Écologie de la reproduction de l'huître Crassostrea gigas en lagune méditerranéenne

Franck Lagarde

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Écologie de la reproduction de l'huître *Crassostrea gigas* en lagune méditerranéenne

Par Franck Lagarde

Thèse de doctorat en Écologie marine

Présentée et soutenue le jeudi 29 novembre 2018

Devant un jury composé de :

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"Vivre comme si l'on devait mourir demain,

Apprendre comme si l'on devait vivre pour l'éternité."

Mohandas Karamchand Gandhi

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Préambule

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[1] Axe 4 : Effet du changement global sur la distribution des espèces, l'accessibilité et la connectivité de leurs habitats et interaction avec la spatialisation de l'exploitation. Action « Comportement, distribution et qualité larvaire d'espèces-clé conchylicoles, *Crassostrea spp.* ».

[2] UMR MARBEC Univ Montpellier, CNRS, IRD, Ifremer

[3] National Research Institute of Fisheries and Environment of Inland Sea, Japanese Fisheries Research and Education Agency

En plus des 4 publications qui forment les chapitres de ce mémoire, ce projet doctoral a fait l’objet de 59 évènements de valorisation des travaux (Tableau 1 ; Annexe 1).

Tableau 1: Valorisation des travaux de thèse

Type	**2015**	**2016**	**2017**	**2018**	**Total**
Conférences internationales, présentations orales	2	2	3	4	**11**
Conférences internationales, posters		1	1	2	**4**
Présentations en colloques au niveau national	1	2			**3**
Présentations en groupes de travail au niveau international	2	2	4	4	**12**
Présentations en groupes de travail au niveau régional	1	4	3	3	**11**
Rapports scientifiques et techniques	3		1		**4**
Jeux de données Seanoe		1		1	**2**
Articles de presse, ouvrages Grand Public			4	1	**5**
Interventions grand public			3		**3**
Participations à des reportages/documentaires télévisuels	1	1	1	1	**4**
Total	**10**	**13**	**20**	**16**	**59**

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Introduction

"La vérité scientifique est une erreur qui a été corrigée". Gaston Bachelard

Contexte

La lagune de Thau est un écosystème méditerranéen dont les principaux usages anthropiques répertoriés par le Schéma de Cohésion Territoriale 2010-2030 sont la conchyliculture, la pêche, le tourisme et les activités nautiques de loisir[4]. La conchyliculture intègre depuis les années 1970, l'élevage de l'huître creuse, *Crassostrea gigas* importée du Japon et du Canada (Grizel & Héral 1991; Buestel et al. 2009; Héral 1989). Les ostréiculteurs méditerranéens cultivent jusqu'à maintenant cette huître en s'approvisionnant principalement en naissains nés sur les côtes atlantiques ou en écloseries puisque cette espèce est connue pour ne pas se reproduire ou de manière très occasionnelle en lagunes méditerranéennes conchylicoles (Héral 1989; Goulletquer 1995; Héral 1986). Cependant, la raréfaction du naissain et la spéculation des prix induites par les épisodes de surmortalité de naissain à partir de 2008 (Pernet et al. 2010; Pernet, Lagarde, Jeanné, et al. 2014; Pernet, Lagarde, Le Gall, et al. 2014) ont amené la filière ostréicole méditerranéenne au souhait de développer un approvisionnement régional en naissain natif.

Pour répondre à ce souhait, plusieurs expérimentations ont été mises en œuvre sur la lagune de Thau dès 2010 dans le cadre des projets régionaux PRONAMED[5] phase 1 de 2010 à 2011 (Rayssac et al. 2012), phase 2 de 2012 à 2014 (Lagarde, Fiandrino, et al. 2015; Lagarde, Gervasoni, et al. 2015) et d'un projet national : l'observatoire national VELYGER[6]. L'objectif scientifique commun à ces projets était d'améliorer les connaissances de la reproduction et du recrutement de l'huître dans le contexte particulier des lagunes méditerranéennes. Les résultats du projet PRONAMED ont mis en évidence le potentiel économique du captage naturel en lagune de Thau, mais avec une forte variabilité dans son succès (Rayssac et al. 2012; Lagarde, Gervasoni, et al. 2015). Les hypothèses avancées pour expliquer cette variabilité étaient diverses sans être précises : les conditions hydrodynamiques de la lagune, son hétérogénéité écologique intrinsèque, ainsi que les conditions trophiques variables induites par les

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[4] http://www.smbt.fr/content/SCOT

[5] PRODuction de NAissains en lagunes MEDitterranéennes

[6] https://wwz.ifremer.fr/velyger/

fluctuations environnementales. Les observations issues du réseau VELYGER démontrent le caractère exceptionnel de la lagune de Thau dans le panorama des bassins conchylicoles français tant sur sa nature hydrobiologique que sur les processus de reproduction (gamétogénèse et ponte) et de recrutement (développement larvaire, fixation et métamorphose) (Pouvreau 2018; Pouvreau 2016).

Il est reconnu que les conditions biotiques (Hjort 1926; Lasker 1975; Rothschild & Osborn 1988) et abiotiques comme la température (Olive et al. 1997) et l'hydrodynamisme (Parrish et al. 1981; Harvey & Bourget 1997; Jonsson et al. 2004) sont déterminantes pour le recrutement des invertébrés marins. Les connaissances sur l'huître creuse, en particulier sur ses stratégies de reproduction et de recrutement, ont été essentiellement acquises dans les écosystèmes atlantiques (His & Seaman 1992; Lagarde et al. 2018; Auby & Maurer 2004; Bernard 2011). Cependant, le fonctionnement des écosystèmes ostréicoles méditerranéens est connu pour être résolument différent des écosystèmes atlantiques en termes de régimes climatiques, de régimes de marée, d'hydrologie, d'hydrodynamique et d'écologie (Pichot et al. 1991; Héral 1989).

Dans ce contexte, l'objectif de cette thèse est donc de décrire et d'analyser les facteurs déterminants de la reproduction de l'huître creuse en écosystème lagunaire méditerranéen à partir des données du projet PRONAMED phase 2. Il s'agit en particulier de comprendre la variabilité du recrutement naturel des larves dans la perspective du développement d'un approvisionnement local en naissain soutenu par les ostréiculteurs méditerranéens.

La lagune de Thau

Géographie et usages

La lagune de Thau fait partie du complexe des lagunes côtières méditerranéennes françaises occupant près de 50% de la côte occitane sur le pourtour du golfe du Lion (Figure 1). Ces écosystèmes récents, âgés de 6 000 à 10 000 ans environ, sont la conséquence de la stabilisation du niveau de la mer au niveau actuel et de dépôts sableux et alluvionnaires. Le découpage des lagunes occitanes et leur configuration actuelle se sont dessinés au cours du 17ème siècle.

La lagune de Thau est un bassin d'une superficie de 6792 hectares, d'une profondeur de 4.0 m en moyenne s'étendant sur un axe NE- SW de Balaruc à Marseillan (Guelorget et al. 1994; Fiandrino et al. 2017). Les caractéristiques hydromorphologiques s'expliquent par sa formation d'origine tectonique avec le bassin en synclinal et un plissement anticlinal formant le massif de la Gardiole au nord-est (Jauzein 2009).

Ce bassin de 295 millions de mètres cubes est alimenté en eau de mer par des canaux artificialisés appelés "Graus" en occitan. Les principaux apports en eau de mer passent par les canaux de la ville de Sète et représentent 90% des apports marins (en moyenne 6 millions de mètres cubes par jour), alors que le grau de Pisses-Saumes, au sud, ne représente que 10% des apports en eau de mer (Fiandrino et al. 2017). Le canal du Midi et le canal du Rhône à Sète représentent des entrées d'eaux saumâtres tandis que les apports en eau douce sont issus d'un bassin versant de 280 km^2 avec deux cours d'eau principaux pérennes (La Vène et le Pallas). Ce réseau hydrographique est composé d'une dizaine de cours d'eau de petite taille, non pérennes pendant la saison sèche. Ces cours d’eau sont sous l’influence directe du climat méditerranéen littoral avec des précipitations irrégulières, pouvant devenir intenses l'été pendant les orages ou à l’automne pendant les épisodes cévenols entraînant des crues aussi violentes que soudaines. D'un point de vue hydrodynamique, le bassin est sous influence d’un régime nanotidal avec une faible influence des marées, un faible taux de renouvellement des masses d’eau et des temps de résidence élevés en moyenne, mais variables d'est en ouest selon les volumes de mélange des intrusions marines avec les eaux de type lagunaire (Fiandrino et al. 2017; Guelorget et al. 1987; Rosello-Tournoud 1991).

Source : IGN, BD Carthage
Réalisation : Observatoire SMBT, 2009

Figure 1: Carte des cours d'eaux et des zones urbanisées du bassin versant de la lagune de Thau (source SMBT)

La lagune de Thau constitue un écosystème réservoir de biodiversité propice aux pêcheries et à la conchyliculture mais également très attractif d'un point de vue touristique. Les usages du bassin versant sont dédiés aux activités agricoles, touristiques et industrielles, avec une urbanisation en phase d'expansion. Cet ensemble d'activités bénéficie des fonctions et services écosystémiques dont le développement durable nécessite une gestion environnementale intégrée.

La conchyliculture a été choisie comme un des usages prioritaires de la lagune par les différents contrats de gestion environnementale, depuis 1995 par le schéma de Mise en Valeur de la Mer, puis en 2005 dans le schéma d'aménagement pour la gestion de l'eau et dans le plan de gestion de la lagune défini par le schéma de Cohésion Territoriale. La gestion environnementale est orchestrée depuis 2005 par le Syndicat Mixte de Gestion du Bassin de Thau.

Ostréiculture

La France est le pays dont la production ostréicole est la plus importante en Europe avec environ 125 000 tonnes d'huîtres creuses produites au cours des années 2015/2016[7]. Cette industrie est fortement représentée sur la façade Atlantique, en Manche et en Méditerranée. Elle est aujourd'hui basée à 99% sur la culture de l'huître creuse *Crassostrea gigas*, dont l'origine est japonaise. Importée en 1970, cette espèce a trouvé une niche écologique favorable dans les écosystèmes atlantiques, en particulier dans les bassins d'Arcachon et de Marennes-Oléron où croissance, survie et reproduction ont permis une viabilité économique immédiate de la production (Grizel & Héral 1991; Debos et al. 1972; Buestel et al. 2009). Mais elle a aussi très rapidement colonisé les écosystèmes au nord de la Loire (Thomas, Pouvreau, et al. 2016) et se reproduit désormais annuellement en rade de Brest (Pouvreau 2018; Pouvreau 2016). Les élevages ostréicoles méditerranéens sont principalement en lagunes à Thau, à Leucate et en mer.

Les premiers essais de mise en culture des huîtres ont été développés pour produire et engraisser des huîtres creuses arcachonnaises ou bretonnes (*Crassostrea angulata*) positionnées sur radeaux dans les canaux de Sète en 1875, puis transférés pour des raisons sanitaires en 1907 en lagune de Thau, dans sa partie Est appelée "l'étang des eaux blanches". En 1908, les premiers essais de mytiliculture ont été mis en place à Balaruc. A partir de 1925, Louis-Antoine Tudesq (entrepreneur de Bouzigues) innove en proposant l'utilisation du plâtre et du ciment pour coller des "huîtres plates" d'Arcachon ou de Bretagne. Puis Pierre-Joseph Benezech utilisa des tuteurs de vignes en bois exotique, le palétuvier.

Les années 1960-70 marquent un contexte de crise ostréicole en France du fait des mortalités de *Crassostrea angulata* (His 1976). En Méditerranée, le développement anarchique des concessions, la surexploitation locale de la capacité trophique et la diminution de la taille commerciale des huîtres s'ajoutent à cette crise de surmortalité (Pichot 1992). La conjugaison

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[7] http://www.cnc-france.com/La-Production-francaise.aspx

de ces phénomènes amène à une diminution des stocks d'huîtres à moins de 2 500 t entre 1965 et 1970 en lagune de Thau (Deslous-Paoli et al. 1998).

En 1970, le remembrement des parcs conchylicoles vise un double objectif pour le renouveau de l'ostréiculture en lagune. Le premier est d'accroître les rendements, en déplaçant les tables vers le large et en les espaçant afin de permettre une meilleure circulation de l'eau, améliorant ainsi la distribution des éléments nutritifs (Hamon 1983; Amanieu et al. 1989). La zone conchylicole passe ainsi de 350 ha à plus de 1300 ha à l'intérieur de laquelle la surface des concessions réservées pour 2816 tables est de 352 ha.

Le second objectif est d'harmoniser les unités de production en créant un type unique de table (50 m de long sur 10 m de large). Ces tables se répartissent d'est en ouest en trois zones (Bouzigues, Mèze et Marseillan) couvrant respectivement 160 ha, 106 ha et 86 ha (Figure 2). Ici encore la diminution d'est en ouest des secteurs cultivables trouvait sa justification dans l'éloignement progressif de la principale arrivée d'eau de mer transitant par les canaux de Sète, considérée comme le principal facteur favorisant d'une manière directe (apport de plancton) ou indirecte (mouvement des masses d'eau) l'approvisionnement en nutriment. Ces mêmes considérations furent à l'origine de la décision de recreuser le Grau de Pisses-Saumes, à l'extrémité sud du Bassin, en 1974, afin de favoriser les échanges entre les eaux de la zone de Marseillan, réputée la moins productive, et la mer.

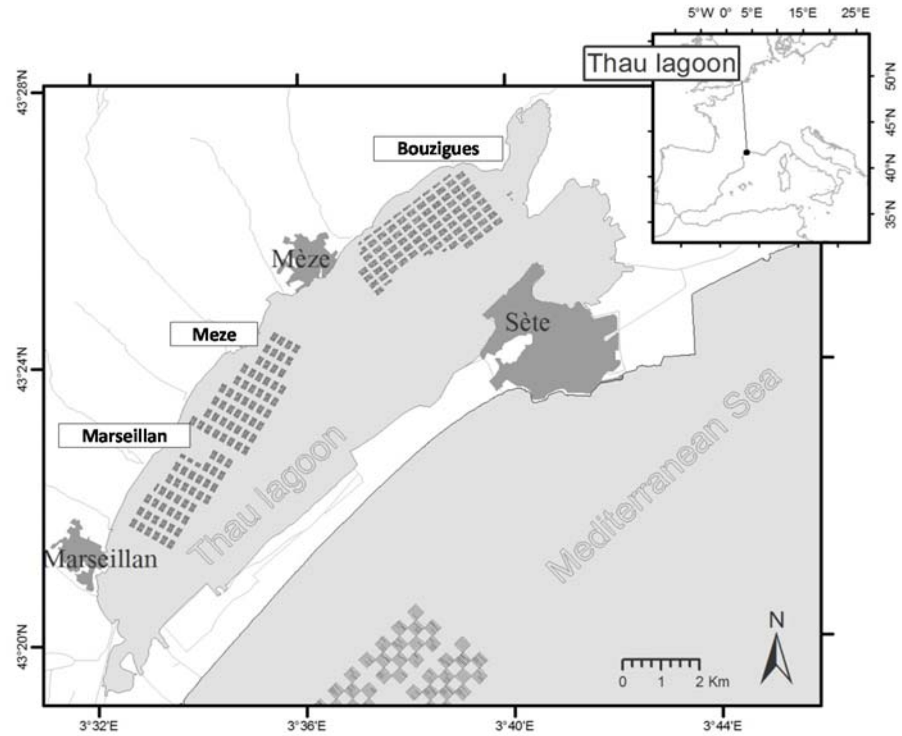

Figure 2: Carte de la lagune de Thau avec en grisé, les trois zones conchylicoles

Par ailleurs, la profondeur moyenne de la zone de Bouzigues (A) étant plus importante que celle des zones Mèze (B) et Marseillan (C), le volume d'eau, et donc la quantité totale de nourriture présente, y sont supérieurs. C'est pour cette raison que les tables ont été installées plus densément dans la zone A, séparées par des "couloirs" de 100 m de large, qu'en zones B et C, où ces "couloirs" atteignent une largeur de 150 m.

Ce remembrement local du cadastre ostréicole associé à l'importation de *Crassostrea gigas* du Japon vers la France a permis un renouveau de l'ostréiculture avec une augmentation des stocks en élevage à partir de 1970 en lagune de Thau pour atteindre un maximum de 22 000 tonnes en 1995 avec une production allant de 9 000 à 15 000 t entre 1993 et 1997.

Les parcs à perches tournantes ont été mis en place dans les années 2000, d'abord à titre expérimental pour des questions de facilitation d'accès aux outils, puis pour faciliter l'exondation des structures d'élevage dans le cadre du développement du prégrossissement. Cette nouvelle pratique culturale a accompagné l'essor des écloseries d'huîtres et est devenue une solution de nursage du naissain de petite taille (de 3 à 30 millimètres) avant la phase de grossissement. Le parcours zootechnique possède alors un cycle de prégrossissement rapide permettant de passer de quelques millimètres à un ou deux centimètres en quelques semaines au printemps, un cycle de grossissement court de 12 à 18 mois, un cycle d'affinage avec l'exondation puis un cycle d'épuration en bassin contrôlé à terre (sanitaire et affinage).

Un plateau au niveau de la production maximale se maintient entre 1999 et 2007 autour de 13 000 t par an (Gangnery 1999; Gangnery 2003; Bec et al. 2018). La lagune est considérée "infertile" ou avec des évènements de captage naturel aléatoires à cause de température et de salinité trop élevées (Héral 1986), cette production intense est soutenue par les approvisionnements en naissain naturel issus des bassins traditionnellement naisseurs comme Arcachon et Marennes-Oléron, et par des approvisionnements issus des écloseries industrielles.

Les années 2007 et 2008 voient l'apparition du phénomène de surmortalité de naissain de l'huître creuse *Crassostrea gigas* en lien avec la présence d'Ostreid-Herpes Virus µvariant (OsHV1-µvar) (Pernet, Barret, et al. 2012; Pernet, Lagarde, Jeanné, et al. 2014). Le phénomène a provoqué une crise ostréicole avec une décroissance rapide des stocks et de la production française entre 2007 et 2010. La production a atteint un minimum en 2010 de 5 600 t mais tend à remonter doucement pour atteindre environ 11 000 t en 2017 (Gervasoni & Giffon 2016). En Méditerranée, cette diminution de la production a été exacerbée par la raréfaction du naissain issu de captage naturel et d'écloserie, de sa spéculation en France et de la dépendance de la filière méditerranéenne aux approvisionnements extérieurs en juvéniles (Figure 3). Cette crise a remis en évidence la dépendance de la production d'huîtres creuses en Méditerranée à l'approvisionnement en naissain issu de captage naturel provenant de la côte Atlantique ou d'écloseries (Lagarde et al. 2017).

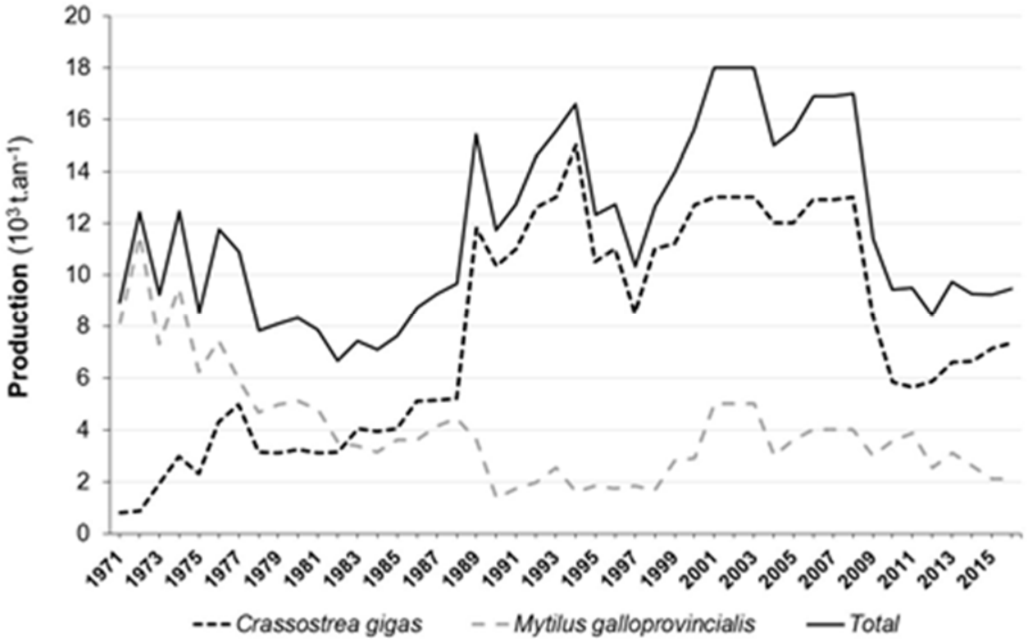

Figure 3: Evolution de la production totale de coquillages en élevage d'huîtres creuses (*C. gigas*) et moules (*M. galloprovincialis*) dans la lagune de Thau de 1971 à 2016 (Bec et al. 2018).

Fonctionnement écologique

Le fonctionnement écologique de la lagune de Thau a été étudié dans le cadre des programmes scientifiques EcoThau (1986-1989), OxyThau (1991-1995) et PNEC (1997-2007) focalisant sur le processus d'eutrophisation puis sur les relations entre l'Homme et l'écosystème. Des programmes de surveillance (Remi, Rephy, Rocch) et d'observation (RSL, DCE, Observatoires conchylicoles) participent également à établir des connaissances générales de son fonctionnement écologique.

Le fonctionnement de la lagune est caractérisé par une juxtaposition de caractéristiques issues de son bassin versant, de sa nature topo-géographique, du contrôle climatique méditerranéen (vent, pluie, ensoleillement, température) associé à une intégration spatiale et temporelle de structures d'origines anthropiques. Le climat et les apports en nutriments déterminent les formes de production primaire à la base du fonctionnement écologique.

La production primaire a été considérée suffisante pour soutenir la conchyliculture à l'échelle annuelle dans les années 1990-2000 (Deslous-Paoli et al. 1998). Néanmoins, des périodes de fortes demandes biologiques en apports trophiques, telles que des phases sans apport venant du bassin versant pendant plusieurs mois, ont mis en évidence un contrôle trophique des organismes filtreurs sur la production primaire (Deslous-Paoli 1996). On parle alors de contrôle trophique descendant (top-down) avec la mise en place des processus de recyclage de matière organique dissoute (MOD). La MOD est recyclée par la boucle microbienne en nutriments qui profiteront principalement aux cellules de petites tailles à forte productivité (picoplancton et bactéries) (Souchu et al. 2001; Mazouni, Gaertner, et al. 1998). Un réseau trophique (protozoaire, microzooplancton et mésozooplancton) se met en place sur la base de la consommation du picoplancton difficilement accessible aux organismes filtreurs (Dupuy et al. 1999; Dupuy et al. 2000). Ce réseau trophique alimenté par la production régénérée accélère le recyclage des nutriments issus des élevages et permet l'augmentation de la taille des particules qui deviennent disponibles pour l'alimentation des bivalves en élevage. Par ailleurs, les pluies d'orages et d'évènements cévenols apportent épisodiquement, parfois de manière torrentielle, les nutriments extérieurs à la lagune, issus du lessivage de son bassin versant, et nécessaires au développement des diatomées pélagiques et d'une part importante de macrophytes (Deslous-Paoli 1996). La production pélagique qui se développe sur ces apports extérieurs, dite production "nouvelle" peut être minéralisée *in-situ* dans la colonne d'eau puis rejoint les sédiments à la mort des organismes, ou peut être consommée par les élevages de filtreurs suspensivores. Ces deux types de fonctionnement de la lagune basés sur la production primaire nouvelle ou sur la production primaire régénérée permettent de soutenir des fonctions écologiques d'alimentation des bivalves (Gasc 1997; Souchu et al. 2001; Mazouni 2004) garantissant une bonne santé des espèces avec survie, croissance et reproduction (Begon et al. 2007) et donc, globalement, le service écosystémique "ostréiculture" (Pernet, Malet, et al. 2012; Dupuy et al. 2000).

