Diversité génétique et phénotypique de populations naturelles ou ensemencées de coquille Saint-Jacques Pecten maximus

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Table des matières

Liste des figures & tabeaux

Avant propos

L'objectif initial de ma thèse était d'explorer les composantes génétiques d'un caractère d'intérêt, la croissance, chez la coquille Saint-Jacques *Pecten maximus* (L. 1758). Cette problématique a été abordée sous deux aspects : une étude de génétique des populations et une étude de génétique quantitative. L'étude de génétique des populations visait à déterminer la structuration génétique spatiale et présumée neutre des populations à large échelle, pour notamment la comparer avec les études phénotypiques existantes sur les patrons de croissance des populations. Parallèlement, pour étudier les composantes génétiques de la croissance, une étude de génétique quantitative, en conditions contrôlées, a été menée en utilisant une production d'écloserie. Les productions d'écloserie sont généralement mise en œuvre à partir d'un nombre réduit de géniteurs ; elles conduisent donc à un faible nombre de familles réduit en comparaison des populations naturelles mais en à effectifs substantiels. Leur impact génétique potentiel en soutien aux populations de la Rade de Brest, a ainsi été étudié. L'introduction générale de ma thèse traitera tout d'abord de la biologie générale de la coquille Saint-Jacques, en ciblant la biologie des populations et la différenciation à l'échelle européenne pour différents des traits de vie, dont la croissance. Dans une deuxième partie, les principes de la génétique de populations seront rappelés, ainsi que l'état des connaissances sur la structure génétique des populations de coquille Saint-Jacques. Dans une troisième partie, les principes de l'approche de génétique quantitative pour évaluer la part génétique d'un trait phénotypique seront présentés, ainsi que leurs moyens d'applications dans le cadre de cette thèse. Enfin, pour terminer, les implications de l'aquaculture des bivalves, et particulièrement des pectinidés, sur la diversité et la structure génétique des populations ensemencées seront présentées.

Chapitre 1

Introduction générale

1.1-Biologie générale

1.1.1 Aire de répartition

La coquille Saint-Jacques est un mollusque bivalve filtreur de la famille des pectinidés. Elle vit sur des fonds sablo-vaseux, et est répartie du nord de la Norvège au nord du Maroc (Fig 1, FAO 2015). Sa distribution n'est pas homogène sur son aire de réartition bien que relativement continue, les coquilles se regroupant en zones de densité plus forte (Shumway & Parsons 2011) séparés par des zones de faible densité. Son habitat optimal se situe entre 5 et 40m de profondeur, mais des coquilles Saint-Jacques ont été trouvées dans des zones beaucoup plus profondes (jusqu'à 100-300m de profondeur) (Shumway & Parsons 2011). Une espèce proche, *Pecten jacobaeus*, a été décrite principalement en Mer Méditerranée (Rombouts 1991).

Figure 1: Aire de répartition de la coquille Saint-Jacques (*Pecten maximus*). FAO, 2015

1.1.2 Cycle de vie

La coquille Saint-Jacques est une espèce à cycle de vie bentho-pélagique (Fig 2). Comme pour la plupart des bivalves, la libération des gamètes et la fécondation se font en pleine eau. Les larves pélagiques dérivent avec les courants pendant 20 à 40 jours en moyenne, en fonction de la température de l'eau (Beaumont & Barnes 1992, Le Pennec *et al.* 2003,). Suite à la métamorphose, les larves se posent sur le fond. Elles développent un byssus pour se fixer au substrat, mais peuvent aussi utiliser ce byssus pour se déplacer avec le courant, un phénomène appelé le *« byssus-drifting »* (Beaumont & Barnes 1992). Les adultes sont généralement localisés sur des fonds sablo-vaseux. Contrairement à d'autres bivalves (huîtres, moules, pétoncles) qui sont fixés au substrat, la coquille Saint-Jacques vit posée sur le fond, à demi-enfouie. Elle peut, en réponse à un stress (par exemple, un prédateur), avoir un comportement de fuite. Ces mouvements sont très coûteux en énergie (Robson *et al*. 2011) et restent peu fréquents. Des estimations ont montrées qu'une coquille Saint-Jacques pouvait se déplacer de 3-10m durant sa vie benthique (Shumway & Parsons, 2011). Il est donc raisonnable de conclure que les phénomènes de migration entre populations se font principalement lors de la phase larvaire et très peu lors de la phase benthique.

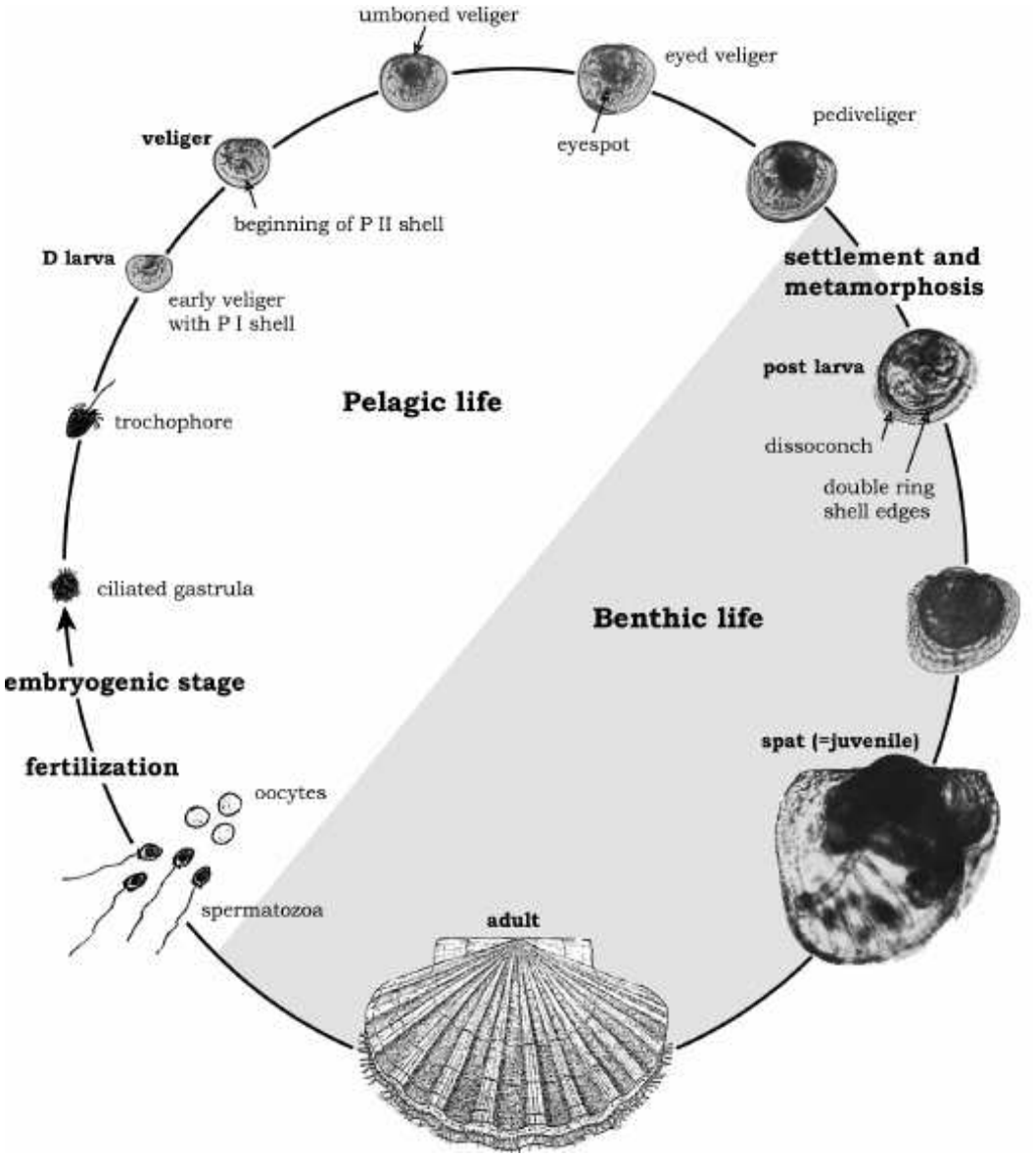


Figure 2 : Cycle de vie de la coquille Saint-Jacques, d'après Le Pennec *et al.* 2003.

1.1.3 Croissance

La coquille Saint-Jacques, comme beaucoup de bivalves, incrémente sa coquille avec une périodicité régulière, tout au long de sa durée de vie. Elle ne grandit pas toute l'année ; l'hiver, sa croissance est fortement ralentie, jusqu'à devenir quasi-nulle, formant ainsi une marque appelée « anneau hivernal » sur sa coquille. Il est donc ainsi possible de lire l'âge des coquilles en comptant leurs anneaux hivernaux (Mason 1957). La saison de croissance recommence aux alentours de début avril, avec une forte variabilité inter-individuelle, qui jusqu'à ce jour n'est pas expliquée. Antoine (1979) a démontré la périodicité journalière de la formation de la coquille ; une strie, observable directement sous loupe binoculaire, est formée par jour (Fig 3). La taille de cette strie est directement reliée aux conditions environnementales du jour de sa formation (Chauvaud *et al*. 1998, Lorrain *et al*. 2000). C'est pourquoi les valves de coquilles Saint-Jacques sont utilisées comme enregistreurs haute fréquences des paléoclimats (Chauvaud *et al.* 2005). Il est possible, par des analyses de croissance journalière mais aussi par des analyses isotopiques, de reconstituer les conditions du milieu dans lesquelles une strie donnée s'est formée (Chauvaud *et al*. 2011, Jolivet *et al*. 2015, Marchais *et al*. 2015).

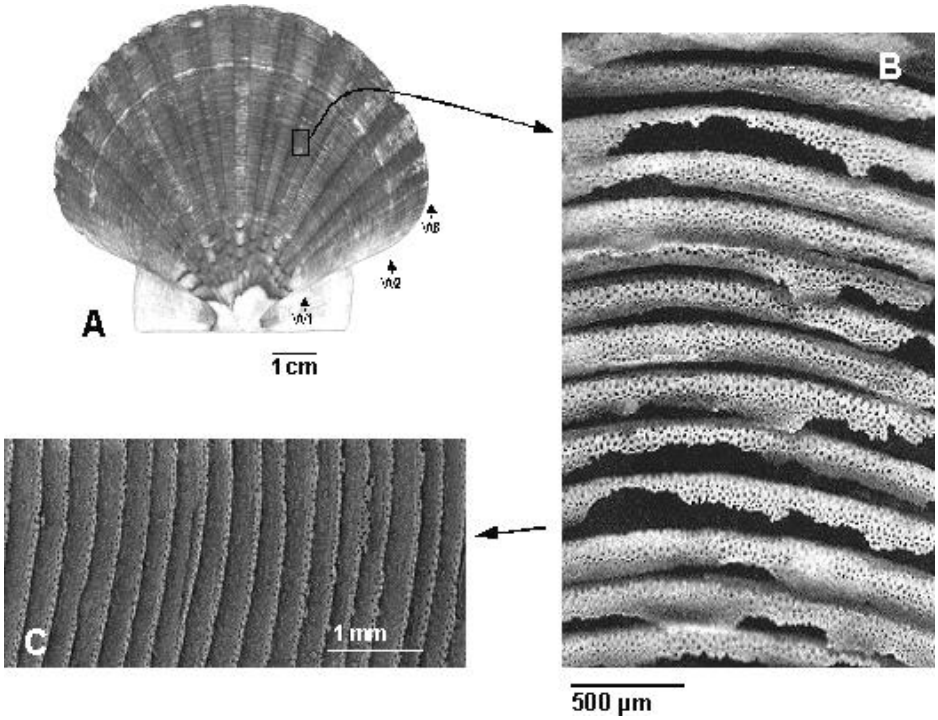


Figure 3 : Illustration des stries de croissance journalières chez *P. maximus,* d'après Chauvaud *et al.* 1998. A : Valve gauche d'un individu âgé de 2 à 3 ans. B: Agrandissement d'une partie de la coquille à la loupe binoculaire, sans traitement. C: Photographie après traitement à l'acide acétique, pour mettre en évidence et mesurer les stries journalières de croissance (Chauvaud *et al.* 1998)

1.1.4 Variabilité phénotypique des populations de coquilles Saint-Jacques

Il existe une variabilité significative des paramètres de croissance de la coquille au sein des populations de coquille Saint-Jacques. Chauvaud *et al*. 2012 ont ainsi montré qu'il existait des différences fortes entres des populations réparties sur l'aire de distribution de l'espèce (Fig. 4). Les populations septentrionales (norvégiennes, écossaises) présentent un taux de croissance plus faible mais une taille asymptotique plus élevée, tandis que les populations méridionales (sud-britanniques, françaises, ibériques) présentent un taux de croissance plus élevée et une taille asymptotique plus faible. Cette observation semble en accord avec la règle de Bergmann-Heisse (Bergmann 1847, Hesse *et al.* 1937, McNab & Brian 1971) qui prévoit cette variation de taille et de croissance suivant la latitude Cependant, les stratégies de croissance des populations de coquilles Saint-Jacques n'évoluent pas de façon continue sur le gradient latitudinal ; il existe en fait une discontinuité entre les deux principaux patterns de croissance en Nord Écosse et à l'entrée de la Mer du Nord (Chauvaud *et al*. 2012). Cette forte différenciation phénotypique est mal expliquée par les paramètres environnementaux, et des hypothèses de divergences génétiques de populations ont été suggérées.

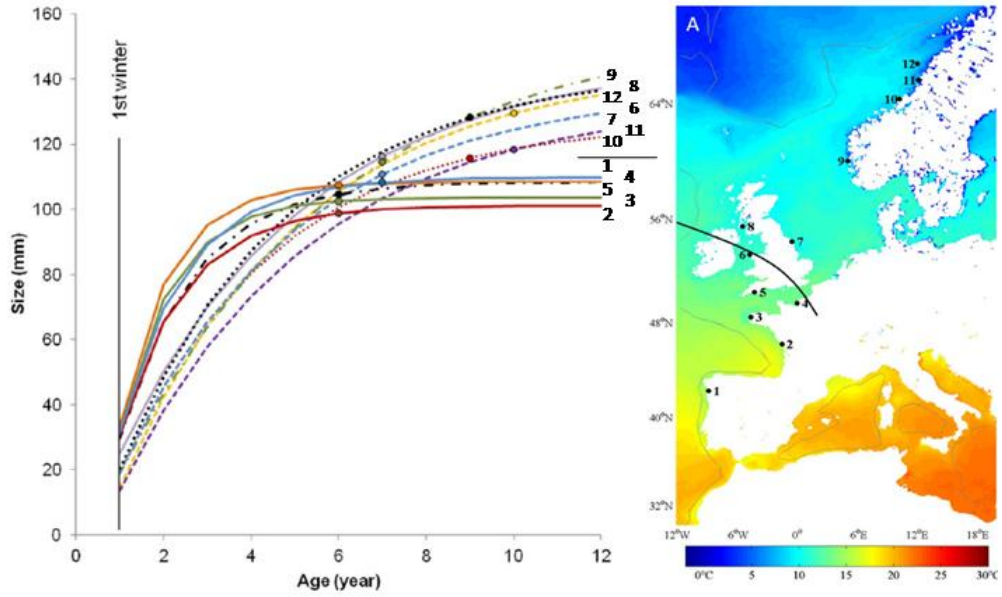


Figure 4 : Trajectoires de croissance de 12 populations de Coquilles Saint-Jacques représentées sur la carte de droite. Fond de carte: température moyenne de surface de la mer. Traits discontinus: populations septentrionales (nord de la limite sur la carte). Traits continus: population méridionales (Sud de la limite sur la carte). Modifiée d'après Chauvaud *et al.* 2012.

La croissance n'est pas le seul caractère phénotypique qui peut différencierles populations de Coquille Saint-Jacques dans son aire de répartition. La stratégie de reproduction varie également. Paulet *et al.* (1988) ont montré qu'il existait deux stratégies de reproduction clairement distinctes entre deux populations géographiquement proches: la Rade de Brest et la baie de Saint-Brieuc. La population de la baie de Saint-Brieuc effectue sa gamétogenèse pendant le printemps, puis effectue une ponte principale début juillet, relativement synchrone entre tous les individus. Suivant la quantité d'énergie stockée pendant la phase de maturation, d'autres évènements de pontes, moins importants peuvent survenir. En Rade de Brest, les individus peuvent être matures tout au long de l'année et il n'y a pas de synchronisme des évènements de ponte, qui peuvent avoir lieu de Mai à Septembre.

Des différences similaires de stratégies reproductrices ont été observées entre populations de l'île de Man -en Mer d'Irlande (Hold *et al.* 2013), et populations norvégiennes (Magnesen & Christophersen 2008) d'une part, et entre des

populations britanniques et françaises d'autre part (Mackie & Ansell 1993, Cochard & Devauchelle 1993). Des expériences de translocations de populations ont montré que les individus gardaient la stratégie reproductrice de leur population d'origine, indiquant une potentielle contrainte génétique ou un conditionnement environnemental précoce (Mackie & Ansell, 1993, Cochard & Devauchelle 1993, Magnesen & Christophersen 2008).

Enfin, Artigaud *et al*. (2014) ont mis en évidence des différences phénotypiques entre des spécimes issus d'une population française et d'une population norvégienne en terme duprotéome du manteau. Une fois de plus, l'hypothèse d'une base génétique est évoquée en éventuel complément de l'effet de conditions environnementales différentes.

1.1.5 Plasticité ou adaptation ?

Des différences phénotypiques significatives ont donc été mises en évidence entre populations de coquille Saint-Jacques à différentes échelles. Les différences phénotypiques observées résultent-elles d'une plasticité phénotypique des individus en réponse à leur environnement, et/ou de différences ancrée dans le patrimoine génétique ? Quelle est la part relative de l'environnement par rapport aux gènes ? Quelques études ont tenté de répondre à ces questions, notamment par des translocations de populations (Mackie & Ansell, 1993, Cochard & Devauchelle 1993), ou des observations en environnement commun (Magnesen & Christophersen 2008). Les individus étudiés ont gardé les caractéristiques de leur population d'origine, ce qui témoignerait plutôt d'une adaptation génétique, au moins pour les caractères liés à la reproduction. Cependant, d'autres explications sont possibles : le conditionnement métabolique précoce et l'épigénétique. Il est possible en effet que les individus, nés dans un milieu donné, soient conditionnés

métaboliquement par ce milieu lors des stades de vie précoces, et en garde ainsi les caractéristiques phénotypiques au cours de leur vie sans que cela soit inscrit dans leur patrimoine génétique. Des études sont donc nécessaires pour étudier les éventuelles bases génétiques de cette différenciation phénotypique entre les populations. La différence entre plasticité phénotypique et adaptation est souvent difficile à mettre en évidence, et demande des expérimentations parfois longues et coûteuses (Kawecki & Ebert 2004). L'objectif de cette thèse n'était pas d'aborder directement ces questions, mais d'apporter des premières informations sur les bases génétiques de différences phénotypiques entre individus de la Rade de Brest. La première approche possible est de mettre en évidence des différences génétiques entre les populations. Nous allons donc explorer, dans un premier temps dans cette thèse, différentes questions : les populations de coquille Saint-Jacques sont-elles génétiquement différentes ? Si oui, quelles sont les forces évolutives qui ont façonnées ces populations actuelles ? Ces problématiques seront abordées par une étude de génétique de populations.

1.2 Génétique des populations de coquille Saint-Jacques

1.2.1 Introduction à la génétique de populations

La génétique de populations est une discipline qui s'intéresse à la distribution de la variabilité génétique au sein des populations, ainsi qu'à leur niveau de différenciation génétique. L'histoire évolutive des populations est inscrite dans leur patrimoine génétique. Il est possible, en utilisant les marqueurs génétiques appropriés, de reconstituer les évènements démographiques, de migration - colonisation, qui ont conduit à la diversité génétique actuelle des populations. Celle-ci est façonnée par quatre forces évolutives majeures : la mutation, la migration, la dérive génétique et enfin la sélection.

La mutation

La mutation est la force évolutive créatrice de diversité génétique à l'échelle moléculaire. Elle se définit comme un changement aléatoire dans l'ADN d'un organisme, souvent produit par des erreurs durant la réplication de l'ADN, qui va être transmis à sa descendance. Chaque mutation créée donc un nouveau variant (appelé allèle) pour un locus donné dans le génome. Il existe de nombreux types de mutations possibles, ayant des effets divers. La plupart des mutations sont dites silencieuses, car elles n'induisent pas de changement d'acide aminé dans les zones codantes ou concernent des zones non codantes du génome. Certaines mutations apparaissent dans des régions codantes et modifient l'expression d'un gène, d'un régulateur de transcription, d'un réseau génique, ou encore modifie directement la structure de la protéine codée par un gène donné. Ces mutations peuvent entrainer un changement phénotypique, le plus souvent délétère, mais parfois avantageux dans un environnement donné. L'apparition d'une mutation et sa propagation dans une population naturelle sont considérés comme un phénomène rare.

La sélection naturelle

La sélection naturelle a été mise en évidence par Darwin, en 1859. Elle est définie comme l'avantage reproducteur qu'on certains d'individus phénotypiquement différents ; cette variation phénotypique étant due à différents patrimoines génétiques. C'est la sélection naturelle qui va permettre à un allèle avantageux de se propager dans une population. Imaginons qu'une mutation non silencieuse apparaisse dans un individu, lui conférant un phénotype plus adapté à un environnement donné ; il va pouvoir se reproduire avec un succès relatif plus élevé que les autres dans sa population, augmentant ainsi la fréquence de cet allèle dans la génération suivante. À l'inverse, un allèle délétère va voir sa fréquence diminuer au sein de la population. A terme, les allèles sélectionnés s'accumulent et peuvent conduire : (1) à des adaptations locales au sein des populations naturelles exposées à des environnements différents, (2) s'il y a isolement reproducteur entre les populations, à une spéciation.

La sélection, agissant sur les phénotypes, n'affecte donc théoriquement que la fréquence des allèles sur les locus non neutres. Il est cependant possible qu'un locus neutre subisse indirectement l'effet de la sélection, par le phénomène d'auto-stop génétique (Ferguson 1994). Si un locus neutre est physiquement proche d'un locus soumis à sélection dans le génome, la ségrégation mendélienne lors de la méiose n'est pas complètement aléatoire, car deux locus proches ont moins de probabilités d'être séparés par recombinaison chromosomique. Les variations de fréquences des allèles d'un locus vont donc être liées à celle du locus sous sélection, il apparait alors un déséquilibre de liaison et un locus supposément neutre se comporte comme répondant à des forces sélectives.

La dérive génétique

La dérive génétique se définit comme la variation aléatoire des fréquences alléliques et génotypiques d'une population, au cours d'une même génération si la taille de celle-ci varie pour cause de mortalités ou entre générations. Les sources de ces variations peuvent être multiples, mais ne sont pas liées à la sélection naturelle des individus. Une des principales sources de variations des fréquences alléliques est une taille efficace réduite. La taille efficace d'une population se définit comme la taille qu'aurait une population idéale se reproduisant sous l'équilibre de Hardy-Weinberg et donnant la même structure génétique de descendance que celle observée. En simplifiant, on peut assimiler la taille efficace au nombre d'individus ayant une descendance viable et un succès reproducteur équivalent à la génération suivante (voir partie 4 de l'introduction pour un développement sur la taille efficace des populations). Si ce nombre est petit, il est possible (et statistiquement plus probable) que les porteurs d'allèles rares n'ai pas de descendance et donc que ces allèles soient perdus à la génération suivante. Plus généralement, une taille efficace faible et donc une dérive génétique forte se traduisent par une variation aléatoire importante des fréquences alléliques au cours des générations successives. Cette variation sera rapide si la taille efficace est petite, et lente si la taille efficace est grande. La dérive génétique est donc un phénomène stochastique propre à chaque population, et peut donc conduire à plus ou moins long terme, à une différenciation génétique neutre (i.e. non adaptative) entre des populations isolées.

La migration

La migration est une force évolutive antagoniste à celle de la dérive. Elle se produit quand un ou des individus (ou leurs gamètes) migrent d'une population vers

une autre, et participent à en suite à la reproduction dans cette population, en y apportant donc leur patrimoine génétique. Si le taux de migration est élevé entre deux populations, leurs fréquences alléliques tendent à s'homogénéiser, et donc la dérive génétique ne peut pas produire de différenciation génétique très marquée. Au contraire, si les flux de gènes son très faibles entre les populations, elles vont alors progressivement diverger sous l'effet de la dérive génétique.

1.2.2 État des connaissances en génétique de populations sur la Coquille Saint-Jacques

Globalement, pour une espèce d'intérêt scientifique et économique, peu d'études de génétiques de populations ont été menées sur la coquille Saint-Jacques. Les quelques études disponibles ont montré une diversité génétique très élevée, mais n'ont pas mis en évidence une structuration des populations le long des côtes Britanniques et françaises, quel que soit le type de marqueur génétique utilisé : allozymes (Beaumont, 1993), ADN mitochondrial (Wilding *et al.* 1997, Rigaa *et al.* 1997, Heipel *et al.* 1998, Ridgway & Dahle 2000), ou microsatellites (Hold 2012). Cependant, quelques populations se différencient, comme Mulroy Bay, en lien potentiel avec des caractéristiques hydrodynamiques très spécifiques (Wilding *et al.* 1997, Hold 2012). Globalement les travaux en génétique de populations ne soutiennent pas les différences phénotypiques et les expériences de translocations présentées dans la première partie de cette introduction.

A l'échelle de la distribution de l'espèce, des indices d'une structuration plus forte entre la Norvège et les populations atlantiques (c'est à dire anglaises, françaises et ibériennes) ont été trouvés, tant avec des marqueurs mitochondriaux (Test de khi-carré sur les fréquences alléliques, $\chi^2=96,5$; $p < 0.002$ Ridgway &

Dahle 2000) que des microsatellites (F_{ST}=0,038 – 0,070 entre la population norvégienne et les autres populations, Hold 2012). Cependant, ces études impliquaient un nombre faible de populations norvégiennes (deux et une, respectivement), ne permettant pas de conclure sur la structuration à l'échelle de la distribution de la coquille Saint-Jacques, ni sur le possible lien entre cette structure et les différenciations phénotypiques observées à cette échelle.

D'autre part, a été décrite en Méditerranée une espèce proche de *P. maximus*, *Pecten jacobaeus* (L. 1758), différenciée phénotypiquement particulièrement au niveau de sa valve gauche, avec des crêtes plus anguleuses et marquées (Rombouts 1991). Le statut taxonomique de *P. jacobeus* reste cependant incertain, certains auteurs considèrant ces deux taxons comme une seule espèce car leur différenciation génétique est plus faible que celle attendue entre deux espèces différentes (Wilding *et al.* 1999, Saavedra & Pena 2004, 2005). La limite entre les deux espèces se situerait le long du front Almeria-Oran (Rios *et al*. 2002). Des expériences d'hybridation ont été menées sous conditions expérimentales, avec succès (Wilding *et al*. 1999, Huelvan, 1985 ; Cochard, résultats non publiés). Les hybrides semblaient viables (taux de métamorphose et de croissance similaires) et présentaient une forme de la coquille et de ses crêtes intermédiaire entre les deux espèces. Cependant, l'expérience n'a pas pu être menée jusqu'à maturité sexuelle des hybrides, pour des raisons techniques. Ces résultats, bien qu'incomplets, confortent l'hypothèse d'une seule et même espèce, mais aucune étude complète avec des marqueurs génétiques pertinents et un échantillonnage suffisant reste à entreprendre.

1.3 La détermination des composantes génétiques de trait : génétique quantitative

1.3.1 Introduction à la génétique quantitative

L'une des approches pour déterminer les composantes génétiques de traits phénotypiques est la génétique quantitative. C'est un domaine d'étude qui trouve des applications dans des disciplines variées : l'agronomie (Gallais & Poly, 1990), l'aquaculture (Gjedrem 1983) ou l'écologie (Wilson *et al*. 2010). Son principe est de déterminer les composantes génétiques et environnementales de la variance phénotypique ; autrement dit, il s'agit de déterminer quelle est la part de la variance d'un phénotype expliquée par la variance génétique entre les individus. Elle se base sur la théorie qui propose que les traits quantitatifs sont contrôlés par une multitude de gènes à effets faibles, qui vont chacun avoir un effet mineur et possiblement (mais pas nécessairement) additif sur le phénotype (Falconer *et al.* 1996, Lynch & Walsh 1998). Il est donc possible d'évaluer l'effet de cet ensemble de gènes inconnus en comparant les phénotypes d'individus plus ou moins apparentés. En effet, si les variations d'un trait est fortement déterminé génétiquement, la variance de ce phénotype sera moins forte entre des individus apparentés (frères, demi-frères…) qu'entre deux individus non apparentés. En termes de variance, la variance totale du phénotype est la somme de la variance génétique, de la variance environnementale et de la variance due à l'interaction entre génétique et environnement. La variance génétique se décompose elle-même en variance génétique additive, en variance due aux effets de dominance et en variance due aux effets épistatiques. Le premier terme nous intéresse particulièrement puisque les effets dus aux gènes additifs sont héritables alors que les autres (dominance et

épistasie) ne le sont pas. Ainsi, en effectuant une analyse de variance, il est possible de déterminer les composantes de variances imputables aux effets génétiques additifs σ_a^2 parmi la variance totale σ_T^2 du phénotype (Falconer *et al.* 1996, Lynch & Walsh 1998).

Héritabilité

La proportion de variance génétique additive parmi la variance totale $\frac{\sigma_a^2}{\sigma_T^2}$ est appelé héritabilité « sens strict ». La proportion de variance génétique totale parmi la variance totale est appelé héritabilité « sens large ». C'est un rapport qui varie entre 0 et 1. Plus l'héritabilité est proche de 1, et plus la variabilité du trait est déterminée génétiquement, dans le sens ou le phénotype d'un individu est prévisible si on connait le phénotype de ses parents (en environnement constant entre ces deux générations). Les analyses de variance de type modèle linéaire mixte (généralement connues sous le nom de modèle animal) utilisée pour calculer cette héritabilité permettent donc de calculer des valeurs reproductives (« *breeding values* ») des individus et de leurs parents. Ces valeurs reproductives sont, pour un géniteur donné et un phénotype donné, l'écart à la moyenne populationnelle du phénotype conféré par le patrimoine génétique de ce parent à ses descendants. Les descendants d'un couple de parents à valeurs reproductrices élevées par rapport à la population totale seront donc plus élevées pour le phénotype mesuré, dans un environnement constant. Il est important de noter que l'héritabilité et les valeurs reproductrices sont des mesures relatives de la contribution du patrimoine génétique, et sont donc dépendantes de l'environnement dans lequel les individus mesurés se trouvaient (Visscher *et al.* 2008). En effet, pour un échantillon d'individus donné, la variance génétique σ_a^2 est fixe, mais la variance environnementale (et

donc la variance totale σ_T^2) est dépendante de l'environnement des individus mesurés, affectant l'estimation de l'héritabilité. Une valeur d'héritabilité n'est donc valable que pour les conditions dans lesquelles elle a été mesurée, et il est possible qu'en changeant d'environnement ou avec le temps cette valeur varie (Visscher *et al*. 2008). L'ampleur de ces possibles variations est caractérisée par les interactions génotype et environnement.

Interactions génotypes et environnement

Avec un plan expérimental adapté, il est possible d'évaluer les interactions entre génotypes et environnement. Prenons l'exemple d'un trait mesuré sur les mêmes génotypes (ou sur des individus très apparentés), dans deux environnements différents. La figure 5 (modifiée d'après Guntrip & Sibly 1998) donne des exemples de résultats qui peuvent être obtenus. Les variations observées sont appelés normes de réaction des génotypes, et représentent ici le phénotype moyen de cinq génotypes, mesurés dans deux environnements. Dans le premier cas, l'environnement n'aucun effet sur le phénotype ; les normes de réactions sont horizontales et parallèles, les génotypes produisent les mêmes phénotypes dans les deux environnements. Dans le deuxième cas, les normes de réaction sont parallèles, mais leur pente n'est pas nulle. L'environnement à un effet fixe, identique sur tous les génotypes, il n'y donc pas dans ce cas d'interaction entre les génotypes et l'environnement. Le troisième cas présente des exemples d'interaction génotypes et environnement : l'environnement a un effet différent sur les phénotypes de chaque génotypes. Les génotypes sont donc reclassés, les plus performants dans un environnement ne sont pas nécessairement les plus performants dans un autre environnement (Bowman 1972). Quand ces interactions sont présentes, l'effet de

l'environnement est un facteur majeur à prendre en compte dans l'interprétation des paramètres génétiques estimés.

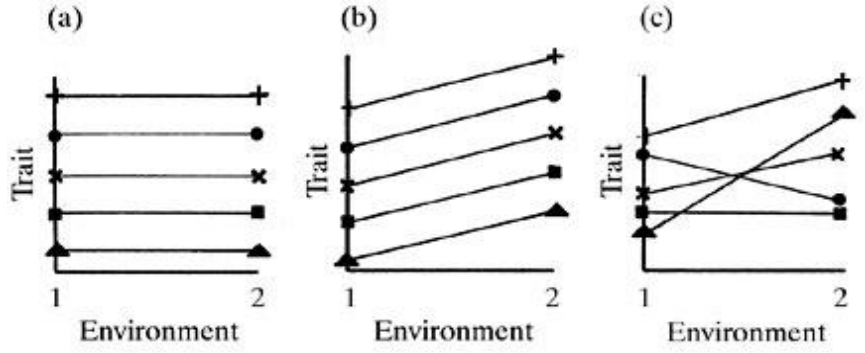


Figure 5: Normes de réactions possibles de 5 génotypes dans deux environnements. Adapté d'après Guntrip & Sibly 1998. (a) Pas d'effet de l'environnement. (b) Effet fixe de l'environnement, identique sur tous les génotypes. Pas d'effet GxE. (c) Interaction entre les génotypes et l'environnement.

Corrélations génétiques entre caractères

Un autre paramètre d'intérêt qu'il est possible d'évaluer avec un design expérimental de génétique quantitative est la corrélation génétique entre différents caractères. Le principe est que, si deux caractères sont mesurés sur les mêmes individus, et que le taux d'apparentement entre ces individus est connu, il est possible d'évaluer la proportion de variance génétique partagée entre les deux caractères (c-a-d la covariance génétique). Si la covariance génétique est différente de zéro, cela suggère que les caractères sont partiellement déterminés par les mêmes gènes et donc que ces gènes sont pléiotropes. Une implication importante des corrélations génétiques concerne les effets de la sélection. En effet, en agronomie et en aquaculture (sélection artificielle) ou en biologie évolutive (sélection naturelle), les populations peuvent subir des pressions de sélection pour certains caractères. Or si ces caractères sont génétiquement corrélés à d'autres, pas forcément souhaitables pour la production ou la fitness darwinienne, effectuer une sélection sur l'un va entrainer des changements sur l'autre et parfois aboutir à des

conséquences inattendues (Gjedrem 1983, Gjerde 1986, Ibarra *et al*. 1999) voire négatives (en cas de corrélations génétiques négatives).

