of this study were to assess for potential proxies and expand environmental knowledge prior to local observational records.

Thirty-eight live- and dead-collected specimens were used to create a chronology spanning 1891 to 2014. When the standardised growth index was correlated with local environmental factors, it showed that the predominant drivers of growth were Elorn inflow, salinity and suspended particulate matter.

Isotopic analysis was undertaken on the juvenile increments of 24 shells. Our results highlight that variations in $\delta^{18}\text{O}_{\text{aragonite}}$ accurately record local sea surface temperatures, leading to the creation of a 45-year SST reconstruction for the Bay of Brest. Correlations between this and climate forcings show that SSTs in this area are controlled by the subpolar gyre and the East Atlantic Pattern.

Five shells (three under the age of 10 and two aged 45) were analysed using a LA-ICP-MS to determine the quantity of strontium and magnesium. Mg and Sr intensities are positively correlated with temperature sub-annually, but on an annual scale there is a negative correlation.

This study conveys the potential of *G. glycymeris* as an archive of climatic and environmental variability in the Bay of Brest. As there are a large number of sub-fossil specimens in the area, this study also provides an anchored base for expansion further back in time.

Keywords: sclerochronology - bivalves - Glycymeris - shell growth - stable isotopes - trace elements

**CONSTRUCTION DE CHRONOLOGIES PLURI-DECENNALES A RESOLUTION ANNUELLE A PARTIR DE
COQUILLES DE *GLYCYMERIS GLYCYMERIS* (BIVALVIA; GLYCYMERIDIDAE) DE LA RADE DE BREST**

RESUME

Ce doctorat repose sur l'utilisation de *Glycymeris glycymeris*, un bivalve de la famille des Glycymerididae, pour reconstruire la variabilité environnementale en rade de Brest (France). Il vise à comparer des données géochimiques archivées dans ces coquilles avec diverses variables biologiques et écologiques. Les objectifs sont d'identifier de potentiels proxys et d'étendre notre connaissance de la variabilité environnementale avant les premières mesures instrumentales.

Au total, 38 spécimens (vivants ou morts) ont été utilisés pour construire une chronologie s'étendant de 1891 à 2014. Les indices de croissance standardisés suggèrent que la croissance coquillière est principalement influencée par le débit de l'Elorn, la salinité et la matière particulaire en suspension.

Des analyses isotopiques, réalisées sur les portions juvéniles de 24 spécimens, mettent en évidence que les variations de $\delta^{18}\text{O}_{\text{aragonite}}$ enregistrent fidèlement la température de surface, permettant sa reconstruction sur 45 ans en rade de Brest. En terme de forçage climatique, ces variations de température en rade semblent contrôlées par le gyre subpolaire et l'East Atlantic Pattern.

Cinq coquilles (3 d'âge inférieur à 10 ans et 2 âgées de 45 ans) ont ensuite été analysées pour leurs concentrations en strontium et magnésium par LA-ICP-MS. Les concentrations en Mg et Sr, quant à elles, sont positivement corrélées à la température de l'eau à une échelle sub-annuelle, mais négativement à l'échelle annuelle.

Cette étude met en lumière le potentiel de *G. glycymeris* comme archive de la variabilité climatique et environnementale en rade de Brest. Du fait du nombre important de spécimens sub-fossiles en rade, cette thèse fournit une base de travail pour l'extension de ces reconstructions dans un passé encore plus lointain.

Mots-clés : sclérochronologie - bivalves - Glycymeris - croissance coquillière - isotopes stables - éléments traces