Au niveau spatial, le fonctionnement de cette lagune est structuré par sa topographie, son hydrodynamisme et la présence de structures d'élevage (tables conchylicoles, couloirs de séparation entre les tables, secteurs d'eau libre au large des secteurs conchylicoles). Les zones d'élevage de bivalves représentent des zones "puits" de matière particulaire. La matière particulaire est retenue par les organismes filtreurs qui enrichissent en nutriment dissous la colonne d'eau de la zone par leurs produits d'excretion favorisant une productivité élevée. Ce fonctionnement caractérise l'ensemble des saisons avec l'été, un contrôle trophique top-down intense créant des déplétions de nano- et microplancton dans les secteurs conchylicoles (Souchu et al. 2001; Bec et al. 2005; Mazouni, Gaertner, et al. 1998). A cette structuration horizontale s'ajoute une stratification verticale qui se met en place dès que les vents faiblissent et les températures augmentent (Frisoni 1984; Jarry et al. 1990; Harzallah & Chapelle 2002). Pendant les périodes les plus chaudes et sans vent, l'apparition de ce genre de stratifications transitoires peut être à l'origine d‘hypoxies voire d’anoxies induites par la boucle microbienne aérobie minéralisatrice au niveau du système benthique dans les zones les plus profondes et sur les bordures de la lagune (Chapelle et al. 2000; Chapelle et al. 2001; Souchu et al. 1998). Les apports en azote et en phosphore relargués enrichissent les eaux de la lagune et sont associés à une plus forte productivité pendant ces évènements (Bec et al. 2005; Mazouni et al. 1996; Bec et al. 2011).

Production autotrophe vs. hétérotrophe

Le développement de la chaîne trophique dans la lagune de Thau est basé sur une production autotrophe ou hétérotrophe selon les conditions environnementales.

Dans le cas de la production primaire autotrophe, les conditions environnementales et les apports en nutriments (azote, phosphore et silice) sont en quantité suffisante pour le développement de biome par photosynthèse. Les principales sources d'azote régénéré sont la diffusion d'ammonium du sédiment vers la colonne d'eau, le biome des installations conchylicoles et la boucle microbienne qui excrètent de l'ammonium et la reminéralisation rapide de la production biologique. Les autres sources d'azote sont issues des apports extérieurs à la lagune, venant du lessivage du bassin versant, apportant essentiellement des nitrates. Ce sont en majorité des diatomées de grande taille qui répondent à ces apports de nitrates (*Dytilum sp*, *Thalassiosira sp* etc.). Les diatomées microplanctoniques (dont *Chaetoceros spp* et quelquefois les péridiniens) constituent alors 1'essentiel de la production nouvelle, effaçant les peuplements nano- et pico-phytoplanctoniques. Le nanophytoplancton et le picophytoplancton (ex. *Ostreococcus tauri*) sont caractéristiques des peuplements de la production régénérée. Ils sont présents toute l’année en réponse au flux diffus d’ammonium plus constants issus de l’ostréiculture que les apports de nitrates par le bassin versant. Ces communautés de petite taille deviennent dominantes en biomasse, particulièrement en été lorsque les pluies sont faibles et la température est à son maximum annuel.

Les résultats du projet Oxythau nous apprennent que la production hétérotrophe est constituée de bactérioplancton, de protozoaires (ciliés nus, ciliés loriqués tels que les tintinnides et flagellés de taille allant de 1 à 5 µm) et de microzooplancton primordiale dans le recyclage des sels nutritifs à partir du stock détritique. La matière organique dissoute (MOD) constitue le plus gros stock de carbone et d'azote (> 70 %) dans la colonne d'eau (Deslous-Paoli 1996). Elle comprend le bactérioplancton dont la gamme de taille se situe principalement entre 0.2 et 0.6 µm et dont le stock représente 1 à 3 % du Carbone Organique Dissous (COD). Plus de la moitié du stock correspond à des composés de taille inférieure à 0,02 µm (colloïdes, composés de faible poids moléculaire, etc.). La production primaire est une source importante de COD. En conséquence, les concentrations de COD atteignent leur maximum en fin de floraison phytoplanctonique. Des concentrations supérieures à 150 µM entraînent une réponse rapide de la communauté bactérioplanctonique qui se traduit par une augmentation de la taille et/ou du nombre de cellules. Les rapports C : N dans la MOD varient de 10 à 40 pour un rapport de 4,5 dans le bactérioplancton (Deslous-Paoli 1996). Ce dernier compense le déficit en azote dans la MOD par une consommation d'ammonium. La production bactérienne dépend donc de la quantité de COD dégradable mais aussi de l'ammonium disponible. Les mesures de production bactérienne effectuées par deux méthodes d'éloignement des prédateurs (filtration et dilution) donnent des taux records supérieurs à 20 µg C 1^{-1} h^{-1} en période estivale, ce qui est du même ordre de grandeur que la production primaire (Deslous-Paoli 1996). Des taux équivalents peuvent être mesurés aux autres saisons à l'issue de floraisons phytoplanctoniques. Ces fortes productions sont liées à la présence récurrente de bâtonnets (vibrions) qui peuvent prendre des volumes cellulaires jusqu'à 2 μm^3. Les mesures sur échantillons naturels montrent que les autres hétérotrophes peuvent consommer de 0 à 80 % de cette production. L'évolution de l'Azote Organique Dissout (NOD) ne se calque pas vraiment sur celle du COD. Ses maximums ont tendance à suivre l’évolution de la communauté planctonique (autotrophes + hétérotrophes) représentée par le Carbone Organique Particulaire (COP). Le NOD a plutôt une origine hétérotrophe (protozoaires, zooplancton).

Les organismes hétérotrophes de la colonne d'eau sont associés en boucle avec le phytoplancton de la production régénérée. Les fortes productions bactériennes entraînent la floculation de la MOD qui est inaccessible aux filtreurs. Les amas bactériens constituent alors une source de nourriture pour les filtreurs (huîtres, ascidies, éponges). L'empaquetage est aussi assuré par la consommation des bactéries et du picophytoplancton par les protozoaires et le microzooplancton. L'ensemble des acteurs de cette boucle voit sa biomasse et sa production augmenter en période estivale.

Le risque chimique depuis 1970

Une perturbation écologique majeure tenant son origine dans l’introduction d’un biocide, le tri-butyl-étain (TBT), a impacté lourdement les productions conchylicoles de 1970 jusqu'aux années 2000, en lagunes méditerranéennes, en France et ailleurs dans le monde (Alzieu et al. 1990; Alzieu 2001; Alzieu & Michel 1991). Bien que le volet "contaminant chimique" ne soit pas directement intégré dans ce travail de thèse, on sait que cette contamination chimique a eu des conséquences sur le fonctionnement écologique de la lagune de Thau et on peut supposer que le recrutement de l'huître creuse a été impacté pendant les périodes de contaminations. Le TBT est une matière active ayant été utilisée dans les peintures « antisalissures » des navires à cette période pour ses propriétés biocides augmentant la longévité et l'efficacité des peintures à base d'oxyde de cuivre. Cette substance toxique a une activité à large spectre dans l'environnement marin (Alzieu & Michel 1991). Les données écotoxicologiques désignent une toxicité particulièrement élevée pour les mollusques, bivalves (dont l'huître *Crassostrea gigas)* et gastéropodes (dont le murex *Hexaplex trunculus)* (Alzieu et al. 1981; Héral et al. 1989). Des concentrations de l'ordre de 10^{-9} $g.1^{-1}$ induisent des défauts de calcification des huîtres adultes et la mortalité des larves, altérant le recrutement dans les bassins traditionnellement naisseurs.

Des travaux (Héral et al. 1989) montrent que des concentrations:

- ~1 ng 1^{-1} : provoquent l'apparition de caractères mâles chez les femelles de nombreux gastéropodes, phénomène connu sous le nom d'imposex. Six stades successifs ont été décrits chez le gastéropode *Nucella lapillus* (Bryan et al. 1986; Gibbs & Bryan 1986), débutant par la formation d'un *vas deferens* et se terminant par un blocage de l'oviducte entraînant la stérilité des individus. L'imposex, observé chez plus de 72 espèces appartenant à 49 genres différents, confirme le caractère de perturbateur endocrinien du TBT pour les gastéropodes ;

- > 1 ng 1^{-1} : limite la division cellulaire du phytoplancton (diatomées) et la reproduction du zooplancton (microcrustacés, copépodes) ;

- > 2 ng 1^{-1} : sont responsables d'anomalies de calcification des coquilles chez l'huître *Crassostrea gigas*, constituées par un empilement de chambres contenant un gel protéique différant de la protéine de calcification (conchyoline) mais se caractérisant par un fort taux de thréonine et une faible représentation des acides aminés qui assurent la liaison calcique (acide aspartique, glycine et sérine);

- > 20 ng l^{-1} : perturbent la reproduction des mollusques bivalves. D'après l'échelle d'effets établie (His & Robert 1985), le développement larvaire de *C. gigas* est interrompu quand la concentration atteint 50 ng/l (mortalité totale des larves au bout de 10 jours).

Dans la lagune de Thau, les analyses d'eau ainsi que les indicateurs biologiques, imposex et chambrage, montrent que sa partie Est, sous l'influence directe du port de commerce de Sète, est la plus exposée aux apports de TBT (Ifremer 2002). Dans les années 1990, les concentrations en TBT dans Thau ont été enregistrées à des niveaux ayant des effets toxiques pour les espèces marines (de 3.4 ng.l^{-1} à 127 ng.l^{-1}) (Michel et al. 1999; Ifremer 1999). La perturbation n'a cessé qu'après la mise en place et l'application d'une interdiction de l'usage de cette molécule par une loi française en 1982 puis une directive européenne (Directive No. 89- 677, 1989) traduite dans le droit français par un décret en 1992 (Décret No. 92- 1074, 1992).

Dans les années 2000, les niveaux de présence de TBT ont été en diminution générale y compris dans le port de Sète. Cependant, la contamination des eaux des ports internes à l'étang avoisinait, de manière anormale, celle trouvée dans certaines zones du port de Sète. L'impact biologique restait notable, puisque le chambrage des huîtres de la zone d'élevage la plus exposée atteignait encore des niveaux élevés, supérieurs à 90 % de la population (Figure 4) et que la majorité des populations de murex *Hexaplex trunculus* de la lagune de Thau restait affectée par le phénomène d'imposex (Ifremer 2002).

Figure 4: Coquille d'huître chambrée issue de l'observatoire Ifremer REMORA, année 2000, source C. Alzieu.

Néanmoins, de manière générale, les effets bénéfiques de la réglementation pour les cultures marines dans diverses régions du monde ont été clairement démontrés (Waite et al. 1991; Dyrinda 1992; Batley et al. 1992) justifiant pleinement la recommandation de l'Organisation Maritime Internationale visant à interdire l'utilisation du TBT pour la fabrication des peintures antisalissures.

Les avancées en terme de chimie analytique et l'arrivée des échantillonneurs passifs ont permis de poursuivre la caractérisation de 141 contaminants chimiques (pesticides, médicaments, alkyphénol, HAP, PCB et métaux traces) en lagunes méditerranéennes (Munaron et al. 2013), montrant une présence ubiquiste de nombreux composés chimiques dans les eaux lagunaires et posant la question de leur impact sur ces milieux. Bien qu'aujourd'hui les concentrations en TBT aient diminué en dessous de sa norme de qualité environnementale, les suivis réalisés en 2010, 2012 puis 2015 au printemps et en été dans le cadre de la Directive Cadre sur l'Eau (DCE) ont classé la lagune de Thau comme étant en mauvais état chimique (Andral & Sargian 2010; Andral et al. 2013). Les Normes de Qualité Environnementale (NQE) ont été dépassées pour différents polluants tels que des insecticides (lindane 13 ng. l^{-1}, lors du suivi 2010) à des concentrations dans l'eau 6 fois supérieures à sa NQE (2 ng. l^{-1}), mais aussi des herbicides (diuron et terbuthryn lors du suivi DCE 2012), des biocides (irgarol en 2012), ou des alkylphenols dans des moules (4-*tert*-OP en 2015). De nombreuses autres substances (herbicides essentiellement, produits pharmaceutiques comme le paracétamol par exemple) ont également été retrouvées lors de ces suivis sans que leur impact ne puisse être véritablement évalué (en l'absence de NQE disponibles pour la plupart). L'évaluation de l'exposition des lagunes aux contaminants chimiques est fondée sur une campagne réalisée tous les 3 ans (soit 2 par plan de gestion durant 6 ans). Et l'évaluation des effets possibles est basée sur une comparaison individuelle de chaque contaminant retrouvé à sa NQE. Or le nombre de NQE définies est réduit par rapport au nombre de produits chimiques présents dans les eaux. De plus l'effet des cocktails de contaminants n'est, à l'heure actuelle, pas pris en considération.

De manière à avoir une idée plus précise du risque lié à la présence conjointe et continue de pesticides dans les eaux de la lagune, un indicateur complémentaire à l'approche DCE, appelé le quotient de risque (Qr), a été défini (Munaron et al. 2017). Cet indicateur écotoxicologique s'appuie sur la théorie de la toxicité des mélanges de produits chimiques ayant des cibles similaires dans l'eau (modèle d'additivité de Loewe). Lors des suivis réalisés en continu au cours de l'année 2015-2016 en un point de la lagune de Thau (zone de Marseillan), la présence de mélanges de pesticides (28 substances), d'usage phytosanitaire et biocide a été révélée. Le quotient de risque calculé pour chaque période de suivi, montre que la période juin-juillet (en 2016) est la plus à risque pour l'écosystème de Thau avec à la fois la présence d'une substance dépassant son seuil d'effet individuel (l'herbicide acetochlor) et des mélanges de substances (herbicides et biocides notamment) ayant des effets cumulés susceptibles de porter atteinte à l'écosystème (Munaron et al. 2017). Au niveau spatial, les rares données obtenues lors des suivis DCE montrent une différence de contamination chimique dans l'eau de la lagune entre le point à l'est (zone Bouzigues) et le point à l'ouest (zone Marseillan), sans toutefois que l'un soit systématiquement plus contaminé que l'autre.

Les sédiments de la lagune de Thau font également l'objet d'une contamination, probablement plus en lien avec le passé industriel de son bassin versant. Cette contamination se caractérise préférentiellement par la présence de métaux traces (plomb, mercure et cadmium), de HAPs et de PCBs. Les concentrations suivies dans le cadre du Réseau d'Observation de la Contamination CHimique du milieu marin (ROCCH) au cours de 3 campagnes (1996, 2006 et 2012) sont néanmois en diminution (Jambou 2015).

Restauration écologique et oligotrophisation

Les lagunes occitanes ont été sous l'influence de l'eutrophisation pendant plusieurs décennies de 1970 jusqu'à 2010 (Mesnage & Picot 1995; Souchu et al. 2010; La Jeunesse 2001). L'eutrophisation est particulièrement exprimée par les lagunes du fait de leurs caractéristiques topographiques, hydrodynamiques et hydrologiques (Leruste et al. 2016). Les lagunes ont en effet des échanges réduits avec la mer, des temps de résidence élevés, des taux de renouvellement faibles, et dans le cas de la lagune de Thau, des volumes de mélange entre mer et lagune réduits au niveau du bassin principal (Fiandrino et al. 2017). Les conséquences de l'eutrophisation sont des états écologiques instables avec une surproduction de biomasse (De Jonge & Elliott 2001; Schramm 1999; Cebrian et al. 2014), une perte de biodiversité et d'habitat, une augmentation des crises hypoxiques et anoxiques (Smith 2006; Carlier et al. 2008; Souchu et al. 1998; Chapelle et al. 2001) voire des efflorescences de phytoplancton toxiques. L'ensemble de ces phénomènes conduit à des modifications de fonctions écologiques, voire à des pertes de services écosystémiques. Depuis les années 2000, la mise en place de la DCE a obligé les états européens à faire des diagnostics des états écologiques de leurs masses d'eau et à adopter des plans de gestion environnementale dans le but d'accéder à un bon état écologique dans un premier temps en 2015, puis en 2021 (Sherrard et al. 2015; Cartaxana et al. 2009). Ces mesures de gestion ont eu pour effet de réduire les apports anthropiques de nutriments, amorçant ainsi le processus d'oligotrophisation avec des changements de communautés phytoplanctoniques (Collos et al. 2009; Leruste et al. 2016) limitant les crises dystrophiques et les efflorescences d’algues toxiques. La lagune de Thau est maintenant dans un état mésotrophe (Bec et al. 2011; Souchu et al. 2010), état favorisant les picocyanobactéries et les communautés phytoplanctoniques de petites tailles (pico et nanophytoplancton) et avec un taux de croissance élevé (diatomées, cryptophytes et algues vertes). Les communautés phytoplanctoniques mixotrophes telles que les dinoflagellés mixotrophes sont également avantagées (Collos et al. 2009; Leruste et al. 2016). L’état écologique de Thau est aujourd’hui reconnu comme bon pour la colonne d’eau et pour le phytoplancton et moyen à bon pour le sédiment selon les critères de la DCE (Derolez et al. 2017).

La reproduction et l’ontogénie de l’huître creuse :

L'huître creuse est un invertébré marin à cycle de vie bentho-pélagique. Son cycle de reproduction suit plusieurs étapes faisant passer l’espèce d'une vie pélagique et vagile (phase larvaire) à une vie benthique et fixée (phase juvénile et adulte) (Figure 5).

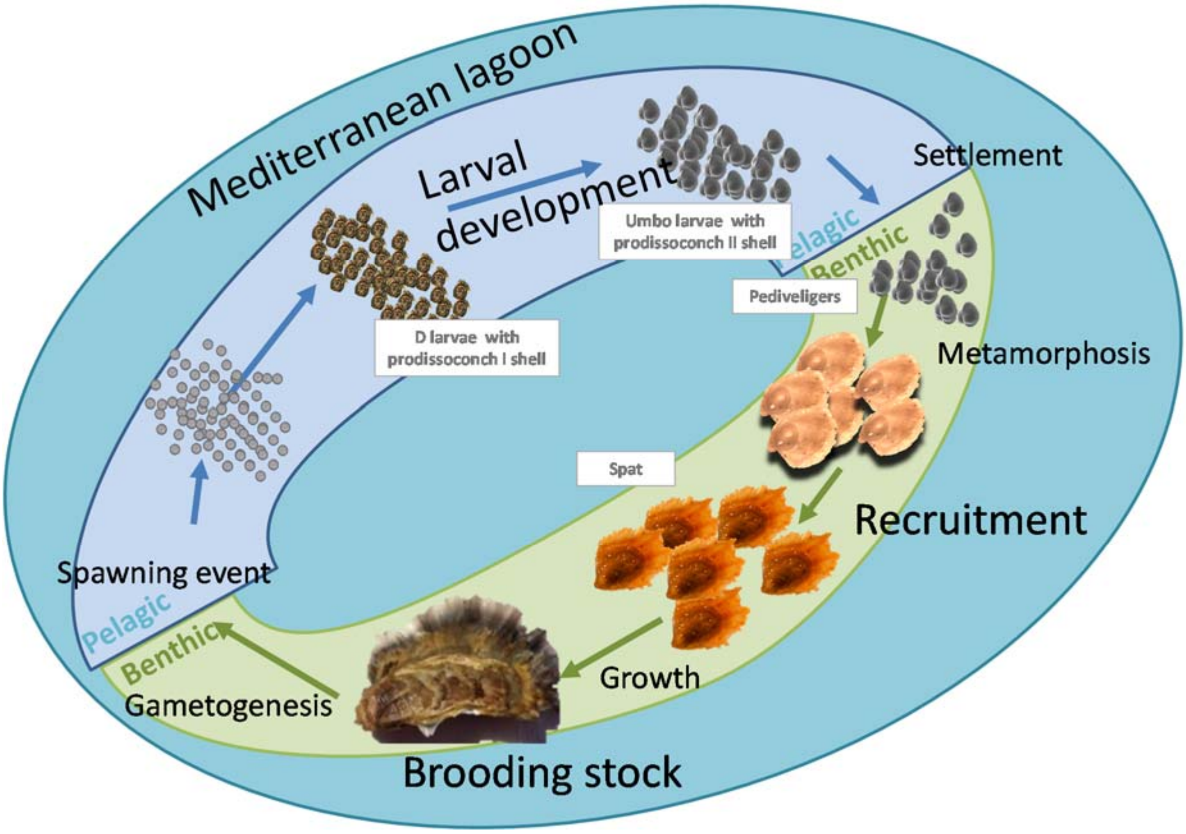

Figure 5: le cycle de reproduction de l’huitre creuse (adapté de His 1976; Pouvreau 2018)

Gamétogénèse et ponte

La reproduction se met en place chez les individus dès la fin du processus de développement de la phase juvénile. La première étape de la repoduction est la gamétogénèse, processus de maturation des gamètes dans les gonades commençant à la fin de l’hiver au moment de l’augmentation des températures printanières (Pouvreau et al. 2006; Bayne 2017). Cette phase de maturation atteint un stade maximum en fin de printemps voire début d'été. L'huître entre alors dans une période pendant laquelle elle devient très sensible aux stimuli extérieurs à l'origine de l'émission des gamètes, appelée couramment "ponte" (Galtsoff 1964; His 1991; Bernard et al. 2016). Une fois les gamètes émises, la fécondation a lieu directement dans la colonne d’eau, il s'en suit l’apparition des larves trochophores à l’origine du développement larvaire.

Développement larvaire

Le développement larvaire (Figure 6) est une phase sensible du cycle de vie des invertébrés benthiques avec des changements physiologiques majeurs (Thorson 1950). Les larves trochophores se transforment rapidement en larves D véligères dotées d’une première coquille d’aragonite, la «prodissoconque I» et d’un système digestif fonctionnel (Gosling 2015). Dans le cas de l’huître creuse, les larves planctoniques sont rapidement planctotrophes se nourrissant de particules de tailles pico et nanoplanctoniques à l'aide de la couronne ciliée du velum.