Ces corrélations génétiques peuvent aussi être établies pour un même caractère dans des environnements différents. Il est alors possible d'évaluer indirectement les interactions génotypes x environnement : la corrélation génétique d'un même trait dans deux environnements devrait être proche de 1 s'il n'y a pas d'interactions entre génotypes et environnement (Falconer, 1952). À l'inverse, une corrélation plus faible (inférieure à 0.8, par convention (Ponzoni *et al.* 2008) indiquerait la présence d'une corrélation génotype x environnement pour ce caractère. L'intérêt de ces corrélations est de renforcer et surtout de quantifier l'intensité des effets génotype x environnement décrit dans la partie précédente (Falconer 1952). Les environnements d'intérêts peuvent être variables dans l'espace (ex : différentes fermes d'élevage, différents sites de semis, etc...) ou dans le temps (ex : changement climatique).

1.3.2 Intérêt de la génétique quantitative pour la coquille Saint-Jacques

L'intérêt de l'évaluation des paramètres de génétique quantitative sur la coquille Saint-Jacques est double. Premièrement, malgré un potentiel aquacole et économique important, aucune étude n'a été menée pour évaluer les possibilités génétiques de développement et d'optimisation de la production (Beaumont & Gjedrem, 2006). Une première approche des paramètres d'héritabilité de caractères d'intérêt pourrait ouvrir des pistes de développement de la filière. Cependant, la motivation première de cette approche est ici de continuer d'explorer la question de la base génétique des différences phénotypiques observées au niveau populationnel, notamment au niveau des paramètres de croissance. En effet, la

génétique quantitative peut être un outil pertinent pour répondre à des questions d'écologie et d'évolution (Kruuk & Wilson, 2008)

L'existence d'une écloserie de coquilles Saint-Jacques commerciale fournit le matériel nécessaire pour une étude de génétique quantitative. En effet, les productions de l'écloserie se font avec peu de géniteurs pour des raisons techniques (voir partie 4), produisant ainsi un grand nombre d'individus apparentés (familles de plein frères, de demi-frères). Ces individus peuvent être maintenus en conditions naturelles (semés sur le fond), semi-naturelles (en cage en mer) ou expérimentales. Les familles peuvent être reconstituées par des méthodes génétiques (voir chapitre 2) et phénotypées pour estimer les paramètres de génétique quantitative décrits ci-dessus, dans l'environnement choisi.

1.3.3 Héritabilité, corrélations génétiques et interactions génotype-environnement chez les bivalves

La littérature sur l'estimation en milieu de production des paramètres de génétique quantitative sur des bivalves d'intérêt aquacole est abondante. Les valeurs d'héritabilité des paramètres de croissance chez les bivalves peuvent présentées des valeurs faibles (<0.1, Nguyen *et al*. 2011), modérées (0.10-0.50, Hadley *et al.* 1991, Jarayabhand & Thavornyutikarn 1995; Toro *et al*. 2004; Li *et al*. 2011), voir même des valeurs fortes (0.7-0.9, Rawson and Hilbish 1990, Toro *et al*. 2004, Wang *et al*. 2011), parfois pour les mêmes espèces, mais dans des conditions différentes. La plupart du temps, les paramètres de croissances (taille, poids, taux de croissance) sont fortement corrélés génétiquement entre eux, à la fois phénotypiquement et génétiquement.

Des interactions génotype-environnement ont été évaluées pour les paramètres de croissance sur l'huitre perlière *Pinctada maxima* dans différentes nurseries (Kvingedal *et al*. 2008), sur la palourde américaine *Mercenaria mercenaria* dans différents sites (Rawson and Hilbish 1990) ou encore l'huitre creuse *Crassostrea gigas* dans différentes conditions de nutrition (Ernande *et al.* 2004)

La plupart de ces études sont réalisées avec des objectifs appliqués d'amélioration d'espèces aquacole, les paramètres sont évalués en milieux contrôlés. La littérature sur les estimations d'héritabilité de paramètres de croissance chez les bivalves dans le milieu naturel, reflétant le fonctionnement d'une population dans son environnement, est pour le moment inexistante, du fait de la difficulté à retrouver suffisamment d'individus apparentés dans les populations naturelles. Les évaluations de génétique quantitative dans le milieu naturel sont principalement réalisées sur des vertébrés (Kruuk *et al*. 2000, 2008, Teplitsky *et al*. 2009). Les bivalves sont des espèces à forte fécondité et à reproduction en pleine eau, et à taille de population effective très grande. Ces caractéristiques compliquent la reconstitution par des marqueurs génétique d'un pedigree d'une population naturelle (Coltman, 2005), car l'échantillonnage à fournir pour avoir suffisamment de familles et d'individus dans chaque famille serait irréaliste. Il apparait donc intéressant d'utiliser la production d'une écloserie (à taux d'apparentement fort et facilement évaluable par reconstruction de pedigree), qui aurait grandi, au moins partiellement, dans le milieu naturel.

Le troisième chapitre de cette thèse explorera cette problématique, en évaluant des paramètres de génétique quantitative sur des individus ayant grandi dans un milieu semi-naturel (cage en mer) puis contrôlé (site expérimental d'Ifremer à Argenton).

1.4 L'aquaculture de la coquille Saint-Jacques et ses impacts génétiques potentiels

1.4.1 L'aquaculture et la pêche des pectinidés

Les principales espèces de bivalves exploitées et cultivées sont, par ordre d'importance, les ostréidés, les vénéridés, les mytilidés et les pectinidés (FAO, 2015). La Chine est le principal pays producteur, assurant à elle seule plus de 80% de la production aquacole mondiale de bivalves (en termes de volume de production). La production de bivalve se fait essentiellement par le grossissement de naissain en milieu ouvert. Le naissain est obtenu soit par captage dans le milieu naturel issu de la reproduction spontanée des espèces, soit par reproduction en écloserie quand le captage est incertain ou non viable économiquement, ou loin des zones de reproduction naturelle de l'espèce (Helm *et al.* 2006).

Parmi les bivalves, la culture des pectinidés est assez diversifiée avec une quinzaine d'espèces exploitées dans le monde (Lovatelli 1988). Les principales espèces sont produites en Chine (*Argopecten irradians*, *Mizuhopecten yessoensis*) et au Japon (*Mizuhopecten yessoensis*). La culture de la coquille Saint-Jacques (*Pecten maximus*) en Europe prend des formes variées. Le captage de naissain a été exploré (Brand *et al*. 1980), et des tentatives de développement à plus large échelle ont été tentées, avec un succès mitigé. La méthode la plus pratiquée en Europe actuellement est la production en écloserie (Robert & Gérard, 1999). Le naissain produit en écloserie peut ensuite grossir de plusieurs façons : en Espagne, en pleine eau soit par « ear-hanging », c'est-à-dire par accrochage par les oreilles le long d'un filin, soit en cage flottante (Cano *et al* 2000), soit semé sur le fond. En Norvège, le naissain est semé sur le fond, parfois entouré de clôtures pour éviter la

prédation (Bergh & Strand 2001). En France, les naissains de coquilles Saint-Jacques sont semés dans les populations naturelles pour soutenir la pêche. Il ne s'agit donc pas à proprement parlé d'aquaculture, mais d'un programme de soutien à la population naturelle.

1.4.2 Le programme de repeuplement de la coquille Saint-Jacques en Rade Brest

L'hiver très froid en 1962-1963 a fortement impacté les stocks de coquilles Saint-Jacques de la Rade de Brest, les débarquements sont ainsi passés de plus de 1000-2000T à 100-300T (fig 6). Durant les années qui ont suivi, malgré l'amélioration des moyens et des techniques de pêche, la production a stagné à un niveau faible. Face à cette observation, les pêcheurs et la communauté scientifique ont menés différentes études pour tenter de soutenir et renforcer les gisements restants de coquille Saint Jacques. Après des essais infructueux de captage de naissain (Robert & Gérard, 1999), une écloserie a été mise en place au port du Tinduff (Plougastel-Daoulas).

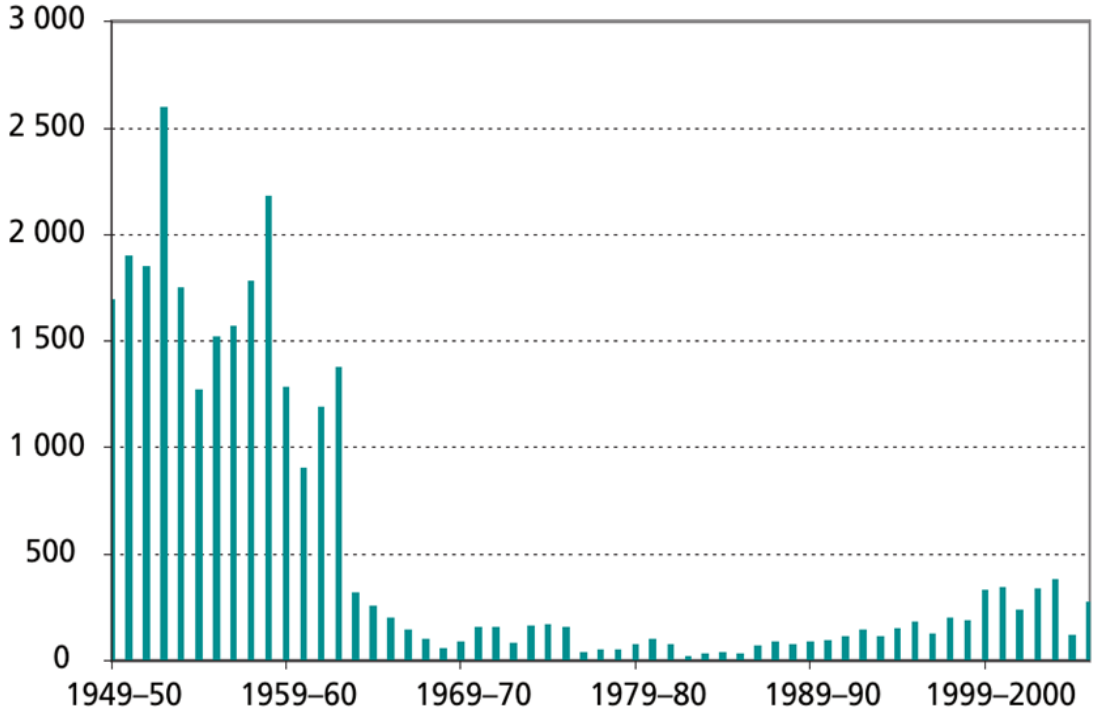


Figure 6: Débarquements annuels de coquille Saint-Jacques en Rade de Brest (en tonnes). Source : Alban & Boncoeur 2008. A noté la légère amélioration des débarquements depuis le début des semis en 1983.

L'écloserie du Tinduff est à ce jour la seule écloserie commerciale française produisant une quantité significative de naissains de coquilles Saint-Jacques. Pour chaque production, les géniteurs utilisés sont prélevés dans la Rade de Brest. Ils sont conditionnés durant environ un mois dans des conditions optimales pour la gamétogenèse : nourriture *ad libitum*, température de l'eau et photopériode estivale. La ponte est ensuite déclenchée par un choc thermique. Les individus sont mis à l'écart dès la constatation du démarrage de la « ponte », pour isoler leurs gamètes. Les coquilles Saint-Jacques émettent d'abord leurs gamètes mâles, puis après un temps variable, les gamètes femelles. Les gamètes ainsi récupérées sont ensuite croisés, avec les pontes de 3-6 mâles pour une femelle. Les larves sont élevées dans les structures de l'écloserie, jusqu'à métamorphose sur tamis. Les post-larves

sont ensuite transférées en mer après une période de grossissement. Elles restent en cage en mer en Rade de Brest ou en Baie de Morlaix, jusqu'à atteindre une taille de 2 à 4cm (Robert & Gérard 1999).

Ces naissains sont ensuite soit semés en Rade de Brest, sur les gisements naturels, soit vendus. Du naissain de l'écloserie du Tinduff est vendu et semé dans les pertuis charentais, en baie du mont Saint-Michel ou encore en baie de Saint-Brieuc (Alban & Boncoeur 2008). Les coquilles Saint-Jacques sont légalement capturables à partir de la taille de 10.5 cm. La contribution des naissains semés à la production totale de la Rade de Brest a été estimée à environ 66% (Fleury *et al*. 2005), contribuant ainsi de manière très significative au maintien économique de la pêche à la coquille en Rade de Brest. La distinction entre coquilles d'écloserie et coquille issues de la reproduction naturelle se fait grâce à une particularité phénotypique : les individus issus d'écloserie subissent un stress important lors du semis, et marquent un anneau sur la coquille. Ils présentent donc, au cours de leur première année, deux anneaux marqués sur leur coquille, l'anneau hivernal et l'anneau du stress de semis, alors que les coquilles issus de la reproduction dans le milieu naturel ne présentent que l'anneau hivernal. Cette méthode dite du « double-anneau » (Fleury *et al.* 2005, Alban & Boncoeur 2008), reste empirique, et n'a pour le moment jamais été validée (voir chapitre 4 de cette thèse pour une étude de la validité de cette méthode par méthodes génétiques d'apparentement).

1.4.3 Implications génétiques du semis de naissains

Les pratiques de semis de naissains peuvent avoir des conséquences génétiques sur les populations receveuses. L'utilisation d'un faible nombre de géniteurs pour produire un très grand nombre de juvéniles, rendue possible du fait de la très forte fécondité, peut entrainer une dérive génétique très importante dans la cohorte produite, source de potentielle perte de diversité génétique (Beaumont *et al*. 2010). Ce phénomène est accentué par la grande variabilité du succès reproducteur des géniteurs dans des conditions d'écloserie qui contribue à réduire la taille efficace de la production (Boudry *et al*. 2002, Appleyard & Ward 2006, Lallias *et al*. 2010b). La taille efficace des populations est un paramètre fondamental à prendre en compte pour les programmes de soutien aux populations (Beaumont *et al*. 2010). Une taille efficace faible, comme souvent observée dans les populations issues de reproduction en écloserie (Waples *et al*. 1999), peut conduire à une diminution de la diversité génétique de la cohorte d'écloserie et à une consanguinité et un apparentement fort entre les individus (Utter, 1998, Araki & Schmid 2010). Les fréquences alléliques étant fortement modifiées par rapport à la population sauvage, elles peuvent conduire à une altération sa structure génétique. Cette perte de diversité a été mise en évidence dans de nombreux cas d'écloserie de bivalve (Taris *et al*. 2007, Lind *et al*. 2009, Lallias *et al*. 2010a; voir Araki & Schmid 2010 pour une revue bibliographique).

Ces caractéristiques typiques des populations d'écloserie peuvent avoir un impact sur les populations naturelles réensemencées. Le but des programmes de repeuplement est d'assurer un soutien à la reproduction par l'introduction de géniteurs potentiels supplémentaires. Or, s'il y a reproduction entre les individus semés et les individus naturels, la perte de taille efficace et de diversité induite par

l'écloserie peut se répercuter sur la population naturelle. Ce phénomène est appelé effet de Ryman-Laikre (Ryman & Laikre, 1991). Cette perte de diversité peut avoir des conséquences néfastes sur la population, ie pouvant conduire à une sensibilité accrue de la population aux perturbations et aux stress (Reed & Frankham 2003, Spielman *et al*. 2004), et à son déclin démographique à moyen à long terme, en contradiction avec l'objectif initial des mesures de gestion (Araki & Schmid 2010).

L'effet Ryman-Laikre et ses conséquences ont été mis en évidence dans le cas de programme de réensemencement de salmonidés (Araki *et al.* 2007, Christie *et al*. 2012), mais est difficile à évaluer sur les populations ensemencées de bivalves. Il est cependant attendu à ce qu'il soit limité chez les bivalves, de fait des caractéristiques de leur reproduction (Gaffney, 2006). En effet, il est possible que les populations sauvages de bivalves avec leur très forte fécondité et leur reproduction en milieu ouvert, présentent une très forte variance du succès reproducteur individuel (l'effet « Hedgecock » (Waples, 1998) ou « sweepstake reproductive success » SRS, (Hedgecock & Pudovkin 2011) qui peut se traduire par la "loterie du succès reproducteur". Quand elle a lieu, cette variabilité de succès reproducteur implique que très peu d'individus ont une descendance à chaque génération, et donc la taille efficace des populations de bivalves est de plusieurs ordres de grandeur plus faible que la taille effective des populations. Introduire une cohorte d'écloserie avec une taille efficace faible n'implique donc pas forcément une réduction de la taille efficace globale, si ces individus ne participent pas significativement à l'effort reproducteur effectif de la population.

De plus, il est possible que les individus d'écloserie aient un succès reproducteur plus faible que les individus sauvages. En effet, des baisses de succès reproducteur ont été démontrées chez des salmonidés d'écloserie (Araki *et al*. 2008,

Christie *et al*. 2012a), résultantes d'une pression de sélection forte en écloserie, provoquant une maladaptation au milieu naturel des poissons nés en écloserie. Cet effet n'a pour l'instant pas été montré chez les bivalves. En effet, les fortes mortalités larvaires souvent observées dans les écloseries de bivalve (Robert & Gérard 1999) laissent à penser qu'il est possible qu'une forme de sélection (non volontaire) s'opère (*e.g.* Taris *et al.* 2007).

Concernant la coquille Saint-Jacques, Hold *et al*. (2012) ont fait une étude par simulation de l'effet du semis d'une cohorte produite par l'écloserie du Tinduff sur la taille efficace des gisements de coquilles Saint-Jacques de l'île de Man (Mer d'Irlande), en utilisant le modèle de Ryman & Laikre (1991). Ils ont démontré que dans la majorité des cas, une réduction de la taille efficace pouvait se produire, mais que, sous certaines conditions de survie et de succès reproducteur des individus semés, il pouvait y avoir une augmentation de la taille efficace globale. Cependant, cette étude se basait sur une unique production de l'écloserie, non représentative de ce qui est effectivement semé. En effet, les individus semés sont souvent issus de plusieurs cohortes, produites avec des géniteurs différents (F. Breton, comm. pers). De plus, la diversité de la cohorte de l'écloserie a été évaluée au stade du semis (naissain de 2-3cm), ce qui n'est pas forcément représentatif de la diversité génétique des coquilles issus d'écloserie participant à la reproduction, après croissance et maturation.

Enfin, il faut noter que la première maturité des coquilles Saint-Jacques correspond environ à leur arrivée à taille commerciale en Rade de Brest (Chauvaud, comm. pers.). Or les pêcheurs connaissent les sites de semis (F. Breton, comm. pers.), et malgré les mesures de gestion misent en place (zones de réserves, Alban & Boncoeur 2008), les coquilles semées subissent probablement une pression de

pêche plus importante, en moyenne, que la population naturelle, réduisant leur succès reproducteur relativement au coquille sauvages.

Pour estimer la taille efficace des populations et répondre aux problématiques mentionnées ci-dessus, il existe des deux catégories de méthodes génétiques. Premièrement, certaines méthodes se basent sur une mesure de la variation des fréquences alléliques dans le temps. Cette variation est une mesure de la dérive génétique, qui est inversement proportionnelle à la taille efficace de la population (Nei & Tajima 1981, Pollack 1983, Jorde & Ryman 2007). Ces méthodes nécessitent un échantillonnage temporel de la population d'intérêt. L'autre catégorie de méthodes se base sur un seul échantillon d'une population, et estime la taille efficace instantanée de la population. Ces méthodes se basent sur des mesures de l'effet de la dérive génétique sur l'excès d'hétérozygote (Zhdanova and Pudovkin 2008), le déséquilibre de liaison (Waples & Do 2008), la structure familiale de l'échantillon (Wang 2009) ou la co-ascendance moléculaire (Nomura 2008). Toutes ces méthodes se reposent sur des hypothèses sous-jacentes importantes (Waples & Do 2010), et l'interprétation des résultats obtenus doit être faite à la lumière de ces hypothèses.

Dans ce contexte, la problématique du chapitre 4 de cette thèse sera d'explorer l'effet de l'ensemencement sur la taille efficace, diversité génétique et structure de la population de la Rade de Brest. Pour cela, deux études ont été réalisées : la première consiste à valider par de l'assignation de parenté la méthode du « double-anneau » de reconnaissance des individus issus de l'écloserie par rapport aux individus issus de la reproduction naturelle. La deuxième utilise cette méthode du double-anneau pour effectuer un suivi temporel, avec échantillonnage de cohorte d'écloserie au moment de leur entrée dans la pêcherie, et

échantillonnage de la cohorte naturelle correspondante. Cet échantillonnage permet d'effectuer des comparaisons de diversité et structure génétique, ainsi que des estimations de taille efficace et de contribution reproductrice relative de chaque cohorte.

1.5 Résumé des objectifs de la thèse

Dans ce contexte, les objectifs de ma thèse étaient les suivants :

- Explorer la structure spatiale et la diversité génétique de la coquille Saint-Jacques à l'échelle européenne, pour reconstituer son histoire évolutive récente et la mettre en parallèle avec les différences phénotypiques (notamment de croissance de la coquille) observées entre les populations.

-Évaluer la part génétique des paramètres de croissance, par une approche de génétique quantitative sur des individus.

-Estimer l'impact génétique des pratiques de réensemencement de coquille Saint-Jacques sur la diversité et la structure génétique des populations sauvages.

Ces trois objectifs font chacun l'objet d'un chapitre de cette thèse, présenté sous la forme d'une ou deux publications scientifiques publiées, soumises ou en préparation. Un court résumé des principaux résultats et conlusions de chaque étude est proposé avant les manuscrits. La thèse se conclue par une discussion générale sur l'ensemble des résultats obtenus et leur implications, suivies des conclusions et perspectives. Enfin en annexe, une publication (également à soumettre) résultant d'un projet parallèle mené au cours de ma thèse est également présentée.

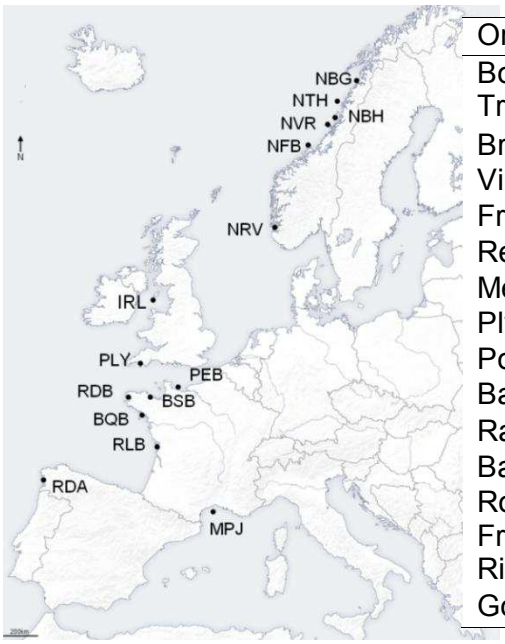
Chapitre 2

Génétique des populations de coquilles Saint-Jacques le long des côtes européennes.

Manuscrit accepté dans *Conservation genetics*

2.1 Principaux résultats et conclusions

Afin d'étudier la structuration génétique des populations de coquille Saint-Jacques, *Pecten maximus*, un échantillonnage sur 14 sites a été réalisé le long de l'aire de répartition de l'espèce (fig.7). Une population d'une espèce décrite comme proche, *Pecten jacobaeus* a également été échantillonnée dans le golfe du Lion en Mer Méditerranée. Ces populations ont été génotypées à 12 marqueurs microsatellites.



Origine	Code	Effectif
Bodø, Grønholmen, Norvège	NBG	24
Træna, Husøya, Norvège	NTH	48
Brønnøysund, Hensteinen, Norvège	NBH	48
Vikna, Ravsholmen, Norvège	NVR	47
Froan, Bordsholmen, Norvège	NFB	48
Rennesøy,Vignesholmane, Norvège	NRV	48
Mer d'Irlande	IRL	29
Plymouth, Angleterre	PLY	29
Port-en-Bessin, France	PEB	48
Baie de Saint-Brieuc, France	BSB	45
Rade de Brest, France	RDB	47
Baie de Quiberon, France	BQB	48
Ronce-les-bains, La Tremblade, France.	RLB	47
Ria de Arousa, Galice, Espagne	RDA	46
Golf du Lion, France (*P. Jacobaeus*)	MPJ	46

Figure 7: Echantillonnage des population de coquilles Saint-Jacques.

2.1.1 Variabilité génétique

La richesse allélique moyenne varie de 6.71 (NBH) à 8.43 (MPJ). Une relation décroissante entre la richesse allélique et la latitude des populations a été mise en évidence (fig 8)

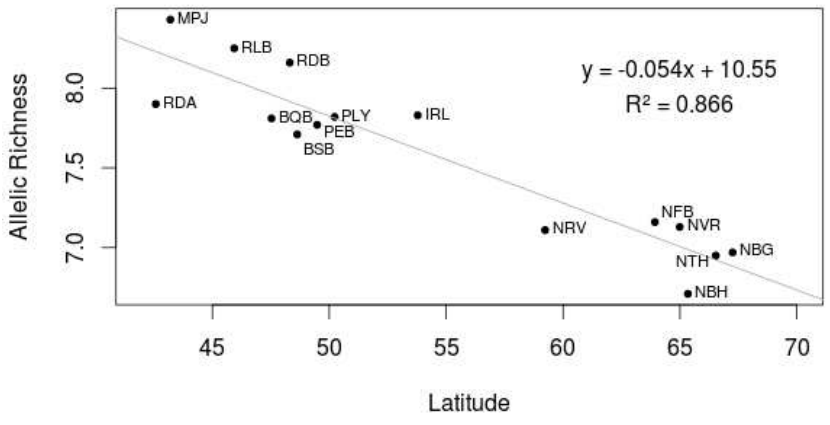


Figure 8 : Richesse allélique moyenne en fonction de la latitude des populations échantillonnées. Une corrélation forte et significative est mise en évidence.

2.1.2 Structure génétique

Deux groupes ont été mis en évidence. Le premier groupe est constitué de toutes les populations norvégiennes, et le second des populations atlantiques, comprenant les populations des îles britanniques jusqu'à l'Espagne. Ces deux groupes sont relativement homogènes génétiquement, mais très différents entre eux (Fig 9). La population méditerranéenne de *P. jacobaeus* se différencie fortement de toutes les populations de *P. maximus* (Fig 9).

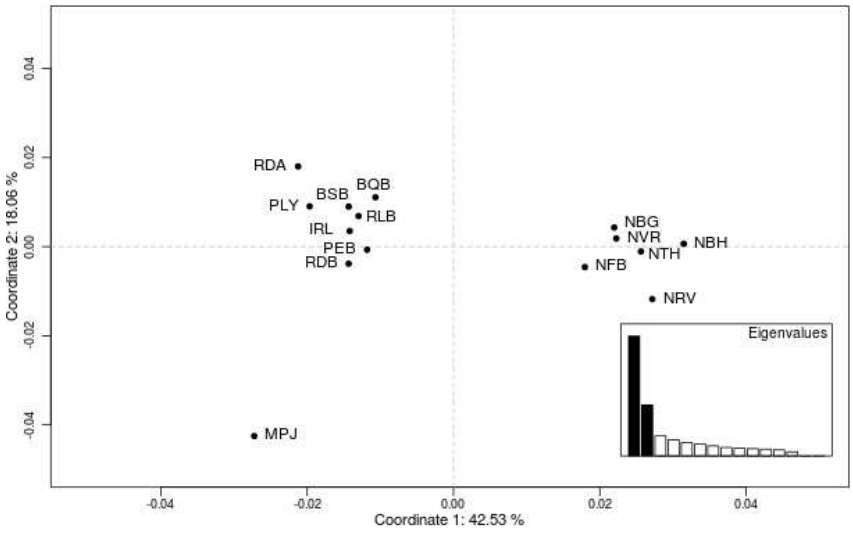


Fig 9 : Positionnement multidimensionnel des populations étudiées. Les coordonnées sont obtenues à partir des valeurs de F_{ST} linéarisées par transformation de Slatkins (1995)

2.2.3 Conclusion

Phylogéaographie des populations de coquilles Saint-Jacques

Les résultats de cette étude mettent en évidence l'existence de deux groupes génétiques de coquilles Saint-Jacques, fortement structuré. Ces deux groupes ont pu se formé lors de la recolonisation suite à la débâcle marquant la fin de la dernière période glaciaire. Deux hypothèses sont proposées : une recolonisation de la façade à partir de deux refuges différents, provoquant cette différenciation génétique ; ou un recolonisation à partir d'un seul refuge, du Sud vers le Nord. Cette deuxième hypothèse permet d'expliquer notamment la perte de diversité avec la latitude, possiblement provoquée par une série d'effets fondateurs. Le plus important semble être celui qui a permis la traverser de la mer du Nord, qui a donné cette différenciation génétique. Quelque soit le scénario plausible, l'isolement entre les deux groupes de populations a ensuite probablement été maintenu par une barrière au flux de gènes. La fosse norvégienne est une bonne

candidate pour cette barrière : elle pourrait contraindre, par sa profondeur et par la courantologie, l'installation et le passage de larves de coquilles Saint-Jacques.

Il est intéressant de noter le parallèle entre la différenciation génétique trouvée ici et la différenciation phénotypique trouvée par Chauvaud *et al.* (2012). Deux groupes sont retrouvés, mais la limite diffère. Alors que les résultats présentés ici et les études autres génétiques (Ridgway & Dahle 2000, Hold 2012) placent la barrière au niveau de la fosse norvégienne, l'étude de Chauvaud *et al.* (2012) place la barrière de l'Écosse à la limite Manche-Mer du Nord. Malgré cette différence, l'existence de ces deux groupes génétiques peut permettre d'expliquer en partie les différences phénotypiques de stratégies de croissance observées.

Diversité génétique de la Rade de Brest

La population de la Rade de Brest a subit un fort déclin démographique à la suite de l'hiver 1962-1963, qui a conduit à la mise en place d'un programme de soutien à la population. Une écloserie a été mise en place, produisant chaque année des millions de naissains, semés sur les stocks naturels (Alban & Boncoeur 2008). Ces pratiques peuvent avoir un impact sur la diversité génétique de la population réensemencées : les naissains, issus d'un petit nombre de géniteurs, peuvent réduire la diversité génétique totale de la population. Les résultats montrent que la diversité génétique de la Rade de Brest est comparable à celle des autres populations du groupe Atlantique, et ne semble donc pas impactée par le programme de soutien à la population. Ce résultat peut être expliqué par plusieurs hypothèses :

-Le succès reproducteur des individus issus d'écloserie peut être moins important que celui des coquilles issues de la reproduction dans le milieu. Ce différentiel pourrait être dû à une pression de pêche plus importante sur les zones semées ou à une fitness moindre des individus d'écloserie, comme observée chez des salmonidés d'écloserie relâché dans le milieu (Araki *et al.* 2008, Christie *et al.* 2012a)

-Il pourrait exister, à l'intérieur ou à l'extérieur de la Rade de Brest, une population source de diversité génétique, qui renouvelle la diversité par un apport important de migrants.

-Enfin, il est aussi possible que les mesures de gestion de la diversité appliquées par l'écloserie permettent le maintien de la diversité génétique de la population naturelle. En effet, les géniteurs utilisés sont renouvelés à chaque production, en prélevant des individus issus de la reproduction dans le milieu. De plus, un grand nombre de géniteurs peut être utilisé (jusqu'à une centaine par an, F. Breton, comm. pers.). Cette problématique sera approfondie dans le chapitre 4.

Statut taxonomique de Pecten jacobaeus.

La population de *P. jacobaeus*, la coquille Saint-Jacques méditerranéenne, est clairement différenciée de toutes les populations de P. maximus. Cependant, l'ordre de grandeur de la différenciation génétique entre la population méditerranéenne et les populations Atlantique est globalement similaire à l'ordre de grandeur de différenciation enter les populations norvégiennes et les populations atlantiques. Ce résultat semble suggérer que la séparation des coquilles méditerranéennes en une espèce différente de *P. maximus* n'est pas forcément justifiée. Ce résultat a été précédemment mis en évidence dans d'autres études (Wilding *et al.* 1999, Saavedra & Peña 2004, 2005), et cette étude conforte cette hypothèse.

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RESEARCH ARTICLE

Genetic structure of a commercially exploited bivalve, the great scallop *Pecten maximus*, along the European coasts

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Abstract The great scallop *Pecten maximus* is harvested in several European countries and fisheries targeting this species are severely regulated by fishing quotas. In addition, hatchery-based population enhancement has been developed in some countries to provide alternative or complementary production. The genetic structure of wild populations of *P. maximus* and the potential impact of aquaculture on the genetic diversity of this species remains poorly documented. In this study, we explored the genetic structure of *P. maximus* using 12 microsatellite markers, considering 14 populations sampled from Galicia (Spain) to the North of Norway, and one population of *Pecten jacobaeus* (L., 1758) from the Lion Gulf (Mediterranean Sea). Results indicated a clear differentiation between Norwegian and Atlantic (from Ireland to Spain) populations, but very little to no difference between populations within these two groups. A decrease of the genetic diversity was observed with latitude. No significant reduction of the genetic diversity was observed in the Bay of Brest, where hatchery-based population enhancement has been performed intensively since 1983. Our results are discussed in the light of the inferred recent evolutionary history, phylogeography and connectivity of populations in Europe, and of the phenotypic variability reported in previous studies between northern and southern populations.

Keywords Great scallop · *Pecten maximus* · Microsatellites · Population genetics · Aquaculture

Introduction

The great scallop, *Pecten maximus* (L., 1758), is a benthic marine bivalve, which is broadly distributed in shallow waters along the European coasts, from northern Norway to southern Spain. This species is of high economical value, mainly in U.K. and France, with landings reaching 63,681 tonnes in 2012 (Beaumont and Gjedrem 2006; FAO 2015). Although dredging of natural beds remains the main method of exploitation, sea-ranching has emerged as an alternative or complementary production solution. This is notably the case in the Bay of Brest (Brittany, France), where sea-ranching *of P. maximus* has been carried out since 1983. Following a particularly cold winter in 1962–1963 (Dao et al. 1999), stocks were severely depleted and sea-ranching was ultimately proposed to revive the local fishery (Dao et al. 1985). Hatchery production of juvenile great scallops is now well mastered, although several aspects could still be optimized (Robert and Gérard 1999; Andersen et al. 2011), and a few commercial hatcheries market significant quantities of scallop seed: 1–5 million seeds are produced yearly by Le Tinduff hatchery

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(Plougastel, France). Similar initiatives are in development in northern Europe.

It is known that hatchery practices can significantly impact the effective population size of enhanced stocks, especially in bivalve species for which variance in reproductive success has been shown to be large (e.g. Boudry et al. 2002). Restocking has been historically low in *P. maximus*, and is now linked to spatially limited aquaculture practices (Beaumont 2000; Minchin 2003). Thus, the impact of transplantations and hatchery stocks on natural populations is therefore presumed to be low (Gaffney 2006; Hold et al. 2012). However, this issue has not been addressed thoroughly, yet. In France, the Bay of Brest is of special interest in terms of conservation, as population enhancement based on seed produced in the local hatchery (Le Tinduff, Plougastel, France) is well developed, and might have a significant impact on the local genetic diversity (Beaumont and Gjedrem 2006). Indeed, landings of hatchery-born scallops are estimated to be around 2/3 of total landings in the Bay of Brest (Fleury et al. 2005).