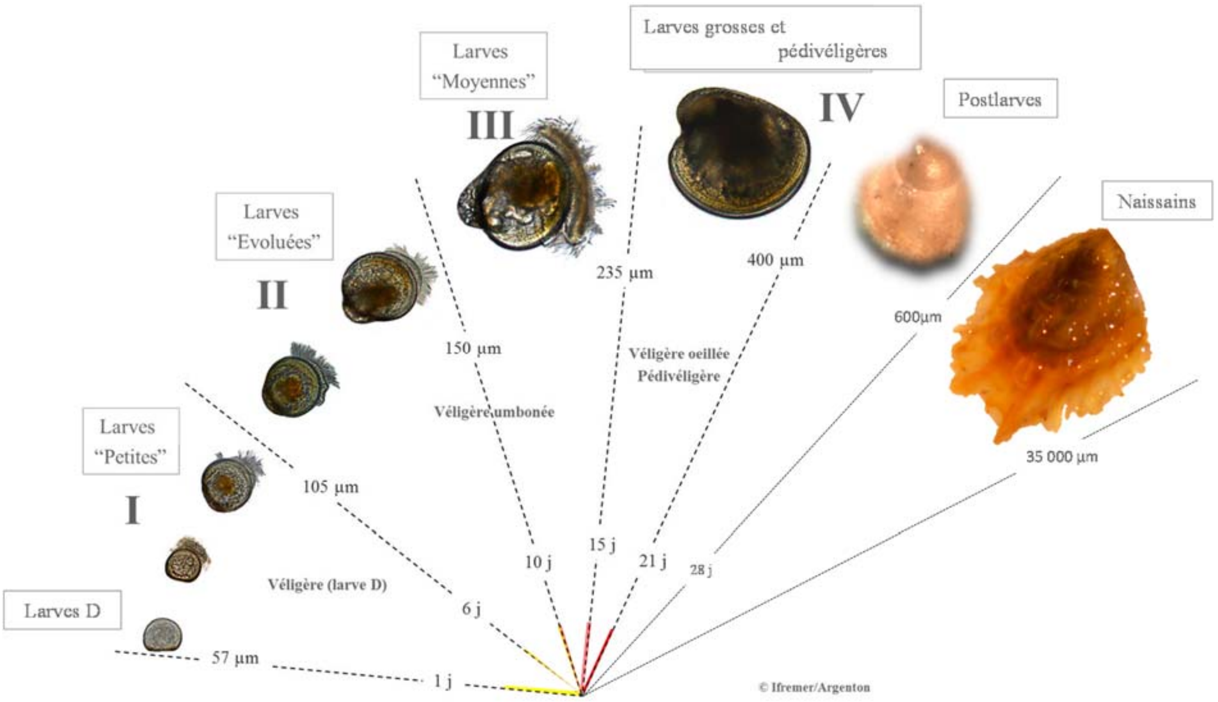

Figure 6 : Stades larvaires, post larvaires et naissains de l'huître creuse, adapté de Pouvreau 2018 et d'après Le Pennec (1978) et His (1991). L'âge est donné à titre indicatif, il est fonction de la température et de la concentration en nourriture

Les facteurs endogènes et exogènes peuvent interagir sur le développement et la survie larvaires impactant directement l'abondance des larves dans la colonne d'eau. Les facteurs défavorables entraînent la réduction du nombre de larves survivantes en rallongeant le temps de résidence dans le pélagos (Widdows 1991; Wing et al. 2003). Plus ce temps de résidence dans le pélagos s'allonge, plus les larves sont dispersées et exposées aux risques relatifs à la vie pélagique (Morgan 1995; Pedersen et al. 2008; Pineda & Reyns 2018). La diminution de l'abondance larvaire devient alors un facteur limitant du recrutement théorisé par le concept de la "*limitation du recrutement*" (Cruz & Harrison 2017; Armsworth 2002; Olafsson et al. 1994). L'abondance de la ressource trophique lors de la vie larvaire est aussi un point particulier qui a été conceptualisé par l'hypothèse du "*Match-Mismatch*" (Burgess et al. 2018; Cushing 1990; Toupoint, Gilmore-Solomon, et al. 2012). Cette hypothèse couvre la période du développement larvaire jusqu'à la métamorphose mais concerne aussi la phase post-larvaire dans certaines études. Cette hypothèse met en relief l'importance du synchronisme entre la nourriture des larves, production primaire en l'occurrence, et leurs cycles de développement.

En outre, l'abondance, la localisation et l'état de santé du stock d'adultes qui prévaut à l'apport larvaire sont aussi des éléments clés dans le recrutement. Le lien entre le stock de reproducteurs, l'approvisionnement en larves et le recrutement benthique a été longuement conceptualisé dans les années 1980-1990 au sein de la théorie du "*supply-side ecology*" (Lewin 1986; Underwood & Fairweather 1989; Grosberg & Levitan 1992a; Gaines & Roughgarden 1985). La relation entre les facteurs environnementaux, la durée de la vie larvaire et le transport pélagique est complexe et a besoin de nouvelles données d'observations rigoureuses pour être approfondie (Pineda & Reyns 2018). Jusqu'à aujourd'hui, l'étude de l'abondance larvaire et de la limitation du recrutement *in situ* reste un champ exploratoire ouvert tant sur l'aspect de la recherche scientifique comme par exemple pour la dynamique des communautés marines benthiques (Hughes et al. 2000) que pour l'aide à la gestion des milieux, de la pêche ou de l'aquaculture (Fairweather 1991; Menge et al. 2009).

Transport physique et dispersion larvaire

Les larves d'invertébrés benthiques sont de petites tailles et ne peuvent pas nager sur de longues distances. La phénoménologie du transport larvaire inclut donc comportement larvaire et mécanisme externe de transport jusqu'à leur habitat final (Thorson 1950). Le transport physique a donc une influence majeure avec ses variabilités temporelles et spatiales sur la dynamique de la population (Pineda 1999).

La dispersion larvaire intègre les processus complexes spatiaux et temporels entre un lieu d'émission de population source et un lieu de fixation de population prenant en compte le transport larvaire, la survie des organismes et leur fixation (Bernard 2011; Pineda et al. 2007; Pérez-Ruzafa et al. 2018).

Préfixation

A la fin de la vie larvaire, la larve devient "pédivéligère" avec l'apparition d'un pied cilié extensible (Pechenik 1990). La coquille se transforme en se bombant au niveau de la charnière (formation d'un umbo) et devient une "prodissoconque II" (PII) caractérisée par des stries de croissance (Bayne 2017; Barbier 2016). Ce stade est aussi appelé "umbo-véligère" et communément "larves grosses" en conchyliculture (Pouvreau 2018). A la fin du développement larvaire, les larves acquièrent une compétence de fixation (Baker 1994; Havenhand 1995) démarrant par un comportement de prospection territoriale très particulier (Marteil 1976; His 1991) leur permettant de passer du stade pélagique à la colonisation du compartiment benthique. Les larves compétentes sont sensibles et réagissent à une hiérarchie de stimulis qui leur permet de tester la qualité de l'habitat incluant biotope et biocœnoses (Kingsford et al. 2002; Carriker 1990; Day & McEdward 1984).

En réponse à l'absence ou à la présence de signaux chimiques et/ou physiques de l'habitat prospecté, les larves se laissent transporter vers un autre habitat ou se métamorphosent et passent à une vie benthique (Hunt & Scheibling 1997a). On parle alors de délais de métamorphose qui reste un sujet de recherche d'actualité compte tenu de la difficulté de suivre les développements de cohortes *in situ*. Le délai de métamorphose est défini comme le maintien de la capacité de fixation et de métamorphose pour une période suivant l'acquisition initiale de la compétence (Coon et al. 1990).

Parmi les différents stimulis supposés, l'hypothèse du déclencheur trophique, d'origine pélagique, de fixation des recrues de bivalves (le TST pour Trophic Settlement Trigger) (Toupoint, Gilmore-Solomon, et al. 2012) suggère qu'une modification qualitative de la ressource trophique pélagique déclencherait la fixation des bivalves. Ce TST agirait donc comme un signal indicateur de conditions trophiques favorables pour ces étapes sensibles de la colonisation et de la métamorphose.

Fixation et métamorphose

Une fois fixée, la larve va ensuite entamer un processus de métamorphose (Coon et al. 1990; Pechenik 2006). Si le taux de fixation larvaire est considéré par certains auteurs comme le facteur déterminant pour le succès du recrutement et donc pour la structure des communautés (Gaines et al. 1985), la métamorphose est aussi une étape à fort risque de mortalité pouvant impacter le déroulement des processus de post fixation et de recrutement (Thorson 1950; Thorson 1966; Gosselin & Qian 1997).

L'étape de la métamorphose est conceptualisée de manière générale pour une grande diversité d'espèces vivantes mais ne correspond pas pour autant aux mêmes transformations morphologiques et/ou physiologiques (Bishop et al. 2006). Ici, la métamorphose est définie comme la transformation d'une larve pélagique compétente en une postlarve fixée benthique accompagnée de changements anatomiques structurels notamment en lien avec l'appareil alimentaire et respiratoire (Beninger & Cannuel 2006; Cannuel & Beninger 2006). Un nouveau type de coquille apparaît, la «dissoconque», qui se différencie des précédentes par sa composition minéralogique à base de calcite.

Pour de nombreuses espèces d'invertébrés marins benthiques, y compris les mollusques, les facteurs influant sur la durée de vie des larves planctoniques, ainsi que la variabilité de la taille à la fixation et à la métamorphose, ne sont pas bien caractérisés, ni compris (Martel et al. 2014). Pourtant, ce sont des caractéristiques clés de l'histoire de vie qui sont importantes pour la recherche et pour les progrès de l'évolution, de l'écologie, de la conservation, ainsi que de l'aquaculture des espèces d'invertébrés marins (Martel et al. 2014).

Recrutement

Par définition, le recrutement représente le nombre d’individus nouvellement admis au sein d’une population donnée ou un stock particulier d'adultes (Cushing 1990; Heath 1992). Chez les invertébrés marins benthiques et sessiles comme les huîtres, il représente le nombre d’individus post larvaire ayant survécu jusqu’à une taille spécifique après leur fixation durant un intervalle de temps donné (Roegner 1991). Globalement, le recrutement est sous la dépendance de facteurs environnementaux qui vont moduler la gamétogénèse, le développement larvaire, la fixation et la métamorphose (Houde 2008).

La qualité de l’habitat offerte par l’écosystème à l’espèce correspond à la niche écologique permettant d’avoir une croissance, une survie et une reproduction optimales (Begon et al. 2007). La niche écologique optimale correspond au domaine de tolérance vis-à-vis des principaux facteurs du biotope (Frontier et al. 2008; Shelford 1931) associés à l’écologie des communautés au sein des biocénoses (Barbault 1992, Figure 7).

Le succès du recrutement est le résultat d’une combinaison de plusieurs processus induits par l’ensemble des facteurs climatiques, hydrologiques et écologiques de son habitat alors appelé la fenêtre écologique du recrutement optimale (Cury & Roy 1989). La fenêtre environnementale optimale du recrutement est définie comme étant le meilleur compromis entre facteurs biotiques et abiotiques permettant la meilleure survie au recrutement (Pineda et al. 2006; Cury & Roy 1989; Arnold & Steneck 2011).

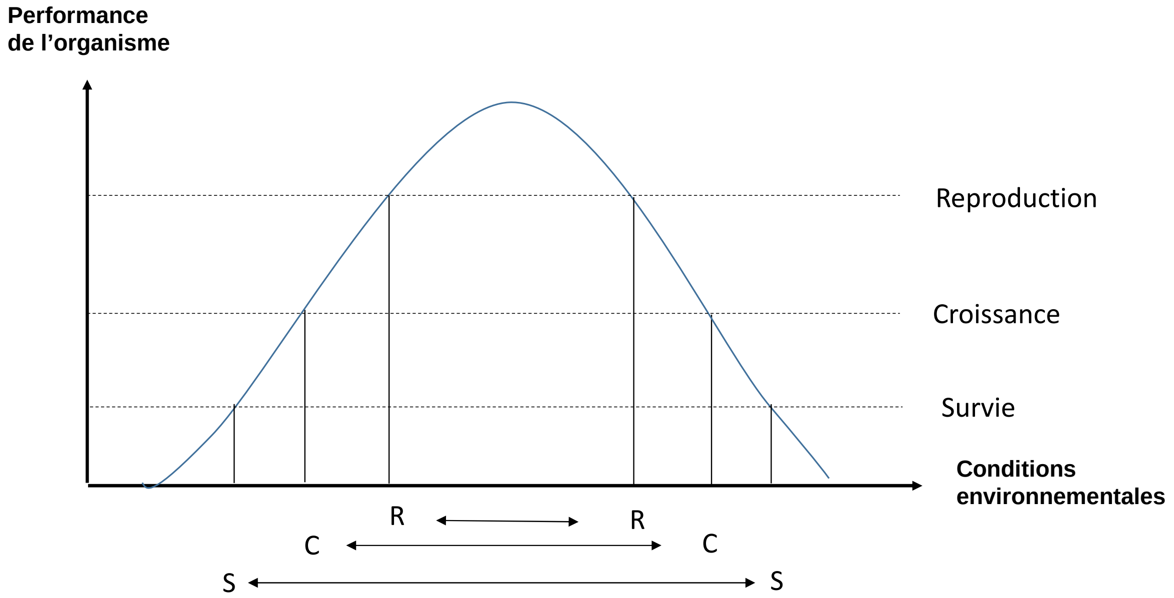

Figure 7: Courbe de réponse illustrant la loi de Shelford avec les effets de l'amplitude de conditions environnementales sur la survie (S), la croissance (C) et la reproduction (R). Les conditions extrêmes sont létales, les conditions moins extrêmes permettent la croissance, seules les conditions optimales permettent la reproduction.

En milieu naturel, le patron spatial du recrutement est souvent contraint par les modes de distribution des larves en aggrégats, essaims ou gradients. Il est aussi sous dépendance de l'habitat offert par l'écosystème (Morse & Hunt 2013; Keough 1983; Broitman et al. 2008). Selon l'hypothèse des "*forces motrices multiples*", les processus abiotiques dominent à grande échelle alors que les processus biotiques dominent à petite échelle (Pinel-Alloul 1995; Thomas et al. 2012). Le patron temporel du recrutement varie en fonction de l'espèce étudiée (Watson & Barnes 2004) mais aussi du type d'écosystèmes favorisant la rétention des larves et l'autorecrutement ou l'export des larves (Swearer & Shima 2010; Swearer et al. 1999; Jones et al. 2009; Hogan et al. 2012).

La compréhension des mécanismes déterminant la variabilité spatio-temporelle du recrutement nécessite donc la prise en compte d'une large gamme d'échelles spatiales et temporelles (Figure 8), adaptées au niveau de variabilité imposée par l'écosystème et le modèle biologique étudiés (i.e. durée de chaque processus larvaire, biogéographie de l'écosystème) (Borcard et al. 2004; Michener & Kenny 1991; Thomas et al. 2012).

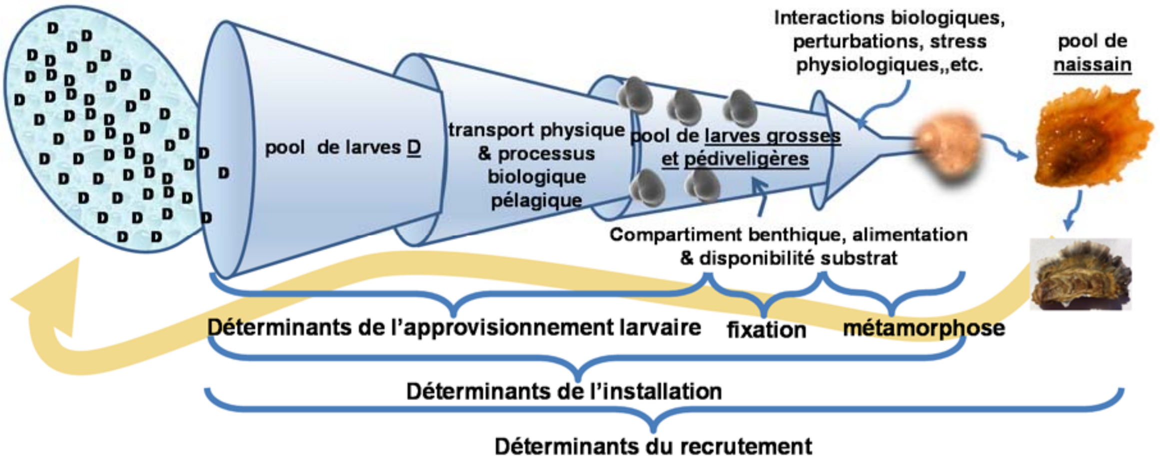

Figure 8 : Les éléments déterminants du recrutement des invertébrés benthiques à cycle de vie benthopélagique (modifié de Pineda et al. 2000)

Connectivité

Le concept de connectivité en écologie (Figure 9) peut être considéré dans l'étude de la variabilité du recrutement des espèces méroplanctoniques (Lewin 1986; Pineda et al. 2007). Il s'agit d'abord de prendre en compte le transport physique (Roughgarden et al. 1988) comme mesurage ou plus spécifiquement comme un indicateur quantifié, i.e. un flux par une approche eulérienne (Pineda & Reyns 2018). La connectivité hydrodynamique permet alors l'intégration du transport physique sur une durée définie par la biologie ou l'écologie.

La connectivité peut inclure les différentes interactions des stades larvaires avec l'environnement durant le cycle de vie pélagique incluant le comportement larvaire (Metaxas & Saunders 2009) et l'adéquation des habitats rencontrés à chaque étape de la vie (Ghezzo et al. 2015). Ainsi, Pineda *et al.* (2007) définit la connectivité comme une fonction de la dispersion larvaire et de la survie larvaire.

La connectivité peut aussi être définie comme un indicateur de la facilité de déplacement dans un milieu donné (Kindlmann & Burel 2008; Watson et al. 2010). Ce concept quantifie l'aptitude d'un environnement à faciliter ou empêcher le mouvement des organismes selon les parcelles écologiques ou biocénoses (Fang et al. 2018; Taylor et al. 1993; Schooley & Wiens 2003). Cette notion établit alors la relation fonctionnelle entre les parcelles d'habitat selon leur distribution spatiale et le mouvement des organismes en réponse à la structure du paysage (Taylor et al. 1993; With et al. 1997).

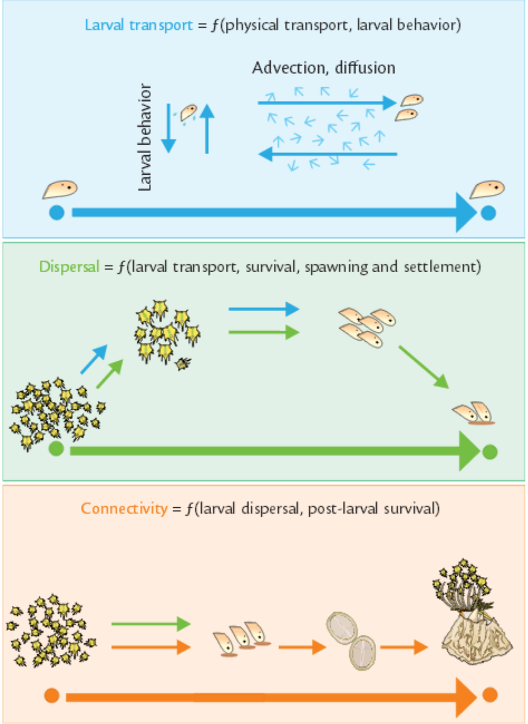

Figure 9 : Les concepts de transport physique, dispersion larvaire et connectivité reproductive d'après Pineda, Hare et al. (2007). Chaque couleur représente un concept.

Objectifs de la thèse

L'objectif général de cette thèse est d'explorer puis de hiérarchiser les éléments déterminant la reproduction et le recrutement de l'huître creuse *Crassostrea gigas* en lagune de Thau, par une approche corrélative multidisciplinaire, s'appuyant sur les processus physiques, physiologiques et écologiques. D'un point de vue appliqué, ces connaissances permettront d'identifier, de caractériser et de déterminer les zones propices au recrutement larvaire, permettant le développement progressif de la pratique du captage naturel par les professionnels de la lagune de Thau.

Le manuscrit de thèse est structuré en quatre chapitres, dont chacun fait l'objet d'un article publié, accepté ou soumis.

Le premier chapitre porte sur la reproduction de l'huître creuse, et en particulier la gamétogénèse conduisant aux déclenchements de la ponte et à l'apparition des larves en lagune de Thau. L'objectif est de décrire ce phénomène dans le contexte méditerranéen en se basant sur une expérience de suivis de cheptels de géniteurs réalisés pendant deux ans sur trois sites d'échantillonnage. Nous avons fait l'hypothèse que les processus décrits pour les bassins atlantiques sont différents en Méditerranée et qu'une hiérarchie de facteurs agit sur le frai soit en l'inhibant, soit en le déclenchant avec une synchronisation optimale allant jusqu'à une amplification du phénomène dans certains cas.

Le deuxième chapitre porte sur le développement larvaire et le recrutement de *C. gigas* dans la lagune de Thau. L'hypothèse posée est que le paradigme "le recrutement est impossible dans la lagune de Thau" est faux ou n'est plus d'actualité. Les objectifs sont de trouver des fenêtres temporelles favorables au recrutement et de fournir les explications du succès du phénomène. Pour cela, les abondances de larves, de jeunes naissains et les paramètres environnementaux associés ont été suivis sur quatre sites d'échantillonnage pendant trois ans (2012-2014). Le processus du recrutement a été replacé dans le cadre du fonctionnement écologique trophique du système.

Le troisième chapitre a pour objectif de caractériser le lien entre la plasticité phénotypique des larves d'huître à la métamorphose (variation des tailles de coquilles Prodissoconque II), l'hétérogénéité du recrutement et le fonctionnement écologique (autotrophe vs. hétérotrophe) de la lagune. Nous posons l'hypothèse que l'environnement trophique influence la taille à la métamorphose et les performances du recrutement. La fixation des larves et le recrutement ont été évalués sur cinq sites d'échantillonnage dans la lagune de Thau lors des principaux événements de recrutement d'été sur trois années consécutives (2012-2013-2014). Des analyses hydrobiologiques et planctoniques ont été effectuées en parallèle sur trois sites d'échantillonnage.

Le quatrième chapitre a pour objectif d'améliorer la compréhension des rôles des facteurs biotiques et abiotiques dans la structuration des patrons spatiaux de recrutement. Sur la base des relations établies entre recrutement observé, connectivité hydrodynamique et facteurs écologiques, nous posons l'hypothèse que l'approvisionnement larvaire dépend de deux éléments de nature différente, la connectivité hydrodynamique et les apports alimentaires des stades larvaires.

Les résultats de ces différents chapitres sont ensuite discutés par rapport à leur portée scientifique et par rapport à leurs intérêts locaux pour le développement du captage naturel d'huître creuse dans la lagune de Thau souhaité par la filière ostréicole méditerranéenne.

Chapitre 1 : Gamétogénèse, comportement de ponte et abondance larvaire de l’huître creuse *Crassostrea gigas* en lagune de Thau : évidence d’une stratégie environnement-dépendant

Ce chapitre fait l’objet d’une publication dans la revue *Aquaculture* 473 (2017) 51-61

Résumé de la publication en français

L’ostréiculture française repose en grande partie sur le captage naturel de naissain le long de la côte atlantique, le reste étant fourni par des écloseries. En Méditerranée, la lagune de Thau fournit 10% de la production française d'huître creuse. Cette production intense est soutenue exclusivement par un approvisionnement extérieur en naissain issu des bassins Atlantique traditionnellement naisseur ou d'écloserie. Malgré cette production intensive de coquillage, le recrutement larvaire de cette espèce dans la lagune de Thau est mal compris et la collecte de naissain est très variable.