Knowledge of the genetic structure of wild populations is a key step to quantify the potential impact of restocking and aquaculture practices on natural resources. Genetic differentiation patterns over the whole geographic distribution of populations are shaped by multiple forces during the evolutionary history of species (e.g. gene flow, genetic drift, selection). In addition to natural forces, the genetic variability of wild populations can be affected by anthropogenic pressures (e.g. pollution, habitat fragmentation, translocations, harvesting) (Smith et al. 1991; Ma et al. 2000). The great scallop, like many bivalve mollusks, can disperse over broad distances during its long planktonic larval phase (Le Pennec et al. 2003). In the open sea, estimates of the planktonic larval duration (PLD) are 18–42 days (Le Pennec et al. 2003). PLD is strongly influenced by water temperature, and larvae take a longer time to achieve metamorphosis in cold water. Hence, in laboratory condition, PLD has been assessed around 24–35 days at 18 °C, and up to 65–78 days at 8–9 °C (Beaumont and Barnes 1992). Thus, larvae clearly display a high dispersal potential, which may be further increased in colder waters. Additionally, newly metamorphosed post-larvae can also migrate via byssus-drifting (Beaumont and Barnes 1992). A larval dispersal model developed by Nicolle et al. (2013) has revealed an important dispersal potential at a small geographic scale. With regards to these results, population genetics could provide insights into gene flow patterns along the latitudinal gradient: gene flow might be higher in the northern than in the southern range of the species. Limited data are available about the population genetic structure of *P. maximus* along its distribution area. Genetic structuring has been described between Norway and the British Isles, using mitochondrial DNA (Ridgway and Dahle 2000). Local studies have also been conducted, revealing a rather consistent pattern of population structuring with a large general homogeneity along Atlantic coasts, using allozymes (Beaumont et al. 1993) or mitochondrial DNA (Heipel et al. 1998, 1999; Wilding et al. 1997). Some hints about small scale structuring have been found with mitochondrial DNA in specific locations, i.e. Mulroy Bay (Wilding et al. 1997; Heipel et al. 1998, 1999) and east of the Isle of Man (Heipel et al. 1998, 1999).

Further studies have recently been conducted on phenotypic traits, suggesting a significant differentiation among great scallop populations. Chauvaud et al. (2012) have described differentiated growth patterns between "northern" and "southern" populations, with individuals from northern populations showing a slower growth but reaching a larger asymptotic size and a lower number of growth days per year. The authors suggested a possible effect of phenotypic plasticity without excluding a possible local adaptation associated with a genetic differentiation of populations. Another type of phenotypic variation, related to the timing of reproduction, has been observed between populations from Scotland, the Bay of Brest and the Bay of Saint Brieuc (France). Features of the reproductive cycle of transplanted scallops remained unchanged following population translocations, suggesting a genetic basis to these traits (Paulet et al. 1988; Cochard and Devauchelle 1993; Mackie and Ansell 1993). A similar maintenance of the natural reproductive cycle has been reported in Norway with scallops transferred between populations along the coast (Magnesen and Christophersen 2008). More recently, a proteomic study has revealed phenotypic differences between two populations sampled in Norway and France (Artigaud et al. 2014). However, the genetic architecture of these phenotypic differences remains to be studied.

A sister species of *P. maximus* has been described in the Mediterranean Sea, *Pecten jacobaeus* (L. 1758). Several genetic studies have suggested that their phylogenetic status is unclear, showing low genetic (allozymes, 16S mtDNA and mitochondrial RFLPs) and phenotypic (shell morphology) differentiation between the two taxa (Wilding et al. 1999; Saavedra and Peña 2004, 2005), leading authors to conclude that they could be considered as a single species. In this context, the relative status of these two taxa remains to be further characterized using microsatellites.

The present study reports a large scale population genetics study of *P. maximus*, based on 12 microsatellite markers. Populations from Norway to Spain, including the Bay of Brest, are considered in the analysis, as well as a Mediterranean population. This study is motivated by two objectives: (1) assessing the genetic structure of *P.*

maximus along the European coasts and, and (2) giving a first insight of the influence of restocking practices on this structure.

Materials and methods

Studied populations

Fifteen populations were sampled from Bodø (Grønholmen, Norway) to the Gulf of Lion (France) (Fig. 1). Twenty-four to forty-eight individuals were collected in each population sample (see supplementary material for details). A fragment of adductor muscle was sampled from each scallop and either preserved in 85 % ethanol or frozen at −20 °C.

In the Bay of Brest, where hatchery-raised scallops are released in the wild, the natural origin of individuals was confirmed by checking the absence of a double growth ring on the shells, characteristic of hatchery-born individuals. A double growth ring results from a period of slow growth due to the stress associated with the drastic environmental change during the transfer from the hatchery cage to the natural sea bed (Fleury et al. 2005).

DNA extraction and microsatellite genotyping

DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen^{TM,} Hilden, Germany), according to manufacturer's instructions. DNA concentrations were estimated using Nanodrop®, and then diluted to 10 ng DNA/mL.

Genetic variation at 12 microsatellite loci was assayed, including two multiplex sets of four markers each from Morvezen et al. (2013) (*mx1* and *mx2*) and one newly developed multiplex of four additional microsatellites named *mx4* (see supplementary material for details). The latter was developed following the procedure described in Morvezen et al. (2013) (see supplementary materials for loci statistics and multiplex parameters). Multiplex PCRs were conducted as described in Morvezen et al. (2013).

Briefly, PCR amplifications were conducted with the Type-it Microsatellite PCR Kit (Qiagen TM) in a 10 µl reaction volume containing 5 µl of Type-it Multiplex PCR Master Mix 2X (including HotStarTaq © Plus DNA Polymerase, Type-it Microsatellite PCR Buffer with $MgCl_2$, and dNTPs), 1 µl primer mix (see Morvezen et al. 2013 and supplementary materials for details), 1 µl

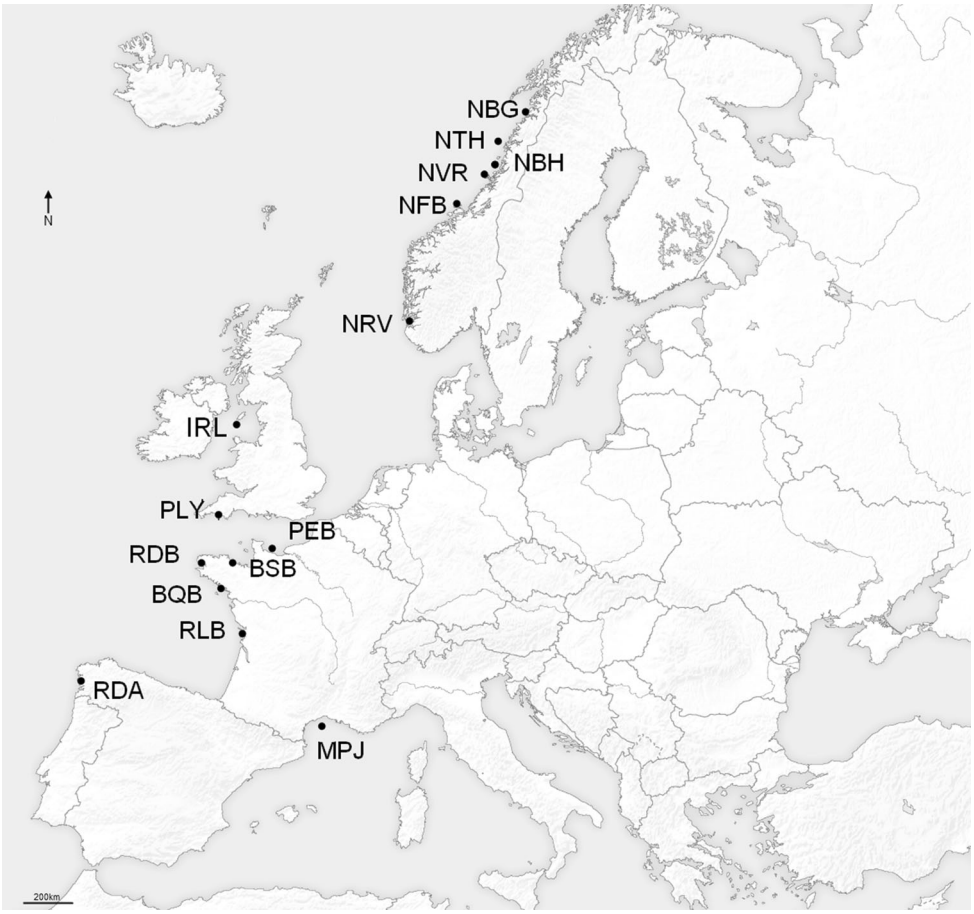

Fig. 1 Sampling map. Population sampling location from south to north: *MPJ* Mediterranean *Pecten jacobaeus* Lion Gulf, France; *RDA* Ría de Arousa, Galicia, Spain.; *RLB* Ronce-Les-Bains, La Tremblade, France; *BQB* Bay of Quiberon, France; *RDB* Bay of Brest, France. *BSB* Bay of Saint Brieuc, France; *PEB* Port-en-Bessin, France; *PLY* Plymouth, England; *IRL* Irish Sea: *NRV* Rennesøy, Vignesholmane, Norway; *NTH* Træna, Husøya, Norway; *NBH* Brønnøysund, Hensteinen, Norway; *NVR* Vikna, Ravsholmen, Norway; *NFB* Froan, Bordsholmen, Norway; *NBG* Bodø, Grønholmen, Norway

Q-solution 5X, 2 µl RNase-free water and 1 µl diluted genomic DNA (10 ng). PCRs included an initial step: 95 °C for 15 min, (94 °C 30 s, 59 °C 90 s and 72 °C 90 s) × 30 cycles, followed by a "nested" step (94 °C 30 s, 55 °C 90 s and 72 °C 90 s) × 8 cycles.

PCR products were mixed with Hi-Di formamide and GeneScan 500-LIZ size standard (Applied Biosystems™ Carlsbad, CA, USA): 1 µl PCR product, 10 µl Hi-Di Formamide, 0.15 µl GS500-LIZ. After five minutes of denaturation at 96 °C and a rapid cooling on ice, PCR products were electrophoresed on an ABI-3130 capillary sequencer (Applied Biosystem™). Fragment lengths and allele scoring were assessed with Genemapper 4.0 software (Life Technologies™).

Data analysis

Within-population diversity

Allelic richness, observed and expected heterozygosities, and locus specific F_{IS} were estimated using Fstat 2.9.3.2 (Goudet 1995). Population specific F_{IS} were calculated using Genetix 4.05 (Belkhir et al. 2001). The significance of F_{IS} estimates was tested using 10,000 permutations, and a false discovery rate (FDR) correction for multiple testing was applied. The presence of null alleles was estimated using Microchecker 2.2.3 (Van Oosterhout et al. 2004). Multi-locus allelic richness, observed and expected heterozygosities were plotted against latitude to explore a possible latitudinal gradient in the genetic diversity, and correlation was tested using Pearson's correlation test with R 2.15 (R Core Team 2012). Linkage disequilibrium for each pair of marker in each population was estimated using Genepop 4.0.5 (Rousset 2008). Effective population sizes were estimated using the linkage disequilibrium method implemented in LDNe 1.31 (Waples and Do 2010); P_{crit} was set at 0.02, as recommended by the authors.

Population genetic structure

Global and pairwise F_{ST} were estimated using Genetix 4.05, and significance was tested using 10,000 permutations. P values were adjusted using FDR correction for multiple testing. A multidimensional scaling (MDS) analysis was computed in R 2.15, using F_{ST} (Weir and Cockerham 1984) linearized according to Slatkin's transformation (Slatkin 1995). Slatkin's transformation (FST/(1−FST)) allow FST to behave more like an Euclidian distance and thus is better suited to do MDS. Exact tests of genetic differentiation were conducted with Genepop 4.0.5 with 1000 dememorisations, 100 batches and 10,000 iterations per batch. Bayesian estimations of F_{ST} were computed with EBFST v1.0 (Kitada et al. 2007).

An Analysis of Molecular Variance (AMOVA) was performed with Arlequin 3.5 (Excoffier and Lischer 2010). Populations were aggregated into two clusters, found with the MDS analysis. The first contained Atlantic populations (RDA, RLB, BQB, RDB, BSB, PEB, PLY, IRL), and the second contained Norwegian populations (NRV, NFB, NVR, NBH, NTH, NBG); the Mediterranean population was excluded from this analysis. The genetic structure was assessed by variance analysis within individuals (F_{it}), among groups (F_{ct}), among populations within groups (F_{sc}) and among individuals within populations (F_{IS}) and the significance of the fixation indices was tested with 10,000 permutations. Allelic richness, heterozygosities and F_{IS} were also compared between those two clusters with Fstat 2.9.3.2.

A Mantel test was performed to test isolation by distance (IBD) with Genetix 4.05 using the same genetic distance matrix as used in the MDS, and a matrix of geographic distances among populations. Geographic distances were assessed following the coast line, or, when not possible, following the shortest marine distance. The Mantel test was performed on all populations (excluding the Mediterranean population because of its outlier status, both genetically and geographically) and within each cluster of populations defined above (i.e. Atlantic and Norwegian populations), and significance was assessed by 10,000 permutations.

Results

Genetic diversity within populations

Multilocus allelic richness (A_r) ranged from 6.71 (NBH) to 8.43 (MPJ) (Fig. 2). Observed multilocus heterozygosity (H_o) varied from 0.60 (NBG, NTH, NBH) to 0.67 (MPJ & RDB). Although all markers were initially developed for Atlantic scallops, the highest mean A_r was observed in the Mediterranean population ($A_r = 8.43$) and the lowest was found in the NBG, NTH and NBH samples ($A_r = 6.71$–6.97; see supplementary material for details).

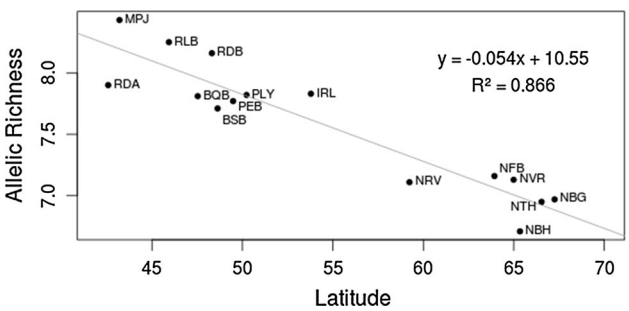

Fig. 2 Allelic Richness plotted against latitude. Site acronym signification is given in Fig. 1

Among *P. maximus* samples, there was a significant decrease in A_r with increasing latitude, as illustrated in Fig. 2 (Pearson correlation test, $r = -0.92$, $p < 0.001$). A similar trend was observed for H_o (data not shown, $r = -0.75$, $p = 0.002$). This latitudinal decrease in genetic diversity was not observed within either the Atlantic and Norwegian groups of populations (as defined below). No significant pattern of linkage disequilibrium was found: less than 5 % of each pair of marker tested on each population gave a significant result.

A moderate but significant heterozygote deficiency was found over all loci (see supplementary material). Five loci in particular showed recurrent heterozygote deficiencies across most populations (after FDR correction for multiple testing): PmRM007 and PmRM027 (five significant F_{IS} estimates), PmRM71 (six significant F_{IS} estimates), PmRM012 (eight significant F_{IS} estimates) and PmRM043 (ten significant F_{IS} estimates) (see supplementary material for details). The presence of null alleles was detected by Microchecker at three loci: PmRM007, PmRM027 and PmRM043. The estimated frequencies of null alleles for these three loci were respectively 0.06, 0.14 and 0.19. Interestingly, no null alleles were detected for PmRM012 and PmRM71.

Confidence intervals of all Ne estimates included infinity, which did not allow us to have a clear estimate of effective population size. However, the method used should not have any difficulties in estimating Ne if it was small (Waples and Do 2010); thus, we can at least conclude that Ne was large to very large in all our populations.

Population genetic structure

The Weir and Cockerham (1984) and the bayesian (Kitada et al. 2007) estimations of F_{ST} gave similar results (data not shown). Only Weir and Cockerham (1984) F_{ST} are described and discussed hereafter.

The global level of differentiation among all population samples was relatively high ($F_{ST} = 0.0291$, $p < 0.001$). We then tested if null alleles had a significant influence on the genetic structure by dropping the three loci with a high proportion of null alleles. Global F_{ST} and confidence intervals were similar (Respectively, global $F_{ST} = 0.0291$ (CI 95 % 0.0157–0.0472) and $F_{ST} = 0.0287$ (CI 95 % 0.0160–0.0424), so all further results are given for all loci. All pairwise F_{ST} comparisons including the Mediterranean population (MPJ) were significant ($0.03 < F_{ST} < 0.08$) and generally higher than estimates among *P. maximus* populations ($-0.05 < F_{ST} < 0.06$) (Table 1). Moreover, all pairwise F_{ST} comparisons between Norwegian and Atlantic *P. maximus* populations were relatively high and significant ($0.02 < F_{ST} < 0.06$). In contrast, pairwise F_{ST} were low (<0.01) and mostly non significant within both Atlantic and Norwegian groups. However, a weak genetic structure without any clear pattern was observed within both groups, as shown by a few moderately significant F_{ST}. Negative values of F_{ST} have been found indicating a slight sampling bias, and should be considered equal to zero (Balloux and Lugon-Moulin 2002). The MDS illustrated the pattern of genetic differentiation shown with the pairwise F_{ST} comparisons: axes 1 and 2 explained respectively (1) the difference between Norwegian and Atlantic populations, and (2) the difference between *the* Mediterranean population and all other populations (Fig. 3). Exact tests of genic differentiation showed a similar pattern, with a highly significant differentiation between the Atlantic and Norwegian groups and the Mediterranean population. Interestingly, exact tests showed also a higher genetic structure within the Atlantic cluster, compared to pairwise F_{ST} (Table 1).

The AMOVA revealed that 95.96 % ($F_{it} = 0.040$) of the genetic variation was explained by variation within individuals, and 3.56 % ($F_{ct} = 0.036$) by variation between groups (Atlantic vs Norwegian). Both were highly significant ($p < 0.001$). Variation among populations within groups ($F_{sc} = -0.005$) and variation among individuals within populations ($F_{IS} = 0.010$) were not significant. These AMOVA results indicated a global genetic homogeneity both within each population and each group, but a significant genetic differentiation between the Atlantic and Norwegian groups.

Comparisons between the two groups of samples with Fstat showed a significant difference for R_a ($p = 0.001$): Norwegian populations showed, on average, 0.9 less allele per locus than Atlantic populations. A slight but significant difference was also detected for H_o ($H_{o\text{-}ATL} = 0.644$, $H_{o\text{-}NOR} = 0.614$, $p = 0.03$). However, no difference was detected in F_{IS} values. The Mantel test for IBD was significant over the whole dataset (excluding the Mediterranean population) ($n = 14$, $r = 0.83$, $p = 0.006$) and also for the Norwegian group, despite our limited number of samples ($n = 6$, $r = 0.73$, $p = 0.03$). In contrast, no IBD was detected in the Atlantic group ($n = 8$, $r = 0.29$, $p = 0.202$).

Discussion

Genetic variability

Overall, the genetic diversity observed in the present study was comparable to those previously reported using microsatellite markers on *P. maximus* (Watts et al. 2005; Hold et al. 2012, 2013), but higher than the diversity found with EST-derived microsatellites (Charrier et al. 2012). Levels of genetic variability were also comparable with

Table 1 Population genetic differentiation

	MPJ	RDA	RLB	BQB	RDB	BSB	PEB
MPJ	–	**0.0582*** **	**0.0474*** **	**0.0544*** **	**0.0349*** **	**0.0525*** **	**0.0482*** **
RDA	**0.0000*** **	–	0.0001	−0.0023	**0.0078***	0.0010	**0.0089*** **
RLB	**0.0000*** **	0.1226	–	−0.0053	−0.0003	−0.0005	0.0046
BQB	**0.0000*** **	0.4781	0.7470	–	0.0033	0.0001	**0.0070***
RDB	**0.0000*** **	**0.0010***	0.0785	**0.0353***	–	**0.0061***	**0.0090*** **
BSB	**0.0000*** **	**0.0157***	0.2210	0.0808	**0.0000*** **	–	−0.0002
PEB	**0.0000*** **	**0.0000*** **	**0.0021*** **	**0.0015*** **	**0.0000*** **	**0.0026*** **	–
PLY	**0.0000*** **	**0.0077*** **	0.4907	**0.0264***	**0.0396***	**0.0237***	**0.0066*** **
IRL	**0.0000*** **	**0.0028*** **	0.2599	0.0538	**0.0100*** **	**0.0029*** **	**0.0000*** **
NRV	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **
NFB	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **
NVR	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **
NBH	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **
NTH	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **
NBG	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **	**0.0000*** **

	PLY	IRL	NRV	NFB	NVR	NBH	NTH	NBG
MPJ	**0.0534*** **	**0.0487*** **	**0.0677*** **	**0.0622*** **	**0.0726*** **	**0.0833*** **	**0.0741*** **	**0.0817*** **
RDA	0.0057	**0.0086***	**0.0553*** **	**0.0388*** **	**0.0388*** **	**0.0581*** **	**0.0479*** **	**0.0356*** **
RLB	−0.0010	0.0003	**0.0412*** **	**0.0290*** **	**0.0290*** **	**0.0420*** **	**0.0335*** **	**0.0248*** **
BQB	0.0055	0.0044	**0.0389*** **	**0.0305*** **	**0.0288*** **	**0.0415*** **	**0.0346*** **	**0.0233*** **
RDB	0.0042	0.0034	**0.0416*** **	**0.0356*** **	**0.0376*** **	**0.0475*** **	**0.0437*** **	**0.0330*** **
BSB	0.0019	**0.0074***	**0.0442*** **	**0.0295*** **	**0.0323*** **	**0.0456*** **	**0.0429*** **	**0.0328*** **
PEB	0.0047	**0.0112*** **	**0.0439*** **	**0.0340*** **	**0.0395*** **	**0.0503*** **	**0.0452*** **	**0.0379*** **
PLY	–	0.0069	**0.0526*** **	**0.0356*** **	**0.0396*** **	**0.0541*** **	**0.0490*** **	**0.0372*** **
IRL	0.1303	–	**0.0450*** **	**0.0333*** **	**0.0335*** **	**0.0451*** **	**0.0370*** **	**0.0298*** **
NRV	**0.0000*** **	**0.0000*** **	–	**0.0064***	**0.0083*** **	0.0023	**0.0100*** **	**0.0081***
NFB	**0.0000*** **	**0.0000*** **	**0.0027*** **	–	0.0014	0.0026	**0.0076*** **	0.0058
NVR	**0.0000*** **	**0.0000*** **	**0.0018*** **	0.0611	–	−0.0017	0.0021	−0.0037
NBH	**0.0000*** **	**0.0000*** **	**0.0123***	0.0901	0.0523	–	−0.0007	−0.0014
NTH	**0.0000*** **	**0.0000*** **	**0.0023*** **	**0.009*** **	0.0801	0.0560	–	0.0020
NBG	**0.0000*** **	**0.0000*** **	0.0811	**0.0267***	0.7343	0.0981	0.2407	–

Above the diagonal: Pairwise F_{ST} for all pairs of populations

Values indicated in bold are significantly different from 0 (permutation test, 10,000 permutations, in bold: significant values (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$) after FDR correction for multiple testing)

Below the diagonal: *p values* obtained with the exact test for genic differentiation implemented in Genepop (Rousset 2008) with 1000 dememorisations, 100 batches and 10,000 iterations per batch

In bold: p-values that remained significant after FDR correction for multiple testing (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

studies on other scallop species (Sato et al. 2005; Kenchington et al. 2006; Arias et al. 2010; Marín et al. 2012).

Null alleles were detected in three out of twelve loci (PmRM043, PmRM007 and PmRM027). One locus (PmRM043) in particular displayed very large F_{IS} values, which were most likely caused by a high frequency of null alleles. Null alleles are frequent in mollusks (McInerney et al. 2010), and they can significantly bias genetic data, e.g. overestimation of F_{ST} estimates (Chapuis and Estoup 2007). However the population structure analysis performed with and without the three loci affected by null alleles gave comparable results (similar global F_{ST}), suggesting that null alleles did not have any impact on the reliability of our results.

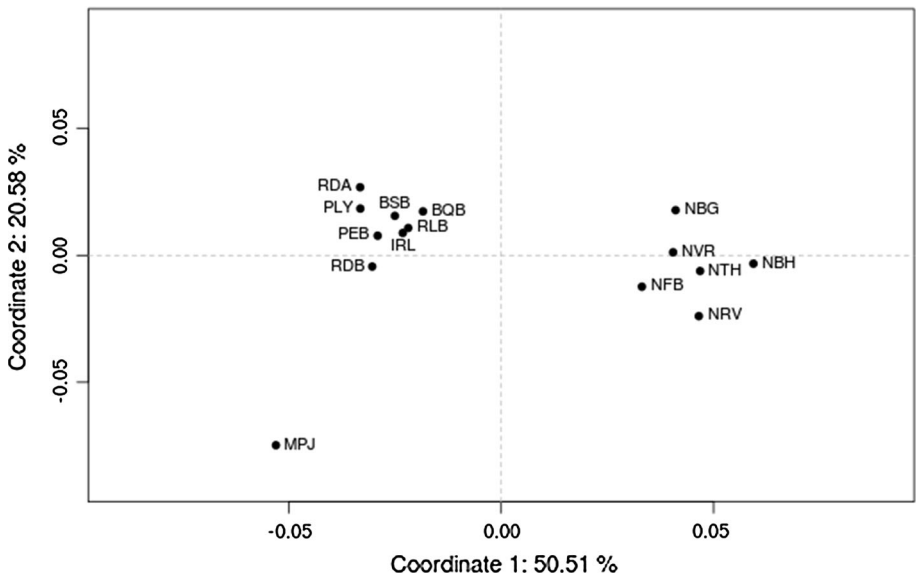

Fig. 3 MDS of linearized F_{ST} (Weir and Cockerham 1984; Slatkin 1995). Acronym signification is given in Fig. 1

Population structure

Overall, microsatellite data showed that populations of *P. maximus* are clearly structured into two groups of populations: a Norwegian and an Atlantic group (the latter being composed of populations from Galicia to Ireland). Allelic richness and observed heterozygosities significantly differed between the two groups, with Norwegian populations displaying 0.9 less allele on average than Atlantic populations and 0.03 lower heterozygosity ($p = 0.001$ and $p = 0.03$ respectively). This difference could be due to a larger proportion of null alleles in Norwegian populations; however, this seems unlikely given the lack of significant differences in F_{IS} values between both groups (a higher proportion of null alleles in Norwegian populations should induce higher F_{IS} values). The clear structuring between the Norwegian and Atlantic populations is likely to be driven by the observed difference in allelic richness. As a result, the significance of the Mantel test over the whole set of populations might reflect this difference rather than overall IBD (see also below regarding IBD within groups). Although the great scallop can have a long PLD (24–78 days in laboratory conditions; Beaumont and Barnes 1992) and therefore great ability to disperse (O'Connor et al. 2007), the genetic difference between the two groups could result, at least partly, from hydrographic features within the northern North Sea, which may constitute a barrier to dispersal between Norway and the North Atlantic (Lee 1980; Huthnance 1991, 1997; Otto et al. 1990; Hislop et al. 2001). In addition, the Norwegian Trench (100 km across, 300–700 m deep; Huthnance 1991) constitutes a barrier for benthic bivalves such as the great scallop (Rosenberg et al. 1996), thus possibly reducing the connectivity between *P. maximus* populations from the western and eastern North Sea.

The population structure assessed with microsatellites is consistent with results from previous investigations. Ridgway and Dale (2000) identified a clear differentiation between two Norwegian samples and twelve British and French samples (data from Wilding et al. 1997) using mitochondrial RFLPs. Discrepancies between levels of population differentiation using nuclear and mitochondrial genomes have often been observed in bivalve species (e.g. in the European flat oyster, Diaz-Almela et al. 2004). Differences in effective population size related to the mode of inheritance, sex-dependant variance of reproductive success or sex-biased dispersal modes have been commonly proposed to explain such differences. In the case of the great scallop, no clear difference in terms of genetic differentiation can be noticed between mitochondrial and nuclear markers (allozymes: Beaumont et al. (1993); present microsatellite data). Hermaphroditism in the great scallop might indeed contribute to reduce eventual discrepancies between nuclear and mitochondrial markers. The congruent genetic structures observed between Norwegian and Atlantic populations for both types of markers support their phylogeographic significance.

A large population group with a very limited structure, ranging from the Iberian peninsula to the British Isles, and genetically differentiated from a more northern group, has been observed in other marine invertebrates: the flat oyster *Ostrea edulis* (Launey et al. 2002), the green crab *Carcinus maenas* (Roman and Palumbi 2004) and the common cockle *Cerastoderma edule* (Krakau et al. 2012). In most cases, a phylogeographical explanation has been proposed, suggesting that this genetic structure may originate from

the existence of two glacial *refugia*: one southern, below the glaciated area and source of the Atlantic recolonization, and one northern, source of the Norwegian recolonization. Following this scenario, the genetic structure found in the great scallop would be thus explained by two independent recolonization events. However, in the case of *P. maximus*, a decreasing genetic diversity with latitude is apparent, thus supporting also the hypothesis of a single southern glacial *refugium*, with successive post-glacial (re)colonization events occurring firstly in the North-East Atlantic and later along the Norwegian coasts. According to this second hypothesis, the lower genetic diversity of Norwegian populations would thus result from founder effects that may have progressively reduced the diversity during successive (re)colonization events toward northern regions.

Norwegian cluster

The observed genetic structure of populations sampled along the Norwegian coast revealed small but significant IBD but no significant gradient in genetic diversity. Along Norway, currents flow northwards following the coast line, from the South (Skagerrak) up into the Arctic Ocean and the Barents Sea (Hopkins 1991). As a result, it is possible that a gradient of genetic diversity exists but our data is not sufficient to detect it. However, longer PLD might blur such a gradient as it has been shown to be longer at low temperature in the great scallop (Le Pennec et al. 2003).

Atlantic cluster

The weak genetic structure observed within the Atlantic group is less expected than within the Norwegian group. A very low level of differentiation from Spain to the British Isles could result from a lack of power of the microsatellite markers, failing to detect weak signals of genetic differentiation. Alternatively, this limited differentiation among Atlantic populations might be explained by regular (albeit low) gene flow over generations. High gene flow is likely to occur along French and British coasts, as *P. maximus* populations are found ubiquitously (Brand 2006). However a stepwise migration model is unlikely between Galicia and France, as there is no significant IBD detected among these regions. There is a gap in the geographic range of *P. maximus* along the northern coast of Spain, as the continental shelf is too narrow (i.e. there is not much area displaying a compatible depth with the maintenance of a functional population of *P. maximus*) and water temperatures are too high (Brand 2006). However, even though distances among populations are often too large to ensure a high connectivity, larvae could occasionally disperse over broad distances during exceptional events (e.g. wind-forced currents, storms). Genetic differentiation is not only driven by migration (or its lack of), but also by genetic drift. In our study, we could not estimate effective population sizes, most likely because they are too large and/or our markers are not sufficiently informative. So the lack of genetic differentiation along all the Atlantic coasts likely results from a combination of gene flow induced by larval dispersal and low genetic drift linked to large effective population sizes.

Comparison with phenotypic studies

The pattern of genetic structuring found in the present study can also be compared with the phenotypic variability observed in natural populations. Chauvaud et al. (2012) reported differences in shell growth patterns between "Southern" (from Spain to Wales) and "Northern" populations (from Scotland to North of Norway), and hypothesized that it could be due to phenotypic plasticity and/or genetic differentiation. We found a similar pattern of differentiation in this study, but the boundary between the two groups was placed slightly differently. Indeed, the combination of our study with those from Hold (2012) have shown that the "Atlantic" group (genetic-wise) expands from Spain to the northern North Sea. Further inquiries are needed to explore the mismatch between the phenotypic and genetic boundaries.

Similarly, phenotypic differences observed in reproductive strategies between Saint-Brieuc Bay and Scottish populations (Paulet et al. 1988; Cochard and Devauchelle 1993) could also have a genetic basis. However, in our study, The Saint-Brieuc Bay population was not clearly differentiated (even if some F_{ST}, albeit low, were significant). The use of non-neutral markers, identified using a genome-scan approach, could be useful for a further exploration of this question. The proteomic study of Artigaud et al. (2014) revealed a differentiation of proteomes between a Norwegian population and an Atlantic one. These results may partly be explained by the genetic structure observed here, although they are likely to be largely influenced by environmental factors (i.e. phenotypic plasticity).

Genetic differentiation of Mediterranean scallops

Aside from their geographic distribution, the two European scallop species can be distinguished on the basis of their shell morphology (Rombouts 1991). Several genetic studies using allozymes (Rios et al. 2002) or mitochondrial DNA (Wilding et al. 1999; Saavedra and Peña 2004, 2005) have shown very little differentiation between the two taxa, suggesting that the separation of *P. maximus* and *P. jacobaeus* into two species may not be justified. Although microsatellites are clearly not appropriate markers to

perform phylogenetic studies, it is interesting to note that no cross-species amplification problems and similar level of allelic richness were observed when genotyping Mediterranean samples using loci initially developed on Atlantic scallops. However, a comprehensive study remains to be conducted to explore thoroughly the taxonomic and phylogenetic distinction between *P. maximus* and *P. jacobaeus*.

Potential genetic influence of hatchery-based population enhancement in the Bay of Brest

The population of the Bay of Brest has potentially undergone a strong bottleneck effect caused by massive mortality in the winter of 1962–1963 (Dao et al. 1999), and has then been heavily stocked by a sea ranching program for approximately 30 years (Beaumont and Gjedrem 2006; Fleury et al. 2005). Interestingly, we have not detected a lower genetic diversity (at any of the measured parameters) compared with other Atlantic populations, or any obvious alteration of the natural population, contrary to what could possibly be expected as a result of hatchery-based population enhancement (Beaumont and Gjedrem 2006; Gaffney 2006). This is clearly shown by the magnitude of genetic diversity in relation to latitude: anthropogenic pressure has not affected the pattern of diversity resulting from the natural evolutionary history of the population from the Bay of Brest. This observation could be explained by several hypotheses: first, gene flow originating from outside the Bay of Brest may maintain a high level of genetic diversity within the population from the Bay. Alternatively, the reproductive success of hatchery-raised individual could be low in the wild, as seen in salmonids (Araki et al. 2007; Christie et al. 2012; Milot et al. 2013). In particular, seeding sites are obviously known by fishermen in the Bay of Brest, and this may result in a higher fishing pressure on hatchery-produced individuals than on wild individuals, thus drastically reducing their reproductive contribution. Furthermore, in some conditions of seeding, size of hatchery broodstock and reproductive success of hatchery-born individuals, Gaffney (2006) hypothesized an increase in effective population size due to the Ryman-Laikre effect (Ryman and Laikre 1991) in mollusk populations with large effective size in the wild, thus preventing any decrease of the genetic diversity. This effect has been confirmed in *P. maximus* in the Isle of Man by a simulation study (Hold et al. 2013), and this may be also the case in the Bay of Brest. Finally, the high level of diversity within the population of Brest may result from good aquaculture practices at the Tinduff hatchery. Indeed, the broodstock used in this hatchery is renewed each year by sampling new genitors in the wild. Moreover, to produce the requested number of spat, up to 100-120 genitors can be used in a single year.