Ce travail a pour objectif d'étudier les caractéristiques reproductives de *Crassostrea gigas* en Méditerranée et d'explorer les déclencheurs de ponte. Pour atteindre cet objectif, le cycle de reproduction de la gamétogénèse jusqu'aux pontes a été suivi sur 2 ans sur différents sites de la lagune de Thau. Le comportement de ponte de l'huître creuse est apparu différent de celui observé sur la côte Atlantique, montrant plusieurs occurrences de pontes de juin à octobre. Les températures minimales pour les pontes massives ont été enregistrées à partir de 23°C, c'est-à-dire une température plus élevée que les températures mentionnées dans la bibliographie pour cette espèce. Une relation significative a été trouvée entre les communautés phytoplanctoniques et l'intensité de la gamétogénèse, cette dernière étant améliorée par un ratio élevé diatomées /dinoflagellés. La température était insuffisante à elle seule pour expliquer les pontes, mettant en évidence d'autres déclencheurs potentiels comme les phases de lune et les occurrences d’orages. Nous suggérons l’existence d'une hiérarchie d’évènements déclenchant les pontes, levant les verrous, jouant sur le synchronisme et amplifiant l’effet de déclencheurs.

Gametogenesis, spawning behaviour and larval abundance of the Pacific oyster *Crassostrea gigas* in the Thau lagoon: evidence of an environment-dependent strategy

Martin Ubertini, **Franck Lagarde**, Serge Mortreux, Patrik Le Gall, Claude Chiantella, Annie Fiandrino, Ismaël Bernard, Stéphane Pouvreau, Emmanuelle Roque d'Orbcastel

Introduction

The aquaculture of the oyster *Crassostrea gigas* in France is largely based on natural recruitment of the species on spat collectors along the Atlantic coast, the remaining spat being provided by hatcheries and nurseries (Pernet et al. 2012). On the French Mediterranean coast, despite the absence of foreshore, natural *C. gigas* spat collection seems possible, particularly in the Thau lagoon (Rayssac et al. 2012). However, in the current state of knowledge, the temporal variability of the natural recruitment within this lagoon did not allow the local shellfish farmers to rely on Thau native spat until now. Massive mortality outbreaks in *C. gigas* oysters were reported in France, Ireland and the Channel Islands since 2008, resulting in a shortage of supplies of the shellfish (Schikorski et al. 2011). In this context, the understanding of the *C. gigas* reproduction within the Thau lagoon is of critical importance to fit professional needs.

The pacific oyster *C. gigas* reproduction has been well studied on the Atlantic coast, fitting hatchery, oyster culture and ecology needs. Since 2008, a national monitoring network that aims to observe the reproductive status of the Pacific oyster over a wide geographic range (see http://www.ifremer.fr/velyger, Pouvreau et al., 2016) helps to understand the spatio-temporal variability in the reproductive performance of this species along the Atlantic coast. On the contrary, the combination of environmental factors involved in oyster reproduction in the Mediterranean is poorly documented. The Mediterranean Thau Lagoon is an important European oyster farming area, representing 9.5% of the French annual production (Robert et al. 2013). This ecosystem is characterized by specific features compared to Atlantic systems: absence of tidal amplitude (oysters are always immerged), higher summer sea water temperatures and salinities (often above 26°C and 36 PSU) and lower phytoplankton concentration enriched in small pico-eukaryotes. In this system, growth rates appear to be among the highest in France (Gangnery et al. 2003), but reproductive performances remain unknown.

It has been long known that gametogenesis of *C. gigas* depends on temperature (Mann 1979)and can be partially formalized through the simple concept of "day-degree". In addition, Auby et Maurer (2004), Fabioux et al., (2005) and Enríquez-Díaz et al. (2009) have demonstrated, in laboratory but also at field, that phytoplankton abundance also plays a key-role, especially to fuel fecundity. Nowadays, several generations of deterministic models on *C. gigas* explain in detail and trough a quantitative way these environment-driven reproductive processes with the help of ecophysiological and bioenergetic approaches (Pouvreau et al. 2006; Bernard et al. 2011; Thomas, Pouvreau, et al. 2016).

After gametogenesis, spawning of Pacific oysters is also dependent on environmental drivers. In natural conditions, if spawning trigger mechanisms remain largely unclear for temperate bivalves (Fournier et al. 2012), it is now admitted that temperature appears as the main cue to trigger *C. gigas* spawning (Dutertre et al. 2009), this parameter being widely used in hatcheries. It acts as a threshold: spawning in *C. gigas* does not occur below a daily seawater temperature of 17-18°C (Cognie et al. 2006; Castanos et al. 2009). The Thau lagoon presents particular conditions compared to the colder Atlantic ecosystems since this thermal threshold is reached for over 5 months (from May to September), making this parameter insufficient in itself to explain *C. gigas* spawning.

Apart from temperature effect, several hypotheses are put forward to explain the triggering of spawning within marine molluscs. Phytoplankton blooms can provoke spawning within marine invertebrate species (Himmelman 1975). The black lip pearl oyster *Pinctada margaritifera* spawning is significantly related to plankton concentration in the water column, the latter acting as a synchronizing factor (Fournier et al. 2012; Pouvreau, Bacher, et al. 2000; Pouvreau, Gangnery, et al. 2000). Maturity and spawning stages had benn related to total chlorophyll levels within the species *C. virginica* (Aranda et al. 2014). The lunar cycle is also supposed to have an influence on spawning synchrony, as it has been demonstrated for corals (Boch et al. 2011) and fishes (Taylor 1984; Takemura et al. 2004). The brightness of lunar light as well as gravitational changes, with maximum gravitational pull occurring when the Moon and Sun are aligned (at periods of new and full moons) may be perceived by animals (Grant et al. 2009). Besides, during the first half of the twentieth century, research was conducted on the possible relationship between the lunar cycle and spawning events within oyster species. No relationship were found between spawning events and lunar cycle within the species *C. gigas* and *C. virginica* (Schaefer 1937; Loosanoff & Nomejko 1951). Regarding *Ostrea edulis*, brooding starts at the spring tides, occurring 2 days after full and new moon (Korringa 1947). More recently, variability of biological rhythms of *C. gigas* had been suggested according to the relative importance of the solar cycle and different lunar cycles associated with tide generation (Tran et al. 2011). Thanks to a dual approach (specific experiments at field and long term data analysis), a relationship had been shown between lunar cycle and the spawning events of *C. gigas* working on several sites on the Atlantic coast, Marennes-Oléron and Arcachon (Bernard et al. 2016). Lastly, punctual events can also trigger spawning. Regarding *Haliotis diversicolor*, a relationship was established between spawning occurrences and typhoon events in the Sagami Bay, Japan (Onitsuka et al. 2007). They hypothesized that those events could be an advantageous phenomenon enhancing larval dispersion in the water column.

In addition to the environmental potential spawning triggers, oysters may have different behaviors in relation to environmental conditions. For example, Aranda et al. (2014) identified two strategies in the spawning behavior of *C. virginica*: (1) seasonal and synchronous, and (2) continuous and asynchronous. They hypothesized that the first strategy occurs when food availability is regular, which could be considered as a "conservative tactic". The second strategy would occur when food availability is irregular; oysters then behave according to an "opportunistic tactic". Considering the ability of oysters to adapt their spawning behavior to environmental conditions (Aranda et al. 2014; Philippart et al. 2014; Jönsson 1997) is, in our opinion, fundamental to better understand the different reproduction strategies of *C. gigas* according to the environment they live in. We think that spawning triggers, instead of being considered individually, should probably be considered together, interacting simultaneously or according to a not obvious hierarchy. The reproduction cycle of *C. gigas* in shellfish ecosystems is truly complex, and needs to be assessed through global approaches, relating the oyster biology to environmental variables in order to apprehend the hierarchy of factors influencing gametogenesis, spawning behavior and subsequent larval abundance in the water column.

In that context, the present study aimed to: (1) acquire some knowledge concerning the reproduction cycle of *C. gigas* within the Thau lagoon and (2) understand *C. gigas* spawning triggers within the Mediterranean. To achieve these goals, temperature, phytoplankton and reproductive features from gametogenesis to spawning and larval abundances were followed during 2 years at 2 locations at a weekly temporal resolution within the Thau lagoon.

Material and Methods

Study site

The Mediterranean Thau lagoon is the largest among the multiple lagoons located in the south of France. This waterbody is 19 km long, 4.5 km wide and 5 m deep on average (Figure 10). The Thau watershed extends over about 280 km^2 and is drained by numerous little streams (3-13 km) with intermittent flows (Plus et al. 2006). Shellfish are cultivated in 3 areas of the lagoon, namely “Bouzigues”, “Mèze” and “Marseillan” from North-East to South-West, the total cultivated area covering about 20% of the total lagoon’s surface; 90% of the regional shellfish production comes from these areas. The lagoon is almost closed, with only narrow connections to the Mediterranean Sea through the Sète channel and other small connections that are negligible in terms of water exchange (Plus et al. 2006). Because of the weak tidal range, the residence time of water masses in the Thau lagoon mainly depends on wind and barometric effects and it has been estimated that the water renewal time is about 3 months.

Within the Thau lagoon, oyster/larvae related collection and analyses were performed in the 3 sites of “Bouzigues”, “Mèze” and “Marseillan”, while environmental variables were recorded from the two extreme sites “Bouzigues” and “Marseillan” from North-East to South-West.

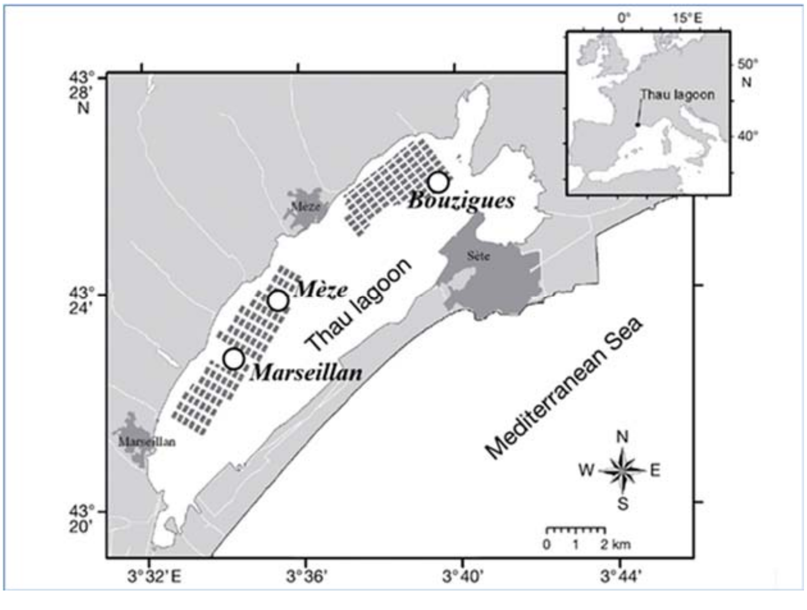

Figure 10 : The Mediterranean Thau lagoon with the location of the 3 sampled stations: oyster/larvae related collection and analyses were collected from the 3 sites, while environmental variables were recorded from the two extreme sites “Bouzigues” and “Marseillan”.

Hydro-climatic parameters monitoring

Wind data (orientation and intensity) as well as atmospheric pressure were acquired through a weather station placed on the Marseillan point. For both Marseillan and Bouzigues stations, temperature was recorded every 10 minutes using an autonomous CTD multiparameter recorder (NKE instrumentation, Hennebont, France) at a 1.5 m depth. Thunderstorms were recorded at the station and lightning strikes over the Thau lagoon were checked using the "meteo60" database (© www.meteo60.fr).

Sample collection and analyses

In early March, 18 months-old oysters coming from Marennes-Oléron were transferred to Thau. These oysters were deployed at the 3 sites (Marseillan, Mèze and Bouzigues) in oyster bags at a depth of 1.5 m, inside oyster rearing areas. At a monthly (spring and autumn) and weekly (summer) frequency, 45 individuals were sampled per station. The first 30 individuals were used for biometric measurements as described by Pernet et al. (2012). Total, tissue and shell wet weight were measured as well as shell length. Tissues and shells for dry weight measurements were placed in pre-weighed aluminium cups, dried for 72 h at 60 °C and weighed on a Mettler precision balance (Mettler-Toledo, Viroflay, France). Then, Condition Index (CI) was calculated according to Lawrence and Scott, 1982, as follows:

CI = Dry tissue weight / (Total wet weight - Dry shell weight)

The remaining 15 individuals were used to precisely define the stages of sexual maturity by quantitative histology (2012: 644 ind., 2013: 942 ind.). A pool (sampling station/date) of whole oyster flesh was stored in a Davidson fixing medium at 4° C until sagittal sections – about 3 mm thick – were made in front of the pericardial cavity. Each individual, placed in a single incremental plastic box was then dehydrated in successive ethanol baths of increasing concentration before being included in liquid paraffin. The trays were placed in the refrigerator for solidification. The blocks obtained were placed on a manual microtome (Leica RM 2115 RT, Nussloch, Germany) to obtain 5 µm cuts, which were deposited on glass slides. They were dried in an oven at 37 ° C for 12 hours. A manual staining of the slides was used in this study by the use of Harris hematoxylin and eosin, a widely used method allowing distinct appearance of the main tissues. After assembly of the slides with a slide glued with resin, each slide was observed under an optical microscope (Zeiss Axioskop – 10 to 100 x, LLC, United States) to determine the gametogenesis stage according to the scale described by Chávez Villalba (2001). To determine the gonado-somatic ratio; the slides were scanned (HP Pro 750V Scanner) and the resulting image processed using Imaq Vision Builder software (v6.0, National Instrument). Total area of the visceral mass and gonadal area was obtained by manually drawing their contour. The ratio between both areas gives the Gonado-Somatic Index (GSI).

Concerning larval abundance, we applied the standard method used by the French Larval Monitoring Network (VELYGER, Pouvreau et al., 2016). To summarize, 1.5 m^3 of seawater was pumped and filtered, twice a week from late May to mid-September with the help of a conic plankton net (mesh size 40 µm). Material retained into the net was conserved in 1 liter sea water bottles (to which were added 10 ml of a 99% alcohol solution). At laboratory and within 48h after sampling, each sample was observed under binocular microscope. *C. gigas* larvae were identified, counted and classified into four classes according to their developmental stage: small (1-6 days, <110 µm), small evolved (6-11 days, 110-190 µm), medium (11-16 days, 190-250 µm), large (16-20 days, >250 µm). We considered significant larval cohorts when the latter classes reached 10000 ind.m^{-3} in the water column.

Phytoplankton concentrations (cell. l^{-1}) were measured by Ifremer's Phytoplankton and Phycotoxin Monitoring Network (REPHY, 2013) at Marseillan and Bouzigues, inside the bivalve farming area, at a depth of 1.5 m. Samples for phytoplankton identification were preserved with Lugol's solution (1 ml. l^{-1}). Ten milliliters were placed in a phytoplankton chamber for at least 6 h for sedimentation, then identified and counted using an inverted microscope (Olympus IMT 2, Olympus, Japan), at 40×. Most species were aggregated at their genus level. Water samples were collected at Bouzigues and Marseillan from March to September in 2012 and 2013, at a bimonthly or weekly frequency. About 5 L of water was collected and pre-filtered through a 200 µm screen to remove any large zooplankton or algal debris. Chl a was extracted from 50mL of seawater filtered on Whatman GF/F filters in acetone 90% for 24 h in the dark, and its concentration was determined using a Perkin–Elmer spectrofluorometer LS50B (Neveux & Lantoine 1993a).

Statistical analyses

Phytoplankton species biovolumes were found in the literature for Thau species (Dupuy et al. 2000) or calculated at the lab following the equations given by Vadrucci et al. (2013). Non-multidimensional scaling (nMDS), based on Bray-Curtis dissimilarity using the R package “vegan” were used to highlight the relationships between stations sampled and phytoplankton communities in terms of biovolumes. A SIMPER (Similarity Percentage) analysis using the R package “vegan” was completed to assess which taxa was primarily responsible for the observed difference between sampling sites.

To characterize the relationships between reproductive activity of *C. gigas* and phytoplankton concentration, we used a correlation analysis between the absolute variation of GSI and the running mean of phytoplankton concentration, similarly to a technique used by Fournier et al. (2012). The absolute variation of GSI between two sampling dates was calculated using the following equation: ΔGSI =GSId – GSId-7, where ΔGSI = GSI variation (%) and GSId is the gonado-somatic index of *C. gigas* at the sampling date d and at the previous sampling date (7 days before GSId = GSId-7). Then, we calculated the running mean of phytoplankton concentration for 4 periods of time (7, 15, 21 and 28 days), assuming that GSI variation could be related to the integration of phytoplankton during the week to the month before. This running mean was done for 5 phytoplankton classes obtained by differential filtration: total (see Lagarde et al., 2017), >20 µm, micro-, nano-, and pico-phytoplankton. Finally, we associated each ΔGSI value with the values of running means calculated for the day corresponding to GSId sampling date and we used a Pearson correlation analysis to test the relationships between ΔGSI and the 4 running means of phytoplankton concentration.

The effect of the lunar cycle was assessed in relation to larval cohorts. Small larvae in the water column were aged from 1 to 6 days, thus the related spawning window was situated within the 6 previous days. A one-way ANOVA was performed using R in order to identify the potential effect of the lunar phases on *C. gigas* larvae abundances. The moon was considered as a binary variable, the spawning window being characterized by the presence/absence of a full/new moon. Then exact Fisher tests were performed using R to determine how spawning events were affected by the moon or by the combination of moon and temperature. Observed distribution of moon-/no-moon related spawning events was compared to a 50/50 theoretical distribution of significant spawning events indicating no relationship at all with the moon.

Results

Environmental factors

Climatic context

As for Atlantic ecosystems with the North Atlantic oscillation, Mediterranean ecosystems can be characterized by a hydroclimatic regime, the Western Mediterranean Oscillation (WeMO), (Martin-Vide & Lopez-Bustins 2006), the index of which was mainly negative for both 2012 and 2013. The negative phase of the WeMO coincides with the Central European anticyclone located to the north of the Italian peninsula and a cyclone, often cut off from northern latitudes, in an extensive area comprising the south-western Iberian Peninsula and the surrounding Atlantic Ocean. This Mediterranean circulation supplies moist warm air to the southern side of the Eastern Pyrenees (García-Sellés et al. 2010), and is responsible for more precipitations.

During 2012-2013, the Thau lagoon was mainly characterized by strong winds coming from the North-West, and marine winds coming from the South (Figure 11). Temperatures followed the same patterns at the 3 sampled stations, but there was a gradient of temperature from North-East to South-West, Bouzigues being the coldest station and Marseillan being the hottest station (Kruskall-Wallis, Khi2 = 247.97, p-value < 0.001). The year 2012 was characterized by a lower temperature than the year 2013 for spring season (Figure 11b). After a rapid temperature increase in June, the temperatures reached those of 2012. Between the beginning of April and the end of September, respective minimal and maximal temperatures were 11°C and 29°C, the minimal 17°C threshold temperature allowing spawning being reached during 5 months for both years. The year 2013 was characterized by more thunderstorms than the year 2012, which appeared more regularly during the reproductive season. This thunderstorm event increase was related to the lower WeMO index in 2013 for the months of July and August.

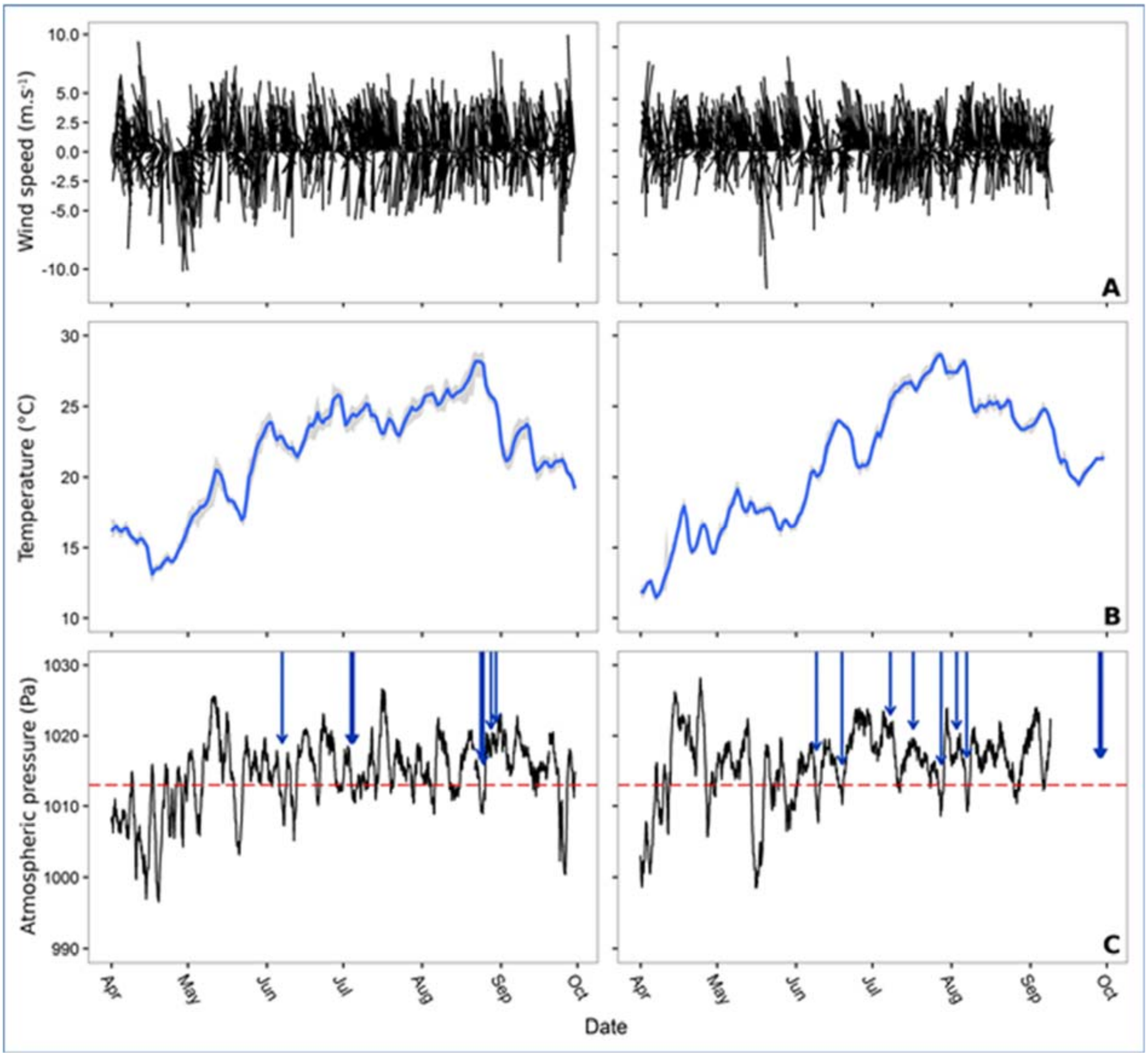

Figure 11 : Wind velocities and direction (A), water temperature, averaged for the 3 sampled stations, min-max range being represented as a shadow area behind temperature (B), and atmospheric pressure at a 3h resolution (C) for both years 2012 and 2013. Blue arrows represent lightening impacts on the Thau lagoon during thunderstorm events. Red dotted lines represent the bi-annual mean for both years 2012 and 2013.