Tettelbach et al. (2002) demonstrated that the potential reproductive success of hatchery-born individuals of *Argopecten irradians irradians* was similar to the potential reproductive success of wild-born individuals, but there was no differential fishing pressure taken into account. Similarly, a lack of impact of aquaculture practices was reported in the Japanese scallop *Patinopecten yessoensis* (Sato et al. 2005) in Japan, but some were found in hatchery production of the same species in China (Li et al. 2007). However, the magnitude of seeding and the renewal of the broodstock were very different than in the Bay of Brest. Moreover, in our study we sampled only the natural population, at one particular year. Sampling both natural and seeded populations, during several different years, will be necessary to continue exploring in details this question.

Conclusion and perspectives

P. maximus population structure appears to reflect both recent evolutionary history and actual pattern of connectivity: two clusters were observed, possibly originating from one or two glacial *refugia*. The observed genetic structure might be maintained by barriers to larval dispersal, like the Norwegian trench. This genetic structure does not seem to be impacted by anthropogenic pressure such as fishing and sea-ranching aquaculture. A more comprehensive study of the impact of sea-ranching practices, particularly in the Bay of Brest, is needed to (1) better understand the genetic and demographic interactions between stock enhancement through hatchery-based seeding and natural recruitment and (2) identify eventual adaptive changes related to hatchery-propagation or local adaption in a rapidly changing environment. Analyzing these issues could help developing a valuable model of a responsible management of an important marine resource, possibly transferable to others marine resources in need of better management. Finally, the limited results on the Mediterranean population analyzed in this study highlight the need for a comprehensive comparative study on the genetics of *P. maximus* and *P. jacobaeus*.

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Chapitre 3

Bases génétiques de la croissance : estimation des paramètres génétiques.

Manuscrit en préparation

3.1 Principaux résultats et conclusions

La croissance de la coquille Saint-Jacques est un caractère d'intérêt pour de nombreuses études scientifiques (*e. g.* Chauvaud *et al.* 1998, 2005, 2011, Lavaud *et al.* 2013, Jollivet *et al.* 2015, Marchais *et al.* 2015) et un paramètre important pour son exploitation. Une étude notable de Chauvaud *et al.* 2012 a montré qu'il existait des différences de stratégies de croissance entre les populations, qui pourraient être expliquées par des différences génétiques. Le chapitre précédent a montré qu'il existait, au sein de l'aire de répartition de *Pecten maximus*, une structure génétique correspondant globalement à ces différences de croissance. Cependant, aucune donnée sur les bases génétiques de la croissance chez *Pecten maximus* n'était encore disponible.

Ce chapitre se propose donc d'explorer les bases génétiques de différents paramètres de croissance chez la coquille Saint-Jacques par une approche de génétique quantitative. Une cohorte produite par une écloserie commerciale (écloserie du Tinduff, Plougastel Daoulas, France) à partir de 18 géniteurs a été maintenu en cage en Rade de Brest par cette entreprise, avant de subir une expérimentation d'un mois et demi en conditions contrôlées (site expérimental d'Ifremer à Argenton). La moitié des individus ont été soumis à une augmentation progressive puis un maintien de la température, de 13,5°C à 21,5°C. L'autre moitié a été maintenue à 13,5°C. A la suite de cette expérimentation, les individus ont été phénotypés (paramètres de croissance (Fig 10) et paramètres métaboliques : réserves, activité enzymatique) et génotypés à 12 marqueurs microsatellites. Le pedigree a été reconstitué par assignation de parenté. Une approche de type « modèle animal » (Wilson et al. 2010) a été utilisée pour évaluer des paramètres d'intérêt pour chaque phénotype : héritabilité, interaction génotype x environnement, corrélations génétiques.

Résultats principaux

Les résultats montrent une héritabilité modérée (0.06-0.29) mais significative pour tous les paramètres de croissance, montrant une base génétique non négligeable dans la variabilité des paramètres de croissance. De plus, une corrélation génétique forte entre certains paramètres de croissance a été mise en évidence, notamment la taille au premier hiver et la date de reprise de croissance, indiquant que les familles ayant le plus grandi durant leur premier hiver sont aussi celle qui ont repris leur croissance le plus tôt. Il n'y a pas de corrélation génétique entre ces phénotypes reflétant la croissance en cage en mer et la croissance mesuré en milieu expérimental, comme reflêté par leréarrangement du rang des familles entre ces deux types

d'environnement. Ces rangs sont par contre conservés entre les conditions expérimentales (pas d'interaction GxE significative détectée). De plus, les paramètres de réserves sont corrélés avec la croissance en milieu expérimental, ce qui suggère que c'est le passage des cages en mer au milieu expérimental qui aurait modifié la physiologie des individus.

Conclusions

L'utilisation d'une cohorte d'une écloserie commerciale présentant un plan de croisement clairement non optimal pour des approches de génétique quantitative nous a tout de même permis d'évaluer des paramètres génétiques grâce à l'approche du modèle animal (Wilson *et al*. 2010). Ce modèle montre qu'il existe une base génétique modérée pour la croissance de la coquille Saint-Jacques, qui semble se conserver dans le temps si l'environnement ne varie pas. Cependant, celle-ci s'exprime différemment suivant le milieu (cage en mer ou écloserie expérimentale), ce qui met en évidence la nécessité d'une prudence pour extrapoler au milieu naturel des résultats biologiques obtenus en milieu contrôlé pour la coquille Saint-Jacques.

Estimating heritability and genetic correlations of growth-related traits under wild and controlled conditions based on reconstructed pedigree in a commercial hatchery cohort of a marine bivalve.

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Keywords: shell growth, heritability, genetic correlations, *Pecten maximus*, aquaculture, temperature.

Introduction

Shell growth in marine bivalves is a phenotypic trait of particular interest for aquaculture (Gjedrem 1983, Grant 1996, Ferreira *et al.* 2007), biological (Bayne 2004) or environmental studies (Bayne *et al.* 1979). Similarly to dendrochronology, in numerous bivalve species, shell rings can allow aging wild individuals (Mason 1957) or even recording their daily growth and the eventual impact of environmental variations (Chauvaud *et al.* 1998). This property has notably been used in the great scallop, *Pecten maximus*, as a high frequency paleoclimate record (Chauvaud *et al.* 2005), as their growth rings are impacted by variations of seawater temperature and availability of trophic resources (Chauvaud *et al.* 1998, Lorrain *et al.* 2000). Isotopic studies along the dorso-ventral axis of the shell have also been conducted, linking daily growth rate, temperature and primary production to stable isotope ratio variations (Chauvaud *et al.* 2011, Thébault & Chauvaud 2013, Jolivet *et al.* 2015, Marchais *et al.* 2015). In most of these studies, variation among populations has been investigated over time or space, but variation among individuals remains to be investigated from a quantitative and evolutionary genetics point of view.

Interestingly, Chauvaud *et al*. (2012) reported different shell growth patterns between great scallops sampled in northern and southern European populations, suggesting distinct evolutionary units. These phenotypic observations were recently partly corroborated by a population genetics study, based on microsatellite markers (Morvezen *et al*. 2015), suggesting genetic bases for the observed traits. However, plasticity across the species range cannot be excluded to explain the observed phenotypic variations in wild populations facing different environmental conditions. In this context, the study of the genetic and environmental bases of shell growth variation of the great scallop is of particular interest.

Significant heritability of growth has been observed in numerous bivalve species under aquaculture conditions (Gjedrem 1983). Growth traits generally display a moderate heritability (0.15-0.40, *e.g.* Jarayabhand & Thavornyutikarn 1995; Ernande *et al*. 2004; Toro *et al*. 2004; Dégremont *et al*. 2007; Li *et al.* 2011), and, for some species under certain conditions, a high heritability (0.7-0.9, Rawson and Hilbish 1990, Toro *et al*. 2004, Wang *et al.* 2010) has been reported. Growth-related traits have been shown to exhibit significant genotype by environment interactions,; for example the Pacific oyster *Crassostrea gigas* and silver-lip pearl oyster *Pinctada maxima* in different nutritional conditions (Ernande *et al.* 2004; Kvingedal *et al.* 2008), the hard clam *Mercenaria mercenaria* in different locations (Rawson and Hilbish 1991).

Body size traits such as length or weight are generally genetically correlated among themselves (Ibarra 1999, Wang *et al.* 2011). Growth has been linked to various physiological and metabolic parameters in bivalves (Thomson & Bayne 1974, Hedgecock *et al.* 1996, Bayne 2004, Huvet *et al.* 2008) and notably in scallops (Wilkins 1973, Heilmayer *et al.* 2004). Conveniently in bivalves, individual growth (total size or weight) is often correlated with shell size although some decoupling between shell growth and soft tissue growth has been observed (Hilbish 1986). However, limited data is available on the genetic basis underlying these links (*i.e.* genetic correlations between growth and physiological or metabolic traits).

In this context, a quantitative genetics approach (i.e. estimating components of variance of phenotypic traits among individuals showing different levels of relatedness) would be most appropriate to investigate genetic bases of variation of daily shell growth in *P. maximus*. In wild mollusk populations, large effective population sizes lead to very low relatedness between individuals within populations

(e.g. in flat oysters (Lallias *et al.* 2010), or in the great scallop (Hold 2012)). The individuals are essentially unrelated and the absence of a pedigree precludes the use of a quantitative genetics approach. However, hatchery seed can be produced for population enhancement programs (Alban & Boncoeur 2008) or aquaculture (Robert & Gérard, 1999, Cano *et al.* 2000, Bergh & Strand 2001) using a limited number of parents. Genetic markers, such as highly variable microsatellite loci, allow identifying related individuals in progenies (Herbinger *et al.* 1995, Thomas & Hill 2000, Wilson *et al.* 2003, Shikano 2007, Morvezen *et al.* 2013).

In the present study, we recorded growth-related traits in one hatchery-produced cohort of juvenile great scallops. Individuals were sampled from aquaculture offshore cages and then maintained under controlled conditions at two temperatures. Genotyping at twelve microsatellite markers was performed to identify related individuals, allowing the estimation of quantitative genetic parameters for the recorded traits.

Material and methods

Sampling

We sampled 563 one-year scallops produced by the crossing of 18 parental individuals in the Tinduff hatchery (Plougastel-Daoulas, France). Great scallops being simultaneous hermaphrodites, most individuals were used both as male and female. Batches of mixed spermatozoa from five to six individuals were used in crosses with each individual female. Overall, 3 individuals were used only as male, and 15 individuals were used both as female and male. These crosses were performed on the same day in spring of 2012 and produced progenies spent their larvae and post-larvae stages in the hatchery facility. They were then transferred in early September 2012 in offshore cages in the Bay of Brest (St Anne du Portzic, Brest, France). One-year old scallop were sampled in late April 2013 and immediately brought to the experimental facility of Ifremer in Argenton (Landunvez, France).

Experimental setup

Scallops were randomly divided in two equal batches, and separated into two tanks side-by-side in the same room with identical water inflow. They were first kept in filtered seawater at 13.5±0.5°C, with 0.8mg.L^{-1} chloramphenicol for two three-day quarantine periods, separated by one day with only filtered/sterilized seawater renewal. This is a common protocol in the experimental hatchery to avoid bacterial contamination of the facility when using bivalves sampled in the natural environment (Holbach *et al.* 2015). Feeding then started with *Isochrisis galbana*, *Chaetoceros gracilis* and *Skeletonema costatum* (Ratio of concentrations: 2/1/1) *ad libitum,* until

the end of the experimentation. One week after the start of feeding, temperature was progressively increased in one of the two tanks, at a rate of 1°C.day^{-1} starting at 13.5±0.5°C, until the final temperature of 21.5±0.5°C. This temperature was maintained for three weeks, and constituted the "warm" condition. The other tank, the "control" condition, was maintained at 13.5°C±0.5°C for the entire duration of the experimentation.

Upon dissection, a fragment of mantle was conserved in 95% ethanol for subsequent DNA analysis, and the adductor muscle and gills were individually flash frozen in liquid nitrogen, and stored in liquid nitrogen for further biochemical analyses (see below).

Pedigree reconstruction

Pedigree was reconstructed by genotyping 12 previously published microsatellite markers (in 3 multiplex PCR: *mx1*, *mx2*, *mx4*, Morvezen *et al*. 2013, 2015) on the 18 candidate parents and 563 offspring. DNA extraction, PCR and genotyping were performed following the protocol in Morvezen *et al.* (2013, 2015).

Parentage assignment was performed with CERVUS v3.0 (Kalinowski *et al*. 2007), using the following parameters: 10 000 replication cycles, 18 candidate parents, 100% of candidate parents sampled, 97% loci typed and a 5% error rate. Only trios (i.e. a pair of parents assigned to an offspring) with the maximum likelihood score were kept.

Growth parameters

For all individuals, three traits were recorded using a digital caliper (fig 10): length along the dorso-ventral axis (L_{dv}), length along the antero-posterior axis (L_{ap})

and ear spacing (L_{EAR}). Total wet weight (W_t) and adductor muscle wet weight (W_m) were measured. Condition index (CI) was calculated using Fulton (1902) equation ($CI = \frac{W_T}{L_{dv}^3}$)). Muscular condition index (CI_m) was calculated similarly as ($CI = \frac{W_m}{L_{dv}^3}$)

Scallops in the Bay of Brest commonly start their growing season at the beginning of April, but variation is observed both between individuals and between years (Chauvaud *et al*. 1998). As we sampled the animals at the end of April 2013, we therefore expected about one month of post-winter growth at sea. This was recorded in the shell because scallops usually display a ring indicative of a stop in growth due to low temperature (i.e. in winter, WR in fig 10) or due to a stress that can result from transfer from one condition to another (SR in fig 10). Consequently, scallops used in this experiment typically displayed two shell rings at the end of our experiment: the outermost (most recent) one caused by the stress of the transfer in the experimental facility: the “stress ring” (SR), and the older, inner one caused by the winter (WR). We were then able to assess separately post-winter growth in the sea (G_{st} in fig 10) and the experimental growth (*i.e*. in tanks, G_{lab} in fig 10). However, due to individual variation of the timing of post-winter growth start in the wild, SR and WR were confounded in some individuals indicating that these individuals had not resumed growth at sea by the time they were transfered in to the experimental tanks (i.e. G_{st} =0).

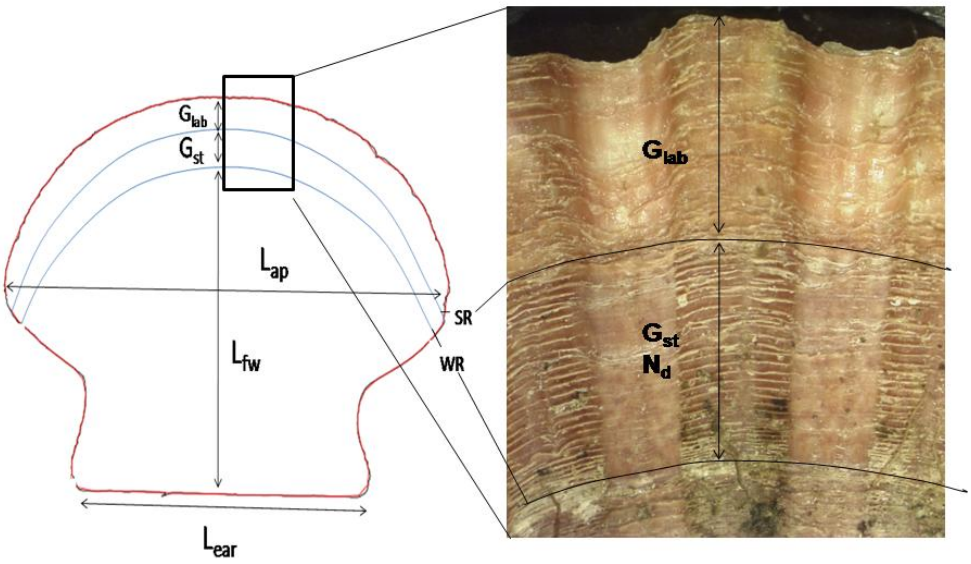

Fig 10: Growth related traits measured on each individual. WR: Winter Ring; SR: Stress Ring. L_{ap}: Antero-posterior length; L_{ear}: length between ears; L_{fw}: Length at the end of first winter; G_{st}: Total growth in semi-natural condition after growth resumption; G_{lab}: growth in experimental conditions. Note that the dorso-ventral length, $L_{dv} = L_{fw}+G_{st}+G_{lab}$

Shell growth under controlled experimental conditions (G_{lab}) was estimated by measuring the length of the total growth between shell margin and the SR, along the dorso-ventral axis, using binocular magnifier and an image processing software (VISILOG). Similarly, post-winter growth at sea (G_{st}) was measured between SR and WR. Daily growth rings, as defined by Chauvaud *et al*. 1998, were easily apparent only for the period of growth at sea, and thus assessed only for this period. Number of days of post-winter growth at sea t (N_d) was obtained for each individual by counting the number of daily growth rings between SR and WR. Since the SR occurred on a fixed date (i.e. the day scallops were transfered from sea cages to the tanks), common to all individuals, N_d is directly related to the individual date of growth resumption post-winter at sea. As mentioned above, for some individuals SR was confounded with WR and therefore both G_{st} and N_d were zero. Mean daily growth rate was calculated as $G_{nm} = \frac{G_{nt}}{N_d}$. Finally, the size of each daily growth ring

was measured individually following Chauvaud *et al*. (1998). For each individual scallop, a vector containing one daily growth measurement for each day of growth at sea was obtained. The length of this vector was indeed variable between individuals, as the date of growth resumption was variable among individuals.

Biochemical parameters

Biochemical parameters were assessed on a subset of the families after pedigree reconstruction in order to study metabolic bases potentially associated with observed heritable growth variation. Full-sib families were selected according to the following procedure. Full-sib families displaying less than five assigned progeny in a given experimental condition (i.e. warm or control) were discarded. Among the remaining families, four full-sib families resulting from parental individuals with the highest and lowest mid-parent breeding values for growth parameters at sea (see below) or under controlled conditions were selected for biochemical analyses. One average full-sib family for all parameters was also studied. For each family, 5-6 individuals in each tank were randomly sampled for further analyses.

Gylcogen content was measured using a weighted fragment of adductor muscle (50-100mg) mixed and diluted to 1/100 (W/V) in distilled water. The homogenate was boiled to inactivate enzymes, and glycogen content was assessed with the Glycogen Assay Kit (Sigma-Aldrich, St Louis, MO, USA) according to manufacturer's instruction. Glycogen content was obtained in mg glycogen/g muscle.

Citrate synthase (CS) activity was measured as a proxy of energetic metabolism using Bergmeyer & Gawehn (1970) protocol, at 21.5°C. Glucose-6-Phosphate deshydrogenase (G6PDH) activity was measured at 21.5°C as a proxy of catabolic metabolism, using Noltmann *et al.* (1961) protocol. Total protein content

was assessed by Bradford quantification (Bradford, 1976). Specific activity was obtained in U/mg proteins.

Statistical analyses

Quantitative genetic models

Models' equations are given in table 1. All models were run with ASREML v4.0 (Gilmour *et al.* 2009). Some traits (G_{st}, G_{sm}, $N_{d,}$ L_{fw}) corresponded to the growth period at sea and therefore could not be influenced by the period under controlled conditions that followed. Those traits are named "sea traits" hereafter. Other traits are named "laboratory traits" (i.e corresponding to our two controlled conditions: warm or control). Heritability estimates were computed using a simple univariate animal model (AM-1) for sea traits (complete data set) and for laboratory traits in separate environment (warm data set or control data set). When no evidence of GxE was found (see below), heritability of laboratory traits were also estimated on the complete data set with a model (AM-1) including a fixed temperature effect T. The significance of heritability was tested with a log-likelihood ratio test, comparing the fit of the full model (including additive genetic variance σ^2_a) to that of the reduced model with only the fixed effects. Twice the difference of the log-likelihood is approximately distributed as a chi-square with 1 degree of freedom (Wilson *et al.* 2010). Fixed effect of temperature was estimating using Wald test implemented in ASREML.Genetic correlations between traits were estimated with a bivariate animal model (AM-2) including a temperature fixed effect for the laboratory traits but not for the sea traits. Significance of the genetic correlation was tested as well with a log-likelihood test, comparing the fit of the full model with that of a reduced model where the additive genetic covariance ($\sigma_{12\,a}$) between trait 1 and 2 was fixed to zero.

GxE effects were first tested with an univariate model (GxE-1). The significance of GxE effects was assessed by comparing the fit of the full model including an interaction term to the fit of a reduced model where the variance of the interaction term was fixed to zero. Then, for experimental traits with which a significant GxE interaction was detected, Falconer bivariate model (Falconer, 1952) was used to estimate the magnitude of the genetic correlation between the same trait measured in the two controlled environments (model GxE-2). A genetic correlation of 0.8 and below is usually taken as indicating significant GxE interaction (Ponzoni *et al.* 2008).

Finally, the daily growth rate data were composed of a series of repeated individual measures over time (one measure each day from the start of the growth post-winter). They were thus analyzed with a repeated measure model (LA-RM) with two time-related fixed effect: D (Date of the growth ring) and TIME (relative period since growth started at sea, i.e. day number 1 being the first day of growth post-winter for this individual). The first fixed effect D was intended to capture any date specific event that would have affected the daily growth rate of every individual (e.g. stormy weather, sudden change in temperature) while the second was intended to capture longitudinal trend among repeated measures (*e.g.* initial daily growth measures just after the resumption of growth tended to be smaller than daily growth measured later on).

Significance of recorded metabolic parameters

Two-way ANOVA were performed in R (R Core team 2013) on metabolic parameters to assess the effects of temperature and family factors. To compare metabolic parameters with growth parameters, family rankings for metabolic parameters were obtained by ranking the means of traits in given environment, and compared to family breeding value ranking calculated for experimental growth(G_{lab}). Rankings between traits were then compared with Spearman rank correlation test (r_s) in R (R core team 2013).

Table 1 : Models used to estimate quantitative genetic parameters.

Name	Traits	Formula	VC	FE	Model outputs
AM1	L_{dv},L_{ap}, L_{ear},L_{fw}, W_t, W_m, CI, CI_m, G_{lab} (lab traits) G_{st}, G_{sm}, N_d, L_{fw} (sea traits)	$y_i = \mu\ (+T) + a_i + \varepsilon_i$	$\sigma_a^2, \sigma_\varepsilon^2$	μ, (T)	$h^2 = \frac{\sigma_a^2}{\sigma_a^2 + \sigma_\varepsilon^2}$
AM2	All pairs possible	$\begin{cases} y_{1i} = \mu_1(+T_1) + a_{i1} + \varepsilon_{1,i} \\ y_{2i} = \mu_2(+T_2) + a_{i2} + \varepsilon_{2,i} \end{cases}$	$\sigma_{1a}^2, \sigma_{2a}^2, \sigma_{12a}$ $\sigma_{1\varepsilon}^2, \sigma_{2\varepsilon}^2, \sigma_{12\varepsilon}$	μ_1, μ_2 (T_1, T_2)	Genetic correlations = $\frac{\sigma_{12a}}{\sigma_{1a} \times \sigma_{2a}}$
GxE-1	Lab traits	$y_i = \mu_1 + T + a_i + d_i + \varepsilon_i$	$\sigma_a^2, \sigma_\varepsilon^2, \sigma_d^2$	μ, (T)	σ_d^2 : variance of interaction deviation (due to GxE effect)
GxE-2	only for Traits with σ_d^2 significantly different from 0 in GxE-1 model	$\begin{cases} y_{1i} = \mu_1 + T_1 + a_{i1} + \varepsilon_{1,i} \\ y_{2i} = \mu_2 + T_2 + a_{i2} + \varepsilon_{2,i} \end{cases}$	same as in AM2	same as in AM2	Falconer Character state effects. GxE effects assessed with genetic correlations across environments (= $\frac{\sigma_{12a}}{\sigma_{1a} \times \sigma_{2a}}$
LA-RM	Daily growth rate	$\log(y)_{ik} = \mu + TIME_{ik} + D_k + a_i + PE_i + \varepsilon_{ik}$	$\sigma_a^2, \sigma_{PE}^2, \sigma_\varepsilon^2$	μ, TIME, D	h^2 for the daily growth rate

i: index of individual i

T: fixed effect of temperature. Only fitted for laboratory traits in complete data set

k: index of repeated measure within i (model LA-RM)

The two compared traits are coded as y_1 & y_2 in model AM-2

For GxE estimations with Falconer character state effect model, traits are coded as y_1 when measured in one environment and y_2 when measured in the other environment (model GxE-2)

Results

Pedigree reconstruction

Only two individuals out of the 563 genotyped could not unambiguously be assigned to a single pair of parents. Assignment revealed 69 full-sib families in our progeny. As expected, family size was highly variable, ranging from 1 to 57 offspring. All 18 potential parental individuals were found to have offspring in the sampled progeny (i.e. constituting 18 half-sib families), but with a very uneven distribution, ranging from 1 to 154 offspring (fig 11).

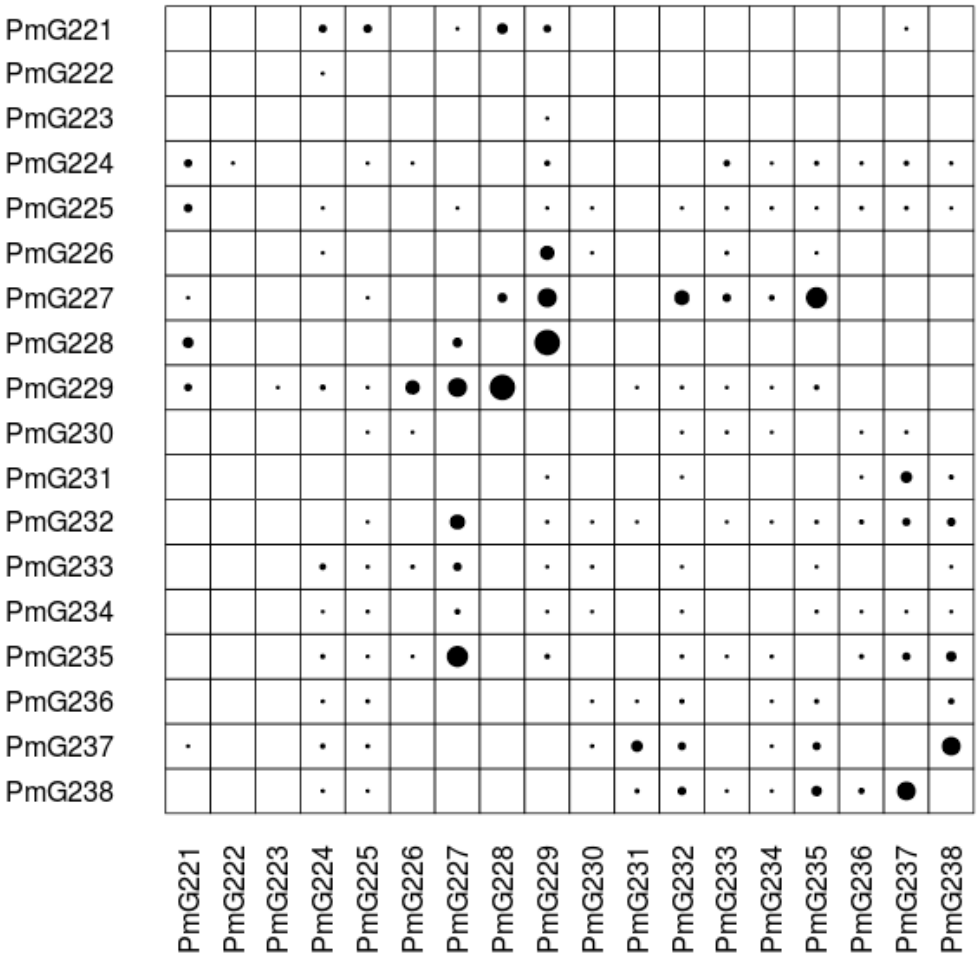

Fig 11: Family sizes variations based on the pedigree reconstructed with microsatellite markers. Row names and columns names are parents used in the hatchery, and the circle size of each case is the relative size of the family.

Heritability estimates

Heritability estimates for each phenotypic trait (model AM1) are given in table 2. When analyzed over the complete data set, significant low to moderate heritabilities were found for all traits (0.06-0.29). When analyzed separately by environment, heritability estimates were globally higher in our warm condition than in the control one, except for G_{lab} and CI_m. Heritability of condition index (CI) was low and non-significant, except when all data was considered together. Temperate effect was significant for all laboratory traits except CI_m (table 2). Heritability of daily growth rate was estimated by LA-RM model at 0.07± 0.04 (s.e.). The repeatability was high (0.42±0.02) indicating a strong permanent environment effect. TIME and date fixed effect were high and significant (Wald's F=121.03 and 2.66, respectively, and p<0.001 for both).

Table 2: Heritabilities, temperature effect and GxE interactions. Heritability estimates and their standard error for each trait, in each experimental environment and overall dataset, were estimated by model AM1. Temperature effect was estimating using Wald F test implemented in ASREmL, for model AM1. GxE effect were estimated by model GxE-1, and significance of the effects were tested with a likelihood ratio test between model GxE-1 and AM1. Note that for "sea traits", estimating heritability, temperature and GxE efects in each experimental environment is non-pertinent. Significant heritability and GxE estimates are given in bold (*: p<0.05, **: p<0.01, ***: p<0.001, Likelihood ratio test)

	control		warm		All data		Temperature	GxE
	h^2	s.e.	h^2	s.e.	h^2	s.e.	Wald's F	
L_{dv}	0.13	0.10	**0.26*** **	0.13	**0.22*** **	0.10	**15.06*** **	-
L_{ap}	0.15	0.11	**0.26*** **	0.13	**0.22*** **	0.10	**15.42*** **	-
L_{ear}	**0.24** **	0.13	**0.26** **	0.13	**0.29*** **	0.12	**16.65*** **	-
W_t	0.12	0.09	**0.28*** **	0.14	**0.21*** **	0.10	**17.74*** **	-
W_m	0.13	0.10	**0.30*** **	0.14	**0.22*** **	0.10	**15.36*** **	-
CI	0.04	0.06	0.10	0.10	**0.06***	0.05	**11.06*** **	-
CI_m	**0.31*** **	0.15	**0.16** **	0.10	**NA**	0.08	2.84	*
G_{lab}	**0.22***	0.13	0.14	0.11	**0.15*** **	0.08	**95.03*** **	-
N_d					**0.15*** **	0.08		
G_{sm}					**0.25*** **	0.11		
G_{st}					**0.25*** **	0.12		
L_{fw}					**0.18*** **	0.10		

Genotype x environment interactions

For experimental traits, the only GxE parameter being significantly different from 0 in model GxE-1 was for the muscular condition index (CI_m) (Table 2). Indeed, the genetic correlation between this trait measured in the two environments (Falconer character state model) was estimated at 0.58, confirming the detection of a significant GxE interaction.

Genetic correlations

Genetic correlations (table 3) between traits were high and highly significant between all "sea traits" (0.84-0.94), as well as between all lengths and weights "laboratory traits" and "sea traits". G_{exp} and CI were not significantly correlated to any parameter. However, CI_m was significantly correlated to W_m, G_{sm} and G_{st}.

Table 3: Genetic correlation between traits, estimated by model AM-2. As they are all highly correlated, only one total length parameter is presented (Ldv). (Significance was tested by a likelihood ratio test against a model with covariance fixed to 0. $^{+}$p<0.1; *p<0.05; **p<0.01; ***p<0.001)

	Wm	Ldv	G_{lab}	CI	CIm	Nd	Gst	Gsn	Lfw
Wt	**0,98*** **	**0,97*** **	-0,15	0,37	0,54	**0,94*** **	**0,94*** **	**0,87*** **	**0,96*** **
Wm		**0,96*** **	0,05	0,32	**0,66***	**0,96*** **	**0,96*** **	**0,82*** **	**0,91*** **
Ldv			-0,12	0,16	0,47	**0,96*** **	**0,96*** **	**0,89*** **	**0,97*** **
G_{lab}				-0,25	**0,73^{+}**	-0,10	0,22	0,12	-0,15
CI					0,48	0,57	0,59	0,77	0,17
CIm						0,68	**0,84***	**0,65***	0,34
Nd							**0,96*** **	**0,85** **	**0,94*** **
Gst								**0,97*** **	**0,90** **
Gsm									**0,87** **

Metabolic traits in selected families

Analysis of variance showed significant family and temperature effects for G_{lab} and glycogen content (table 4). No significant effects were detected for enzymatic activity traits, although the p-values were low, and close to 0.05 in the case of G6PDH. This might indicate a limited power to detect possible effects because of small sample size. Family ranks were significantly correlated for glycogen content and G_{lab} in both environments, indicating that families with higher growth also had higher glycogen content in adductor muscle (table 5). Glycogen content rankings were significantly correlated with Citrate

Synthase activity in the control environment, indicating that family with higher energetic metabolism also had higher reserves in that environment. G_{lab} and glycogen content rankings were correlated between environments, but not enzymatic activities.

Table 4: Two-way Anova results for metabolic traits and G_{lab} measured on the 5 selected families. D.F : degree of freedom ; F : F-value ; p : p-value. See fig 10 and main text for phenotype details

	D.F.	G_{lab}		Glycogen		CS		G6PDH	
		F	p	F	p	F	p	F	p
Temperature	1	**6.88**	**0.013**	**16.9**	**0.000**	2.30	0.137	3.38	0.07
Family	4	**5.07**	**0.002**	**2.8**	**0.039**	2.09	0.10	2.26	0.08
Temperature x Family	4	1.18	0.33	0.17	0.95	1.76	0.16	0.6	0.66
Residuals	37								

Table 5: Family Rank correlation for metabolic traits. Above diagonal: warm environment. Below diagonal: control environment. Diagonal: correlation of the same trait in both environments. In bold: value significantly different from 0. * p<0.05, **p<0.01, ***p<0.001

	G_{lab}	Glycogen	CS	G6PDH
G_{lab}	**1.0***	**1.00*****	-0.5	0.7
Glycogen	**0.9***	**0.9***	-0.5	0.7
CS	0.8	**0.9***	-0.2	0.2
G6PDH	0.1	0.3	0.0	0.4

Discussion

Pedigree reconstruction and power to estimate genetic parameters using a highly unbalanced dataset.

The set of markers used in our study was sufficiently powerful to assign almost all offspring to a pair of parental scallops. The commercial hatchery progeny used in our study displayed a highly unbalanced family structure, indicating high genetic variance in reproductive success between parents. This observation is common in bivalve progenies resulting from controlled reproduction in hatcheries (e.g. Boudry *et al*. 2002, Lallias *et al*. 2010). Despite the observed unbalanced parental contributions in the studied progeny, the difficulties to balance the crosses between hermaphroditic parents and the overall low sample size (563 progenies), the use of Linear Mixed Models (*i.e.* the animal model) to estimate heritability and other quantitative genetic parameters proved robust. All models converged reliably and results were overall consistent. Statistical power was limited when considering each experimental condition separately; the heritabilities of experimental traits estimated in separate laboratory conditions were non-significant for lower values (h^2<0.20, table 2, but see below). Similarly, moderately high genetic correlations (between ~0.5 and ~0.8) were generally not significant (table 3). This is likely a result of sampled family sizes being too small in our limited pedigree to reliably estimate low heritabilities or low to moderate genetic correlations between traits. Significance of the genetic correlations found with our suboptimal dataset should be further confirmed using a larger set of families. Nevertheless, our study demonstrates that useful quantitative genetic parameters can be obtained using microsatellite-reconstructed pedigree of commercial cohorts.

Heritability estimates of growth in relation with environmental conditions

Our results showed low to moderate but significant heritability for all traits measured when analyzed in the large combined data set (Table 2). This is consistent with previous study of growth in bivalves (Jarayabhand & Thavornyutikarn 1995; Toro *et al*. 2004; Li *et al* 2011, Dégremont *et al*. 2007) and show that there is potential for improvement of aquaculture production through selective breeding or for response to eventual natural selection in wild populations. Interestingly, heritability estimates were higher in our warm condition relative to the control, suggesting that higher phenotypic variance resulting from higher temperature led to increased variance between families, potentially revealing unfavorable conditions despite higher growth (Hoffman & Merilä 1999).

Heritability estimates can vary when measured in different environmental conditions, notably between natural and controlled conditions, although they can generally be considered as good approximations (Weisenberg & Roff 1996). In our study, quantitative genetic parameters of growth in semi-natural conditions (i.e. cages in open sea) and in experimental laboratory conditions have been estimated successively. Interestingly, our heritability estimates for those two types of traits are globally similar, showing that there is significant genetic background influencing the growth of the great scallop. This observation could indicate that the genetic hypothesis to explain growth differences between scallop populations at the European scale (Chauvaud *et al*. 2012) may be plausible. However, caution must be taken when extrapolating results to different populations. In our study we only had scallops produced with parents from a single population (the Bay of Brest). Genetic basis of growth might be different in scallops with different genetic background, such as Norwegian scallops (Morvezen *et al*. 2015).

Phenotypic and genetic correlations between integrative traits resulting from growth during both semi-natural and controlled periods.

When interpreting results of between environment effects (notably GxE effects), it is important to consider that all animals have shared a common environment (a cage in the bay of Brest) for 8 months prior to spending 1.5 months under different laboratory conditions. So growth-related traits such as lengths and weights (L_{dv}, L_{ap}, L_{ear}, W_t, W_m) that we recorded at the end of experimentation might have been driven by individual differences experienced in the initial common environment. This hypothesis is comforted by the high genetic correlations between traits recorded under semi-natural conditions (number of growth days, total and mean daily growth) and experimental traits such as lengths and weights. The global lack of genotype x environment interactions for lengths and weights measured at the end of the experiment indicated that rankings between families were similar in both controlled environments, and could be explained by these correlations. Indeed, if total length or weight is mainly driven by what happened before the experimentation, the experimental settings are expected to not have a strong influence on those phenotypes. A longer period of contrasted environmental conditions might have been necessary reveal genotype x environment interactions due to temperature.

Phenotypic and genetic correlations between traits only resulting from growth during the laboratory growing periods.

Some phenotypes are not necessarily expected to be directly correlated with previous common environment. Growth increment in experimental settings (G_{lab}), for example, was not significantly genetically correlated with any other parameters, except loosely with the muscular condition index ($p = 0.06$). This result indicated that re-ranking of families performance occurred when transferred in the experimental settings. However, since no GxE effect was found for G_{lab} and families breeding values were correlated

between environments, rankings between both temperature conditions were consistent. This result suggested that it was the experimental settings and not the temperature changes that changed family performance. Similar results are found for the condition index and muscular condition index, except that a significant genetic interaction is found between temperatures for that last trait. It is difficult to interpret condition indexes, as they are calculated using highly correlated traits (total weigh, muscle weigh and length), that are themselves largely influenced by previous common natural environment (see above). Muscular condition index seemed to show an intermediate response between other "laboratory traits" and "sea traits": it still was significantly correlated with several natural traits, but not all. If the experiment had been run longer, it is probable that CI_m would have behaved more like G_{lab}, and would not have been found to be correlated with any traits measured in the natural milieu previous to experimentation.

Metabolic basis of heritable growth variations during the controlled growing periods.

Significant family and temperature effects were revealed by our ANOVA analysis on experimental growth G_{lab} and glycogen content of the adductor muscle, but not on enzymatic activity. This is probably because our sample sizes were limited and might be biased by the fact that enzymatic activities were measured at a single temperature. The observed family ranking correlation between glycogen and growth might be indicative of different feeding capacities among families. Glycogen accumulation as often been linked to somatic growth and maturation in preparation for gametogenesis in bivalves (Thompson, 1977, Barber and Blake 1981, Pazos *et al* 1997, Strohmeier *et al*. 2000) and associated to food intake. Our results confirms the correlation between growth and reserves accumulation at the genetic level; in the same food availability conditions, families with higher growth rate also accumulated more reserves, suggesting that differences between families might be explained by different feeding rates. However, this

observation has been made in experimental settings, and scallops held under experimental conditions are easily stressed. They tend to close their valves frequently so that their shell growth rings are disrupted. This movement is very costly, and can hinder growth and other processes (Robson *et al* 2011). Therefore, differences between families in their susceptibility to disruption could also explain the correlation between glycogen content and growth. Note that disruption could also explain why daily growth appeared erratic and could not clearly be measured in laboratory condition.

The correlation between the energetic metabolism and glycogen in the control environment but not the warm environment could also be explained by the same phenomenon. The absence of a correlation in the warm environment could be indicative of genotype by environment interaction for metabolic traits, as indicated by the absence of correlation for enzymatic activities between environments. The increase in temperature might induce physiological tradeoffs between maintenance, reserves and growth (Angilletta *et al*. 2003), creating the possible genotype-environment interaction observed here. However, as only five families were measured, this remains a putative explanation at present. A proper quantitative genetic study of metabolism and reserves in relation to feeding rate and growth is needed to assess genetic relations and potential tradeoffs between them (Angilletta *et al*. 2003).

Growth-related traits recorded at sea

Heritability of daily growth rate at sea, estimated by model LA-RM, was lower than estimates for other growth-related traits. This could be explained by the very large permanent environment effect found. A permanent environment effect implies that there is strong correlation between repeated measures of a given individual, that is not genetically based (Visscher *et al*. 2008, Wilson *et al* 2010).This permanent environment effect could reflect variability of local environmental conditions at sea, such as food availability or

hydrodynamic parameters influenced by the scallop placement in the cage. For example, scallops closed to the cage border could have greater food availability than scallop in the center of the cage. To refine heritability estimate for the daily growth rate, this potential position effect could be minimized with a lower density of scallops in the cage, or when seeded on natural beds where scallops could experience less potential competition for food availability.

There is a strong genetic correlation between growth-related traits recorded under semi-natural conditions. Families showing larger size at the end of autumn (L_{fw}) started to grow earlier the following spring. Consequently, genetically based growth performance appeared conserved over time (*i.e.* between growing periods). However, it is important to remember that scallops were not in completely wild, natural conditions, as they were maintained in cages. The apparent conservation of family rankings for growth traits could in fact be reflective of a genetic differential tolerance to maintenance in semi-artificial conditions, in a manner similar to what might have been observed for laboratory traits (see above). This has implication for the aquaculture industry, as this would indicate selection potential for adaptation to artificial condition and domestication. However such selection, intended or not, could also have implication for the fitness of the seeded and the wild animals in the natural milieu. Loss of fitness has been documented in hatchery produced salmonids (Araki *et al*. 2007, Christie *et al*. 2012) and, although never (to our knowledge) observed in a bivalve species, caution must be advised. Proper assessment of reproductive success and fitness of hatchery produced scallops seeded on wild stocks should be conducted.

Conclusions and perspectives

The present study estimated genetic parameters for growth-related traits in a commercial cohort of great scallop originating from the Bay of Brest. Similar studies should be made at a larger scale, with scallops from a different populations (Norway, Mediterranean Sea) in common garden experiments with hybridization between lineages, or with reciprocal transplants to evaluate the adaptive nature of recently reported phenotypic differences (Chauvaud *et al* 2012).

In our study there were considerable phenotypic differences between growth in experimental settings and semi-natural conditions. Consequently, it remains uncertain to predict how natural or artificial selection on growth-related traits might shape natural and aquaculture populations and how they could interact in the context of population enhancement programs.

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Chapitre 4

Le programme de soutien des stocks de coquilles Saint-Jacques en Rade de Brest : soutien à la population ou pacage marin ?

Premier manuscrit à soumettre à *Aquaculture*
Deuxième mauscrit soumis à *Heredity*

4.1 Principaux résultats et conclusions

Partie 1 : Double anneau

Pour évaluer la proportion des coquilles Saint-Jacques originaire de la production de l'écloserie dans les débarquements issus de la Rade de Brest, pêcheurs et gestionnaires utilisent la technique dite du « double anneau » (Fleury et al. 2005, Alban & Boncoeur 2008). Cette dernière se base sur l'observation d'un anneau surnuméraire sur la coquille des individus semés. Cet anneau est dû à l'arrêt de croissance induit par le stress lié au semis, et est comparable à un anneau hivernal. Les coquilles originaires d'écloserie présentent donc deux arrêts de croissance dans leur première année, l'un du à l'hiver et l'autre au stress du semis (Fig 12). Cette méthode, empirique, restait à valider.

La première partie de ce chapitre se propose de vérifier la validité de la méthode du « double anneau » par une assignation de parenté aux géniteurs utilisés par l'écloserie du Tinduff pour a cohorte correspondante. Pour cela, des coquilles provenant de deux sites de semis (Rade de Brest et Pertuis Charentais) ont été échantillonnées, âgées et leur origine potentielle a été identifiée par la méthode du « double anneau ». Les géniteurs utilisés pour les semis correspondant à la période d'échantillonnage ont été échantillonnée de la même manière. Toutes les coquilles ont été génotypées avec des marqueurs microsatellites, et une assignation de parenté a été menée.

Les résultats montrent un bon taux d'assignation pour les coquilles identifiées avec un double anneau en Rade de Brest, mais pas dans les Pertuis Charentais. Les coquilles identifiées sans double anneau n'ont pas été assignés aux géniteurs d'écloserie, dans aucun des deux sites. Ces résultats suggèrent, d'une part, que l'absence de double anneau est un bon indicateur que la coquille est née en milieu naturel, et d'autre part, que la technique du double anneau semble valide pour la Rade de Brest, mais pas pour les Pertuis Charentais. Ce résultat peut être dû à une plus grande variabilité environnementale dans les Pertuis, conduisant à de nombreuses périodes de stress marquant la coquille et rendant l'identification d'un double anneau formé par le semis incertaine.

Partie 2. Suivi temporal de la population de la Rade de Brest

Les semis d'individus issus de reproduction en écloserie peuvent avoir des conséquences génétiques importantes sur les populations naturelles (Araki & Schmid 2010). La deuxième partie de ce chapitre se propose d'évaluer ces impacts par un suivi génétique temporel de la population de la Rade de Brest. Cette population est ensemencée depuis le milieu des années 1980 dans un but de soutien à la pêche par l'apport d'une biomasse de reproducteurs potentiels supplémentaire (Alban & Boncoeur 2008). Cependant, peu d'information sont disponibles sur la contribution effective des semis à l'effort reproducteur total de la population. L'étude présentée ici propose, par des méthodes d'analyse de diversité génétique, de taux d'apparentement et des calculs de taille efficace d'estimer le succès reproducteur des individus semés.

Des coquilles identifiées comme originaire de semis (avec double-anneau) et issues de la reproduction naturelle (sans double-anneau) ont été échantillonnée en Rade de Brest, trois années différentes. Elles ont été génotypées avec des marqueurs microsatellites. Les résultats de diversité, taux d'apparentement et taille efficace (estimée par différentes méthodes) sont donnés dans le tableau 6.

Les résultats montrent une baisse modérée de richesse allélique observée, mais une altération assez forte des fréquences pour les cohortes d'écloserie. Cependant, aucune tendance temporelle n'est observée dans la population naturelle. Au contraire, les estimations de taux d'apparentement montre une évolution temporelle, tant pour les cohortes d'écloserie que les cohortes naturelles (fig 13). Enfin, les estimations de taille efficaces et de succès reproducteur indiquent une contribution potentiellement significative des semis à la reproduction de la Rade de Brest.

Ces résultats semblent indiquer que l'objectif initial du programme de soutien à la population est atteint : l'augmentation temporelle de taux d'apparentement, les estimations de taille efficace et de succès reproducteur. Cependant, la diversité génétique ne semble pas affectée. Cela pourrait être dû à la période de temps limité de cette étude, ne permettant pas de mettre de mettre en évidence cette baisse. Des flux de gènes, apportant de la diversité génétique de l'extérieur ou d'une sous-population non exploitée à l'intérieur de la rade (zone militaire), pourraient aussi expliquer ce résultat. Enfin, il est également possible que les pratiques de l'écloserie du Tinduff soient adaptées à un maintien relatif de la diversité génétique. Les géniteurs sont renouvelées tous les ans en échantillonnant des individus issus de la reproduction naturelle (sans double anneau) et plusieurs cohortes sont produites et mélangées pour chaque opération de semis.

Tracing hatchery-born great scallops seeded to enhance wild stocks: parentage assignment reveals that shell rings are not always reliable.

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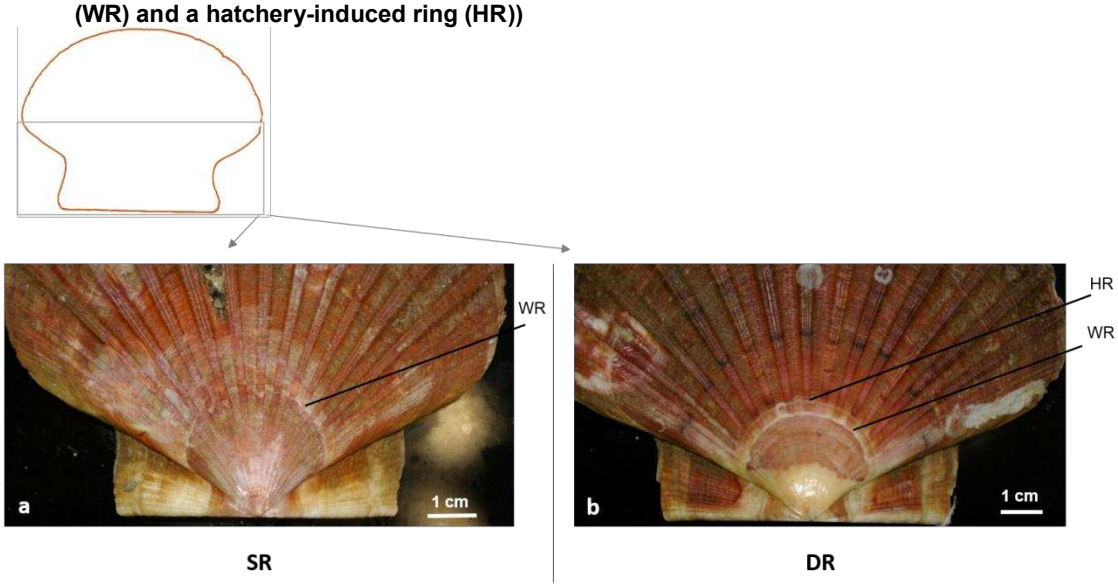
Introduction

One of the major concerns in assessing the efficiency of a seeding program is evaluation of survival, reproductive success and overall demographic contribution of the individuals released in open waters (Bell *et al*. 2008). A first step to assess such parameters is to be able to distinguish between hatchery-born and wild-born individuals, as they are often phenotypically similar. Different methods have been proposed for a variety of species, such as isotopic analysis of fish otoliths (Gao *et al*. 2008), genetic tagging (Sekino *et al.* 2005) or parentage assignment (Vera *et al*. 2010; Lu *et al*. 2011), but they are generally expensive and time consuming, and therefore often not applicable for the management of stocks. However, in some species, this distinction is easy as it can be recorded visually on individuals. For example, abalones display a clear green mark on their shell that has been used to identify hatchery-born individuals (Schiel, 1993). For the great scallop, *Pecten maximus*, hatchery individuals seeded in the Bay of Brest were reported to display a stress ring on their left shell, in addition to the winter ring, formed by a stop in growth rate caused by the stress related to the change of environment (Fleury *et al*. 2005, Fig 12). This identification method has been called the “double-ring (DR) method” and used to assess the origin (hatchery *versus* wild) of dredged individuals, and therefore the success rate of stock enhancement in the Bay of Brest (Dao *et al*. 1999, Alban & Boncoeur, 2011). However, stress rings on the shell can also be formed by other non-anthropogenic factors, such as environmental stress (Chauvaud *et al,* 1998; Lorrain *et al*. 2000), and if the timing of the stress coincided with the time of seeding, wild-born scallop would then be miss-identified as hatchery-born. Conversely, the growth some hatchery-born scallops may not always be impacted at seeding, and the double ring would then not be visible. Those potential sources of uncertainty in the DR method raised questions about its reliability, especially in stocks undergoing high environmental variations.

In this context, our study aimed to assess the efficiency of the double ring method in two seeded sites: the Bay of Brest and the Pertuis Charentais. Microsatellite-based parentage assignment to previously-sampled parental individuals was used to distinguish seeded and wild individuals.

Fig. 12: Phenotypic differentiation between (a) scallops with one winter ring (WR), identified as wild-born scallops (SR for single-ring identification) and (b) scallops identified with a double ring (DR) (a winter ring (WR) and a hatchery-induced ring (HR))



Materials and Methods

Sampling

Scallops (aged 2+ by counting winter marks as described by Mason, 1957) were sampled from two seeded sites: the Bay of Brest (BB) and the Pertuis Charentais (PC). For the Bay of Brest, sampling was done in 2009 (*i.e.* 2007-born scallops), and double rings were recorded by the staff of the Tinduff hatchery. In most individuals, presence ("DR") of absence of double ring ("SR" for Single Ring) was determined without ambiguity. In the Pertuis Charentais, sampling was performed in 2014 (*i.e.* 2012-born scallops) and double-ring identification proved to be difficult, even for trained readers and was thus recorded by three independent readers for each individual. As a result, scallops were classified as follows:

- ‘PC-DR’: the three readers agreed in the presence of a double ring (*i.e.* phenotypically hatchery-born scallop),
- ‘PC-SR’: the three readers agreed in the absence of a double ring (*i.e.* phenotypically wild-born scallop),
- ‘PC-U’ when readers were not congruent about reading of rings (*i.e*. phenotypically uncertain scallop).

Sample sizes are given in Table 1. In order to validate the DR method using parentage assignment to hatchery broodstock, all parental individuals (n = 49) used by the Tinduff hatchery (Finistère, France) to generate the 2007 progeny seeded in the Bay of Brest were sampled. Similarly, the 24 parental individuals used as broodstock in 2012 to generate the progeny seeded in the Pertuis Charentais was also sampled. For all parental individuals, an adductor muscle or mantle fragment was individually collected and preserved in 70-95% ethanol for further DNA analyses.

Microsatellite genotyping

Scallops sampled in the Bay of Brest were genotyped for 10 loci as described by Morvezen *et al.* (2013). Among them, locus PmGC05 was discarded due to missing data caused by poor PCR amplification. For the Pertuis Charentais sample, one multiplex (mx3) also proved to be unreliable in terms of PCR amplification. It was thus replaced by another set of 4 microsatellite markers, named ‘mx4’, described in Morvezen *et al.* (2015).

Data Analysis

Genetic diversity (*i.e*. allelic richness and gene diversity) was computed using FSTAT v2.9 (Goudet, 2001). Significance of differences between samples was tested using paired Wilcoxon tests for the two samples in the Bay of Brest, and Friedman test for paired samples for the three Pertuis Charentais using in R 3.0 (R Core Team, 2013),

followed by paired Wilcoxon tests for all pair of samples. No differences between sites could be tested, as marker sets did not completely overlapped.

Parentage assignment was conducted using CERVUS v 3.0, (Kalinowski *et al.* 2007), using the following parameters: 10 000 replication cycles, 49 (BB) or 24 (PC candidate parents, 100% of candidate parents sampled, 95% loci typed and a 5% error rate. Only trios (*i.e*. a pair of parents assigned to an offspring) with the maximum likelihood score were kept. Colony was also used to reconstruct full-sib and half-sib dyads without the parental genotypes.

Results

Results are summarized in Table 6. No significant difference in terms of allelic richness or gene diversity was observed among our two BB samples (paired wilcoxon test, W=46, p=0.2324) nor the three PC samples (Friedman rank sum test, Friedman chi-squared = 0.1818, p=0.91).

Parental assignment was much high in the BB-DR sample compared to others (91 versus 1-10%). Family structure (*i.e*. number of full-sib and half-sib dyads) was also higher in the BB-DR sample (among all possible dyads, 1% were full-sibs and 11% were half-sibs) compared to others (0-0.3% full-sibs and 3.9-6.7% half-sibs).

For the PC sample, a slightly higher assignment rate was found for the PC-DR sample compared to two other PC samples (10.4% *vs* 2.0-4.4%), indicating that it might comprise more hatchery-born scallops than the two others.

Table 6: Genetic diversity, assignment rate and family structure of each sample. *N*: sample size, A_R: Alleclic richness, ± standard error ; *H* gene diversity± standard error; AS: assignment success given by Cervus; FS dyad: number of full-sib dyads in each samples calculated by Colony; HS dyads number of half-sib dyads in each samples calculated by Colony. FS dyads and HS dyads percentage are calculated relatively to the proportion of possible dyads D, with $D = \frac{N(N-1)}{2}$

	BB-DR	BB-SR	PC-DR	PC-U	PC-SR
N	55	94	67	91	49
A_R±s.e.	9.32 ± 1.16	9.71 ± 1.08	9.27 ± 1.36	8.88 ± 1.31	9.36 ± 1.57
H±s.e.	0.79 ± 0.05	0.75 ± 0.07	0.70 ± 0.07	0.69 ± 0.08	0.68 ± 0.08
AS (%)	50 (91)	1 (1.1)	7 (10.4)	4 (4.4)	1 (2.0)
FS dyad (%)	13 (1.0)	2 (0.0)	4 (0.2)	3 (0.1)	3 (0.3)
HS dyad (%)	161 (11)	170 (3.9)	149 (6.7)	216 (5.3)	70 (5.9)

Discussion

Our study clearly suggests that most of the DR-identified samples in the Bay of Brest were hatchery-born. They displayed an expected familial structure and a high parentage assignment rate to the presumed corresponding broodstock. Scallops classified as SR (*i.e.* presumed to wild-born) sampled in the Bay of Brest showed characteristics of a wild population: non-significant parentage between individuals and close to zero assignment rates to hatchery broodstock. Thus, the double ring method seemed to be reliable in the Bay of Brest to identify hatchery-born individuals, at least for this particular year.

However, our results were clearly different concerning scallops sampled in the Pertuis Charentais. Assignment rates were overall low, albeit slightly higher in the “PC-DR” individuals. One possible explanation could be a problem with the parentage assignment in this sample, such as an error in the presumed hatchery broodstock or in the aging of sampled scallops. However, scallops were aged without particular difficulty and have been classified as “DR” by all three readers. Moreover, the hatchery staff guaranteed that a

single cohort from the sampled broodstock was seeded that year in the Pertuis Charentais, so those possible sources of errors are low. There could also be limited statistical power of parental assignments or genotyping errors. All genotypes have been double checked as described in Morvezen *et al.* (2013), and simulation performed using Cervus gave a good expected assignment rate as previously reported (Morvezen *et al*. 2013). We thus are confident in this method, and were able to assign hatchery-born individuals sampled in BB to a single pair of parents, so we should also be able to assign hatchery-born individuals sampled in PC if they were present.

These results call for caution in using the double ring method to identify hatchery born great scallops outside of the Bay of Brest. The method appears reliable for the studied 207 cohort. However, performance of the DR method was very low in the Pertuis Charentais, probably caused by environmental stress leading to different shell growth patterns. The PC stocks are presumed to be more exposed to a more variable environment: although the mean water temperature is higher than in the Bay of Brest, scallops commonly take one more year to reach their commercial size 10.5cm in width (Chauvaud, comm. pers). Many environmental factors can alter the growth and survival of scallops: temperature, algal blooms (Chauvaud *et al*. 1998), predators (Robert *et al*. 2006), as well as anthropogenic stresses such as dredging (Caddy, 1973). Indeed, dredging is common all year around, targeting different species, but by-catches are common (Leaute & Coupeau 2013). A scallop that is dredged and then release will be stressed and might therefore display a shell ring, either caused by the stress alone or by shell damage. Those marks could be confused with a seeding ring, generating a double ring, and thus make the method uncertain. We recommend in the PC a close monitoring of seeded scallops to assess their survival and growth as initiated by Robert *et al*. (2006). These authors reported that the one-year survival rate of scallops seeded was 0.05 to 0.16, which is consistent with our study. Our results suggest that sampling hatchery-born scallops in the

PC was difficult, despite good knowledge of seeded sites and active search for them. This could reflect a high mortality of seeded scallops in the Pertuis Charentais during their growth as previously quantified by Robert *et al.* (2006). Finally, although the double ring method appeared trustworthy in the Bay of Brest, caution must be taken before using it in other sites were environmental stressors, such as toxic phytoplankton blooms or dredging, might affect shell growth. We do not recommend using the double ring method outside the Bay of Brest without proper assessment of its reliability, by parentage assignment or other technique (e.g. stable isotope profiling, as scallops born in hatchery might display a different isotope signature than those born in the wild.)

Conclusion

Parentage assignment proved to be a useful tool to monitor the hatchery-born scallops in wild populations, and could thus be implemented in other scallop seeding programs. Sea-ranching of scallops has developed in Norway and in the UK, mainly by growing animals in cages (Bergh & Strand, 2001). In some cases, experiments of seeding hatchery-born scallops have been made in Scotland and Norway (Laing, 2002). Parentage-based monitoring and, when possible and reliable, DR-identification could be used to better track survivability, growth and catch rate of hatchery seeded scallops. Parentage-base tagging is already well implemented in the monitoring and evaluation of hatchery-released salmonids (Steel *et al*. 2013) and, combined with the DR-method, could be useful tools to monitor scallops and other mollusks sea ranching programs.

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Stock enhancement or sea ranching? Insights from monitoring the genetic diversity, relatedness and effective size in a seeded great scallop population (*Pecten maximus*).

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Introduction

The mass release into the wild of hatchery-produced juvenile individuals of commercial species has commonly three main purposes: restocking, stock enhancement or ranching. It concerns terrestrial or aquatic species and is now a common practice answering a depletion of exploited marine species (Bell *et al.* 2008). The primary objective of restocking programs is to re-establish locally extinct or nearly extinct species using non local or captive broodstock. On the other hand, when wild populations of marine species are declining but not extinct, due to overfishing or degradation of environmental conditions, the mass release of cultivated individuals aims at increasing population size (sea-ranching) and/or improving natural recruitment (stock enhancement).

Restocking and stock enhancement programs raise numerous questions related to their efficiency in terms of local recruitment and their eventual impact on the genetic diversity of the resulting population. First, components of the fitness of cultured *versus* wild individuals might differ, due to unintentional domestication (Araki & Schmid, 2010) or poor adaptation to local environmental conditions (e.g. Waal *et al.* 2013). Secondly, the effective size (Ne) of cultured stocks is commonly much lower than in the wild, potentially leading to a global depletion of the local genetic variability due to strong genetic drift. This is particularly the case in marine bivalves, where very high fecundities associated with high variance in reproductive success lead to small Ne in cultured populations (Boudry *et al.* 2002, Appleyard & Ward 2006, Lallias *et al.* 2010b). As a consequence, enhancing natural populations with hatchery-born individuals can induce a reduction of their effective size. This phenomenon is known as the Ryman-Laikre effect, which corresponds to the consequence of mixing populations with different effective sizes (Ryman & Laikre 1991, Ryman *et al.* 1995). This effect has been documented in a wide range of species (Utter & Epiphanio, 2002) but has appeared to be minimal in bivalve mollusks in the light of their population biology (Gaffney, 2006). The risk of significantly reducing the effective size of

an enhanced population due to a Ryman-Laikre effect strongly increases as the ratio between the number of seeded individuals and the census size of the recipient population increases, and depends also on the respective effective sizes (Gaffney, 2006).

Stock enhancement can also lead to a significant increase in relatedness among individuals and, ultimately, inbreeding depression. Although negative effects of inbreeding have been documented in shellfish for a variety of fitness-related traits under experimental or aquaculture conditions (*e.g.* Saavedra & Guerra 1996, Bierne *et al.* 1998), no comprehensive evaluation of the fitness of released mollusks in the wild have been performed so far. Overall, Araki & Schmid (2010) found ample evidences of negative impacts of hatchery-rearing on the fitness (evaluated as reproductive success of released animals) and diversity of cultured individuals. The vast majority of studies revealing a significantly lower reproductive success of hatchery-reared individuals have been conducted in fish (mainly salmonids and flatfishes, see Araki & Schmid (2010) for details). However, similar studies on cultivated marine invertebrates, such as mollusks, are still scarce. In any case, it is recommended that a population enhancement program should take into account the maintenance of genetic diversity, notably by using the largest possible broodstock, by renewing it regularly, and by performing a genetic monitoring of the recipient populations (Bell *et al.* 2008). Examples of beneficial seeding programs in marine population are rare in the literature, but see Gonzales *et al.* (2008) for an example of apparent demographic recovery of the fishery of the black sea bream in Hiroshima Bay (Japan).

The great scallop *Pecten maximus* is a benthic marine bivalve of high economical value. It is mainly exploited in the United Kingdom and in France. Total landings amounted to 65 632 T in 2013 (FAO, 2015). The main exploitation method consists in dredging on naturals beds and sea-ranching supplementation has been implemented in specific locations to complement the natural production. It is notably the case in the Bay of Brest

(Beaumont & Gjedrem, 2006), where a seeding program has been initiated since the early 1980's following the dramatic collapse of the local stock caused by a particularly cold winter in 1962-1963 (Dao *et al.* 1999). To that aim, a commercial hatchery has been implemented in Le Tinduff harbor (Plougastel, France) since 1983.

The Tinduff hatchery produces between five and ten millions spats per year (F. Breton, com. pers). The vast majority is seeded on natural beds in the Bay of Brest. The broodstock is renewed each year by sampling new genitors locally. More than 100 spawners can be used per year. Juveniles are then seeded on natural scallop beds of the recipient populations, to be exploited by dredge when they reach commercial size (10-10.5 cm in length). Hold *et al.* (2013) simulated the impact of seeding a Tinduff-produced cohort in populations of the Isle of Man, to assess their potential genetic impact using the Ryman-Laikre approach. They found that under certain conditions, only a limited impact was to be expected. Morvezen *et al.* (2015) found no significant difference in terms of genetic variability between the heavily seeded population of the Bay of Brest and neighboring unseeded or low-seeded natural populations. However, a precise estimation of the genetic impact remained to be conducted, notably assessing a possible Ryman-Laikre effect on the effective size of seeded populations.

In this theoretical and empirical framework, the present study aims at evaluating whether the seeding program of the great scallop in the Bay of Brest is actually a population enhancement program with detectable impacts on the genetic diversity and effective size of the natural seeded population, or a sea-ranching program with limited genetic consequences.

Material and methods

Samples collection

Three different year-classes, born in 2007, 2009 and 2012, were sampled at the age 2+ (estimated by counting annual winter ring on the shell, (Mason 1957)) in the bay of Brest, by dredging on natural seeded beds. For each year-class, hatchery-born scallops and wild-born scallops were differentiated by the double-ring method: hatchery-born scallops display a stress ring caused by the seeding procedure in addition to the winter ring in their first year (Fleury *et al.* 2005, Alban & Boncoeur, 2008). Each individual valve was checked by two to three independent readers, to allow a robust assessment of the presence or absence of the double ring. Samples sizes are given in Tab 1. For all individuals, a fragment of adductor muscle or mantle was collected and preserved in 70-95% ethanol for further DNA analyses.

DNA extraction and microsatellite genotyping

DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen^{TM,} Hilden, Germany), according to manufacturer's instructions. DNA concentrations were estimated using Nanodrop ®, and then diluted to 10 ng DNA/mL. Genetic variation at 12 microsatellite loci was assayed using three multiplex PCR amplifications (mx1, m2, mx4), as described in Morvezen *et al.* (2013, 2015).

Statistical analysis

Allelic richness (A_r) and gene diversity (H_e) were assessed for each locus and each population using FSTAT v2.93 (Goudet, 2001). Null allelic frequencies were estimated using MICROCHECKER v2.2 (Van Oosterhout *et al.* 2004). Significant differences between samples were tested using a Friedman Chi-squared test for non independent data, followed by pairwise paired-wilcoxon tests, with FDR correction for multiple testing in

R (R Core Team, 2013). Pairwise F_{ST} (Weir & Cockerham 1984) were calculated with GENETIX v4.05 (Belkhir *et al.* 2001), and heterogeneity in allelic frequencies between pairs of samples was tested with GENEPOP v4.2 (G-test; 1,000 dememorisations, 100 batches and 10,000 iterations per batch; Rousset, 2008).

Relatedness was calculated with COANCESTRY v1.0 (Wang, 2011), using the triadic likelihood method described by Wang (2007). This estimator was chosen because it is least biased when data contain lot of unrelated individuals, as expected in natural populations of marine mollusks. Significance of mean differences in relatedness between samples was assessed by 10,000 permutations in COANCESTRY.

Effective size (Ne) was estimated according to seven different methods. NEESTIMATOR v2.01 (Do *et al.* 2014) was used to apply the heterozygosity method (Zhdanova & Pudovkin, 2008), the linkage disequilibrium method (Waples & Do, 2008), the coancestry method (Nomura, 2008), as well as three temporal methods (Nei & Tajima, 1981, Pollack, 1983, Jorde & Ryman, 2007). The sibship method (Wang, 2009) was calculated using COLONY.

Temporal methods were calculated on the three possible time-frames (*i.e.* 2007-2012, 2007-2009, 2009-2012), and then decomposed into estimates of yearly effective number of breeders (Nb) using the method implemented in SALMONNB v1.1 (Waples et al 2007). This method has been developed for the Pacific salmon, a semelparous species, but could be applicable in our case (Waples, pers. com., and see discussion for expanded explanation).