Trophic environment

For both years 2012 and 2013, chlorophyll fractions (size class) and phytoplankton assemblages were different between both stations (Figure 12). To characterize trophic resources within the Thau lagoon, the Northern station Bouzigues and the Southern station Marseillan phytoplankton assemblages considered as a function of their biovolumes were computed in a non-Metric Dimensional Scaling (nMDS) analysis (Figure 13).

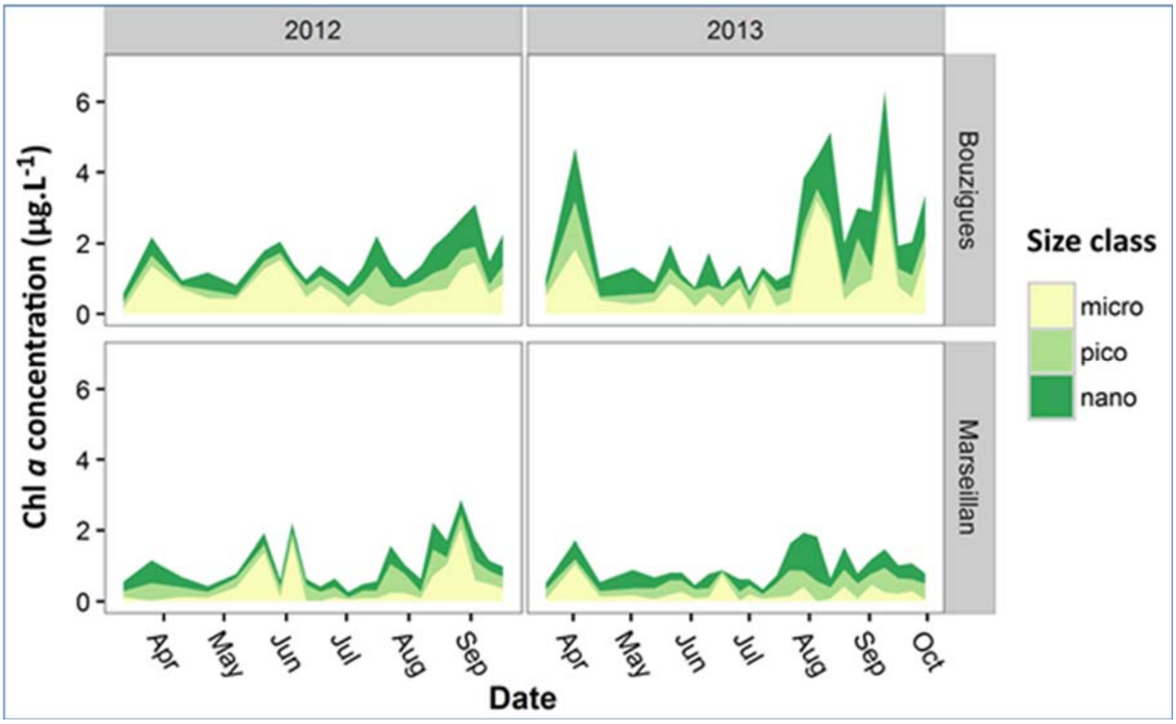

Figure 12 : Relative Chl a concentration for the 3 size-class phytoplankton cells for both years 2012 and 2013 at the 2 stations Bouzigues and Marseillan.

In 2012, phytoplankton species were well distinguished by types, with a pattern dominated by dinoflagellates while the rest of the analysis space was dominated by diatoms. The different samplings realized in the summer showed that most of the Marseillan samplings were characterized by higher dinoflagellates abundances than Bouzigues. In the same way, 2013 was characterized by 2 distinct patterns between dinoflagellate and diatom dominance, with most of the Marseillan samplings being located close to the dinoflagellate pattern. Among the 10 phytoplankton species which contributed the most to the analysis dissimilarities between Bouzigues and Marseillan datasets (Tableau 2), five species were common to both years 2012 and 2013: *Rhizosolenia setigera*, *Gonyaulax*, *Peridinium*, *Thalassionema* and *Gymnodinium*. For both years, the diatom *Rhizosolenia setigera* was the species that contributed most to the dissimilarity between Bouzigues and Marseillan datasets.

Tableau 2 : SIMPER analysis related to phytoplankton species contribution to the dissimilarity between the 2 stations “Bouzigues” and “Marseillan” per year. Shaded lines are common to both years 2012 and 2013.

2012	Family	Genus-Species	Contribution (%)	Cumulated sum
	Diatoms	*Rhizosolenia setigera*	58.4	58.44
	Dinoflagellates	*Gonyaulax spp*	9.18	67.58
	Diatoms	*Skeletonema costatum*	7.64	75.22
	Dinoflagellates	*Peridinium spp*	4.31	79.53
	Diatoms	*Rhizosolenia spp*	1.73	81.26
	Diatoms	*Pseudo-nitzschia spp*	1.48	82.74
	Dinoflagellates	*Hemiaulus spp*	1.35	84.09
	Diatoms	*Thalassionema spp*	1.07	85.16
	Dinoflagellates	*Gymnodinium spp*	0.95	86.11
	Dinoflagellates	*Prorocentrum spp*	0.57	86.68
2013	Family	Genus-Species	Contribution (%)	Cumulated sum
	Diatoms	*Rhizosolenia setigera*	32.61	32.61
	Diatoms	*Chaetoceros spp*	16.72	49.33
	Diatoms	*Thalassionema spp*	8.48	57.81
	Dinoflagellates	*Gonyaulax spp*	7.81	65.62
	Diatoms	*Rhizosolenia hebetata*	4.39	70.01
	Dinoflagellates	*Prorocentrum spp*	2.67	72.68
	Dinoflagellates	*Gymnodinium spp*	2.37	75.05
	Dinoflagellates	*Peridinium spp*	1.97	77.02
	Diatoms	*Striatella spp*	1.75	78.77
	Dinoflagellates	*Diplopsalis spp*	1.22	79.99

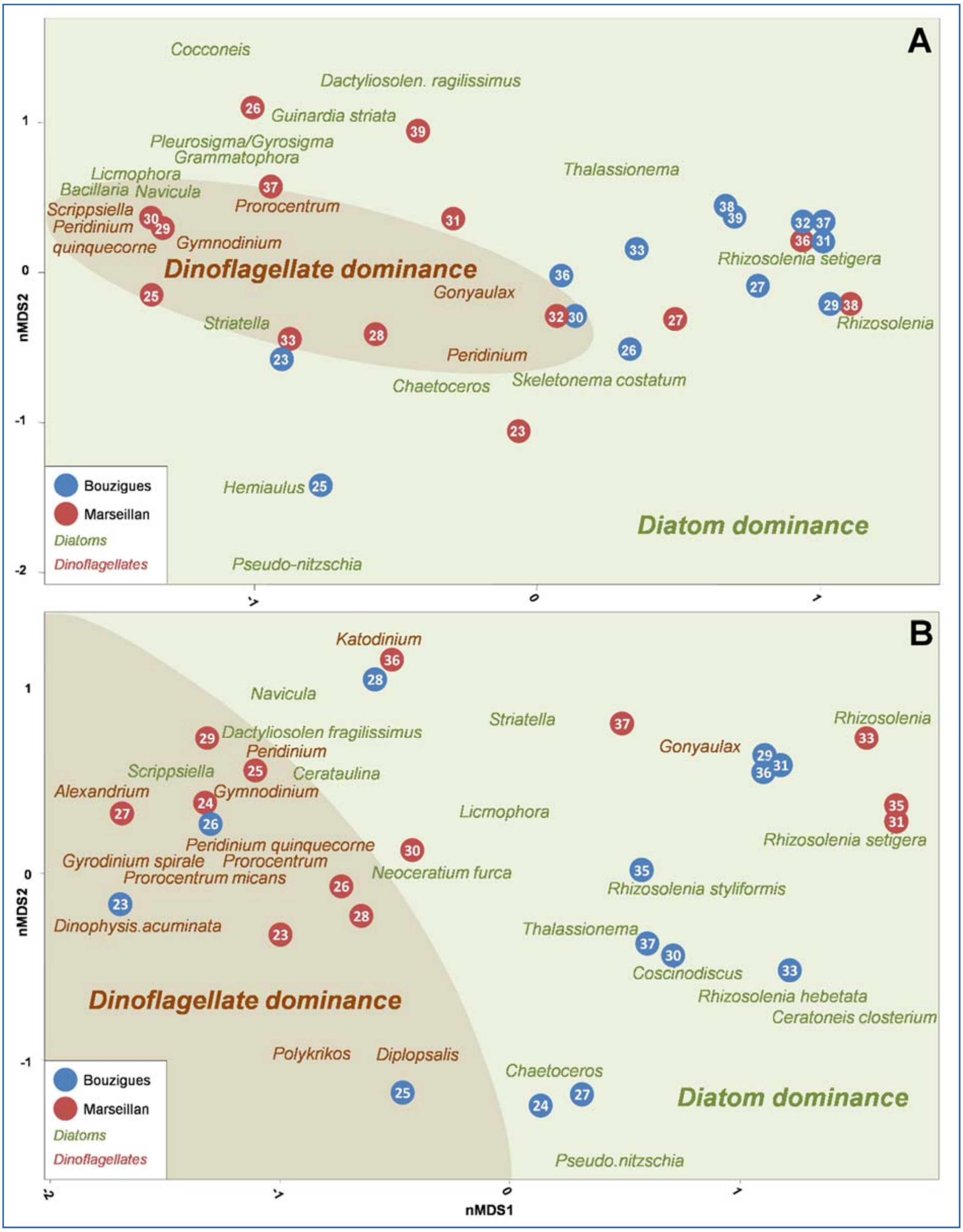

Figure 13 : Non-metrical Multi Dimensional Scaling (*nMDS*) analysis showing the relationship between summer samplings at the stations “Bouzigues” and “Marseillan” and phytoplankton species considered by biovolume in 2012 (A) and 2013 (B). Stress = 0.08 for both analysis, R^2 computation between ordination distance and observed dissimilarity = 0.97 for 2012 and 0.98 for 2013. Species (genus labels) were considered in the analysis when they represented at least 4% of the total biovolume at a sampling date (numbers).

Spatio-temporal variability in gametogenesis

The condition index (CI, %) was measured on *C. gigas* in 2012 and 2013, showing different patterns for both years and between stations (Figure 14). In 2012, CI evolution was asynchronous between the 3 sampled stations, with a first peak for Marseillan in mid-May, for Meze at the beginning of June and for Bouzigues at the beginning of July. In 2013, we observed a more synchronized pattern between the 3 stations, with a first increase of the CI from March to the beginning of May followed by a second increase from May to mid-July, and a third peak observed at the end of summer. Gonado-Somatic Index (GSI, %) was evaluated through histological analysis for each individual during both reproduction seasons (Figure 15). Gametogenesis started in April for both years, with class [20-40 %] appearing at the beginning of June in 2012 and at the end of April in 2013. The different stations had the same global evolution pattern regarding the distribution of maturation stages (Figure 15). However, in 2012, we observed 2 major gonad class regressions at Bouzigues, 1 in Marseillan and 3 in Mèze. In 2013, the 3 stations showed a more synchronized evolution in terms of gametogenesis cycles. We observed multiple gonad maturation events from March to the end of September, indicating asynchronous maturation within oyster groups and/or multiple maturation events. Gametogenesis appears to be possible during the whole summer reproductive season in this ecosystem, with spatial variability between sites and a possible rematuration process of the gonad.

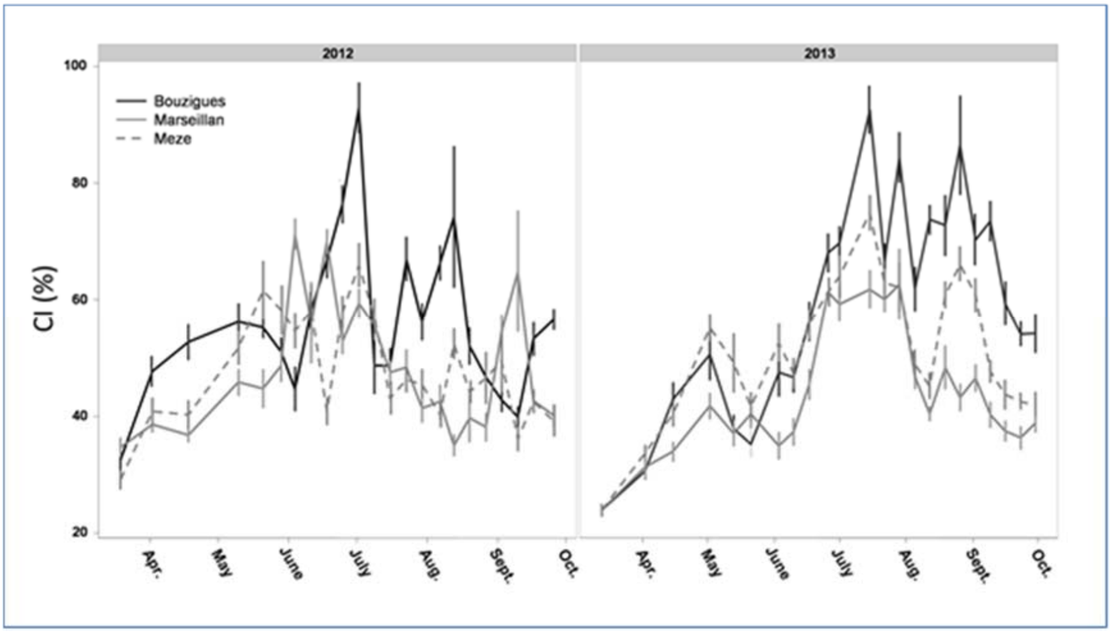

Figure 14 : Condition index (CI) measured on *C. gigas* individuals for each sampling site in 2012 and 2013 (±SE).

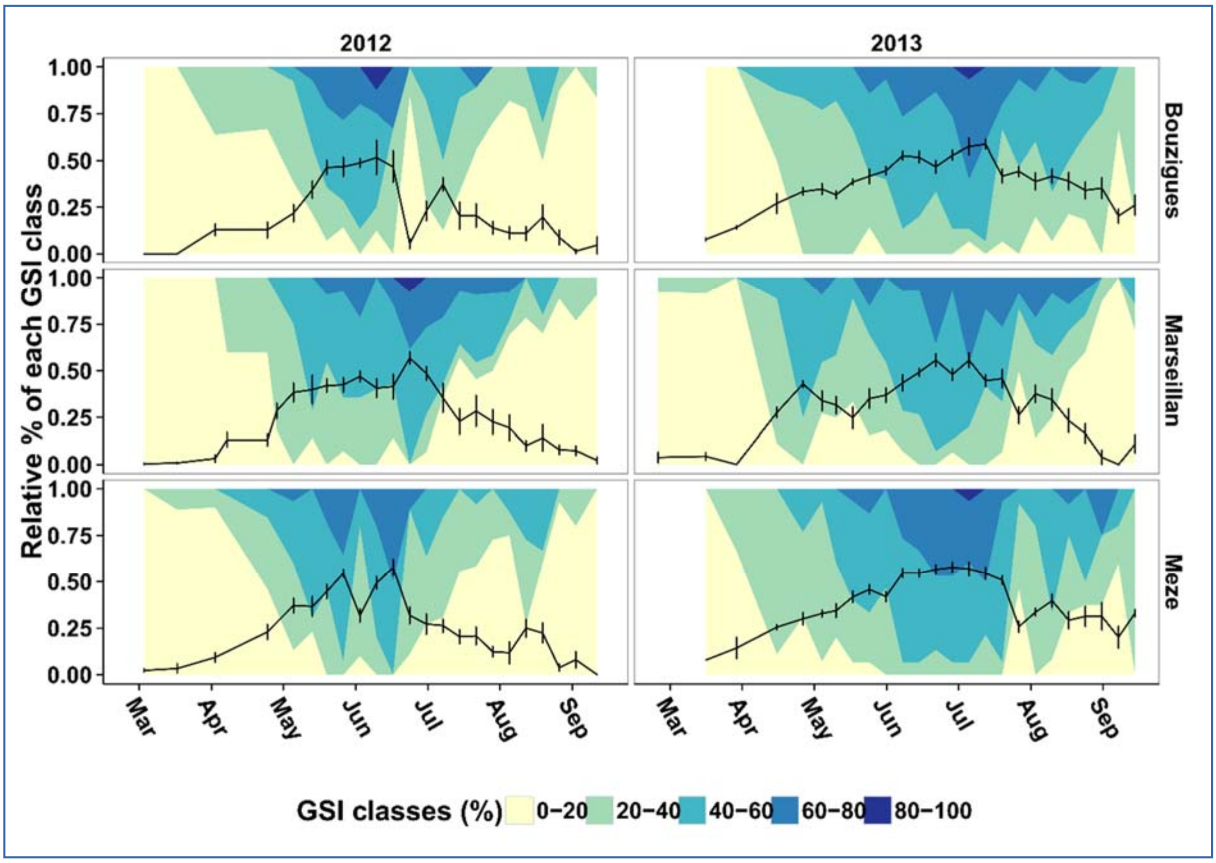

Figure 15 : Relative contribution (%) of *C. gigas* individuals to the gonado-somatic index class determined by quantitative histology for each sampling site in 2012 and 2013. The black line represents the mean GSI (±SE).

Larval abundance

During the 2 reproduction seasons, larvae at various developmental stages were observed from the beginning of June to the end of September (Figure 16). All stages were present through the reproduction season, showing a complete development of the *C. gigas* pelagic larvae in the lagoon for both years. During that 4-months period, multiple peaks of small larvae were observed for both years 2012 and 2013, indicating multiple spawning events in the 3 sectors during summer. Altogether, we observed respectively a total of 14 significant pics of small larvae (1-6 days-old) in the water column (>10000 ind.m^{-3}). First spawning events appeared at the end of Spring for both years 2012 and 2013, with small larvae observed on the 21th of June 2012 at Marseillan (27466 ind.m^{-3}) and on the 17th of June 2013 at Mèze (12333 ind.m^{-3}). The highest small larvae abundances were observed on the 5th of July 2012 at Mèze (414633 ind.m^{-3}) and on the 22th of July 2013 at Bouzigues (169000 ind.m^{-3}), even if the abundance of larvae was 4 times lower the second year.

The last significant larvae peaks were observed on August 8, 2012 at Marseillan (11933 ind.m^{-3}) and on August 26, 2013 also at Marseillan (21933 ind.m^{-3}). Larval abundances decreased at each stage (from small to large larvae) in the water column. The loss of larvae from the beginning to the end of their pelagic life is of the order of a factor log 2, determined thanks to a well-identified cohort on the 5th of July 2012.

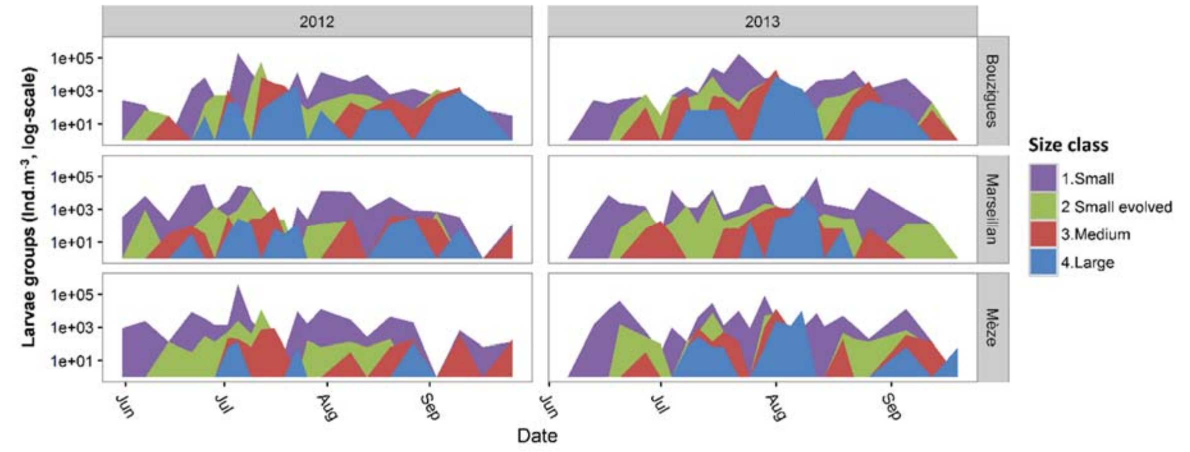

Figure 16 : *C. gigas* larvae groups from water sampled within reproduction season for 2012 and 2013. The sampling weeks spread from mid-May to mid-October. Four stages of the larvae were differentiated during counting and are represented by colors: 🟪 = Small larvae, 🟩 = Small larvae evolved, 🟥 = Medium larvae, 🟦 = Large larvae.

Environmental spawning triggers

Temperature

The first potential spawning trigger is presumably linked to water temperature. No larval cohorts were observed below 20°C (Figure 17, Tableau 3). Even if small cohorts were observed between 20 and 22°C at the end of the reproduction season, the first cohorts only appeared at temperatures over 22°C. The more significant larval cohorts (>10000 ind. m^{-3}) appeared in the water column when water temperatures reached 23°C.

Tableau 3 : Summary of the principal larvae cohort observations at the 3 sampled stations for the years 2012 and 2013. Daily temperatures are given as mean of the day ± SD (n = 24).

Station	Observed larval cohorts	2012			2013		
		Date	Ind.m^{-3}	Daily mean T°	Date	Ind.m^{-3}	Daily mean T°
Bouzigues	First	05/07	187766	23.82 ± 0.33	15/07	25466	26.53 ± 0.38
	Biggest				22/07	169000	27.19 ± 0.33
	Last	30/07	13533	24.47 ± 0.25	22/08	17733	24.45 ± 0.19
Mèze	First	05/07	414633	24.76 ± 0.15	17/06	12333	23.48 ± 0.39
	Biggest				29/07	86466	28.03 ± 0.20
	Last	30/07	12866	24.78 ± 0.36	05/09	13333	24.51 ± 0.31
Marseillan	First	21/06	27466	24.89 ± 0.45	04/07	14933	22.86 ± 0.28
	Biggest	25/06	35900	24.36 ± 0.35	12/08	97133	25.04 ± 0.46
	Last	08/08	11933	25.24 ± 0.29	26/08	21933	23.91 ± 0.26

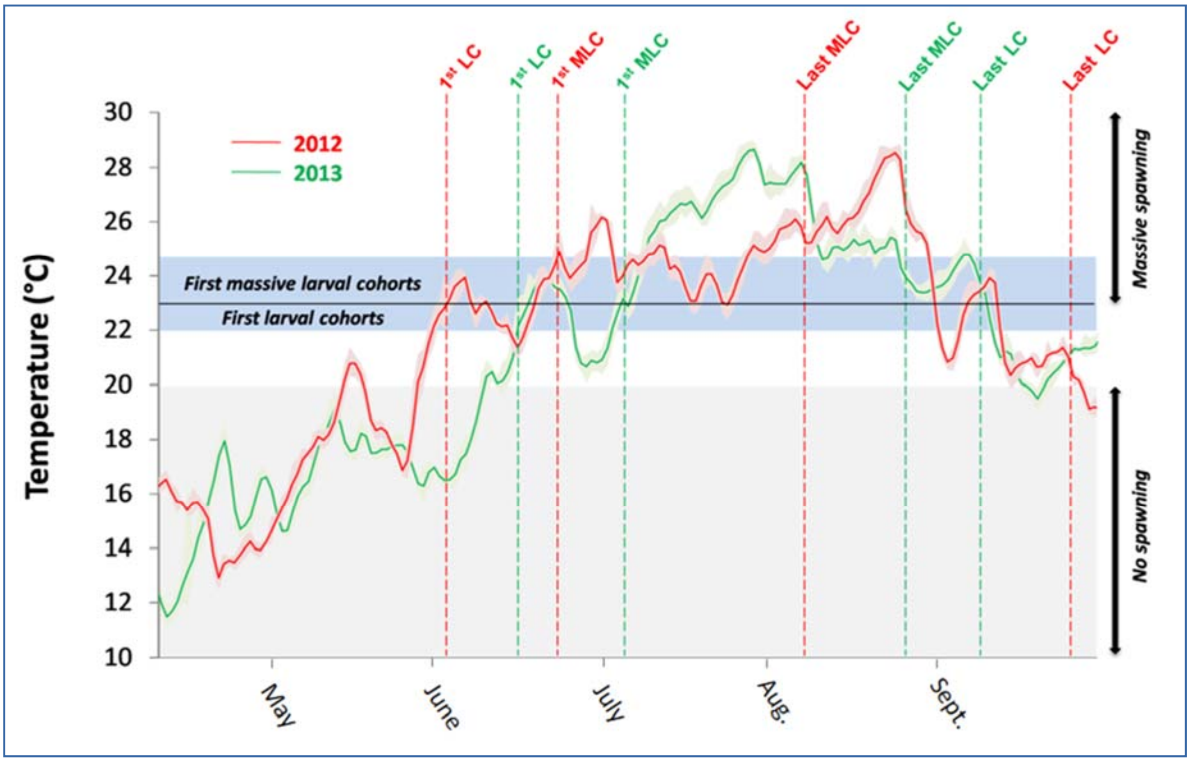

Figure 17 : Relationship between temperatures and associated temperature daily ranges and small larvae cohorts for both years 2012 and 2013. Temperature curves represent the mean temperature observed between the 3 sampled stations Bouzigues, Mèze and Marseillan. Vertical dotted lines indicate first/last larvae cohorts (1st LC/Last LC) and first/last massive larvae cohorts (1st MLC/Last MLC) observed in the water column.

Phytoplankton

The relationship between phytoplankton concentration and *C. gigas* spawning was assessed by exploring the potential linkage between Chl *a* concentration and GSI fluctuations. Results differed between the 2 years: 2013 was characterized by a significant negative relationship between GSI fluctuations and different Chl *a* concentration classes. A good correlation was found for the 2 weeks mean total Chl *a* concentration at Bouzigues (ρ Spearman

= -0.65, p-value <0.01) and the 3 weeks mean total Chl *a* concentration at Marseillan (ρ Spearman = -0.67, p-value <0.01). Thus, in 2013, GSI decreased meanwhile Chl *a* increased in the water column and conversely. The inverse relationship was found in 2012, with GSI and 3 week mean micro Chl *a* concentration being positively related at Marseillan (ρ Spearman = 0.54, p-value <0.05). GSI and 2 week mean total Chl a concentration were also positively related at Bouzigues (ρ Spearman = 0.56, p-value <0.05).

Lunar cycle

Both full moon and new moon appearance during the spawning window affected the number of larvae in the water column with significant higher larvae abundances (ANOVA, F = 4.783, p-value = 0.030). Among the 28 significant cohorts observed for both years at all stations, 19 larval cohorts were characterized by a full/new moon appearance during the related spawning window. The relationship between significant larval cohort appearance and moon phases was not significant (χ2, p-value = 0.277).

Lunar cycle associated to temperature

The lunar cycle happens whatever the temperature is during the whole year; on the contrary, the temperature appeared to have a critical value of 23°C under which we did not observe significant spawning. We thus made the hypothesis that a full/new moon could trigger spawning only if temperature was over 23°C during the spawning window, and that a spawning window characterized by both full/new moon and a temperature below 23°C would lead to a delayed spawning when temperature would increase a new over 23°C. In order to test our hypothesis, we thus explored the remaining 9 significant spawning events that were not characterized by a full/new moon appearance during the related spawning window. Among them, 6 were in line with our hypothesis, with previous full/dark moon characterized by a temperature under 22°C. We tested that new condition regrouping moon and moon delayed spawning together, which appeared to be accurate to explain significant spawning (χ2, p-value = 0.003).

Thunderstorms

As for the lunar cycle, lightening impacts on the Thau lagoon were considered as a binary variable, appearing or not during the 6 days preceding a cohort apparition in the water column. Lightning impacts – 14 during the studied period – did significantly affect the number of larvae in the water column (ANOVA, F = 9.553, p-value = 0.002). When considering only significant spawning events, lightning impacts did have a significant effect on larvae concentration (ANOVA, F = 7.185, p = 0.012), with more larvae in the water column after a lightning impact within the 6 day potential spawning period.

Discussion

Phytoplankton as a fuel for gametogenesis

Settlement and recruitment dynamic are known to be largely affected directly by competent larvae supply, transport processes, availability of space and habitat selectivity but also indirectly by adults population, individual gametogenesis, fecundity and spawning strategy (Rodriguez et al. 1993). One of the Thau lagoon's specificity is the absence or quasi-absence of wild oyster broodstock and reproductive effort relies only on cultivated oysters. From one year to another, cultivated oyster stocks may vary on both (1) quantitative and (2) qualitative ways, since farmers may switch between diploid and triploid-sterile cultures. But, we can make the reasonable hypothesis that stocks of oysters were nearly the same all along the present study. In such context, the gametogenesis step could be of crucial interest to better analyze reproduction success of *C. gigas* in the Thau lagoon.

Gametogenesis is known to be highly dependent on environmental conditions. For instance, it may suffer from possible (1) trophic limitation at the lagoon scale as well as (2) rapid temporal variations. Biometry revealed that the Bouzigues station allowed a higher growth than the Marseillan station (CI), but also a higher reproductive effort (GSI). However this reproductive effort is difficult to assess since multiple spawning events happen in the summer. Influence of phytoplankton on gametogenesis has been demonstrated within many oyster species (Pouvreau, Gangnery, et al. 2000; Fabioux et al. 2005; Fournier et al. 2012; Aranda et al. 2014). More precisely, temperature was the main factor driving the *Crassostrea gigas* gametogenesis rate, food availability was responsible of gametogenesis intensity(Enríquez-Díaz et al. 2008). Our observations are totally in line with these authors, since we observed similar kinetics of gametogenesis between the two studied sites (Marseillan and Bouzigues) but different intensities probably due to differences in terms of phytoplankton communities and abundances. For instance, we observed more diatoms at "Bouzigues" than at "Marseillan", *C. gigas* being known to feed mainly on diatoms (Dupuy et al. 2000; Pernet, Malet, et al. 2012). In fact, *Rhizosolenia setigera* contains 4310 pg C.cell^{-1} (Dupuy et al. 2000), making this species interesting in terms of food supply. That difference in terms of phytoplankton communities between the 2 points must be linked to (1) the confinement gradient of the lagoon (Fiandrino et al. 2017), Bouzigues being more linked to the sea and thus being characterized by a better water renewal and (2) the fact that new water coming from the North-East has already been filtered by oysters. The better growth in 2013 at the Bouzigues station during August and September can be clearly linked to the dominance of large diatoms such as *Coscinodiscus*, *Rhyzosolenia*, *Thalassionema* during these months (Figure 13).

An asynchronous spawning strategy

This study is the first to highlight the reproduction strategy of *C. gigas* in a Mediterranean lagoon. We showed that the reproduction window within the Thau lagoon is stretched from the beginning of June to the end of September, and is characterized by multiple, asynchronous and partial spawning events for both studied years. These results show that gametogenesis and spawning are much more asynchronous in the Thau Lagoon compared with what has been observed on the French Atlantic or Channel coasts (Royer et al. 2008; Enríquez-Díaz et al. 2008; Dutertre et al. 2009) both places being under macrotidal regime. Besides synchronicity, Dutertre et al. (2009) observed during 2005 and 2006 two spawning events per year, between 18°C and 22°C, but Royer et al. (2008), Enriquez et al. (2009) and Bernard et al. (2016) observed only one synchronous spawning event per season. In any case, our observations are different from the previously cited literature, since we observed asynchrony between sites within the same ecosystem as well as within sites. There is little literature concerning *C. gigas* reproduction cycle in non-tidal ecosystems, except the study performed by Dridi et al. (2007) in the lagoon of Bizert (Tunisia). Authors show that spawning occurred from June to September at temperatures spreading from 23°C to 27°C, a temperature window and threshold which are rather in accordance with our study. The apparent difference in terms of synchronicity of the reproduction between tidal on non-tidal systems may be related to different hypothesis: (1) In tidal ecosystems, synchronicity of the reproduction is all the more important that gametes are washed away during ebb-tide, while non-tidal lagoons offer more chances for gamete encountering; (2) Mediterranean lagoons, because of a lower water renewal than tidal systems, are characterized by a more spatially heterogeneous biocoenosis, increasing the spatial heterogeneity of the whole ecosystem and thus the heterogeneity of gonad maturation within the lagoon. Our results, in complement of the review given by Castaños et al. (2009) regarding

C. gigas reproduction, underline the species reproduction plasticity of the Pacific oyster to different kinds of ecosystems.

Hierarchy of spawning triggers

Numerous spawning triggers have been mentioned within marine bivalve species. Among them and for the species *Crassostrea gigas*, the main trigger seems to be played by the temperature threshold. It is reported in the literature to vary between 17°C (Castaños et al. 2009) and 18°C (Cognie et al. 2006, Dutertre et al. 2009) among several ecosystems. In the Thau lagoon, for the years 2012 and 2013, we observed that first spawning events occurred at a temperature of 22°C. This 4-5°C difference with the previously cited literature may have two explanations. The likeliest hypothesis is the difference in terms of temperature stress between Atlantic and Mediterranean ecosystems. In fact, during the flow within Atlantic tidal ecosystems in the summer, animals are exposed to a temperature stress resulting of the difference between the warm air and the incoming colder water. On the contrary, within Mediterranean ecosystems, there is no such stress because of the absence of noticeable tidal amplitude. Moreover, the gonad maturation in the Thau lagoon could be insufficient at 17-18°C to ensure internal triggering of spawning processes. In fact, the greatest number of mature individuals corresponded to the first appearance of significant larval cohorts. The latter was observed in the water column at a temperature exceeding 23°C, optimal temperature for larvae development being superior to 22°C (Rico-Villa et al. 2009). Thus, once the gonad maturity is reached, temperature must be – as for many other ecosystems – an important trigger for gamete release. However, reproduction within the Thau lagoon is characterized by multiple spawning events during the wide favorable temperature window, making the temperature itself insufficient to explain accurately spawning strategy in this ecosystem.

Once conditions are met to release gametes in the water column, oysters need to rely on synchrony for increasing the gamete matching chances. We used the same analysis as Fournier et al. (2012) for *Pinctada margaritifera* in order to identify the potential effect of phytoplankton concentration as a spawning trigger for the *C. gigas* species. While they identified it for the species *P. margaritifera*, we were not able to identify it for *C. gigas*, at least not for both years, as they showed opposite results.

The moon influence on marine species spawning has been widely studied, especially for corals (Nozawa 2012). Moon influence on spawning events within the species *Ostrea virginica* has been well studied by Loosanoff & Nomejko (1951), who found no clear relationship between moon phases and spawning. More recently, Tran et al. (2011) and Bernard et al. (2016) found a strong relationship between the rhythms of valve behavior – related to gamete release – and the association of the sun-earth-moon orbital positions. The extremely moderated neap-spring tide cycle (~40 cm) associated to the permanent immersion of followed individuals allowed us to highlight the moon influence as a synchrony trigger for gamete release within the Thau lagoon. In our study, among the 28 significant cohorts observed for both years at all stations, 19 larval cohorts were characterized by a full/new moon appearance during the related spawning window. Further investigations about this result should deserve a particular attention.

In addition to major triggers affecting presence/absence and synchrony of spawning events, punctual additional triggers may add a degree of complexity to the *C. gigas* spawning strategy. The punctual nature of these events makes them difficult to analyze and they are thus poorly documented, whereas local farmers often consider them as determinant environmental factors. Localized thunderstorms, typical during the summer over the Thau lagoon, could be potential punctual spawning triggers. They notably affected the number of larvae in the water column even when considering only significant spawning events, and the largest small larvae cohorts in the water for both years 2012 and 2013 were preceded by a thunderstorm in the 5 previous days (Tableau 4). Even if they may have confounded effects with moon and/or temperature, thunderstorms could be additional signals triggering spawning events.

Tableau 4 : Relationship between largest small larvae cohorts observed in the water column and potentially related thunderstorm occurrences. The delay in days is indicated between brackets.

Station	year	Date of observed cohorts	Date of thunderstorm
Bouzigues	2012	05/07	04/07 (d-1)
	2013	22/07	18/07 (d-4)
Mèze	2012	05/07	04/07 (d-1)
	2013	29/07	28/07 (d-1)
Marseillan	2012	25/06	-
	2013	12/08	07/08 (d-1)

Conclusion

Understanding the whole reproductive cycle from gametogenesis of adults to settlement of progeny has always represented a challenging issue in marine invertebrates. Of course, environmental driving variables are numerous and may be different from one ecosystem to another. In that context, our study aimed to give some answers within the species *C. gigas* and in a particular the Mediterranean lagoon where *C. gigas* has been introduced artificially for shellfish purposes. It appears from our study that the reproduction window within the Thau lagoon stretches from the beginning of June to the end of September, and is characterized by multiple, asynchronous and partial spawning events. From a quantitative point of view, gametogenesis intensity (GSI, fecundity) was correlated to the abundance of diatoms. Once gametogenesis is complete, spawning can occur according to 3 types of spawning triggers: (1) temperature should be considered as a “locking trigger” with a threshold value above 22°C; (2) the moon (full and new) appeared as a “synchrony trigger” maximizing chances of gamete matching; (3) a last additional trigger like thunderstorms may increase the gamete release, acting more like a stressful trigger. These new findings should play one's part in the conceptual pattern of the *C. gigas* reproduction in non-tidal Mediterranean.

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We would like to thank France-Agrimer, Conseil Régional d’Occitanie/Languedoc-Roussillon, Conseil départemental de l’Hérault, Comité Régional de la Conchyliculture en Méditerranée, Cepralmar and Ifremer for their financial support in the scientific part of PRONAMED 2 project (2012-2015). This work was made possible thanks to the collaboration of the LERLR team and special thanks go to Hélène Cochet, Béatrice Bec, Marie Boj, Axel Leurion and Solenn Soriano for their availability for the 2012 and 2013 campaigns. The authors are also grateful to the Ifremer staff of the RESCO, VELYGER and REPHY networks, through which parts of the field data were gathered. Finally, we would like to thank the 3 reviewers who helped us a lot in improving the quality of this article.

<u>Chapitre 2 :</u> Recrutement de l'huître creuse (*Crassostrea gigas*) en lagune méditerranéenne exploitée par la conchyliculture : découverte, facteurs influents et fenêtre de recrutement favorable

Ce deuxième chapitre fait l'objet d'une publication en Feature Article dans la revue *Marine Ecology Progress Series* 578 (2017) 1-17

"Quand on ne dispose pas de connaissance scientifique permettant d'expliquer les choses, il ne reste plus qu'à se reposer sur des arguments qui semblent avoir du sens."
Peter J.Bentley,
Extrait de : livre des nombres : Leur histoire et leurs secrets, des origines à nos jours.

Résumé de la publication en français

Dans le cadre actuel de demande sociétale pour une restauration environnementale des milieux, les systèmes aquatiques exploités peuvent être amenés à évoluer dans un contexte d'oligotrophisation. C'est le cas des lagunes méditerranéennes françaises, en particulier du bassin conchylicole de Thau, où nous avons étudié le recrutement de l'huître creuse (*Crassostrea gigas*). Les naissains d'huîtres et les paramètres environnementaux ont été suivis sur plusieurs sites d'échantillonnage pendant trois ans (2012-2014) en utilisant une méthode originale de déploiement temporel de collecteurs avec chevauchement pour étudier les processus avant et après la fixation/métamorphose et identifier les meilleures conditions de recrutement. Contrairement au paradigme établi "*Pas de reproduction des huîtres creuses en lagune méditerranéenne*", notre étude a montré que le recrutement de cette espèce introduite est en réalité possible dans la lagune de Thau à des niveaux comparables à ceux des autres bassins de reproduction traditionnels français. Nous avons identifié des conditions favorables pour le recrutement, caractérisées par une température de l'eau élevée (> 26,5 °C) et des abondances élevées en nanophytoplancton et *Chaetoceros spp.* (> 4,3 10^6 cellules l^{-1} et 345 10^3 cellules l^{-1}, respectivement). Dans ces conditions favorables, nous suggérons que l'écosystème fonctionne comme un système autotrophe, par opposition à un système hétérotrophe qui caractérise des conditions défavorables. Dans les conditions hétérotrophes, une forte abondance

d'organismes mixotrophes et hétérotrophes (ciliés et dinoflagellés) limite la métamorphose des larves de *C. gigas*, entraînant un faible recrutement. Cette étude apporte de nouvelles connaissances sur la reproduction de l'huître creuse dans un lagon méditerranéen dans le contexte du réchauffement et de trajectoire écologique d'oligotrophisation. En outre, ce travail apporte à l'industrie conchylicole la mise en évidence de gisements de naissain utiles pour développer de nouvelles pratiques de captage naturel respectueuses de l'environnement et ainsi limiter les risques de transferts avec d'autres zones d'élevage.

Recruitment of the Pacific oyster (*Crassostrea gigas*) in a shellfish exploited Mediterranean lagoon: discovery, driving factors and favorable environmental window

Franck Lagarde, Emmanuelle Roque d'orbcastel, Martin Ubertini, Serge Mortreux, Ismaël Bernard, Annie Fiandrino, Claude Chiantella, Béatrice Bec, Cécile Roques, Delphine Bonnet, Gilles Miron, Marion Richard, Stéphane Pouvreau, Christophe Lett

Introduction

Ecosystems change over time under the constraints of combined global warming and anthropogenic impacts. More visible than global warming, marked local effects due to coastal urban development and/or pollution lead to environmental changes that seriously affect ecological processes. Hence, the increase in nutrient intakes in recent decades has led to the eutrophication of aquatic ecosystems (De Jonge & Elliott 2001; Nixon 1995; Smith & Schindler 2008). Now, in the context of increasing demand for the recovery of ecosystem services (Bullock et al. 2011) and good environmental status (Vethaak et al. 2017), these ecosystems face the challenge of evolving under oligotrophication (Yanagi 2015; Duarte et al. 2009; Jeppesen et al. 1998; Jeppesen et al. 2005).

Oligotrophication (Boesch 2002; Cloern 2001) caused by ecosystem and wastewater management (Leruste et al. 2016) and increasing temperature (Collos et al. 2009) are two major trends observed in Mediterranean lagoons. Oligotrophication causes a shift in the structure of the phytoplankton community from diatoms, cryptophytes and green algae to mixotrophic dinophytes (Leruste et al. 2016; Gowen et al. 2015). The combination of global warming and reduced nutrient loads may lower phytoplankton biomass (Collos et al. 2009; Saeck et al. 2013; Lie et al. 2011). This reduction would affect the breeding stocks of bivalve suspension-feeders in shellfish basins (Dame 2011), possibly jeopardizing the sustainability of their production if the shellfish biomass is not well balanced with the carrying capacity of the ecosystem.

The Pacific oyster (*Crassostrea gigas*) is one of the most economically important invertebrate species in aquaculture. The world declared production in 2014 was estimated at around 625 925 tons according to FAO Fishery Statistics and global production could exceed 4 million tons. This oyster species was introduced in France in 1970 for social and economic reasons (Grizel & Héral 1991) and was highly productive during the 1980-2000 period in the Atlantic and Mediterranean shellfish farmed basins (Deslous-Paoli et al. 1993; Héral & Deslous-Paoli 1991). About 10% of the French oyster production comes from the Mediterranean Thau lagoon (Robert et al. 2013), located in southern France.

C. gigas has been widely studied all over the world and its life history (reproduction, larval cycle and recruitment phase) is relatively well described in both controlled environments (Fabioux et al. 2005; Enríquez-Díaz et al. 2008; Rico-Villa et al. 2010) and coastal tidal ecosystems (Dutertre et al. 2010; Bernard et al. 2016; Thomas, Pouvreau, et al. 2016). Fewer studies have been conducted in nanotidal environments such as Mediterranean lagoons (Tagliapietra & Ghirardini 2006). The gametogenesis and spawning behavior of *C. gigas* were only recently explored in the Thau lagoon (Ubertini et al. 2017). According to these authors, the spawning behavior of *C. gigas* within Thau lagoon appeared to be slightly different from the one of the Atlantic coast, showing several spawning events from June to October. The minimal temperature observed for spawning was 23°C, which is much higher than the temperature mentioned in the literature for this species. Additionally, a strong relationship was found between phytoplankton communities and gametogenesis, the latter being improved by higher diatom/dinoflagellate ratio.

The Mediterranean oyster industry is based on spat supplied from Atlantic nursery basins (mainly Arcachon and Marennes-Oléron) and hatcheries (Buestel et al. 2009). The massive spat mortality observed in France since 2008 (Pernet et al. 2012; Pernet et al. 2010; Pernet et al. 2014) emphasized the dependence of the Mediterranean industry to outside sources, showing the need of native supply. However, reproduction of *C. gigas* is believed to be impossible (Drullion 2002; Debos et al. 1972) or irregular (Goulletquer 1995) in the Thau lagoon, possibly due to unfavorable hydrological conditions (*e.g.* temperature, salinity, insufficient food and high concentrations of antifouling paint) (His et al. 1986; His & Robert 1985; Deslous-Paoli et al. 1982).

A limited number of studies on marine invertebrates suggested that recruitment in the Thau lagoon is spatially highly heterogeneous, potentially related to plankton depletion due to the intensive shellfish culture (Lam-Hoai et al. 1997; Souchu et al. 2001) and hydrodynamic circulation (Borsa & Millet 1992). However, recruitment information on *C. gigas* in Thau lagoon remains extremely limited and the aim of the present work was to fill this gap.

To this end, several larval and oyster spat monitoring sites were created at different locations in the Thau lagoon to precisely monitor both pelagic larval phases and benthic settlement of *C. gigas* over a period of three years. The general hypothesis of this study is that the ‘no-recruitment paradigm in Mediterranean lagoons is false and that lagoon ecology offers recruitment windows, supporting several theories like supply side ecology (Grosberg & Levitan 1992b), match-mismatch (Cushing 1990), transport and retention (Bishop et al. 2006). Our objectives are to (i) identify the patterns of oyster recruitment in space and over time, (ii) characterize variations within different pelagic and benthic larval stages, and (iii) explore the effects of environmental factors on larval development and recruitment success. Using a correlative approach, we paid particular attention to the feeding sources known to play a major role in the development of bivalve mollusk larvae (His et al. 1989; His & Seaman 1992).