All Ne estimates were combined using an unweighted harmonic mean, as suggested by Waples & Do (2010). Variance-weighted harmonic mean would have been preferable (Waples & Do 2010), but variance estimates could not be obtained for all estimators, as some were equal to infinity.

Finally, the relative reproductive success (x) of hatchery-born scallops was estimated using the Ryman-Laikre equation (Ryman & Laikre, 1991) for two possible time-frames (1/ combining 2007 wild and hatchery samples to produce 2009 wild samples, and 2/ combining 2009 wild and hatchery samples to produce 2012 wild samples), as well as over all data (2007-2012) using combined Ne estimates. Estimates of the relative reproductive success were used to predict Ne resulting from crossings between 2012 wild and hatchery cohorts.

Results

Genotype scoring and null alleles

One locus (PmRM007) was excluded from further analysis because of inconsistencies in allele scoring. Null alleles were detected at three (PmRM027, PmRM012, PmRM043) out of the eleven remaining loci, which is consistent with previous studies using the same markers (Morvezen *et al.* 2013, 2015). Most analyses (except the most computationally intensive, COANCESTRY and COLONY, for technical reasons) were performed with and without those loci and provided similar results. Further results are thus given for all eleven loci.

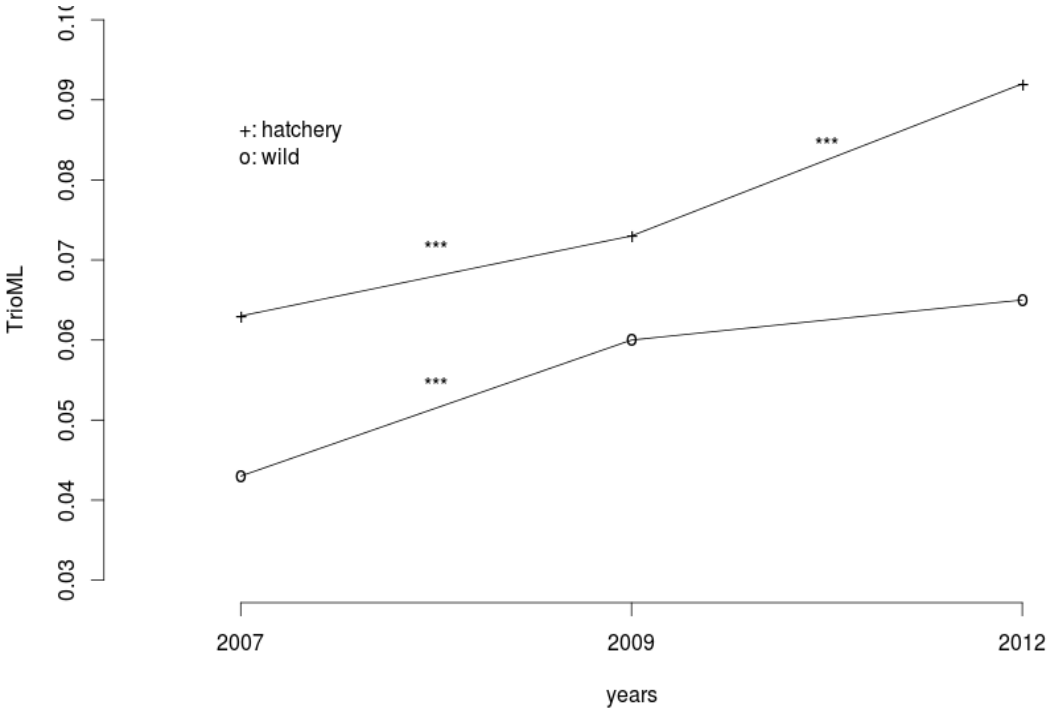
Genetic diversity

Wild-born samples displayed the highest allelic richness values (Ar = 8.97-9.29), whereas hatchery samples showed a lower Ar, which was approximately 0.5-1.5 lower (Tab 6). Allelic richness differed significantly among samples (Friedman Chi-squared = 14.32 df=5 p = 0.013). However, no significant difference was detected in pairwise comparisons after FDR correction for multiple testing (paired Mann-Whitney test, p > 0.05 for all comparisons). Observed heterozygosity appeared relatively similar among all samples (Ho ~ 0.70), without any significant difference (Friedman Chi-squared = 1.45 df = 5 p = 0.917). No significant differentiation was detected among wild cohorts (F_{ST} non-significantly different from zero, no significant heterogeneity in allelic frequencies (G-test, p > 0.05) Tab 7). All pairwise F_{ST} involving a hatchery cohort were significantly greater than zero (0.0049 < F_{ST} < 0.0265), except for the pair H07-N09 (F_{ST} = 0.0045, p = 0.087). The highest F_{ST} values were found among hatchery cohorts. All pairwise G-tests involving a hatchery sample were significant, indicating a strong heterogeneity in allelic frequencies.

Relatedness

Relatedness was globally higher within hatchery cohorts (Fig 13). A small but notable trend to the increase of relatedness with time was observed in natural as well as hatchery cohorts. This temporal increase in relatedness was significant (10,000 permutations, $p<0.001$), except between 2009 and 2012 for the wild cohorts.

Figure 13: Temporal evolution of relatedness in natural and hatchery cohorts.



Effective population sizes and reproductive contribution of the hatchery seed

Effective size estimates varied largely among methods (Tab 8). However, Ne estimates were systematically higher in wild cohorts than in those from hatchery, with combined estimates varying respectively between 58.62 and 302.47, and between 25.56 and 32.80. A high variability in Ne was observed among years in natural cohorts, with a particularly low Ne was found in 2009 (Ne = 58.62). The relative reproductive contribution of the 2007 hatchery cohort was estimated to be x = 0.67 in the 2009 natural cohort,

meaning an estimated reproductive output of 67% for hatchery-born scallops for this year. It was however not possible to estimate x for the 2009 hatchery cohort in the 2012 natural cohort, because Ne increased in 2012 compared to 2009, which is not mathematically possible if we assume that 2009 wild and hatchery samples are the breeding populations producing 2012 wild sample (see discussion). Over all data (2007-2012), *x* was estimated at a value of 0.34. With *x* ranging from 0.34 to 0.67, the predicted Ne for the breeding population composed by 2012 wild and hatchery cohorts was found to be $Ne_{predicted}$ = 55-143.9.

Tab 7: F_{ST} (below diagonal) and G-test result (above diagonal) for all pairs of populations. Significance of F_{ST} are calculated by 10,000 permutations. *: p < 0.05; **: p < 0.01;*: p < 0.001. N: Natural sample (without double ring); H: Hatchery samples (with double rings). Year of birth are given for each sample after the origin.**

	N07	H07	N09	H09	N12	H12
N07	-	***	NS	***	NS	***
H07	**0.0054****	-	***	***	***	***
N09	0.0013	0.0045	-	***	NS	***
H09	**0.0077****	**0.0107*****	**0.0068***	-	***	***
N12	-0.0006	**0.0049***	0.0048	**0.0064****	-	***
H12	**0.0198*****	**0.0261*****	**0.0212*****	**0.0265*****	**0.0161*****	-

Table 8: Genetic diversity, effective size for each sampled cohort. Ar: Allelic richness; He: gene diversity; s.e.: standard error; LD: estimation of Ne with the single sample method of linkage disequilibrium method (Waples & Do, 2008); Het_ex: heterozygote excess method (Zhdanova & Pudovkin, 2008); Sibship: parentage method (Wang, 2009); Coancestry: coancestry method (Nomura, 2008); Pollack: Estimation of Ne with the temporal method of Pollack (1983), then transformed in yearly Nb with Waples *et al.* (2007) method; Nei & Tajima: *idem* with Nei & Tajima (1981) method; Jorde & Ryman: *idem* with Jorde & Ryman (2007) method; Harmonic mean (unweighted): Harmonic mean of Ne estimators.

		N07	H07	N09	H09	N12	H12
N		96	55	40	100	69	76
A_r ± s.e.		9.08 ± 1.44	8.46 ± 1.47	9.29 ± 1.45	8.48 ± 1.25	8.97 ± 1.44	7.42 ± 1.03
H_e ± s.e.		0.69 ± 0.08	0.70 ± 0.07	0.68 ± 0.08	0.69 ± 0.08	0.70 ± 0.07	0.70 ± 0.06
N_e (single sample)	LD	932 (313.6-∞)	39.4 (32-49.9)	∞	28.7 (25-32.8)	∞ (255.7-∞)	27.6 (23.0-33.5)
	Het_ex	∞	∞	∞	∞	∞	∞
	Sibship	95 (75-130)	35 (22-59)	62 (41-99)	50 (33-80)	95 (68-137)	30 (19-51)
	Coancestry	29.9(10.4-59.5)	10.9(3.8-21.8)	20.9(6.3-44.2)	14.9(4.8-30.4)	105.8(0-531.2)	11.5 (6.1-18.7)
N_e (temporal)	Pollack	-127.2	NA	71.0	NA	161.7	NA
	Nei & Tajima	-131.5	NA	66.2	NA	156.2	NA
	Jorde &Ryman	-155.4	NA	38.1	NA	155.7	NA
	Harmonic mean (unweighted)	302.47	27.45	58.62	32.80	179.55	25.56

Discussion

Genetic diversity

Although hatchery cohorts displayed a lower allelic richness than wild-born ones, the difference was weak and not strongly supported statistically. This suggests that the genetic diversity of hatchery seed was not much reduced compared to the recipient wild population, contrary to what is often observed in hatchery production of bivalves (Taris *et al.* 2007, Lind *et al.* 2009, Lallias *et al.* 2010a). This might result from the care taken by the Tunduff hatchery in their crossing procedure (F. Breton, pers. com.): great scallop being a simultaneous hermaphrodite, most individuals are commonly used both as male and female and batches of mixed spermatozoa from five to six individuals are used to individually fertilize ovocytes from each female. This is likely to maximize parental contributions in the resulting progenies. However, F_{ST} and G-test results indicated a high genetic differentiation between natural and hatchery cohorts, as previously reported in other mollusks such as the European flat oyster (Lallias *et al.* 2010a) and abalone (Hara & Sekino 2007). This differentiation reflects a strong alteration of allelic frequencies in the hatchery that is most likely due to genetic drift resulting from the limited size of the broodstock (commonly 30 to 60 individuals) combined with high variance in reproductive success (Boudry et al., 2002, Morvezen *et al.*, 2013).

Even if limited, the reduction in allelic diversity and alteration of allelic frequencies could potentially accumulate over generations, thus gradually eroding the genetic variability of the great scallop population in the Bay of Brest. However, no trend was observed over time in sampled wild cohorts (*i.e.* stability in allelic richness, no significant F_{ST} or G-Test among wild samples), as would be expected if significant genetic erosion was occurring. This result must be interpreted cautiously, and the study should be extended over a longer period of time, in order to be able to detect a significant temporal trend. Moreover, analyzing years pre-dating the first seedings (i.e. before 1980's) or

corresponding to the first seeding events (early 1980's) would be ideal but is unfortunately hampered by the absence (to our knowledge) of historical samples. According to previous results (Morvezen *et al.* 2015), the level of genetic diversity in the natural population of the Bay of Brest is very similar to those in neighboring wild scallop populations, thus supporting the temporal stability found in the present study among wild cohorts.

The temporal stability displayed by the wild population in terms of genetic diversity could be explained by four alternative (and not mutually exclusive) hypotheses. First, regular gene flow from natural populations surrounding the Bay of Brest may maintain a high diversity in the wild population harbored in the bay. Such gene flow might be promoted by the large tidal amplitude in the bay of Brest, leading to strong renewal of the water during each tidal cycle (~40%; Delmas & Trégier 1983) and is congruent with observed F_{ST} estimates between populations along the French Atlantic coasts and the English Channel (Morvezen *et al.* 2015). Second, military areas closed to fishing may harbor unexploited sub-populations which could ensure the retention of genetic diversity within the bay by contributing significantly to the total reproductive output of the Bay. Third, the reproductive success of hatchery-propagated scallops could be limited in the wild, as previously reported in salmonids (Araki *et al.* 2007; Christie *et al.* 2012; Milot *et al.* 2013), thus avoiding negative genetic impacts in the natural seeded population. However, to our knowledge, this observation has never been made in bivalves. However, this hypothesis is contradicted by estimates of the relative reproductive contribution of hatchery cohorts provided in the present study (see below). Fourth, practices implemented in the local scallop hatchery may limit the loss in genetic diversity: the broodstock is fully renewed every year by random dredging natural adults (identified according to the double-ring method) in the wild population (Alban & Boncoeur 2008). However, this practice could not avoid completely the erosion, even limited, of the genetic variability (Waples 1999).

Relatedness

As expected, relatedness was higher in hatchery cohorts than in the wild ones, due to the inherently strong familial structure caused by the low number of breeders. The increase in relatedness over time in the wild population, albeit small, may reflect some hybridization between highly related hatchery individuals and their wild counterparts. This temporal increase is also observed in hatchery cohorts, possibly because the broodstock originates from the local wild population, thus amplifying the effect with hatchery crosses over time. Again, this observation must be interpreted with caution, as only three time points over five years have been studied, and the increase is weakly supported statistically (notably between 2009 and 2012). To strengthen the results, the genetic monitoring should be extended over a longer time.

Effective sizes

The observed lack of consistency among the different Ne estimators is not surprising. Despite the increase in research interest, statistical and software refinements, and the development of new genetic markers and methods, estimating effective population size as Wright proposed in 1938 remains a challenge. This is particularly the case with organisms such as the great scallop, which displays complex life-history traits. Indeed, most methods for estimating effective size have been developed for a narrow scope of life-history traits: non-overlapping generations, relatively small variance in reproductive success, separated sexes (Waples & Do, 2010). Those criteria are not met with the great scallop, and as a consequence, using and interpreting most methods for estimating Ne should be done with caution in this species.

In a complex case like the great scallop, the best estimation of Ne is usually the combination (via harmonic mean) of all available methods into one value, to dilute the differential biases associated with each method, and thus improve the accuracy of Ne

estimate (Waples and Do, 2010). However, this methodology can be challenging, especially combining estimates from single sample methods and temporal methods, as they do not apply to the same time frames (Waples and Do, 2010; Nomura, 2008). One approach for combining single sample and temporal Ne consists in considering temporal Ne as equivalent to the harmonic mean of the effective number of breeders per generation (*i.e.* yearly Nb) for the generations engulfed in the time period of interest. This oversimplification is more realistic when generations are not overlapping (as in semelparous species) and with a fixed age-structure of breeders (Waples *et al.* 2007). Although this might not seem realistic for a marine bivalve such as the great scallop, the case of the Bay of Brest is peculiar: scallops are harvested just after their first reproductive season (i.e. 2+), and because of the high fishing pressure, individuals may reproduce only once (Jollivet, pers. com.). Therefore, *Pecten maximus* can be functionally considered as a quasi-semelparous species in the Bay of Brest, with limited age structure among mature individuals. Thus the method developed in salmon for estimating yearly Nb from temporal Ne estimates appears viable in the present study (Waples, pers. com.).

For the hatchery broodstock, combined Ne estimates (which can be assimilated to yearly Nb) appear slightly lower than usual broodstock sensus size. This result can be explained by the high variance in reproductive success that strongly reduces the effective number of breeders. High variance in reproductive success is often observed in bivalve hatcheries (Boudry *et al.* 2002, Lallias *et al.* 2010b), including in the great scallop (Morvezen *et al.* 2013). For the wild cohorts, Ne estimates are much lower than expected in a bivalve displaying large population census-sizes and mass-spawing with fecundation in the open sea. Small Ne/N ratios have been often reported in the literature (Frankham 2007), sometimes even as low as 10^{-6} in the Pacific oyster (Hedgecock *et al.* 1992). In the present study, estimating Ne/N is difficult because of a lack of data concerning the census size of the great scallop population in the bay of Brest. However, considering the total

annual landings for this stock (~100-300T, Alban & Boncoeur, 2008), the census size should be at least in the order of magnitude of 10^6-10^7, thus giving an approximation of Ne/N in the order of magnitude of 10^{-4}-10^{-5}. A small Ne/N ratio could be caused by sweepstake reproductive success where a small number of individuals contribute to the majority of the next generation (Hedgecock and Pudovkin, 2011). Although such a phenomenon could result in a strong variance in allelic frequencies through generations and induce chaotic genetic patchiness (Larson and Julian, 1999), a stable genetic diversity was found over the three successive natural cohorts that we studied, as discussed above. The estimation of Ne for the 2009 wild cohort is the most surprising. It is three-five folds lower than Ne estimates for both 2007 and 2012 wild cohorts. This could be explained by a strong sampling bias, as the 2009 natural sample is the smallest in the data set and Ne estimators are sensitive to sampling errors (Waples and Do 2010). It could also be an indicator of a strong Ryman-Laikre effect, due to a large reproductive contribution of hatchery-born individuals during this particular year (see below for Ryman-Laikre effect discussion).

Overall, the results suggest that the annual effective number of breeders is small and variable in the wild, which is coherent with a sweepstake reproductive success (Hedgecock & Pudovkin, 2011). Moreover, this effect could be amplified by the genetic drift resulting from hatchery propagation if seeded scallops contribute significantly to the reproduction.

Relative reproductive contribution of hatchery and wild-born cohorts

Estimates of the relative reproductive contribution were highly variable and sometimes not possible. This could indicate that the simplistic hypotheses underlying the Ryman-Laikre model might not be realistic enough to be informative in the case of the great scallop. In particular, the relative contribution could not be assessed for the 2009-

2012 period, clearly showing that the hypothesis of a simple reproductive mixing between 2009 hatchery and natural cohorts is not sufficient to explain the observed Ne in 2012. Nevertheless, estimates of x (0.34-0.67) suggest a Ryman-Laikre effect, with a high contribution of hatchery stocks to the total reproductive output of the natural population. Accordingly, the increase in relatedness over time is also an indicator that seeded individuals appear to contribute to some degree to the reproduction of great scallop population in the Bay of Brest (see above). The Ryman-Laikre effect might be the cause for the relatively small Ne observed in the natural population (in particular in 2009) but seem to be counter balanced by other processes, as Ne appears to be able to increase even after a dramatic reduction (in 2012). Again, as explained above, gene flow from neighboring populations as well as undetected population sub-structure could explain both the retention of genetic diversity and the apparent recovering of effective size.

Conclusion: sea-ranching or stock enhancement?

The present study suggests that hatchery-born scallops may significantly contribute to the reproduction of the natural population, thus fulfilling the objective of the population enhancement program conducted in the bay of Brest, which is providing supportive breeding. In spite of the relative alteration of the genetic diversity and lower effective size in the hatchery cohorts, the genetic variability appears relatively stable over time in the natural population supplemented with hatchery seed. In particular, gene flow from surrounding populations and/or the reproductive input of putative unexploited sub-populations within the bay may buffer the Ryman-Laikre effect and ensure the retention of the local genetic variability. Although the first goal of the supportive breeding program is to enhance the local recruitment and improve the productivity of the scallop population in the bay of Brest, the stock is far from having recovered to its historical levels (Alban and Boncoeur, 2008). Various factors may strongly limit the demographic growth of the local population, such as trophic competition with non-native invasive species like *Crepidula fornicata* (Thouzeau *et al.* 2000) or *Crassostrea gigas* (Lejart & Hily, 2011) increased predation or emerging environmental pressures (eutrophication, toxic algal bloom Anderson *et al.* 2002, 2012). Genetic monitoring of the population in the bay of Brest should be continued over a longer period of time, and extended to other seeded scallop populations. This would provide opportunities to better assess the extent of the reproductive success of hatchery-born scallops in the wild, and its impact on the effective size and genetic diversity of seeded populations. Moreover, further investigations are required to evaluate whether the reproductive contribution of hatchery cohorts may affect the local adaptation of wild populations, and their adaptive potential to environmental changes (see Laikre *et al.* 2010). This issue appears particularly crucial to ensure the long-term persistence of enhanced populations, particularly in a context of global changes.

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Chapitre 5

Discussion générale

5.1 Bases génétiques de la croissance de la coquille Saint-Jacques

Le premier objectif de ma thèse était d'évaluer la part génétique de la croissance chez la coquille Saint-Jacques, par deux approches : une étude de la structuration génétique des populations à l'échelle européenne pour la comparer aux différences phénotypiques observées (chapitre 2), et une approche de génétique quantitative pour évaluer l'héritabilité, les corrélations génétiques et interactions Génotypes x Environnement (GxE) (chapitre 3).

Les deux études présentent des résultats convergents. Tout d'abord, deux groupes génétiques et deux groupes phénotypiques (Chauvaud *et al.* 2012) ont été observés le long de l'aire de répartition de la coquille Saint-Jacques. L'étude de Chauvaud *et al.* (2012) a placé la frontière entre les deux groupes entre l'Écosse et l'entrée de la Mer du Nord. Cette limite marque notamment une discontinuité dans les patterns de croissance ; les populations nordiques présentant une perte du nombre de jour de croissance d'une année à l'autre plus faible que les populations méridionales (voir figure 5b de Chauvaud *et al.* 2012). Cette capacité à maintenir une période de croissance plus longue pourrait être le résultat d'une adaptation des populations, mais en l'absence de données génétiques, la variation phénotypique due à la plasticité est confondue avec la variation adaptative (Stillwell 2010).

Les résultats présentés au chapitre 2 donnent une première idée de la variation génétique existante sur une échelle comparable à celle de l'étude de Chauvaud *et al.* 2012. Deux groupes génétiques sont retrouvés et sont relativement concordants avec les données phénotypiques. La limite entre les deux groupes ne correspond cependant pas parfaitement ; les données phénotypiques tendent à placer la limite plutôt de l'Écosse à l'entrée de la Mer du Nord. L'étude présentée dans le chapitre 2 ne permet pas de placer aussi précisément une limite.

On remarque par ailleurs que la fosse Norvégienne pourrait constituer une barrière physique et écologique pour les bivalves benthiques (Rosenberg *et al.* 1996). De plus, en tenant compte des données bibliographiques sur la différenciation génétique entre populations norvégiennes et atlantiques (Ridgway & Dahle 2000, Hold 2012), la limite génétique semble plutôt être placée au Nord Est de la mer du Nord, au niveau de la fosse Norvégienne. En effet, lors de sa thèse sur la génétique de la coquille St Jacques, N. Hold (2012) a pris en compte des échantillons collectés au centre de la mer du Nord, non présents dans l'étude du chapitre 2. Ces échantillons se regroupaient plutôt avec les autres populations plus à l'ouest, qui correspondraient au groupe génétique « Atlantique » défini ici, appuyant au final l'hypothèse d'une séparation par la fosse Norvégienne.

Les variations génétiques et phénotypiques ne sont certes pas parfaitement concordantes géographiquement, mais l'existence des deux groupes phénotypiques comme génétiques suggèrent que les variations phénotypiques observées pourraient refléter des processus adaptatifs. Il faut cependant rester prudent, car outre l'incertitude de placement de la limite des deux groupes, les études génétiques du chapitre 2 et de la littérature se basent sur des marqueurs neutres. Ces marqueurs reflètent typiquement des processus de différenciation génétique associés à la dérive génétique et la migration, qui ne sont pas forcément en lien avec des processus adaptatifs. Il est donc nécessaire, pour confirmer ou infirmer la base génétique de la croissance chez la coquille Saint-Jacques de mener d'autres expérimentations, tel que des analyses de type Q_{ST}-F_{ST} (Merilä & Crnokrak 2001, Leinonen *et al.* 2013), des expériences de jardin commun (« common garden », Sanford & Kelly 2011 ; voir ci-après la partie Perspectives) ou des études de génétique quantitatives tel que celle du chapitre 3.

Le chapitre 3 présente un exemple d'étude de génétique quantitative visant à estimer la part héritable des caractères de croissance de la coquille Saint-Jacques. Les résultats montrent que tous les paramètres mesurés présentent une héritabilité modérée,

notamment la date de reprise de croissance ou la croissance en milieu semi-naturel. C'est un résultat qui semble être compatible avec l'hypothèse d'une variation de nature adaptative des phénotypes entre les populations. En effet, une valeur d'héritabilité se définit comme la proportion de variance phénotypique expliquée par la variance génétique. Le fait que cette proportion de variance soit différence de zéro montre qu'il y a une composante génétique, sans exclure qu'il existe une certaine plasticité phénotypique.

Cependant, l'étude présentée au chapitre 3 ne concernait qu'une seule population, issue de la Rade de Brest, dans des conditions non strictement naturelles (cages de pré-grossisement en milieu ouvert). Même si les estimations d'héritabilité en milieu contrôlés sont souvent une bonne approximation de la valeur de cette héritabilité dans le milieu naturel (Weisenberg & Roff 1996), le chapitre 2 montre que le patrimoine génétique des populations norvégiennes diffère significativement de celui des populations atlantiques, et il est difficile d'extrapoler les résultats obtenus avec des coquilles Saint-Jacques brestoises aux coquilles norvégiennes. Cette étude suggère donc qu'il existe un potentiel évolutif des caractères liés à la croissance de la coquille Saint-Jacques dans la Rade de Brest. Des études complémentaires, portant sur d'autres populations (notamment au niveau de la limite entre les deux groupes et dans le groupe norvégien) avec d'autres marqueurs et méthodologies sont nécessaires pour mettre en évidence une adaptation locale des populations (voir section Perspectives).

5.2 Impact de l'activité de réensemencement sur la diversité génétique et phénotypique

Le chapitre 4 met en évidence une probable contribution des coquilles nées en écloserie à la reproduction en Rade de Brest (33 à 66%). Ces estimations sont cependant à prendre avec beaucoup de précautions, car elles se basent sur (1) un suivi génétique de courte durée ; (2) des estimateurs de taille efficace et le modèle de Ryman-Laikre qui se basent sur des hypothèses sous-jacentes peu réalistes dans le cas d'un bivalve hermaphrodite à fécondation externe, avec un certain degré de chevauchement des générations (Ryman & Laikre 1991, Waples & Do 2010). Cependant, ces estimations, combinées avec l'observation de l'augmentation de la consanguinité, suggère tout de même que les individus semés contribuent à la reproduction totale de la population. Cette partie se propose de mettre en relation cette conclusion du chapitre 4 avec les résultats de l'étude de génétique quantitative du chapitre 3, pour inférer des possibles conséquences phénotypiques et adaptatives des semis sur la population de la Rade de Brest. En effet, des exemples existent d'introgression génétique dûe à des programmes de soutien à la population, amenant des individus mal adaptés qui diminuent la *fitness* globale de la population (Frankham *et al.* 2002, Kostow, 2009, Araki & Schmid 2010).

Le chapitre 3 montre qu'un changement d'environnement (ici passage de cage en mer à des structures expérimentales) peut avoir un effet sur l'expression des bases génétiques de la croissance. En particulier, il n'y a pas de corrélation génétique observée entre les paramètres de croissance en milieu semi-naturel et la croissance en milieu expérimental. Cependant, la croissance en milieu expérimental est corrélée à la teneur en glycogène du muscle adducteur. Ces résultats semblent indiquer que le métabolisme des individus est modifié en milieu expérimental : les rangs (*i.e.* les performances) des familles sont changés. L'estimation d'héritabilité de la croissance en écloserie expérimentale reste

du même ordre de grandeur (0.15 vs 0.15-0.25 en conditions semi-naturelles), mais cette variabilité génétique se traduit par une variabilité phénotypique différente de celle du milieu semi-naturel. La corrélation des rangs de familles entre la croissance en milieu expérimental et le taux de glycogène suggère que ce différentiel phénotypique s'exprime au niveau de la capacité de nutrition (Thompson 1977, Barber and Blake 1981, Pazos *et al* 1997, Strohmeier *et al*. 2000). Plusieurs hypothèses sont possibles : tout d'abord, la nourriture en écloserie est complètement différente par sa composition et son abondance de la nourriture disponible en milieu naturel. Certaines coquilles peuvent se voir avantagées par ces conditions, du fait d'une capacité à sélectionner, ingérer et digérer la nourriture. En effet, les coquilles Saint-Jacques sont soupçonnées de pouvoir sélectionner leur nourriture (par exemple des cellules phytoplanctoniques ou des particules de matière organique, in Lavaud *et al.* (2013)), et d'avoir une nourriture diversifiée, pouvant comprendre du phytoplancton, du microphytobenthos, du nanoplancton et des bactéries (Chauvaud *et al.* 2001, MacDonald *et al.* 2006). Il est possible qu'il y ait ici une capacité différente des familles à se nourrir dans différents environnements, qui se traduirait par une interaction génotype x environnement entre les différentes conditions de nourriture, comme observées chez d'autres bivalves (Toro & Paredes 1996, Ernande *et al.* 2007). Cette interaction n'a pas pu être mesurée ici, car les deux environnements (cage en mer *vs* station expérimentale) n'étaient pas simultanés mais consécutifs, et des traits différents ont été mesurés dans chaque environnement. Cependant, il serait intéressant de déterminer si cette possible interaction génotype x environnment est effectivement présente entre des coquilles dans des conditions de nourritures différentes (voir perspectives).

Une autre hypothèse possible serait qu'il existe des différences entre familles dans leurs capacités à répondre aux conditions expérimentales. En effet, les coquilles Saint-Jacques sont très sensibles aux perturbations acoustiques ou lumineuses (Chauvaud,

comm. pers.) et peuvent adopter un comportement de fermeture des valves ou de fuite. Le comportement de fuite consiste en un battement rapide et répété des valves, expulsant de l'eau vers l'arrière et permettant la propulsion de l'individu (Cheng *et al.* 1996). Ce comportement peut être particulièrement couteux en énergie. Robson *et al.* (2011) ont montré par des mesures d'accéléromètres que la coquille Saint-Jacques passe environ 0,1% de son temps à bouger, mais ce mouvement correspond à 16.8% de son budget énergétique journalier en milieu naturel, et 41,8% en condition d'écloserie expérimentale considérées donc comme a priori stressantes . Ce comportement donc couteux en énergie pourrait avoir des effets significatifs sur la croissance et les réserves des individus (Robson *et al.* 2011). Il a été montré chez *Argopecten purpuratus* que la capacité de nage était héritable (h^2= 0.36–0.57, Brokordt *et al.* 2012). Les différences familiales observées ici sont donc peut être dues à une susceptibilité différente aux perturbations en milieu expérimental, qui ne sont pas présentes en milieu semi-naturel, créant ainsi ce changement observé dans le rang des familles.

5.3 Implication pour la diversité phénotypique et la fitness des semis.

Le naissain de coquille Saint-Jacques subit au moment du semis un changement d'environnement ; il entre en contact avec le sédiment et l'environnement benthique après avoir passé la première partie de sa vie en écloserie, puis quelques mois de grossissement en pleine eau (cages). Ce changement de conditions de vie pourrait provoquer un phénomène similaire à celui mis en évidence dans le chapitre 3 : la variabilité de la base génétique de la croissance qui s'exprime différemment entre les deux environnements. Une sélection par la taille (volontaire ou involontaire) est souvent pratiquée dans les écloseries de bivalves (*e.g.* Langdon *et al.* 2003, Taris *et al.* 2007). Or

ici, cette sélection pourrait s'effectuer sur des individus à fort potentiel de croissance en conditions non-naturelles, potentiel qui ne s'exprimerait pas forcément en condition naturelle après semis. Cette éventuelle sélection non-intentionnelle pourrait avoir des conséquences sur la fitness des individus semés : croissance et accumulation de réserve plus lentes entrainant une gamétogénèse moins efficace et donc un succès reproducteur et une *fitness* réduite. Des réductions de *fitness* d'individus issus d'écloserie ont été montrées chez des salmonidés nés en écloserie par rapport à des salmonidés issus de la reproduction naturelle (Araki *et al.* 2007, 2008, 2010, Christie *et al.* 2012a, Neff *et al.* 2015), mais pas encore chez les bivalves. Les mécanismes entrainant cette baisse de *fitness* peuvent être liés partiellement à la consanguinité (Christie *et al.* 2013), à des effets maternels (Matos 2012), ou une sélection pour l'adaptation aux conditions d'écloserie (Araki *et al.* 2007, 2008, Christie *et al.* 2012). Une augmentation temporelle de de la consanguinité de la population de la Rade de Brest a été observée au chapitre 3. Cette augmentation peut avoir un impact sur le succès reproducteur des individus, mais devrait être limitée (Christie *et al.* 2014). Une évaluation précise du succès reproducteur des individus semés devrait donc être entreprise.

Le chapitre 4 répond partiellement à cette problématique. En utilisant une approche populationnelle d'évaluation de la taille efficace, cette étude suggère que les individus d'écloserie contribuent significativement à la reproduction en milieu naturel. Cependant, cela ne permet pas de conclure sur une éventuelle perte de *fitness* due à l'écloserie. Il est possible que même si les individus nés en écloserie sont moins féconds, le simple fait d'en introduire un grand nombre (relativement au nombre de coquille Saint-Jacques nées de la reproduction en pleine eau) peut permettre à la cohorte d'écloserie d'avoir une contribution reproductrice significative malgré une fitness individuelle potentiellement plus faible que les individus sauvages. C'est notamment illustré par le fait que dans les débarquements,

deux tiers des coquilles pêchées présentent un double anneau et sont donc probablement originaire de l'écloserie (Fleury *et al.* 2005).

Malgré cet apport important de géniteurs, la productivité de la Rade de Brest reste faible par rapport à ce qu'elle a été avant le déclin de 1962-1963 (100-300 T contre 1000-2000 T) (Alban & Boncoeur 2008). L'introduction de géniteurs avec une *fitness* sous-optimale dans la Rade pourrait être un facteur expliquant cette non-reprise de la production depuis le début des semis, mais pas uniquement. D'autres facteurs comme la compétition trophique avec des espèces envahissantes (*Crepidula fornicata* (Thouzeau *et al.* 2000)*, Crassostrea gigas* (Lejart & Hily, 2011)), ou de nouvelles pressions environnementales (comme l'eutrophisation, les efflorescences de phytoplancton toxique (Anderson *et al.* 2002, 2012), peuvent également avoir un impact.

5.4 Conclusion

Cette thèse a mis en évidence

- L'existence d'une structure génétique des populations de coquilles Saint-Jacques, partiellement concordante avec la variabilité phénotypique précédemment observée, malgré une légère différence de limite entre les deux groupes.

- une base génétique significative de la croissance de la coquille Saint-Jacques en Rade de Brest, et donc un probable potentiel adaptatif derrière les stratégies de croissance différentielles entre les populations. Le même type d'évaluation doit être mené sur d'autres populations pour répondre plus précisément au caractère adaptatif des variations phénotypiques observées.

- L'impact apparemment limité mais significatif en terme de diversité génétique du programme de repeuplement par semis de naissains en Rade de Brest, malgré une contribution significative des semis. Des données à plus long terme sont cependant nécessaire pour déterminer avec plus de confiance un éventuel impact.

- L'impact important des conditions expérimentales sur la physiologie et la croissance de la coquille Saint-Jacques, l'extrapolation de résultats expérimentaux au milieu naturel devant donc être considérée avec précautions.