Materials and methods

Study site

The Thau lagoon is the largest nanotidal lagoon in the Occitanian region in southern France (Figure 18). It covers an area of 7,500 hectares (19 km × 4.5 km) on a north-east / south-west axis, and has a mean depth of 3.5 meters. Seawater inputs from the Mediterranean Sea enter by artificial channels. Four spatfall sites (Figure 18) were monitored to assess pre-settled oyster larvae and post-settled spat abundances in pelagic and benthic habitats: three inside shellfish farming zones (Bouzigues, Meze and Marseillan) and one outside (Listel).

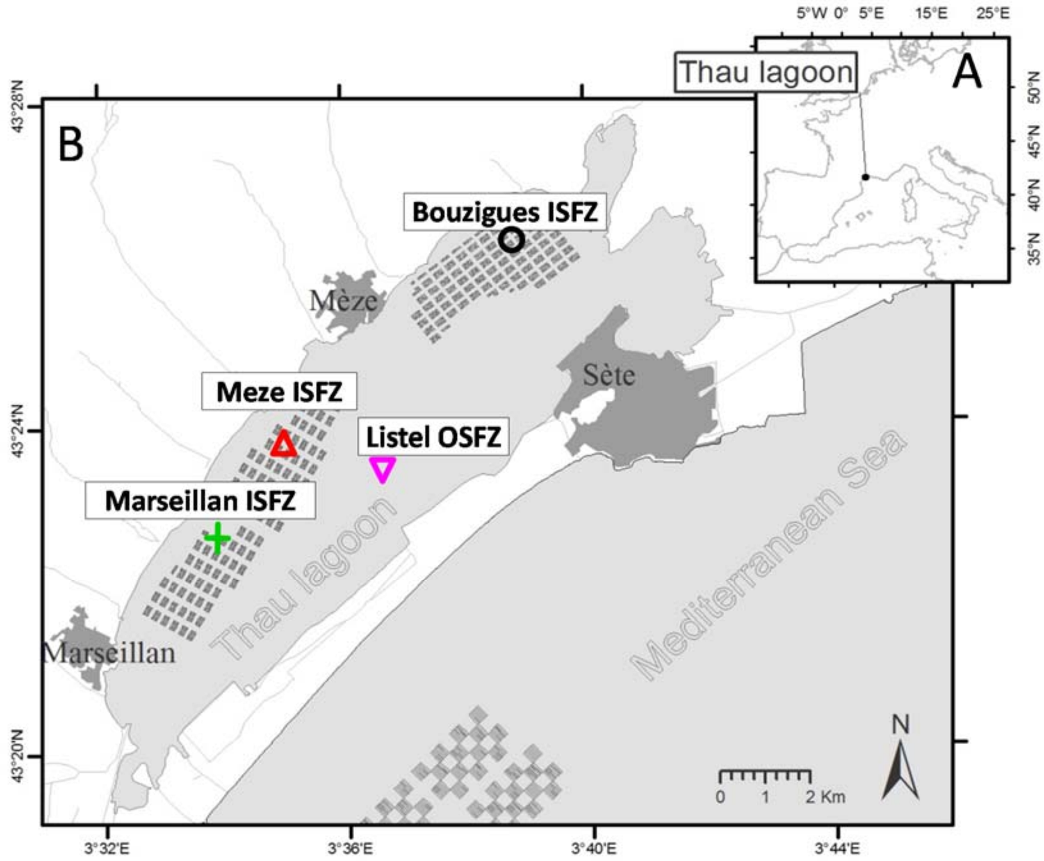

Figure 18: Location of A) The Mediterranean Thau lagoon in France, B) sampling sites in the Thau lagoon. Rectangle symbols show the location of four sampling sites (Marseillan, Meze, Bouzigues and Listel) where the pelagic and benthic oyster larvae, spat abundances, hydrological and plankton data were monitored. ISFZ: Inside the shellfish farmed zone, OSFZ: outside the shellfish farmed zoned. The grey boxes represent bivalve farms.

Larval and spat abundances

Oyster pelagic and benthic larval abundances were assessed in 2012, 2013 and 2014 from June to September. Pelagic larvae assessments were carried out twice a week using a standard protocol provided by the National Larval Network (Pouvreau, Petton, et al. 2013; Pouvreau et al. 2016) where the size of “D-Larvae” varies between 60 and 100 µm and “large umbo larvae” between 180 and 300 µm. A sampling volume of 1.5 cubic meter was pumped and filtered through a 40 µm plankton net.

Benthic oyster abundances were estimated every two weeks at three different settler~~s~~ stages: pre-settled larvae, young postlarvae and newly-settled spat (Arakawa 1990b). An original method with a temporal overlap deployment of collector was used (Arnold & Steneck 2011; Hughes et al. 2000).-To collect these benthic stages, the sites were equipped with three replicated sets of two collectors (Figure 19). Each collector was composed of 44 white plates (15 cm in diameter, 250 cm^2), measuring 110 cm (Figure 19). Collectors were vertically submerged two meters below the surface, suspended under shellfish farming structures or, outside farming structures, on specially designed mooring systems (Figure 19a). The mooring systems were positioned so that the top of the uppermost collector was 2.5 meters below the surface. The systems were designed to support two sets of collectors, one immersed for two weeks to assess pediveliger and postlarvae abundance and the other immersed for four weeks to assess oyster spat abundance. The collectors were replaced every two weeks throughout the summer. Pediveligers were observed on the collectors' plates, with prodissochonch 2 shell size ranging from 180 µm to 300 µm (Figure 20). This pediveliger stage precedes metamorphosis, a vulnerable phase of their life cycle (Pechenik 2006; Coon et al. 1990). The main physiological transformations of metamorphosis are complex (Bishop et al. 2006). It basically convert the larval body plan into an adult body plan (Wray 1995). In bivalves, the evolution of important organs such as gills during metamorphosis affect remarkably the feeding mechanism~~s~~ (Cannuel & Beninger 2007; Beninger & Cannuel 2006; Cannuel & Beninger 2006). Metamorphosis is defined as the transformation of pediveliger into postlarvae. Postlarvae are strictly benthic and cemented with a dissoconch shell. Their size ranges from 300 µm to 1000 µm. Oyster spat take over from postlarvae at 4 weeks of age (maximum) and their size ranges from 1 mm to 8 mm.

Each collector was sampled at three vertical levels: close to the top (39th plate), middle (22th plate) and bottom (5th plate). Both sides of the plates (above and below) were examined under a binocular microscope to assess the mean abundance of pediveliger, postlarvae and newly settled spat, or macroscopically when possible. On each occasion, counting was carried out on subunits, i.e. from 1 to 4 basic subunits (14 cm^2 to 56 cm^2)-, replicated three times per side (Figure 19b), to have 54 counts by date and site (3 collectors × 3 levels × 2 sides × 3subunits). The abundance per plate was averaged from subunit counts and converted into total individual abundances per plate.

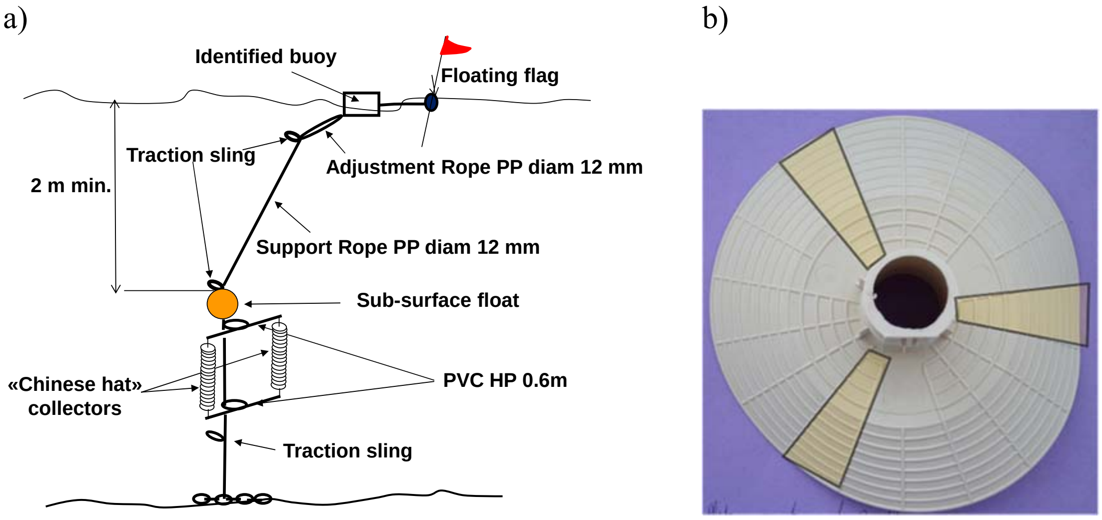

Figure 19: a) Diagram of the mooring system used in the present study. The gear supported a set of two "Chinese hat" collectors with plates deployed outside shellfish farming structures in the Thau lagoon. The first collectors was immersed for 2 weeks and the second for 4 weeks before being replaced by a new collector. The sampling period lasted from June to October. b) Top view of a collector plate with counting subunits in grey, in this case: 14 cm^2.

The pediveliger and spat abundances were classified in five categories according to a recruitment scale proposed by Pouvreau et al. (2013) for the French coast: zero (0 individuals $plate^{-1}$), low (1 to 20 individuals $plate^{-1}$), medium (21 to 200 individuals $plate^{-1}$), high (201 to 2000 individuals $plate^{-1}$), overabundant (>2000 individuals $plate^{-1}$). An additional qualitative recruitment factor was created to compare pediveliger (Pedi) and spat (Spat) abundances (Figure 20). The recruitment factor terms "Pedi –" and "Spat –" summed the abundances from 0 to 20 individuals per plate, and "Pedi +" and "Spat +" summed the abundances higher than 20 individuals per plate. The recruitment factor combined terms to define three conditions: "Pedi-" indicated low pediveliger supplies, "Pedi+Spat-" indicated metamorphosis failure, "Pedi+Spat+" successful metamorphosis.

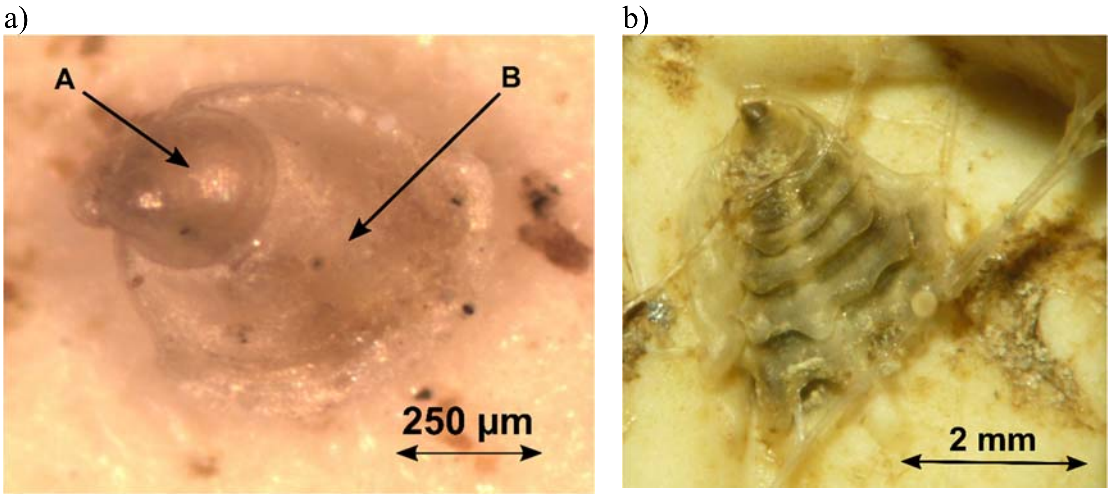

Figure 20 : Settled oyster larvae on a collector plate. a) Shell of pre-settled larvae (Pediveliger) (A) and shell of young postlarvae after metamorphosis (B) on collectors immersed for two weeks, b) newly-settled spat on a collector immersed for four weeks.

Environmental measurement

Environmental parameters (hydrological and plankton samples) were recorded every Monday morning from first of June to the end of September in 2012, 2013 and 2014. Samples were taken on spatfall sites for Listel and Mèze. Bouzigues and Marseillan spatfall sites were located less than one kilometer from where environmental measures were monitored (sampling sites for REPHY French network of phytoplankton monitoring to which these data were used in this study). For Bouzigues and Marseillan, hydrological and plankton samples associated to spatfall sampling sites were in biocoenosis characterized by the same phytoplankton, zooplankton and benthic populations (Jouffre & Amanieu 1991; Guelorget et al. 1994; Jarry et al. 1990).

Hydrological monitoring

Temperature and salinity were measured twice a week and weekly averaged with WTW® probes positioned between 1 and 1.5 m below the surface. Oxygen concentrations were measured once a week at the Bouzigues site at the bottom of the water column. Data were collected from June to September in 2012, 2013 and 2014.

Plankton monitoring

Our sampling strategy provided 135 observations: 3 sampling sites X 15 weeks X 3 years. Each Phytoplankton and protozooplankton sample was collected by sampling site and date using subsamples from a 4 liters sample. Samples were weekly sampled using a Standard

Water Sampler acc. to Ruttner (Hydro-Bios Apparatebau GmbH) at three sampling sites per year. No analytical replicate or sampling replicate was used. The “Bouzigues”, “Meze” and “Marseillan” sites were sampled in 2012 while the “Bouzigues”, “Listel” and “Marseillan” sites were sampled in 2013 and 2014.

For total chlorophyll *a* [Total Chla] measurements, seawater samples (200 mL) were filtered (Bec et al. 2005; Bec et al. 2011) under vacuum (<10 cm Hg) on Whatman GF/F membranes (0.7 µm porosity) and stored in glass tubes at –20 °C. Filters were ground in acetone (90%) and extracted for 24 h at 4 °C in the dark. Chla biomass were also determined after size fractioning (200ml for picophytoplankton and 200ml for nanophytoplankton from the 4 liters sample) through Nuclepore membranes (3 and 20 µm) to determine the contribution of picophytoplankton (PICO, <3 µm), nanophytoplankton (NANO, 3-20 µm) and microphytoplankton (MICRO, >20 µm) to total phytoplankton biomass. The pigment content (µgChla l^{-1}) was measured using a spectrofluorometer (Perkin-Elmer LS50b)(Neveux & Lantoine 1993b).

Abundances of picocyanobacteria (CYAN, < 1 µm), autotrophic picoeukaryotes (PEUK, <3 µm) and nanophytoplankton (NANO TOT, 3-20 µm) were determined on the basis of a sampled volume of 1 mL with a Becton Dickinson FACSCalibur flow cytometer (Bec et al. 2011). Total picophytoplankton (PICO TOT) abundances were estimated by the sum of the CYAN and PEUK abundances. Among nanophytoplankton, Cryptophytes (CRYPTO) were distinguishable from other photosynthetic organisms (NANO) by their strong orange fluorescence and their size. Abundances were expressed in 10^6 cells l^{-1}.

To measure the bacterial abundance, samples (1 mL from the 4 liters sample) were fixed with prefiltered (0.2 µm) buffered formaldehyde (2% final concentration) and stored in liquid nitrogen. Abundances were determined by a FACSCalibur flow cytometer Becton Dickinson method (Marie et al. 1997). The procedure was slightly modified as higher concentrations of fluorochrome (SYBR Green I) were used (Bouvy et al. 2016). One milliliter of fixed samples was incubated with SYBR Green I (Molecular Probes) at a final concentration of 1/375 for 15 min at 4 °C in the dark. Stained bacterial cells excited at 488 nm were determined according to their side-scattered light (SSC) and green fluorescence (FL1) collected using a 530/30 nm filter. Fluorescent beads (0.94 µm, Polysciences Inc.) were added to each sample.

Protozooplankton (heterotrophic flagelates 30 mL sample and ciliates 100 mL sample) and total phytoplankton flora (10 mL sample) were estimated using the standard Utermohl method NF-EN-152014, 2006. For the total phytoplankton flora, two main "Diatoms" (aggregating 52 taxa) and "Dinoflagellates" (aggregating 38 taxa) classes were first used to explore the dataset. In addition, we paid particular attention to the diatom genus of "*Chaetocoeros spp.*" because of its high abundance and its known role in the biological cycle of oyster recruitment (Blanchard et al. 2008; Rico-Villa et al. 2006; Ben Kheder et al. 2010). Protozooplankton and total flora were expressed as the number of individuals per liter. Total phytoplankton flora were not monitored in 2012 at the "Meze" sampling site.

For analysis of the heterotrophic flagellate (HF), the 30 ml samples were preserved in an 8% formaldehyde solution and stored in a cold room at 4 °C in the dark until analysis. A 10-ml subsample was stained using 4^0, 6-diamidino-2-phenylindole (DAPI) at a final concentration of 2.5 µg ml^{-1}. The HF counts were performed using an epifluorescence microscope (Olympus AX70) with UV illumination (Sherr et al. 1993). For the naked ciliate and tintinnid counts and analyses, 100 ml samples were preserved in 2% Lugol's iodine solution and kept in a cold room at 4 °C in the dark until analysis. Naked ciliates and tintinnids were identified, measured and counted with an inverted microscope (Olympus IX70) after a 100 ml sample was left to settle in an Utermohl chamber for 24 h (Utermöhl 1931).

Mesozooplankton were sampled using subsurface horizontal net tows. AWP2 net type with a mesh size of 80 µm and an opening diameter of 50 cm was used. In general, this plankton net was towed at an average speed of 3 km h^{-1} for 2 to 3 minutes. The volumes of water filtered by the net averaged 20 to 30 m^3 and were calculated precisely for each trawl, taking into account the speed of the boat and the time of immersion of the net. Once the net had been brought back on board, the contents of the collector were sieved over 80 µm in order to concentrate the sample and then transfer to a 250 ml sample bottle fixed with 4% stabilized formaldehyde. The samples were then stored at room temperature until analysis (diversity and abundance). Abundances of mesozooplankton considered as potential predators and trophic competitors of *C. gigas* larvae were estimated using a binocular microscope with taxonomic identification (Rose 1933). The group of "trophic competitors" was determined as the sum of copepods nauplii, annelids and barnacle larvae, ascidia and gastropod larvae. "Potential predators" were assessed as the sum of cladocerans *(Penilia avirostris, Podon spp., and Evadne spp.)*, decapod larvae, mysids and hydrozoa (*Obelia spp.*). Mesozooplankton are expressed as individuals per cubic meter.

Data analysis

All data analyses were performed with R statistical software (Core Team 2015). Decimal logarithm and square root transformations were used to tend towards linearity of response variables and to linearize the relationship with explanatory variables (Tableau 5).

An ANOVA was performed to test the effect of “Year” and sampling “Site” on the recruitment of observed spat abundances with Power Box-Cox transformation (lambda=-0.63). Normality and heteroscedasticity of residuals were checked by visual inspection. Oyster spat recruitment was graphically described using comparison of means with 95% confidence intervals.

The relationships between abundances of larvae at different stages (i.e. small, large, pediveliger, postlarvae and newly settled spat) were studied using a series of plot, statistical tests, namely non-parametric Spearman correlation tests and linear model. In addition, we used the ratio of pediveliger to spat abundance to quantify successful metamorphosis. The “Listel” sampling site was excluded from the D-larvae analysis because it is located outside the geographic location of shellfish farming, thus inducing very few small larvae supplies.

To study the influence of environmental (hydrological and planktonic) variables on the different life stages, we integrated the data over periods that matched the time scale of the process under study. Hence, to characterize the environment of the pelagic larval and settling phases, we averaged respectively the environmental variables over two periods: over a 22-day period before retrieval of the collector (suffix variable LARV for LARVal phase) and over a 14-day period preceding retrieval of the collector to characterize the environment during metamorphosis (suffix variable MET for METamorphosis). Small and large pelagic larvae abundances are represented with their maxima in both the LARV and MET periods.

A decision tree method was used to explore the links between target variables (abundance of pediveliger, abundance of spat and survival after metamorphosis) and environmental variables, with the package (party) provided by CRAN-R Project, with conditional inference trees (ctree) (Hothorn, Hornik & Zeileis 2006; Hothorn, Hornik, van de Wiel, et al. 2006). As only the first splitting path of the decision trees was significant, we split the dataset into two and performed simple graphical and statistical tests such as boxplots to observe the quantitative effects of significant variables on the dataset.

A Principal Component Analysis (PCA), based on plankton data with “Recruitment” factor representation, is presented to explore and illustrate interactions between the Pediveliger (“Pedi”) and “Spat” classes. Kruskall and Wallis tests were used to assess significant environmental factors on the classes “Pedi-”, “Pedi+”, “Spat-”,“Spat+” and significant “Favorable” or “Unfavorable” parameters of the recruitment windows to identify the optimum recruitment windows.

Tableau 5: Variables characterizing the interactions between the environment and larvae. Each environmental variable was averaged over a 22-day period preceding retrieval of the collector (suffix variable LARV for LARVal phase i.e. log_nano_LARV), and over a 2-week period preceding the retrieval of the collectors to characterize the environment for metamorphosis (suffix variable MET for METamorphosis i.e. log_nano_MET)

Variables	Description	Unity	Transformation	Abbreviation
Target variables				
oyster spat	abundance	ind. plate^{-1}	$\log_{10}(X+1)$ or power Box-Cox (lambda=-0.63)	log_spat or pbc_spat
postlarvae	abundance	ind. plate^{-1}	$\log_{10}(X+1)$	log_post
pediveligers	abundance	ind. plate^{-1}	$\log_{10}(X+1)$	log_pedi
Environmental variables				
max small larvae	maximum small pelagic larvae abundance	ind. m^{-3}	$\log_{10}(X+1)$	log_max_SL
max large larvae	maximum large pelagic larvae abundance	ind. m^{-3}	$\log_{10}(X+1)$	log_max_LL
pediveliger/spat survival	ratio of pediveliger abundance on oyster spat	-	-	-
oxygen concentration	daily average	mg l^{-1}	-	-
temperature	daily average	°C	-	-
salinity	daily average	No unit	-	-
bacteria	abundance	10^6 cell. l^{-1}	-	log_bact
total picoeukaryotes	abundance	10^6 cell. l^{-1}	square root(X)	sqrt_peuk_tot
total cyanophycae	abundance	10^6 cell. l^{-1}	square root(X)	sqrt_cyan
picoeukaryotes+ cyanophycae	abundance	10^6 cell. l^{-1}	$\log_{10}(X+1)$	log_pico_tot
nanophytoplankton	abundance	10^6 cell. l^{-1}	$\log_{10}(X+1)$	log_nano
cryptophycae	abundance	10^6 cell. l^{-1}	$\log_{10}(X+1)$	log_crypto
nanophytoplankton + cryptophycae	abundance	10^6 cell. l^{-1}	$\log_{10}(X+1)$	log_nano_tot
heterotrophic flagellates	abundance	cell l^{-1}	$\log_{10}(X+1)$	log_HF
ciliates	abundance	cell l^{-1}	$\log_{10}(X+1)$	log_ciliates
tintinnidae	abundance	cell l^{-1}	$\log_{10}(X+1)$	log_tinti
diatom	abundance	cell l^{-1}	$\log_{10}(X+1)$	log_diatom
dinoflagellates	abundance	cell l^{-1}	$\log_{10}(X+1)$	log_dinoflagellates
Chaetoceros	abundance	cell l^{-1}	$\log_{10}(X+1)$	log_chaetoceros
total chlorophyll *a*	biomass	µgChla l^{-1}	-	Total_chloa
picophytoplankton	biomass	µgChla l^{-1}	-	pico
nanophytoplankton	biomass	µgChla l^{-1}	-	nano_3_20
picophytoplankton+ nanophytoplankton	biomass	µgChla l^{-1}	-	nano_low20
microphytoplankton higher than 20µm	biomass	µg l^{-1}	-	micro
competitors	abundance	ind. m^{-3}	$\log_{10}(X+1)$	log_comp
predators	abundance	ind. m^{-3}	$\log_{10}(X+1)$	log_pred

Results

Remarkable abundance of Pacific oyster spat were observed each year at each of the four sites sampled in the Thau lagoon (Figure 21). Over the three years, we assessed 12 events of “medium” spat recruitment (21 to 200 individuals per plate), 19 events of “low” recruitment (1 to 20 individuals per plate^{-1}) and 65 events of “null” recruitment. The analysis was performed using the whole dataset over the three-year period but spat abundances did not differ significantly between years (Tableau 6). In contrast, the "Site" factor had a significant effect on oyster spat abundances (pbc_spat, lambda = -0.63, pvalue < 0.05) (Tableau 6).