5.5 Perspectives

Plasticité vs adaptation

La comparaison de marqueurs génétique neutres avec des phénotypes pour inférer des potentielles adaptations locales des populations est possible par une approche de type Q_{ST}-F_{ST} (Merilä & Crnokrak 2001, Holderegger *et al.* 2006). Il s'agit de comparer des indices de différenciations phénotypiques (Q_{ST}) avec des indices de différenciation génétique neutre (F_{ST} ou équivalent). Si la différenciation phénotypique est supérieur à la différence génétique neutre, il est possible d'émettre l'hypothèse d'une pression de sélection sur ce phénotype (voir Leinonen *et al.* 2013 pour une revue bibliographique). Cette approche a été appliquée avec succès sur diverses espèces marines tel que l'épinoche (*Gasterosteus aculeatus*, Leinonen *et al.* 2006), un copépode (*Tigriopus californicus* Edmands & Harrison 2003) ou le buccin commun (*Buccinum undatum* Mariani *et al.* 2012). Cette approche pourrait être utile dans le cas de la coquille Saint-Jacques pour comparer formellement les études phénotypiques avec les études génétiques, notamment avec un échantillonnage plus fin autour de la limite des deux groupes phénotypique et génétique. Cette méthodologie est cependant sujette à caution et sa validité est parfois remise en cause (Pujol *et al.* 2008 Edelaar *et al.* 2011a, b).

Une autre façon de comparer les deux groupes génétiques et phénotypiques serait d'effectuer une expérience de jardin commun (« *common garden* »). Pour cela, il faudrait comparer en conditions expérimentales ou mieux en semi-naturelles similaires des coquilles en provenance des deux groupes génétiques, de préférences ayant partagées le même environnement depuis leur naissance. Les phénotypes développés en environnement commun pourraient ensuite être comparés pour évaluer la part de plasticité par rapport à l'adaptation. Le principal phénotype d'intérêt à évaluer en environnement commun serait la durée de la période de croissance, l'un des paramètres permettant de mieux différencier les stratégies de croissance des deux groupes de populations

(Chauvaud *et al.* 2012). Il faudrait donc pour cela mener une expérimentation sur plusieurs années. Une solution serait d'utiliser une approximation de cette durée, comme par exemple la date de reprise de croissance (voir chapitre 3). Une autre solution serait d'opter pour une expérience de translocation de populations, en élevant des coquilles Atlantique (par exemple brestoises) en Norvège, et inversement. Les valves de coquille Saint-Jacques présentent l'opportunité de mesurer *a posteriori,* précisément, des paramètres de croissance, permettant à moindre coût de déterminer les phénotypes d'intérêt sans avoir à effectuer un suivi régulier. Il serait donc possible de semer des coquilles brestoises en Norvège et inversement, et de les échantillonner deux à trois ans après. Toutefois, cette opération présente un certain risque car le taux de survie des semis n'est pas garanti (Fleury *et al.* 2005).

Étude de génétique quantitative dans le milieu naturel

Afin de continuer à explorer les bases génétiques des paramètres de croissance, il serait intéressant de mesurer les paramètres de génétique quantitative dans des conditions naturelles contrastées, pour mettre en évidence des potentiels adaptatifs (Kruuk 2004). En milieu marin, ces études sont rares à cause de la nature très dispersive du milieu, et des tailles efficaces des populations importantes, qui rendent très difficile le suivi de pedigree. Ces approches sont explorées plus communément en milieux terrestres, notamment sur des populations d'oiseaux suivies par capture-marquage-recapture sur plusieurs générations (Postma & Charmentier 2007, Teplitsky *et al*. 2009). Les semis de coquilles Saint-Jacques offrent une opportunité nouvelle d'étudier des individus apparentés, produits en écloserie, facilement reconnaissables (grâce à la méthode du double anneau) et dont l'âge peut être déterminé avec précisions, ces individus grandissant en milieu naturel. Il faudra toutefois être prudent pour extrapoler les résultats aux populations naturelles par rapport aux éventuelles pressions de sélections

(involontaires ou volontaires) induites par l'écloserie, qui pourraient modifier la structure génétique des individus semés et leur représentativité phénotypique relativement à la population naturelle.

Impact du programme de réensemencement en Rade de Brest

La première étude présente ici a montrée qu'il y avait une potentielle contribution des semis à la reproduction, mais avec un impact sur la diversité génétique qui semble limité. Cependant, le suivi n'a été effectué que sur une courte période, ce qui ne permet pas de conclure sur des éventuelles conséquences à long terme. Il paraît donc particulièrement pertinent de prolonger le monitoring génétique la population de la Rade de Brest, et de suivre parallèlement la *fitness* relative des individus semés par rapport aux individus issus de la reproduction naturelle (Araki & Schmid 2010).

La poursuite du suivi est particulièrement importante à la lumière des nouvelles perturbations environnementales qui apparaissent dans la Rade. La saison de pêche a été annulée en 2014-2015 en raison d'efflorescences de phytoplancton toxique (*Pseudo-nitschia sp.*). Il est important pour le suivi de la population d'estimer les effets de ces efflorescences sur la physiologie et la reproduction des coquilles Saint-Jacques. Si ces efflorescences impactent la fitness individuelle de façon limitée, elles conduisent souvent à une réduction de l'effort de pêche ; elles pourraient donc être bénéfiques à la démographie de la population, en augmentant la longévité des géniteurs dans le milieu, et donc la biomasse féconde. Il serait alors très pertinent d'étudier l'effet de cet arrêt soudain de la pêcherie sur la variabilité génétique de la population, et sur sa démographie.

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Annexe

Projet parallèle à la thèse :

Deleterious effects of bioactive extracellular compounds and paralytic shellfish toxins produced by *Alexandrium minutum* on growth and behaviour of juvenile great scallops *Pecten maximus*.

Manuscrit en préparation

Deleterious effects of bioactive extracellular compounds and paralytic shellfish toxins produced by *Alexandrium minutum* on growth and behaviour of juvenile great scallops *Pecten maximus*.

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Abstract

Effects of harmful algal blooms (HAB) of two strains of *Alexandrium minutum* were assessed on behavioural and physiological traits of great scallops *Pecten maximus*. Scallops were exposed for a week to three different conditions: *Isochrysis galbana* (clone T-*iso*) Parke (1949) and *Chaetoceros neogracile* VanLandingham (1968) (=*C. muelleri*) (food algae), a paralytic shellfish toxins (PST) producing *A. minutum* with food algae and a second strain of *A. minutum* producing bioactive extracellular compounds (BEC) that may have allelopathic, ichtyotoxic, cytotoxic or haemolytic effects with food algae. Escape response to predators, daily shell growth, histological effects and sensitivity to accumulation of PST were recorded after a week exposure and after two extra weeks of only food supply, without any *A. minutum*. Daily shell growth was delayed three days in scallops exposed to BEC *A. minutum* strain, probably on the three first days of the exposition phase. An increase of reaction time to predators, after exposure, was observed in scallops exposed to the BEC condition. This suggests that bioactive extracellular compounds might have altered the feeding and recognition processes. After exposure, a less efficient escape response and muscular damages in scallops exposed to PST was observed, which could reflect the effects of paralytic toxins on nervous system of scallops. However, after depuration, king scallops seemed to recuperate their initial performance up to control condition. This study highlights dissimilar effects of the several toxic compounds produced by *A. minutum* on marine organism. The nature of the compounds and the understanding of their effects on marine organisms are important factors to take into account for a better management of the marine resources.

Introduction

Harmful Algal Blooms (HABs) develop mainly in near coastal waters (Sellner *et al*., 2003) and have been reported worldwide (Van Egmond *et al*., 1993; Bricelj & Shumway, 1998; Sellner *et al*., 2003; Lassus *et al*., 2006). The genus *Alexandrium* Halim (1960) is one of the most prominent in terms of intensity of HABs, diversity and distribution worldwide (Anderson *et al*., 2012). Some *Alexandrium* species such as *A. minutum* or *A. catenella* among others, are known to produce neurotoxins, which include saxitoxin (STX) and its derivatives (21 congeners), also known as paralytic shellfish toxin (PST). These neurotoxins present a very acute toxicity for human consumers (Lassus *et al*., 2006; Anderson *et al*., 2012) but are also potentially toxic to aquatic organisms (Landsberg, 2002). PSTs are responsible for the "Paralytic Shellfish Poisoning" (PSP) syndrome that can modify the trophic structure of marine food webs, by causing intoxication or death of several organisms (Anderson *et al*., 2012). The STX blocks the passage of sodium ions in the nerve cell sodium channels, leading to severe neuromuscular disorders, especially among consumers of intoxicated flesh (Château-Degat, 2003).

Dinoflagellates of the genus *Alexandrium* are also known to produce others toxic molecules which are excreted extracellularly (Emura et al., 2004; Ma et al., 2009). These compounds can have allelopathic, ichtyotoxic, cytotoxic, haemolytic or growth inhibition impacts on marine organisms, such as neighbouring phytoplanctonic cells, filter feeders, fishes etc. (Arzul et al., 1999; Landsberg, 2002 ; Lelong *et al.,* 2011, Haberkorn *et al.,* 2010).

Great scallop *P. maximus* is usually found into shallow depression in the seabed at the surface of the sediment or buried (Baird, 1958). P. maximus appears to have three responses to disturbance (Thomas and Gruffydd, 1971 ; Brand, 1991) : closing valves, jumping reaction, vigorous swimming reaction. Many of these responses are chemosensory. King scallops are regularly subjected to harmful algal blooms. However, despite an important commercial value of king scallops, little information is available regarding their response to a toxic phytoplankton bloom and their accumulation of toxins. Numerous studies have assessed the impact of *A. minutum* on toxin accumulation, filtration and feeding activities, valve activity, as well as other physiological parameters, such as immune response, anti-oxidant response, tissue damages or reproduction on mussels or oysters (Bougrier *et al*., 2003; Fabioux et al., 2015; Haberkorn *et al*., 2009, 2010, 2011; Tran *et al*., 2010, 2015). A recent study demonstrated that an exposure of the scallop *Argopecten purpuratus* Lamarck (1819) was exposed at cultures of *A. catenella* (Whedon & Kofoid) Balech (1985) modified scallop escape response to their predator, the seastar *Meyenaster gelatinosus* Meyen (1834) (Hégaret *et al*., 2012). The escape response of scallops appears as a good physiological indicator, as it represents a stereotyped and consistent response (Ortiz *et al*., 2003),

consisting of an alternation of adduction/abduction movement propulsing the scallop by expulsing water from its dorsal side (Brokordt *et al.*, 2006).

Some previous studies highlighted an alteration of the growth of *P. maximus* in the Bay of Brest, which was associated to effects of microalgal extracellular compounds. Chauvaud *et al.,* (2001) demonstrated growth failure in *P. maximus* following a bloom of the toxic dinoflagellate *Gymnodinium* cf. *nagasakiense* Takayama & Adachi (1985) (=actual *Karenia mikimotoi* (Miyaka & Kominami *ex* Oda) Hansen & Moeastrup (2000)). Two other studies also showed that *Karenia mikimotoi* bloom, caused growth rate alteration and inhibition, as well as mortality in juvenile *P. maximus* scallops (Chauvaud *et al.,* 2001; Erard-Le-Denn *et al*., 1990; Thébaud *et al.*, 2005). This algal species contains several extracellular compounds presenting allelopathic and haemolytic characters (Gentien & Arzul, 1990 ; Arzul et *al.*, 1999; Gentien 2006 ; Gentien et al., 2007), which are indeed suspected to be responsible for these altered deleterious effects.

The present study aimed to assess the impact of both PSTs, but also extracellular compounds produced by *A. minutum* on growth, escape and histological responses of juvenile scallops *P. maximus*. Scallops were exposed to two strains of *A. minutum,* one producing both PSTs and Bioactive Extracellular Compounds (BEC), the other producing only BEC, in order to assess the effect of these toxins on physiological responses of juvenile *P. maximus* scallops.

Materials and methods

Biological material

Scallops (7-8 gr; 38-40 mm) were obtained from the Tinduff hatchery (Plougastel-Daoulas, France) and maintained in experimental system for 45 days before the start of the experiment.

Seastars *Asterias rubens* were obtained from the Bay of Brest by scubadiving three days before the "escape response assays" and maintained in a 120 L tank in running seawater (14°C).

Algal cultures of *Isochrysis galbana* (clone T-*iso*) Parke (1949) and *Chaetoceros neogracile* VanLandingham (1968) (=*C. muelleri*), two commonly used species used in bivalve hacheries, were produced as the principal diet. T-*iso* was first cultivated in continuous culture and *C. neogracile* in batch culture, with Conway media (Walne, 1970; Blancheton, 1986) in 1 µm filtered, UV sterilised seawater. These strains were cultured in 10 L bottles, before being inoculated into 150 L cylinders maintained at 18°C cultured in batch. To optimize growth conditions, cylinders were aerated with a mix of air and CO_2 and the cellular density of the mixture (T-*iso* + *C. neogracile)* fluctuated around 2.04 x 10^7 cells per day per scallops ± 2.71 x10^5 cells per day per scallops.

Two strains of *A. minutum* were used in our study. The first one, AM89BM hereafter named "PST" for convenience, was isolated from a bloom in Bay of Morlaix, Brittany, France (Erard-Le-Denn *et*

al., 1990). This strain is known for its high PST content (Haberkorn et al. 2010), but has also been shown to produce some bioactive extracellular compounds (BEC) (Lelong *et al*., 2011). The second one, CCMI1002 hereafter named "BEC" for convenience, was isolated from a bloom in Ireland (Tillmann & John, 2005). This strain does not produce any PST, but has been shown to produce a large amount of BEC (more than the AM89BM strain) with known allelopathic and cytotoxic activity (Lambert et al. 2013). Both *A. minutum* strains were grown with L1-medium made with autoclaved, 1-µm-filtered seawater (Guillard & Hargraves, 1993). The microalgae were cultured in 2L, 6L and 10L glass bottles to be inoculated in 300 L cylinders at 15°C with photoperiod of 12h/12h. Aeration, without CO_2 supplement, was maintained in the cylinders, and pH was constantly checked to supply favourable conditions for growth. Cultures were not free of bacteria and grown without antibiotics.

Allelopathic assay

Production of allelopathic compounds by the two *Alexandrium minutum* strains was tested on *Chaetoceros neogracile* according to the protocol developed by Lelong *et al*. (2011).

When *A. minutum* cultures reached end of exponential phase, they were centrifuged and resuspended in clean, new medium in triplicate at 10^4 cell.ml^{-1}. Analyses of allelopathic effects were performed after 24h after inoculating cultures, to standardize production of allelopathic compounds.

Before each sampling, cultures were mixed by gentle, manual shaking.

Supernatant from *A. minutum* cultures was separated by centrifugation (10 min, 800×g, 18°C) and filtered to 0.2 µm (acetate cellulose filters, Minisart, Sartorius, Göttingen, Germany) to eliminate bacteria (Lelong *et al*., 2011). The supernatant was prepared just before the experiment and added directly after filtration to *C. neogracile* cultures. For each condition, 2ml of the supernatant were added in 3 ml total with a final concentration of 10^5 cell.ml^{-1} of *C. neogracile* in exponential growth phase. Un-inoculated F/2 medium was used as a control.

Allelopathic effect experiments were performed in triplicate in 15-ml, pre-sterilized falcon tubes maintained in culture conditions for 3,5hours prior to analyses and assessed by measuring the quantum yield and the chlorophyll fluorescence of *C. neogracile*.

Quantum Yield (QY), a measure of the efficiency of the photosynthesis, was measured using the PAM AquaPen-C AP-C 100 fluorometer (Photo Systems Instruments, Czech Republic), on cells of *C. neogracile* after 20 min of dark adaptation at 16◦C according to the formula:

$$QY = Fv/Fm = (Fm - F0)/Fm,$$

where F0 and Fm are the minimum and maximum fluorescence of cells at 455 nm, respectively. Chlorophyll fluorescence was assessed using flow cytometry, a FACSCalibur (Becton Dickinson) flow cytometer, with an argon blue laser (488 nm). Red fluorescence (FL3) is linearly correlated with chlorophyll content of cells and was used to discriminate living microalgal cells.

Experimental design for the exposure experiment

1620 scallops (7-8 gr; 38-40 mm) were randomly distributed into six 120 L tanks (270 per tank). Two replicate tanks were used per condition. Each tank was filled with three smaller replicate trays containing each 90 juvenile king scallops. The tanks were supplied with running seawater, from Bay of Brest, filtered successively at 10 µm and 5 µm at a continuous flow of 200 $mL.min^{-1}$.

Juvenile scallops were first acclimated for 1.5 month and fed a regular diet of *C. neogracile* and T-*iso*. Food diet was mixed at the inlet of seawater (T-iso and *C. neogracile:* 50/50 dw per dw of scallops). Scallops were maintained at 10.5 °C for the first week. Then, every three-four days, the temperature and photoperiod were increased respectively by 0.5°C and 30 min day light to reach 14°C and 14 hours of day light after 3 weeks and simulate a gradual increase of the spring photoperiod and temperature.

Then, scallops were exposed to two strains *A. minutum* for one week. This supply was supplemented to the regular diet in 4 of the tanks and inserted in parallel (25 $mL.min^{-1}$ per tank, 4,17 $mL.min^{-1}$ per bin at the concentration of 3×10^4 cell/ml).

Three different conditions were run in our experiment:

1. A control condition, where scallops were fed with food diet only, containing a mix diet of *T-iso* and *C. neogracile*, hereafter named "CON"
2. Scallops subjected to "PST" *A. minutum*
3. Scallops subject to "BEC" *A. minutum*

A daily portion was registered at 2.33×10^6 ($\pm$ 3.45×10^4) cells per day per scallops for the PST strain and 2.08×10^6 ($\pm$ 7.14×10^4) cells per day per scallop for the BEC strain. At the end of exposition phase, supply of *A. minutum* was stopped for an additional two weeks of depuration and recovery, where scallops were fed the regular food "control" diet.

For the purpose of this study, only 120 scallops distributed in the several trays (5 scallops per tray, *i.e.* 15 scallops per tank, *i.e.* 30 scallops per condition) were analysed over the course of the experiment. These scallops were individually identified with an RFID chip and were weighted and measured once a week.

After the acclimation phase, a group of 9 king scallops were tested for escape response and dissected for further analyses. Similar analyses were performed on 45 king scallops (15 per

conditions) after the exposition phase and on 36 king scallops (12 per conditions) after the depuration phase.

Estimation of algal cell consumption

All algal cells (T-iso, *C. neogracile* and *A. minutum*) from the inflow and outflow of the tanks from all conditions were monitored during the exposure phase of the experiment. Cell counts were performed with a FACSalibur (Becton Dickinson) flow cytometer, with an argon blue laser (488 nm) and three fluorescence detectors: FL1 (green, 530 nm), FL2 (orange, 585 nm), FL3 (red, 670 nm). The scattered light informed about the morphology and the structure of the cells, the FL3 detector allowed for chlorophyll fluorescence detection, thus permitting to differentiate the three algal species.

Clearance rate (CR) was evaluated by calculating consumption of food algae according to the formula:

$$CR = F * ((Ci-Co)/Co)$$

Where F corresponds to the flow rate in $L.h^{-1}$

Ci the cell concentration in the inflow, in $cell.mL^{-1}$

And Co the cell concentration in the outflow, in $cell.mL^{-1}$

During the exposure phase, bio deposits were collected three-time a week and observed by fluorescence microscopy.

Evaluation of escape response

Each tested scallop was placed individually in a 25 L flat rectangular container with filtered seawater at 14°C, following a protocol adapted from Brokordt *et al.* (2006).

The mantle of king scallops was stimulated with an arm of sea-star *A. rubens*. Several indicators of escape response were evaluated (adapted from Brokordt et *al*., 2006):

1. Reaction time (Rt): the time elapsed between the first contact of the sea-star arm with the mantle edge of great scallop and the first clap.
2. Clapping time (Ct): duration of the clapping response.
3. Total time (Tt): total duration of experiment (=Rt+Ct).
4. Number of claps (Nc): number of claps performed by the king scallop to escape the sea-star.
5. Clapping rate (Cr): number of claps per second (=Nc/Ct).

After exhaustion, when the scallop stopped clapping, it was allowed to recuperate for 5min before being challenged again. Finally, the same predator stimulation was reiterated one last time on the same king scallop, 1 minute after the previous challenge.

Containers were rinsed after 2-3 assays to eliminate all residues derived from interactions between the sea-star and the king scallops (piece of flesh or molecules). Sea-stars were changed after 2 or 3 assays, but the same sea-star was used for the three successive stimulations of the same scallop.

At the end of the escape response challenge, scallops were dissected for further physiological measurements.

Condition index

Condition index of scallops was calculated using the wet flesh weight. Different indexes were chosen.

The first is the weight condition index (WCI) adapted of Fulton *et al.*, (1911) based on the individual total weight (Twi) and of Ernst *et al.*, (1991) based on the length (L) of shell:

$$WCI = \frac{Twi \times 10^4}{L^3} \quad \frac{g}{mm} \quad \% \text{ of total weight}$$

The other, the muscular condition index (MCI) is also an adaptation Fulton *et al.*, (1911) and Ernst *et al.*, (1991), calculated from the weight of adductor muscle (Wam):

$$MCI = \frac{Wam \times 10^4}{L^3} \quad \frac{g}{mm} \quad \% \text{ of muscle weight}$$

Measurements of toxins

Quantification of PSTs in scallops was performed after both exposure and depuration phase. Following the manufacturer's protocol, Paralytic Shellfish Toxins (PST) accumulation in the digestive gland, which is the organ where the accumulation is maximal (Manfrin *et al.*, 2012), was estimated using a Saxitoxin (PSP) ELISA kit (Microtiter Plate, Abraxis, Warminster, Pennsylvania, USA). After dissection, digestive glands were preserved in liquid nitrogen before being homogenized and preserved in hydrochloric acid (HCl 0.1 M). Samples were then centrifuged, and hydrolysed by heating at 104°C before being analysed with the ELISA kit.

Histological assessments

14 scallops per condition were examined macro- and micro-scopically to search for the eventual occurrence of tissue damages at the end of the exposure period. Individual scallops were prepared for histological analyses as follows: after examination for gross abnormalities, a section of soft-tissues (5mm thick), including gills, mantle, digestive glands and adductor muscle was excised and fixed in Davidson's solution (Shaw and Battle, 1957) for 48h at 4°C before being preserved in 70° ethanol. Tissue sections were dehydrated in ascending ethanol solutions, then cleared with claral

and embedded in paraffin wax. Five µm thick sections were stained with Harris' hematoxylin and Eosin (Howard *et al*., 2004). Histopathological lesions were observed under a light microscope. Intensity of each histopathological observation was rated using a three-level semi-quantitative scale ranging from 0 to 3.

Evaluation of shell growth

The shell growth was assessed 20 days after the depuration period, in order to readjust the curves, for all conditions. External shell growth rings were examined individually on the surface of the valves (Clark, 1974a ; Chauvaud et *al.*, 1998) of the juvenile scallops. Scallops (class 0+), spawn in April 2013 (class A), were brought in the laboratory in January 2014 after half a winter in natural environment in Bay of Brest. Shells thus presented an early winter bulge characterised by a tightening of growth rings (Antoine, 1978). Shell growth rate was measured from the outside to the inside (bulge) of the shell, according to a protocol adapted from Chauvaud *et al*., (1998) and Lorrain *et al*. (2002), which preconizes to read the rings on the dorsal-ventral axis only. The growth rings were read in triplicate on three separate axis of the shell and were counted on the external face of the left valve. To improve the reading of shell growth rings, excessively developed ridges of daily growth rings were removed by a 1min bath in 10% acetic acid.

The distance between rings was measured under a microscope using image analysis. The distance (D_{AB}) between any two adjacent rings (A and B) was determined using the equation: $D_{AB} = \sqrt{(X_A - X_B)^2 + (Y_A - Y_B)^2}$ (where X and Y are coordinates point). The pixel measurements were converted into distance (µm) after calibration with known measurement unit (0.4 mm).

Statistical analyses

Data following a normal law (Shapiro test) and showing homogeneity of variances (Bartlett test) were analysed using analysis of variance (ANOVA). A Tukey post-hoc test was further performed in order to compare the different treatments. Data which did not follow a normal law and/or homogeneity were analysed using nonparametric Kruskal-Wallis test and compared by a multiple comparison test (Wilcoxon test).

To compare the dependant data (same condition, different times) an ANOVA with repeated measures was used (if normality and/or homogeneity of data) or a Friedman test (if no normality or homogeneity). After these tests, if the H1 was respected ($p<0.05$) a Tukey post-hoc test was used.

For histopathology results, a unilateral Mann–Whitney U test ($\alpha= 0.1$) was used to assess the effect of of algal treatment after seven days of exposure to *A. minutum*.

Pearson correlation test was used to fit all growth curves between conditions, issued from shell growth ring measurements.

Results were all analysed with the R software v. 3.1.2. (Boston, Massachusetts, United states, 2013).

Results

Production of allelopathic compounds by the two strains of A. minutum

Results indicated a significant effect of supernatants of both *A. minutum* strains on *C. neogracile* on both chlorophyll pigments (FL3) and efficiency (QY) (Table 1). The BEC, non-PSP producer, *A. minutum* strain appeared to be the most allelopathic strain, as it triggered the highest decrease of quantum yield, chlorophyll fluorescence and morphological characteristics in *C. neogracile* after only 3h30 of incubation (Table 1). The PSP *A. minutum* strain also appeared to produce some extracellular bioactive compounds with allelopathic effects, but to a lesser extent than the BEC strain (Table 1).

Impact of A. minutum *on scallop physiology*

Consumption of food and A. minutum during exposure

Microscopic observations of faeces of scallops showed that intact cells of *A. minutum* had been ingested but not totally digested (Fig. 1). No *A. minutum* cells were observed in CON condition.

Monitoring of algal cells in the inflow and outflow of the tanks showed no significant differences of the amount of T-iso and *C. neogracile* concentrations between the treatments in the different inflows and no *A. minutum* cells were detected in CON condition.

Clearance rate of scallops exposed to both *A. minutum* species were significantly lower than the CON condition (Table 2). This decrease in clearance rate was stronger for scallops exposed to the BEC condition. After five days of exposure (21/02/2014), the clearance rate of scallops exposed to both *A. minutum* strains remained lower that the controls, however, it started to slightly increase for scallops exposed to the BEC strain (Table 2).

Impact of A. minutum on escape response

Escape response of scallops exposed for 7 days to toxic *A. minutum* and after two extra weeks of depuration are presented in Table 3. After 7 days of exposure, the reaction time of scallops exposed to the BEC condition was significantly longer than the control condition for all challenges (Fig 2.A-C, Table 3), whereas PST-exposed scallops showed an intermediate reaction time (Fig 2.A-C, Table

3). The number of claps (Fig 2.D-F, Table 3) was significantly lower for PST condition than for CON and BEC conditions for all challenges. The clapping time (Fig 2.G-I, Table 3) was also significantly lower for PST condition, than for BEC condition, which was also significantly lower that the CON condition for all challenges. No significant difference in clapping rate was observed in any of the treatments regardless of the challenge (Fig 2.J-L, Table 3).

After exposure, scallops also showed a faster reaction time after the two last challenges than for the first challenge, which corresponded to the first contact (Fig 2.A-C, Table 3). Significant differences between the several challenges after exposure could also be observed in the number of claps (Fig 2.D-F, Table 3) and the clapping time (Fig 2.G-I, Table 3) in the BEC condition, with a significant increase for the second challenge compare to the others.

After two additional weeks of depuration, all the differences observed between the different feeding treatments as well as between challenges after exposure disappeared. No significant difference could thus be observed between the feeding treatments for any of the tested parameters. Finally, some significant differences could also be observed between after exposure and after depuration. Indeed, a significant reduction of the reaction time of scallops exposed to the BEC and CON condition could be observed for the first challenge (Fig 2.A, Table 3). The number of claps also significantly increased from after exposure to after depuration for the first challenge in BEC condition (Fig 2.D, Table 3), the first and second challenge for PST condition (Fig 2.D-E, Table 3) as well as for the third challenge in CON condition (Fig 2.F, Table 3). The clapping time also appeared significantly longer for the third challenge in scallops exposed to the CON condition after depuration compare to after exposure (Fig 2.I, Table 3). Finally, the clapping rate also significantly increased between exposure and depuration for the first challenge in BEC condition (Fig 2.J, Table 3) and the second challenge for PST condition (Fig 2.K, Table 3).

Condition index

No significant difference could be observed for weight condition index (WCI) and muscular condition index (MCI) between conditions (CON, PST, UEM) or phases (after exposure and depuration).

Paralytic shellfish toxin (PST) accumulation in king scallops exposed to A. minutum

No PSTs were detected in CON and BEC conditions. Scallops from PST condition accumulated toxins at 4.4 (± 1.87) µg STX.g^{-1} digestive gland and still contained 1.16 (± 0.16) µg STX.g^{-1} digestive gland after two additional weeks of depuration, corresponding to a none significant decrease.

Histological assessment

Histopathological observations in scallops exposed for 7 days to toxic *A. minutum* are presented in Table 4 and show a significant effect of both *A. minutum* strains compare to the control. Despite some melanisation and hemocyte infiltration, which could be observed in gills of scallops regardless of the treatment, no significant effect could be noticed (Fig.3.A,B,C). Melanisation and hemocyte infiltration were however higher in the mantle of scallops exposed to both *A. minutum* strain (Fig.3.D,E,F). The BEC strain seemed to cause higher melanisation of tissues (Fig.3.F). Overall, scallops exposed to both BEC and PST conditions showed higher hemocyte infiltrations, hyalinisation of muscle fibers and atrophy of adductor muscles (Fig.3.G,H,I) . Hemocyte infiltrations observed in muscle was particularly higher in BEC condition. Presence of brown cells, hemocyte infiltrations and alterations could be observed in digestive tubules of all scallops, alterations were however higher in scallops exposed to the PST strain.

Impact of A. minutum on daily shell growth

Shell growth (number and size of growth rings) appeared equivalent for PST and CON conditions. However, a difference of three daily growth rings could be observed for scallops exposed to the BEC conditions. The pearson test allowed the maximum fit to be attained at a 3 day shift (Pearson correlation, r= 0.95 p-value= 6.82×10^{-6}). It was thus assumed that this lack of growth appeared for the first three days of exposure of scallops to the BEC condition, which restarted their growth after these 3 days (Fig.4).

Discussion

This study investigated the impact of *A. minutum* on physiology, growth and behaviour of juvenile great scallops *P. maximus*. Ingestion rate, toxin accumulation, tissue alteration growth rate and escape response were investigated after exposure of *P. maximus* to two strains of *A. minutum*. This present study also aimed to discriminate the impact of PSTs versus the impact of extracellular bioactive compounds, both produced by *A. minutum*, on scallop physiology.

This first step of this study consisted in finding strains of *A. minutum* producing either PST or BEC. The "PST" AM89BM *A. minutum* strain has already been shown to produce PST, which accumulated in bivalves (Haberkorn et al., 2010; 2011). Conversely, the "BEC" CM1002 *A. minutum* strain is known to be non-PST producing (confirmed by our results, data not shown), but has been previously shown to largely impact oysters *Crassostrea gigas* and clams *Rudiptapes philippinarum* feeding behaviour (Contreras, 2011), which were hypothesized to be due to BEC. The results of this preliminary experiment showed that both strains actually produced extracellular bioactive compounds with allelopathic effects, but that the "BEC" *A. minutum* strain had much higher allelopathic effect, thus suggesting a much higher production of BEC. The bioactive

extracellular compounds of these two strains have already been shown to impact other cell types, such as hemocytes or spermatozoa (Lambert et al. 2013), with the similar intensity as observed for the allelopathic effects in our study. Several studies have already demonstrated the allelopathic, ichtyotoxic or hemolytic impacts of *Alexandrium* sp. independently from PST production (Arzul *et al*., 1999) and demonstrated that *A. tamarense* producing low level of PSP toxins showed a strong hemolytic activity due to unknown compounds (Emura *et al*., 2004; Simonsen *et al*., 1995).

Behavioral and physiological effects on scallop resulting from exposure to BEC

Our study showed a 3-day growth delay in BEC condition, which could not be observed with the two other conditions. Antoine (1979) and Chauvaud *et al*. (1998) demonstrated that daily growth rings of scallops *P. maximus* appeared food-dependent, in terms of quality and quantity. In the latter study, growth delays were potentially associated to either the sedimentation of a *Rhizosolenia delicatula- Chaetoceros sociale* bloom, thus clogging scallop gills with the large aggregates formed by these diatoms, or to a toxic *Gymnodinium* cf *nagasakiense* (syn. *Karenia mikimotoi*) bloom. Indeed, this algal species produces cytotoxic molecules, which have already been shown to be cytotoxic (Chang, 2011), lytic (Dafni & Shilo, 1966), allelopathic and autotoxic (Gentien *et al.,* 2007) and produce bioactive extracellular compounds (Arzul *et al*., 1995; Satake *et al*., 2002). These compounds may indeed have had deleterious effects on the gills or mantle of scallops, which thus stopped feeding, directly affecting their daily growth ring. Nielsen and Strømgren (1991) also found that shell growth (measured as shell length) of *Mytilus edulis* was reduced after feeding on the toxic algae *Gyrodinium aureolum* (=syn. *K. mikimotoi= G. nagasakiense)*, as well as *Alexandrium ostenfeldii, Chrysochromulina polylepis*, and *Gymnodinium galatheanum,* related to the toxic effect of algae leading shell valve closure, and reduced filtration.Most of these species (*A. ostenfeldii, Gyrodinium aureolum, Gymnodinium sp*) have indeed been shown to produce bioactive compounds (Smolowitz & Shumway, 1997; Li et *al*., 2002; Gentien et *al*., 2006; Krock et *al.,* 2006). This suggests that bioactive extracellular compounds produced by *A. minutum* may have stopped the growth of king scallops inducing a decrease in filtration.

During the exposition phase, results indeed demonstrated a reduced filtration rate in great scallops exposed to the BEC strain, which were constantly open with their tentacles retracted (pers. comm.). This behaviour was not observed in scallops exposed to PST strain, which still showed an intermediate decreased filtration rate. Li *et al.* (2002) showed that different PSP toxin levels had no significant effect on clearance rate on mussels *Perna veridis*. Feeding rate of *Placopecten magellicanus* was also unaffected by the presence of PSP-producing *A. tamarense* (Shumway &

Cucci, 1987; Gainey & Shumway, 1988a,b ; Wilkens, 2006), thus suggesting that the intermediate response to PST condition could be associated to the BEC produced by the "PST" AM89BM *A. minutum* strain (as shown by the intermediate allelopathic effect).

Two hypotheses can be provided to explain this decrease in filtration rate.

a. The first one relies on the fact that the bioactive compounds excreted by both *A. minutum* strains may have allelopathic impact on food cells (T-*iso* and *C. neogracile*), either affecting their nutritional value or inducing the formation of undigested aggregates. Tillman et al. (2007) indeed demonstrated the lytic capacity of *Alexandrium* sp. More specifically, Arzul *et al.* (1999) showed inhibition of the growth of *C. neogracile* by *A. minutum.* Lelong *et al*., (2011) also highlighted morphological changes in shape, cytoplasmic characteristics and cell-surface, as well as decrease of fluorescence linked to Chl-*a* content or function, and an alteration of photosynthetic abilities to produce O_2 of *C. neogracile* exposure to *A. minutum* extracellular bioactive compounds. Additionally, as mentioned previously, BEC could also induce the production of Transparent Exopolymeric Particles (TEPs) by food algae (Villacorte *et al*., 2012), which could reduce the food intake by clogging of gills due to the formation of aggregates. All these studies showed that extracellular bioactive compounds can have numerous effects on environing sympatric microalgal community, thus directly affecting scallop filtration and feeding rates.

The second hypothesis is that the cytoxicity could act directly on scallop tissues, affecting the gills for example, reducing filtration rate, and thus food intake. Contreras (2011) showed a stronger inhibition of filtration rates in oysters *C. gigas* and clams *Ruditapes philippinarum* under the effect of non-PST strain of *A. minutum* (CM1002), compared to an *A. minutum* producing PST (AM89BM); the two same strains used in our study. They also observed an inhibition of clearance rate of *C. gigas* exposed to the non PST-producing CM1002 strain (our "BEC" strain). Authors hypothesized that this was mainly associated to the effects of extracellular compounds on tissues. Overall, melanisation in both gills and mantle was significantly higher in king scallops exposed to BEC strain that for both other conditions, but a significant impact of the PST strain was still observed on the mantle. Hégaret *et al.* (2012) already described a melanisation of mantle and gills in scallops *A. purpuratus* exposed to *A. catenella.* Basti et al. (2015) showed that *A. catenella* produced lytic compounds, either as exudates or located on its cell membrane, with lytic activity on oyster eggs. The melanisation was associated with hemocyte infiltrations, probably as a response to the direct contact with the extracellular compounds produced by both strains. Moreover, Haberkorn *et al.* (2010) showed a production of mucus in gills of oysters and suggested that the origin of this irritation could be due either to PSTs or more likely to the presence of extracellular bioactive

compounds produced by *A. minutum*. Lush *et al.* (1998) observed damages on gills of juvenile greenback flounder (*Rhombosolea taparina*) exposed to *A. minutum* whole cell suspension and attributed this effect to a fast acting toxin distinct from PSTs. Some studies on *Aureococcus anophagefferens,* another harmful algae responsible for brown tides, demonstrated an alteration of the feeding activity of gills and the clearance rate of *Mytilus edulis* and *Mercenaria mercenaria,* via inhibition of ciliary feeding currents in bivalve larvae and adults, associated to the presence of putative uncharacterized toxins, suspected to be unknown extracellular polysaccharids (Tracey, 1988; Bricelj & Lonsdale, 1997 Bricelj et al., 2001). Additionally, Ward & Targett (1989) showed that this toxic effect required direct contact with brown tide cells. The similarity between the effect of the unknown bioactive metabolites of *A. anophagefferens* cells and the BEC produced by *A. minutum* suggests (1) that the tissues of king scallops may be altered by unknown extracellular compounds produced by *A. minutum* strains, (2) requiring a direct contact with *A. minutum* cells.

The significantly increase in reaction time of scallops exposed to BEC strain clearly highlighted an impact of bioactive extracellular compounds. The escape response of BEC exposed scallops seems to be less effective in contact with predator. Chemosensitivity is involved in escape response of scallops, and the behavioural reflex of escape is dependent on tactile and chemical stimulus. This stimulus is perceived by ciliated epithelial cells of mantle, particularly on tentacles (Wilkens, 2006) suggesting that the recognition of predator is disrupted by direct tissue damages (such as melanisation) caused by bioactive extracellular compounds. The reaction time decreased after the first challenge for all conditions, suggesting a faster recognition of predator after the second contact. The effect of extracellular compounds however remained significant for the three challenges and the data showed an intermediate reaction time in scallops exposed to PST strain, thus clearly suggesting the involvement of extracellular bioactive compounds which are produced in smaller quantity than in BEC strain.

Results also showed an increase of clapping time in BEC condition, which could be explained first, by the involvement of the smooth adductor altered by bioactive compounds thus causing a longer clapping time. Hemocyte infiltrations were indeed significantly observed in muscle of BEC exposed scallops, as previously observed in *A. ventricosus* exposed to *Gymnodinium catenatum* (Escobedo-Lozano et al. 2012), which could be the result of a defence mechanism in order to evacuate bioactive extracellular compounds and avoid an excessive alteration of muscle. The escape mechanism is associated to valve adduction movement which allows a quick shell closure and

creates a jet-propulsion of water from the mantle cavity (Wilkens, 2006). Jumps, swims and valve adductions are rapid by the activation of fast adductor muscle at the beginning of escape (Wilkens, 2006). But, during swim episodes, it is the smooth adductor, which reopens gradually the valves (Wilkens, 2006). A second hypothesis relies on exposures to *A. minutum* (strain producing PSTs and BEC), which have previously been shown to increase micro-closures of shell after 1h of exposure of *C. gigas* to *A. minutum* (Tran et *al*., 2010,Haberkorn et al., 2011, Mat et al. 2013). Authors suggested that there could be a cytotoxic effect of *A. minutum* directly on gills. Haberkorn et al. (2011) showed a correlation between the increase of micro-closures and the *A. minutum* concentration in water, which was not associated to PSP accumulation in digestive gland. This suggests that the effect on micro-closures is due to *A. minutum* BEC and not PST. The increased number of micro-closures is a static response which requires a certain energetic cost (Robson *et al*., 2015), potentially leading a longer clapping time.

Behavioral and physiological effects associated to exposure to PST

Toxin accumulation in scallops exposed to the PST strain clearly indicates that some microalgae were ingested. Conversely scallops exposed to BEC producing *A. minutum* strain or control did not accumulate any toxin, confirming the absence of PSTs in these algal strains. This study allowed the differentiation between the effects of BEC from paralytic shellfish toxins.

Effect on escape response and role of valve activity

Exposure to PST strain did not affect overall scallop growth but affected their escape response, potentially increasing king scallop vulnerability to predator. The number of claps and clapping time of scallops exposed to PST strain were significantly lower than with other conditions. Hégaret *et al.* (2012) showed a decrease in the number of claps of *A. purpuratus* exposed to *A. catenella* when challenged by seastar *M. gelatinosus*, but this difference was only significant after scallops had been challenged twice (i.e. after 5min recuperation). As for clapping time, a significant decrease was observed due to *A. catenella*, compared to control (Hégaret *et al.*, 2012) suggesting an exhaustion of northern scallops or a modification of valve activity. Unfortunately, this study did not assess valve gape or force of clapping, but the data still suggest an alteration of the locomotory system which, in normal operation, leads to a swimming response to escape predator by valve activity. Numerous studies showed a modification of valve activity after exposition to toxic microalgae. May *et al*. (2010) showed a reduction of valve gape in three shellfish species (*M. mercenaria, C. virginica, P. viridis*) exposed to *A. monilatum*. Mat et al. (2013) correlated a reduction of valve-opening amplitude of *C. gigas*, to the amount of *A. minutum* paralytic shellfish

toxins (STX and derivates) accumulated in the digestive gland. Haberkorn et al. (2011) also correlated an increase of the frequency of opening duration with PST concentration in digestive glands. Similarly, valve movement was modified after exposure to PST producers in the scallop *N. subnodosus* (Estrada et *al*., 2010). Shumway & Cucci (1987) showed that the swimming/clapping activity patterns was shorter during exposure to *Protogonyaulax tamarensis* (=syn. *A. tamarense*). All these studies showed a modification of valve activity, which is very likely due to alteration of adductor muscle and paralysis.

Indeed, results showed a myopathy of adductor muscle in scallops exposed to both *A. minutum* strains, compared to the control), characterised by myoatrophy and hyaline degeneration of the muscle fibers. Similar results were already observed in scallops *A. purpuratus* juveniles exposed *A. catenella* (Hégaret et al., 2012), or in oysters C. gigas exposed to *A. minutum* (AM89BM), which also showed a severe myopathy of muscles, characterised by myoatrophy, hyaline degeneration and wavy-pattern degeneration (Haberkorn *et al.* 2010). The latter hypothesized the direct effect of paralytic shellfish toxins on muscle fibers. Bricelj *et al*. (2005) showed that burrowing of *Mya arenaria* was not affected by exposure to *A. tamarense* strain (CCMP115) which is of negligible PSP toxicity, but showed that the incapacity to burrow was toxin-induced in clams and resulted in muscle paralysis (Bricelj *et al*., 1990; 2005). Burrowing in *M. arenaria* is a hydraulic process, involving several muscular systems that work simultaneously (Trueman 1966, Checa & Cadée 1997, MacQuarrie & Bricelj, 2008). Each of these is under nervous control and thus can be affected by PSTs (MacQuarrie & Bricelj, 2008). The link between accumulation of PSTs and paralysis of adductor muscle has been already observed in several bivalve species such as *C. gigas, M. edulis* or *A. ventricosus* exposed to PST producing dinoflagellates (Hégaret et *al*., 2007; Galimany *et al*., 2008; Escobedo-Lozano et *al*., 2012). Estrada *et al.* (2010) demonstrated paralysis of scallops *N. subnodosus* induced by the injection of gonyautoxin (GTX, a saxitoxine-like toxin), produced by *G. catenatum* into the muscle. The paralytic effects of PSTs are due to STX and derivates, which have effects on sodium channels (Bricelj et *al*., 2005) and can blocked impulses nerves. Moreover, the locomotory system depends on neuromuscular system. Adductor muscle and muscle fibers are involved in the mechanisms of contraction and relaxation of the adductor muscle and a nervous information is sent to sodium channels to initiate movement by muscle contraction (Catterall, 1992). The decrease in the number of claps and clapping time observed in this study, could highlight the action of STX on scallops by the blocking the impulse nerve altering the capacity of the muscle to move.

Conclusion

Results obtained in the preliminary experiment demonstrated the impact of extracellular compounds on *C. neogracile*, thus highlighting that *Alexandrium* species can produce different types of toxic compounds, such as paralytic shellfish toxins, but also allelopathic compounds, which are not always related to each other. These results clearly highlight the importance of working with different strains of HABs when assessing their possible impacts on marine organisms. Indeed, an *Alexandrium* species, producing high level of PST may not be as toxic to other organisms as a strain producing high level of bioactive extracellular compounds.

Physiological and behavioural responses of scallops were clearly different between the two *A. minutum* strains. Scallops exposed to the "PST" strain, showed a decrease in the number of claps and clapping time, thus suggesting an effect of paralytic shellfish toxins on the muscle, affecting the coordination of locomotion system and causing a paralysis in scallops. On the contrary, paralytic shellfish toxins did not seem to affect the recognition processes when facing a predator.

Conversely, altered recognition processes could be observed in scallops exposed to the non-PST strain, with an increased time of reaction when facing a predator. This could be associated to the alteration of tissues responsible for this recognition (gills, mantle, and tentacles). These alterations can be explained by the cytotoxic effects of extracellular bioactive compounds released by *A. minutum*. These extracellular compounds are also likely responsible for the reduction of filtration rate, thus causing cessation of growth by altering the tissues.

This study highlights the importance to better characterize these extracellular bioactive compounds in order to better understand their roles and impacts on sympatric organisms, such as phytoplankton species via allelopathy, but also other marine organisms (copepods, bivalves, fishes etc.) via ichtyo- or cytotoxicity.

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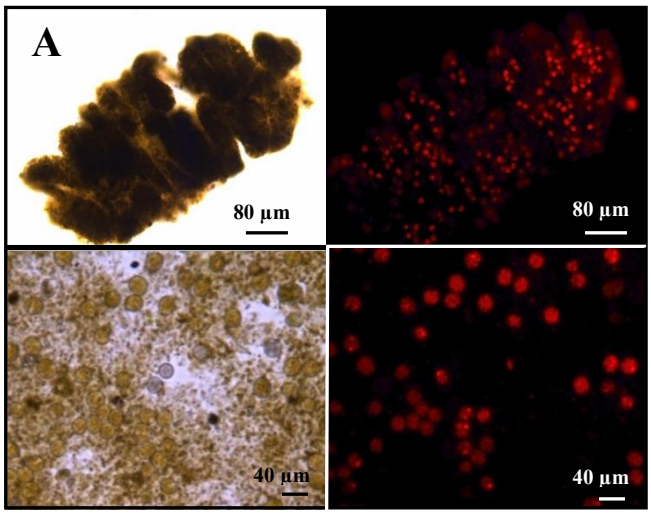
FIGURES

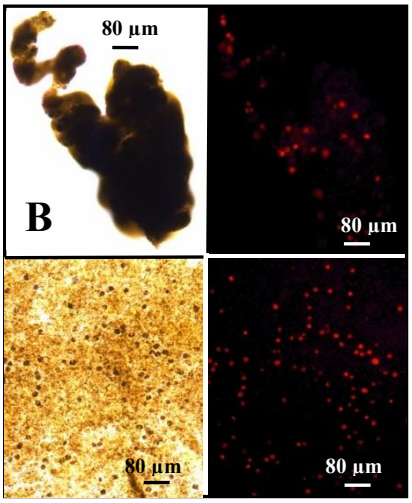
Fig.1 : Light and epifluorescent pictures of fecal pellets of king scallops *Pecten maximus* containing intact *A. minutum* cells of PST condition (A) and BEC condition (B)

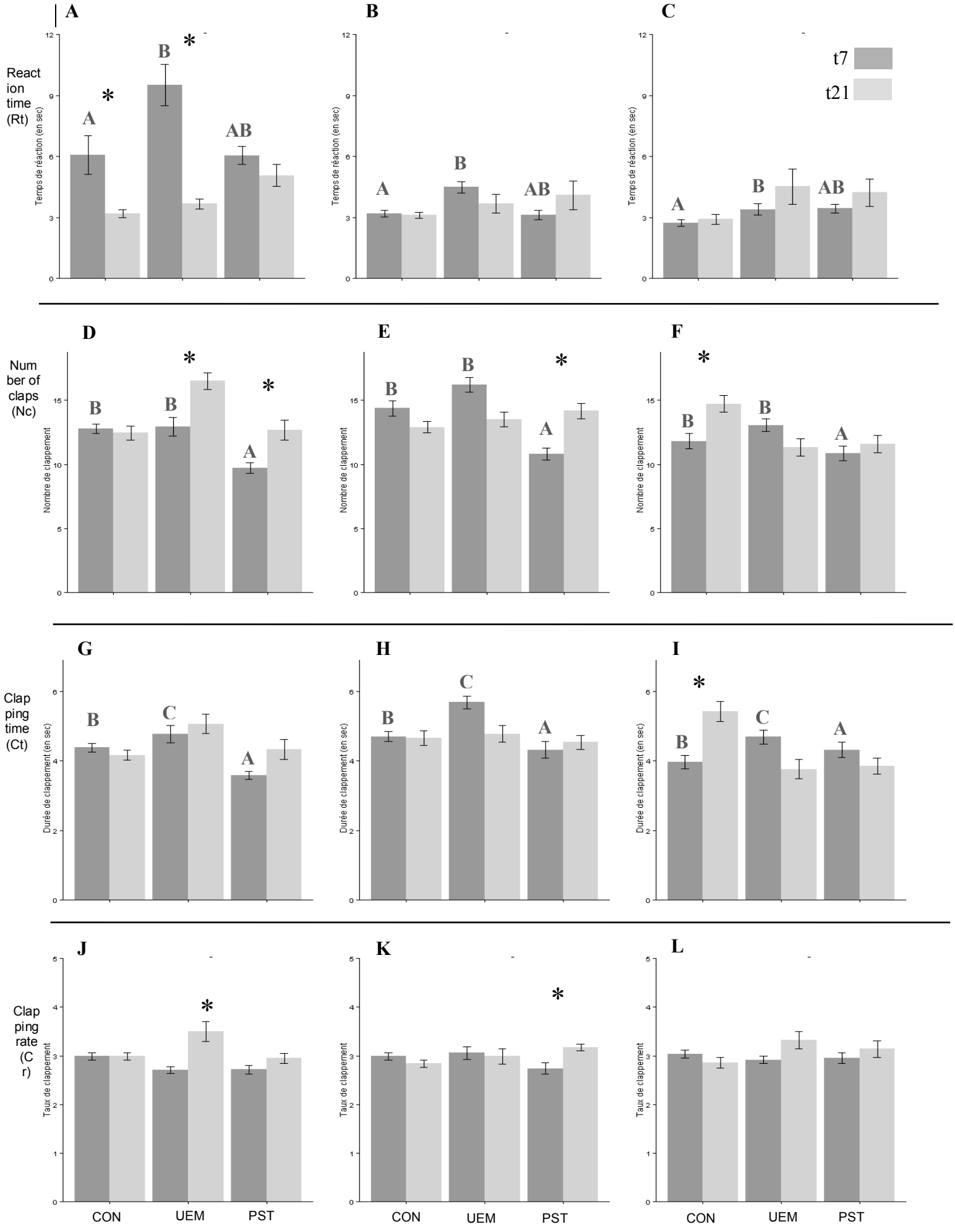
Fig. 2 : Escape response of *P. maximus* to predator; fed the control diet (CON) or exposed to BEC *A. minutum* strain (CCMI1002), or PST-*A. minutum* strain (AM89BM),in terms of reaction time (Rt), Number of claps (Nc), Clapping time (Ct) and Clapping rate (Cr). (ANOVA, P>0,05; Mean +/- SE).

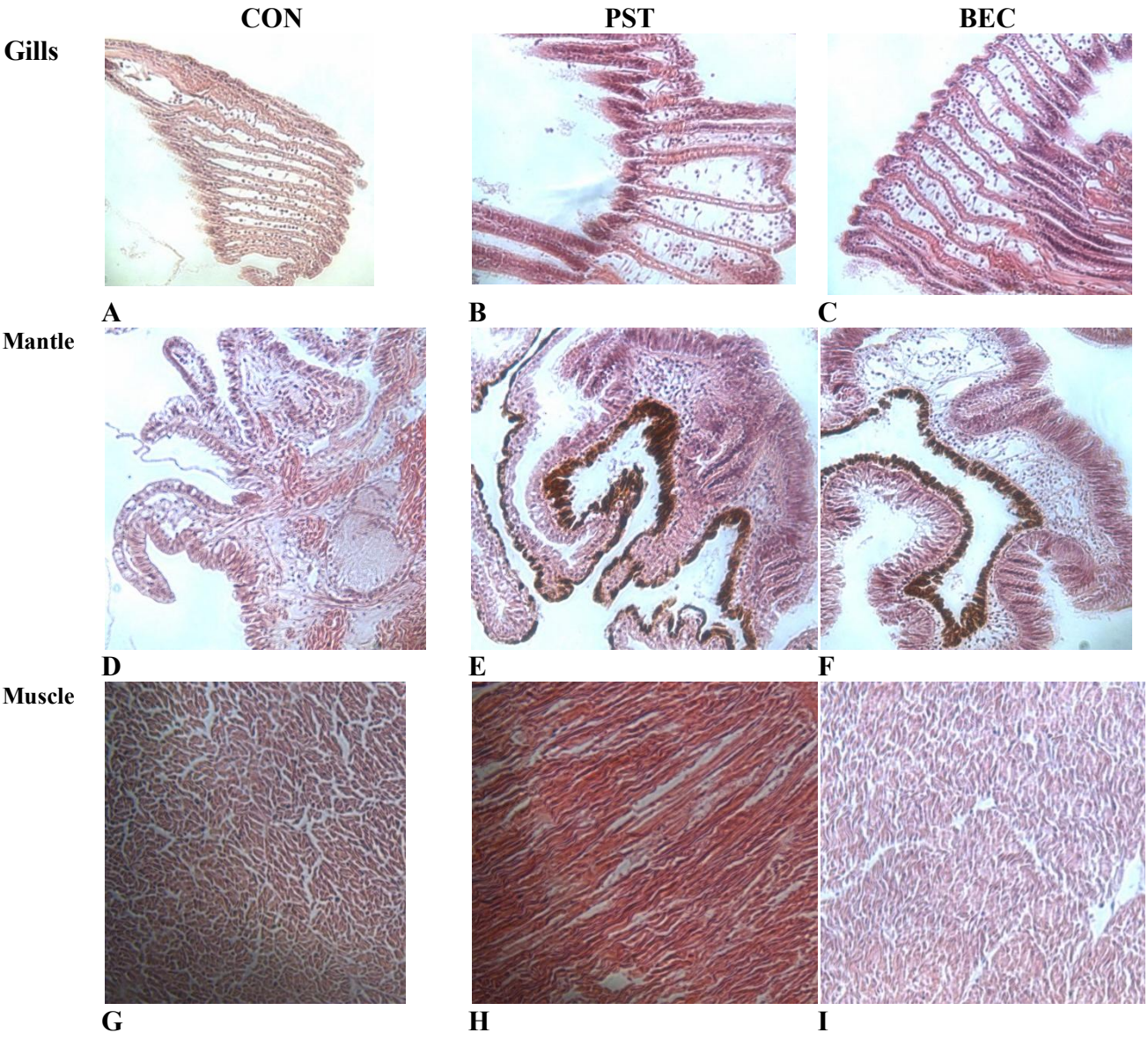
Fig. 3 : Histopathological observations in CON (A, D, G), PST (B, E, H) and BEC (C, F, I) conditions on several tissues : gills (A-C), mantle (D-F), muscle (G-I).

Fig. 4 : Growth curves of CON, PST and BEC conditions


A
80 µm
80 µm
40 µm
40 µm


80 µm
B
80 µm
80 µm
80 µm


A
B
C
Reaction time (Rt)
Temps de réaction (en sec)
t7
t21
A
B
AB
*
D
E
F
Number of claps (Nc)
Nombre de clappement
B
C
A
G
H
I
Clapping time (Ct)
Durée de clappement (en sec)
J
K
L
Clapping rate (Cr)
Taux de clappement
CON
UEM
PST


CON
PST
BEC
Gills
A
B
C
Mantle
D
E
F
Muscle
G
H
I

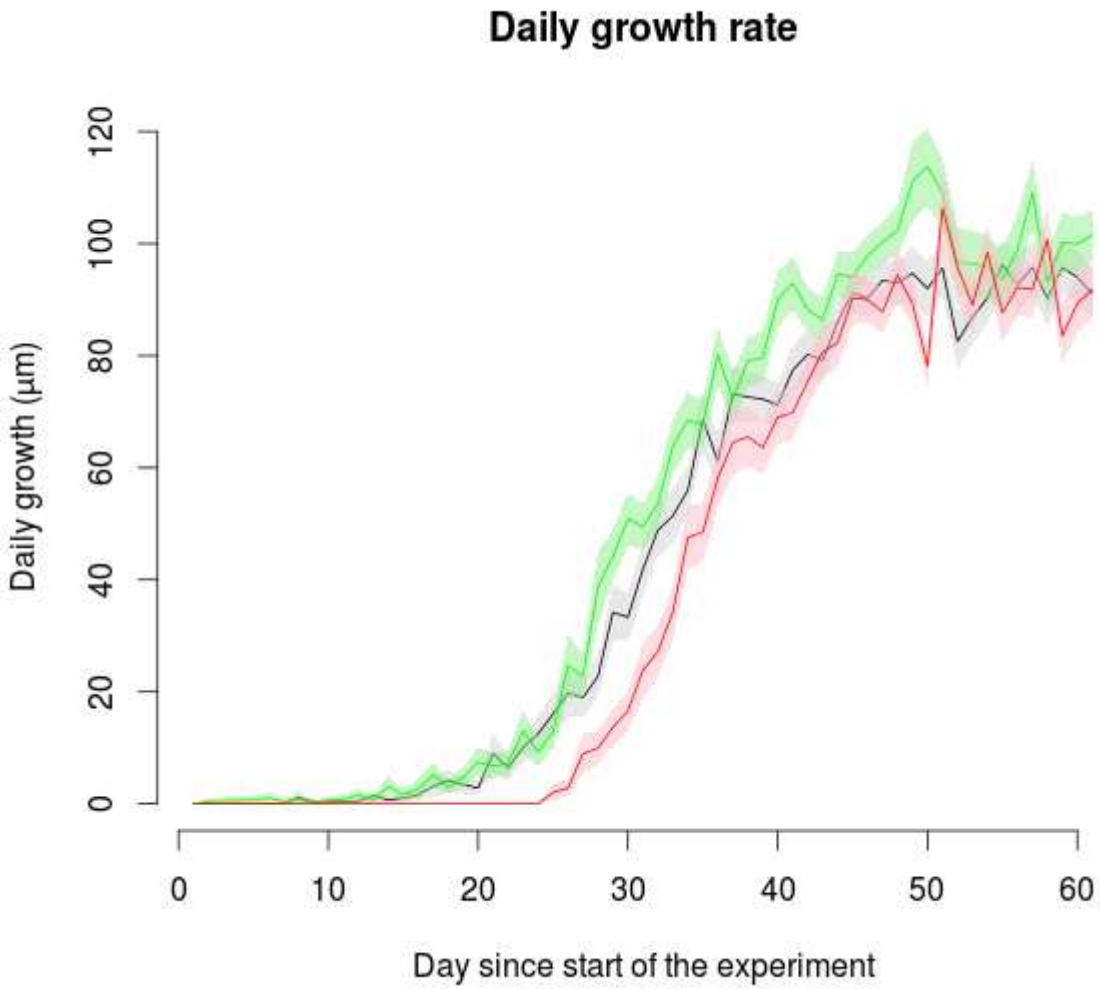

Daily growth rate
Daily growth (µm)
0
20
40
60
80
100
120
0
10
20
30
40
50
60
Day since start of the experiment

Table 1: Impact of supernatant of two strains of *A. minutum* (PSP and BEC) or a L1-medium control on quantum yield and "FL3" chlorophyll fluorescence of the diatom *C. neogracile* (n=3, ANOVA, Mean ± SE, letters a,b,c apply to significant differences among treatments)

	P-value	CON	BEC	PST
Quantum Yield	P<0,01	0,67 (0,010) (a)	0,42 (0,005) (c)	0,61 (0,033) (b)
FL3	P<0,01	319,0 (9,88) (a)	214,1 (3,56) (c)	280,6 (6,56) (b)

Table 2: Clearance rate ($L.h^{-1}.ind^{-1}$) of scallops *P. maximus* after three (Feb 19th) and five (Feb 21st) days of exposure the control diet (CON) or exposed to BEC *A. minutum* strain (CCMI1002), or PST-*A. minutum* strain (AM89BM). (ANOVA, Mean ± SE; , letters a,b,c apply to significant differences among treatments).

Date	P-value	CON	BEC	PST
Feb 19th	P<0,05	4,12±0,87 (a)	0,29±0,007 (c)	2,03±0,1 (b)
Feb 21st	P<0,05	3,67±0,17 (a)	1,01±0,1 (bc)	1,94±0,29 (b)

Table 3 : Impact of the three feeding treatment on escape responses of great scallops P. maximus after exposure and depuration phase (Mean ± SE ; ANOVA * p<0,05 and ** p<0,01; letters a,b,c apply to significant differences among treatments for each challenge ; Letters α, β, γ apply to significant differences among challenges for each treatment).

		Exposition phase				**Depuration phase**		
	Challenge	**CON**	**BEC**	**PST**	**P-value**	**CON**	**BEC**	**PST**
Rt	**Cha 1**	6,07 (a,β) ±0.96	9,51 (b,γ) ±1.02	6,04 (ab,β) ±0.44	*	3,19 ±0.20	3,67 ±0.26	5,07 ±0.54
	Cha 2	3,18 (a,α) ±0.17	4,49 (b,β) ±0.27	3,12 (ab,α) ±0.23	*	3,11 ±0.14	3,69 ±0.47	4,09 ±0.71
	Cha 3	2,72 (a,α) ±0.14	3,4 (b,α) ±0.30	3,44 (ab,α) ±0.22	*	2,91 ±0.26	4,52 ±0.90	4,23 ±0.69
P-value		*	*	**		NS	NS	NS
Nc	**Cha 1**	12,77 (b) ±0.36	12,93 (b,α) ±0.73	9,74 (a) ±0.41	*	12,45 ±0.56	16,50 ±0.69	12,66 ±0.80
	Cha 2	14,38 (b) ±0.60	16,22 (b,β) ±0.57	10,81 (a) ±0.45	*	12,90 ±0.47	13,50 ±0.58	14,16 ±0.61
	Cha 3	11,81 (b) ±0.59	13,07 (b,α) ±0.50	10,88 (a) ±0.56	*	14,72 ±0.68	11,33 ±0.67	11,58 ±0.68
P-value		NS	*	NS		NS	NS	NS
Ct	**Cha 1**	4,38 (b) ±0.12	4,76 (c,α) ±0.24	3,58 (a) ±0.11	*	4,16 ±0.14	5,06 ±0.29	4,33 ±0.28
	Cha 2	4,7 (b) ±0.14	5,67 (c,β) ±0.18	4,31 (a) ±0.24	*	4,65 ±0.21	4,77 ±0.25	4,53 ±0.20
	Cha 3	3,96 (b) ±0.19	4,69 (c,α) ±0.20	3,88 (a) ±0.22	*	5,42 ±0.29	3,76 ±0.29	3,84 ±0.23
P-value		NS	*	NS		NS	NS	NS
Cr	**Cha 1**	2,99 ±0.08	2,71 ±0.07	2,72 ±0.09	NS	2,99 ±0.07	3,5 ±0.21	2,95 ±0.10
	Cha 2	2,99 ±0.07	3,06 ±0.13	2,74 ±0.12	NS	2,83 ±0.08	2,99 ±0.16	3,17 ± 0.07
	Cha 3	3,04 ±0.08	2,91 ±0.08	2,94 ±0.11	NS	2,85 ±0.12	3,31 ±0.19	3,14 ±0.17
P-value		NS	NS	NS		NS	NS	NS

Table 4 : Impact of two strains of *A. minutum* (PSP and UEM) on tissues of king scallops compare to control (n=14 per condition, Kruskal-wallis, *: $p < 0.05$, #: $p < 0.10$; , letters a,b,c apply to significant differences among treatments)

Tissues	Histological features	Control	BEC	PST	P-value
Gills	**Melanisation**	0,86 ±0.18	1,38 ±0.13	0,97 ±0.17	NS
	Hemocyte infiltration	2.07 ±0.16	1.93 ±0.19	2 ±0.19	NS
Mantle	**Melanisation**	0,73 (a) ±0.16	1,46 (b) ± 0.19	1,5 (b) ±0.22	*
	Hemocyte infiltration	1.21 (a) ±0.11	2.17 (b) ±0.22	1.93 (b) ±0.24	*
Muscle	**Hyalinisation of muscle fibers**	0.68 ±0.12	1.30 ±0.25	1.13 ±0.26	NS
	Atrophy	0.59 ±0.19	1 ±0.26	1.33 ±0.31	NS
	Hemocyte infiltration	0 (a)	0.34 (b) ±0.16	0 (a)	*
	Hyalinisation + Atrophy + Hemocyte inf	1.18 (a) ±0.29	2.65 (b) ±0.41	2.47 (b) ±0.36	*
Digestive tubules	**Presence of brown cells**	1.5 ±0.15	1.54 ±0.17	1.5 ±0.17	NS
	Hemocyte infiltration	1.29 ±0.17	1.36 ±012	1.32 ±0.13	NS
	Alterations	0 (a) ±0	0.27 (a) ±0.26	1 (b) ±0.20	#
All tissues	**Mean of all melanisation**	1.58 (a) ±0.24	3 (b) ±0.25	2,5 (a) ±0.30	#
	Mean of all hemocyte infiltration	4.5 ±0.25	5.54 ±0.46	5.31 ±0.45	NS
	Mean of all histological features	6.8 (a) ±0.17	9.14 (b) ±0.70	9.80 (b) ±0.71	#

Diversité génétique et phénotypique de populations naturelles ou ensemencées de coquille Saint-Jacques *Pecten maximus*

Romain MORVEZEN

Résumé

La coquille Saint-Jacques (*Pecten maximus*, L.) est un bivalve d'intérêt scientifique et économique. De nombreuses études ont notamment porté sur sa croissance en réponse aux variations temporelles ou spatiales de l'environnement, mettant à profit l'incrément journalier de la croissance coquillère. Certaines populations naturelles font l'objet de soutien à la pêche par semis de naissain produits en écloserie, pour compenser les baisses de productivité constatées depuis la seconde moitié du XXème siècle. Cependant, très peu de données génétiques sont disponibles sur cette espèce, ne permettant pas d'estimer l'impact de ces pratiques en termes de diversité et de potentiel adaptatif. Dans ce contexte, ma thèse s'est articulée autour de trois axes principaux : (1) une étude de génétique des populations à l'échelle de l'aire de distribution; (2) une étude de génétique quantitative pour estimer les bases génétiques de la croissance; (3) le suivi temporel d'une population ensemencée en Rade de Brest, pour déterminer l'impact génétique du programme local de soutien aux populations exploitées. Les résultats montrent qu'il existe une forte différentiation entre deux groupes de populations : norvégiennes et atlantiques (de l'Angleterre à l'Espagne), globalement concordante avec des différences de croissance précédemment observées, ce qui suggère un base génétique à ces dernières. L'étude de génétique quantitative soutient également cette hypothèse, montrant une héritabilité modérée mais significative des paramètres de croissance en rade de Brest. Enfin, un suivi temporel de cohortes en rade de Brest montre un impact limité du programme de réensemencement sur la diversité génétique, malgré une estimation des contributions reproductrices des semis relativement élevée. L'ensemble souligne l'intérêt de poursuivre l'étude du potentiel adaptatif des populations de cette espèce exploitée et de l'impact des réensemencements sur les populations naturelles.

Genetic and phenotypic diversity of natural and seeded populations of the great scallop *Pecten maximus*

Abstract

The great scallop (Pecten maximus, L.) is a marine bivalve of economic and scientific interest. Numerous studies have been done on its growth, notably on growth variation according to temporal or geographic variations based on daily shell growth rings. Some populations are undergoing enhancement programs, by the seeding of hatchery-born juveniles on natural beds, aiming to compensate for the decline observed since the middle of the 20th century. However, little information was available on the genetics of P. maximus, as a background for evaluating the impact of such programs. In this context, my thesis consists in 3 chapters: (1) a population genetics study along its area of distribution; (2) a quantitative genetics study to estimate genetic basis of growth -related traits; (3) a temporal genetic monitoring of a seeded population in the Bay of Brest to estimate possible impact of the enhancement program. Results showed a strong genetic structure between Norwegian and Atlantic (from U.K. to Spain) populations, coherent with phenotypic differences of growth parameters, suggesting a possible adaptive component of these traits. The quantitative genetic study hints to a similar result by revealing a moderate heritability of growth parameters. Finally, genetic monitoring revealed a limited impact of the population enhancement program, despite relatively high estimations of reproductive contribution of seeded individuals. Overall, these results highlight the scientific interest and the necessity to further study the evolutionary potential of this exploited species and the impact of population enhancement programs.