Tableau 6: Results from an ANOVA examining the effect “Year” and “Site” factors on spat abundance per plate (Power Box-Cox transformation (lambda=-0.63, n = 96). Df: degree of freedom, SS: Sum of squares, MS: Mean square.

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Site	3	2.528	0.8425	3.353	0.0227*
Year	2	0.080	0.0400	0.159	0.8529
Site:Year	6	3.210	0.5351	2.130	0.0582.
Residuals	84	21.105	0.2512		
Signif. codes:	'***'0.001	'**'0.01	'*'0.05	'.'0.1	' '1

In 2012, two "medium" intensity spatfall events occurred, the first in mid-August, with 126 and 47 individuals per plate at the Listel and Bouzigues sites, respectively, the second at the end of September with 45 and 21 ind. plate^{-1} at Bouzigues and Listel sites, respectively (Figure 21). In 2013, there was a single “medium” spatfall event lasting from the middle to the end of August, but at all four sites, with maximum values of 188, 187, 91 and 49 ind. plate^{-1} at Bouzigues, Marseillan Listel and Meze, respectively. In 2014, a “medium” spatfall event occurred at Listel only, but lasted longer, from the end of August to the end of September. To summarize, “medium” spatfall events were observed each year at Listel, in two years at Bouzigues and only in one year at Marseillan and Meze. It is interesting to note that “medium” spat recruitment occurred at any time from August to September, but never in July nor October.

Significant correlations were found between the abundance of small D larvae and the abundance of pediveligers, specifically in the shellfish farmed area (Figure 22a, $n = 75$, $\rho = 0.44$, P- value < 0. 001). At the lagoon scale, a significant correlation was found between large larvae and pediveligers (Figure 22b, $n = 100$, $\rho = 0.45$, P-value < 0.001). The relationship between large larvae and pediveliger abundance, assessed using a linear model, was strong (pediveligers = large larvae$^{0.864}$ on average).

Pediveliger and postlarvae abundances were on average significantly correlated (Figure 22c, $n = 100$, $\rho = 0.47$, $R^2 = 31\%$, P-value < 0.001) and the relationship between pediveligers and postlarvae (Postlarvae = Pedi$^{0.41}$) was successful or ranged from low to zero (failure of metamorphosis). Among 69 harvest events with less than two spats (“Spat -”), 26 (38%) had a relatively high number of pediveligers two weeks before (>20 ind. plate^{-1}, i.e. “Pedi +”) whereas 43 (62%) had not (<20 ind. plate^{-1}, i.e., “Pedi -”).

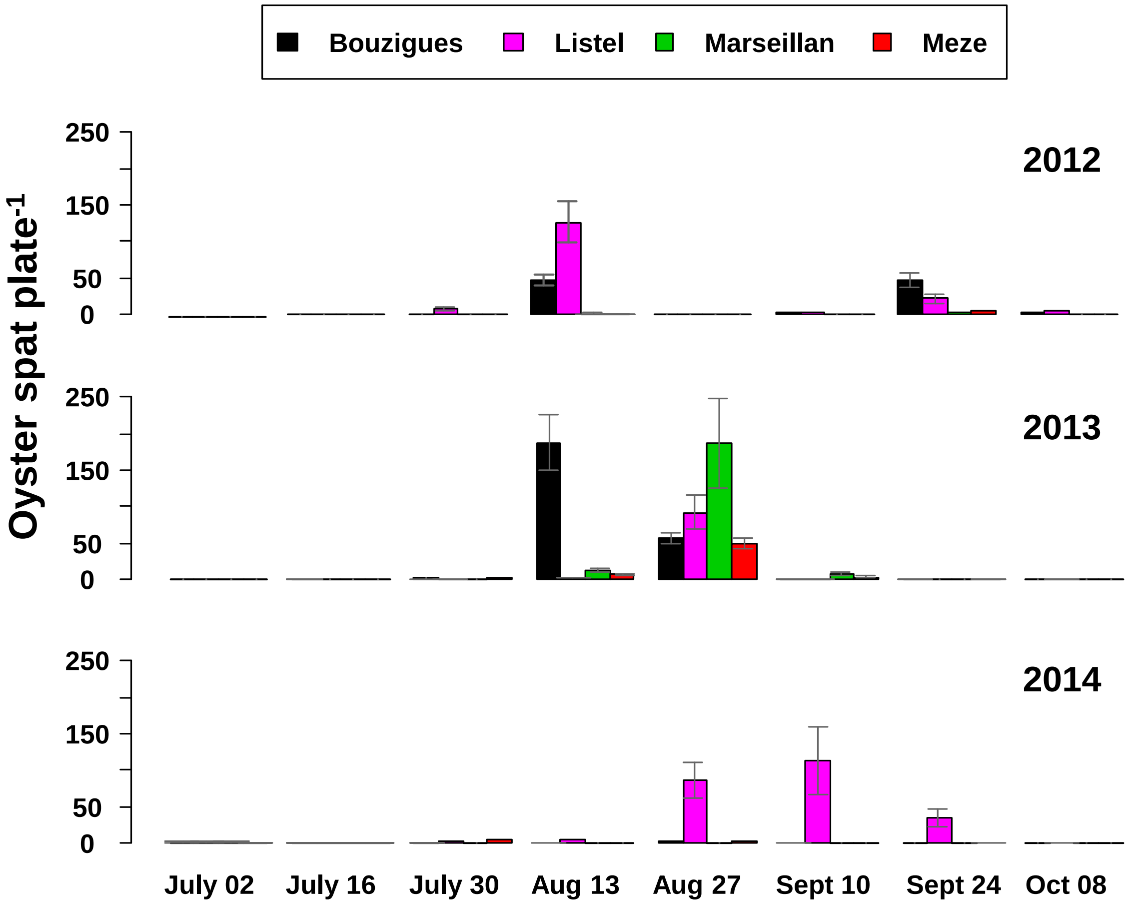

Figure 21: Barplot of means and error bars (95% confidence intervals) of spat abundance per plate observed at the four spatfall sampling sites (Bouzigues, Listel, Marseillan and Meze), at two week intervals throughout the summer (2012, 2013, 2014). Spat abundances were estimated after one month of immersion (n=54 per date & sampling site).

Oyster "spat" abundances were highly correlated with "postlarvae" abundances (Figure 22d, $n = 96$, $\rho = 0.69$, $R^2 = 65\%$, P-value < 0.001). The linear model between postlarvae and oyster spat was, on average, defined as $Spat = Postlarvae^{0.59}$.

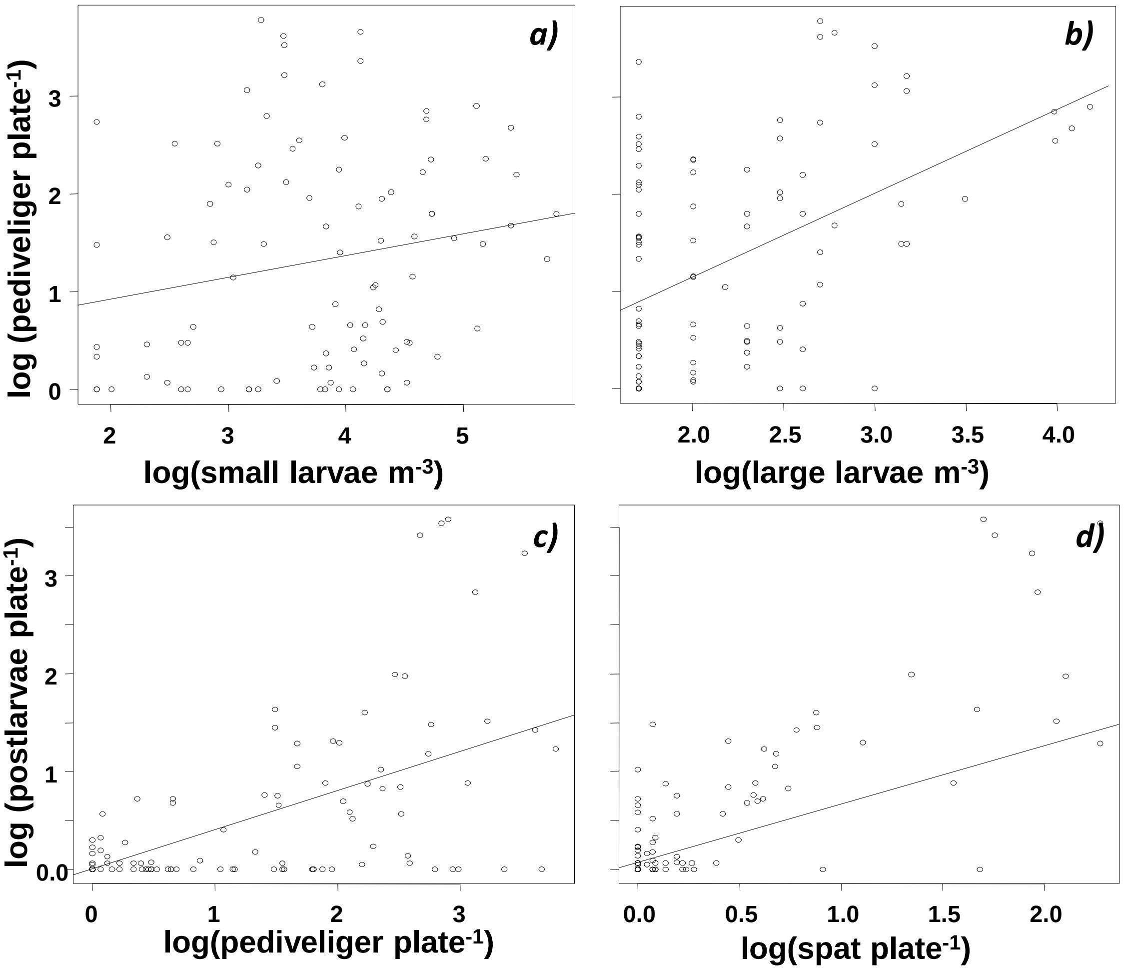

Figure 22: Scatterplot and linear model of relationships between "pediveliger" and (a) "small larvae", (b) "large larvae", and "postlarvae" and (c) “pediveliger” or (d) "spat" abundances for the years 2012, 2013 and 2014.

Concerning environmental drivers and based on analyses of the decision trees, we found that (1) spat abundance was significantly higher (p-value = 0.034) when the temperature (throughout the larval pelagic period-LARV) was above 26.5°C, (Figure 23a); (2) the abundance of pediveligers was significantly higher (p-value = 0.012) above a threshold value of *Chaetoceros* abundance (~ 345 10^3 cells l^{-1}, Figure 23b) during the larval cycle and; (3) the success of metamorphosis survival was significantly higher (p-value = 0.014) with high nanophytoplankton biomass (throughout the period of metamorphosis (MET), >4.3 10^6 cells l^{-1}, Figure 23c).

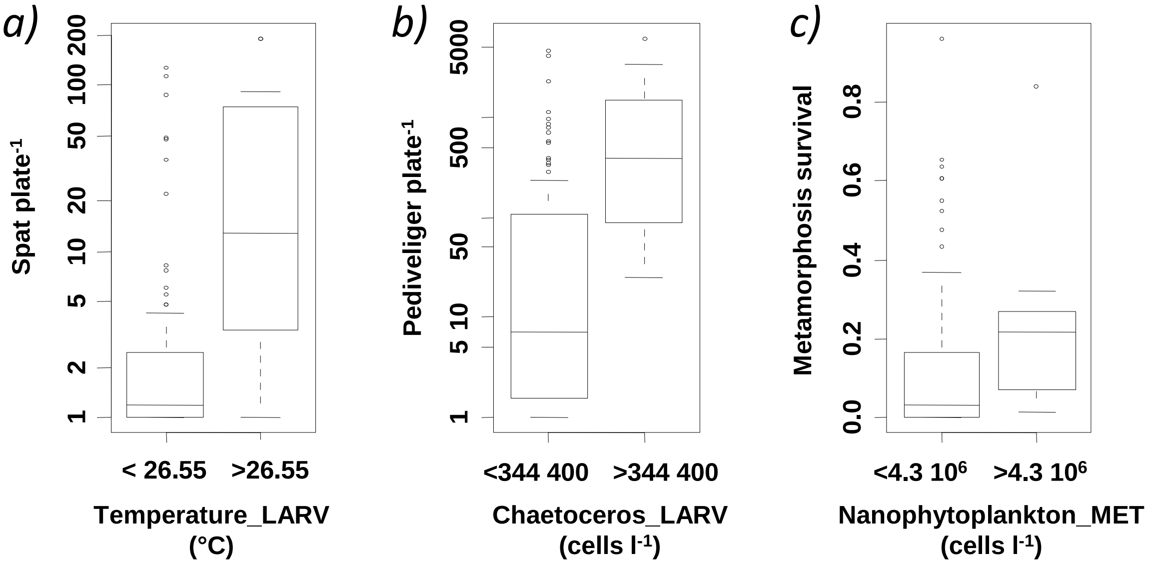

Figure 23: Boxplots of environmental and planktonic variables that have a significant effect on pre-and post-settled stages. Effect of (a) temperature averaged over the larval period (Temperature_LARV) on spat abundance, (b) *Chaetoceros* abundance averaged over the larval period (Chaetoceros_LARV) on the abundance of pediveliger and (c) Nanophytoplankton abundance averaged over the metamorphosis period (Nanophytoplankton_MET) on metamorphosis survival.

A Principal Component Analysis of the environmental dataset with the "recruitment" factor (categorized as Pedi-, Pedi+Spat-, and Pedi+Spat+ terms) showed that the first two axes explained 49.4% of the total inertia of our dataset (Figure 24). The first axis was built with the contributions of nanophytoplankton biomass (nano_low20^{B}, nano_3_20^{B}) and abundances (log_nano^{A}, log nanotot^{A}) and [chlorophyll *a*] biomass (total chla^{B}). This first axis can be seen as a temporal gradient representative of fauna, flora and hydrological successions (Figure 24). The second axis separated picophytoplankton abundance (sqrt_peuk_tot^{A}, log_pico^{A}), biomass (pico^{B}) and cryptophyte abundance (log_crypto^{A}) from microphytoplankton >20 µm biomass (micro^{B}) and *Chaetoceros* abundance (log_chaetoceros^{A}). On this axis, ciliate and dinoflagellate abundances are also opposed to picoeukaryote abundances, heterotrophic flagellate abundance and picophytoplankton biomass. Their contribution to the building of the axis was however, low. The second axis can be seen as a spatial gradient reflecting the effect of lagoon confinement and the effect of the shellfish biocoenosis on hydrobiology.

The barycenters of our recruitment factors "Pedi-", "Pedi-Spat-", "Pedi+Spat+" occurrences are well differentiated on the first PCA axis (Figure 24). Pediveliger abundance

(Pedi-, Pedi+) was mostly correlated with the second axis, with positive effects of *Chaetoceros* abundance (Figure 24a), microphytoplankton and nanophytoplankton, and negative effects of picophytoplankton (abundance and biomass) and cryptophyte abundance. The success of metamorphosis, *i.e.*, the "Spat-" and "Spat+" terms, was mostly differentiated on the first axis, with positive effects of nanophytoplankton biomass.

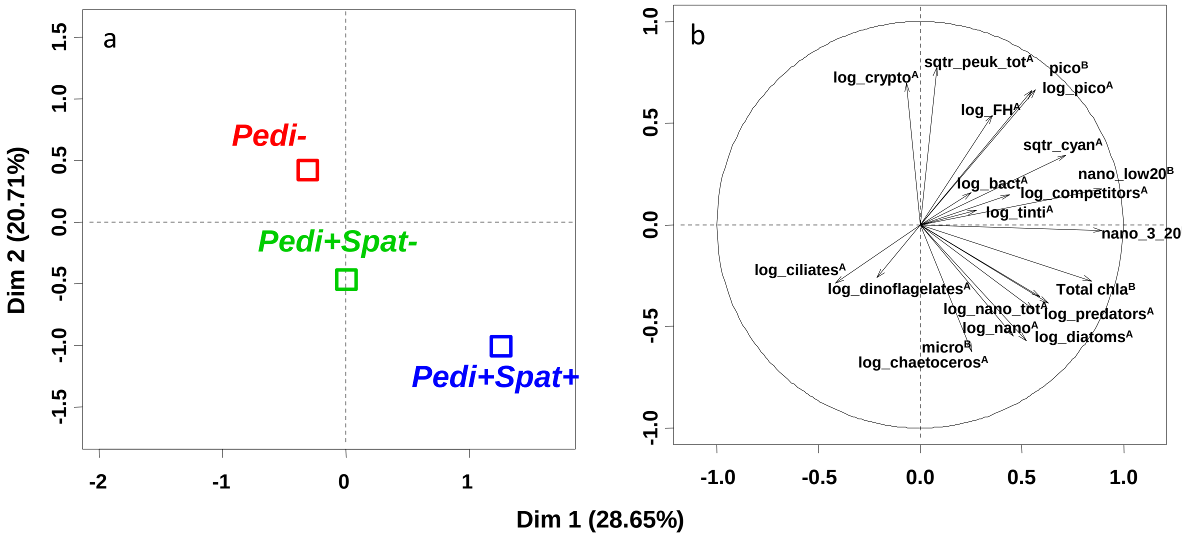

Figure 24: Principal component analysis plots of (a) recruitment factors and (b) correlation circle of environmental transformed data integrated over a 22 d period before retrieval of the collector (superscript A: abundance; B: biomass). Recruitment factor categorizes Pacific oyster pediveliger and spat abundances 'Pedi−' and 'Spat−' by aggregating the abundances from 0 to 20 ind. plate−1, and 'Pedi+' and 'Spat+' by aggregating abundances with >20 ind. plate−1

Non-parametric analyses (Kruskall and Wallis test) of the whole dataset distinguished seven variables with significant effects on "recruitment" (p-value<0.05). All seven variables were integrated over the 3-week pelagic larval phase (i.e., _LARV).

High pediveliger abundance and/or successful metamorphosis were observed when picoeukaryote abundance was low (Figure 25a), or when total nanophytoplankton levels (Figure 25b) or diatom levels (Figure 25c) were high. Seventy nine percent of the variability of diatom abundances (P-value < 0.001) was explained by *Chaetoceros* abundances. The effect of microphytoplankton abundance on recruitment (not shown) was similar to the effect of diatoms.

Concerning hydrological variables, we found no effect on pediveliger supply but a significant effect of oxygen concentrations and temperature on recruitment, with more

"Pedi+Spat+" occurrence at lower oxygen concentrations (Figure 26a) and higher temperatures (Figure 26b).

The changes in recruitment over time (aggregated over the three sampling years) in each of the three recruitment categories "Pedi-", "Pedi-Spat+", "Pedi+Spat+" defined above suggested a strong summer pattern independent of the year (Tableau 7): no "Pedi+Spat+" occurrences were observed in weeks 24 to 29 (June-July) whereas weeks 31 to 37 (August and September) saw the occurrence of "Pedi+Spat+". Conditions were unfavorable at the end of the season (> week 38), with no "Pedi+Spat+" occurrence.

Tableau 7: Evolution of changes in recruitment categories over time. Recruitment factor categorizes pediveliger and spat abundances Pedi – and Spat – by aggregating the abundances from 0 to 20 individuals per plate, and Pedi + and Spat + by aggregating the abundances with more than 20 individuals per plate. See Figure 24 for definitions

		Timeline															
Months		June		July				August					September				Oct
Weeks		24	26	27	28	29	30	31	32	33	34	35	36	37	38	39	41
Recruitment factor	Pedi-	4	4	0	4	0	3	2	2	3	5	3	7	0	5	1	3
	Pedi+ Spat-	4	4	4	4	4	4	0	1	1	2	1	0	2	3	3	1
	Pedi+ Spat+	0	0	0	0	0	1	2	5	0	1	0	1	2	0	0	0
Recruitment windows		Unfavorable					Favorable								Unfavorable		

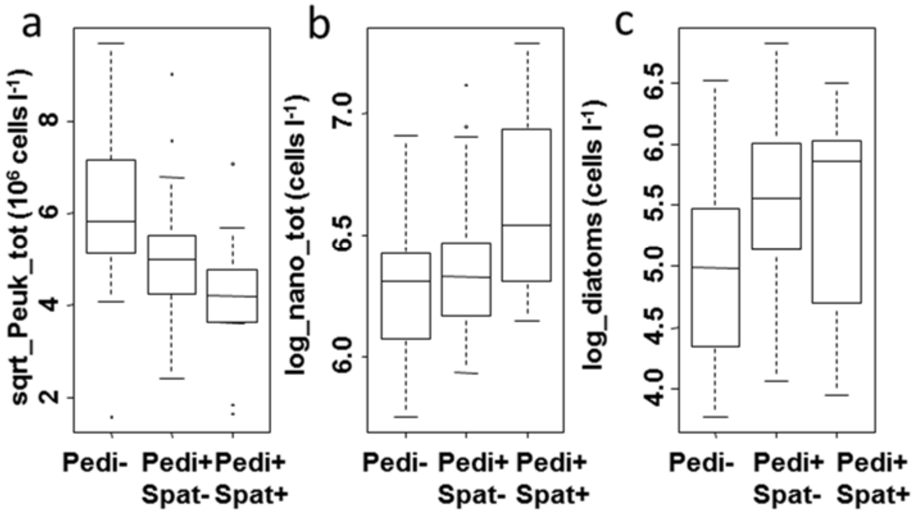

Figure 25: Plankton variables that had a significant effect on oyster "Recruitment". The values on the y-axis are (a) the square root values of total picoeukaryotes abundance and (b, c) the log to base 10 values of total nanophytoplankton and diatoms abundances. ($n_{Pedi-} = 37$, $n_{Pedi+Spat-} = 26$ and $n_{Pedi+Spat+} = 9$)

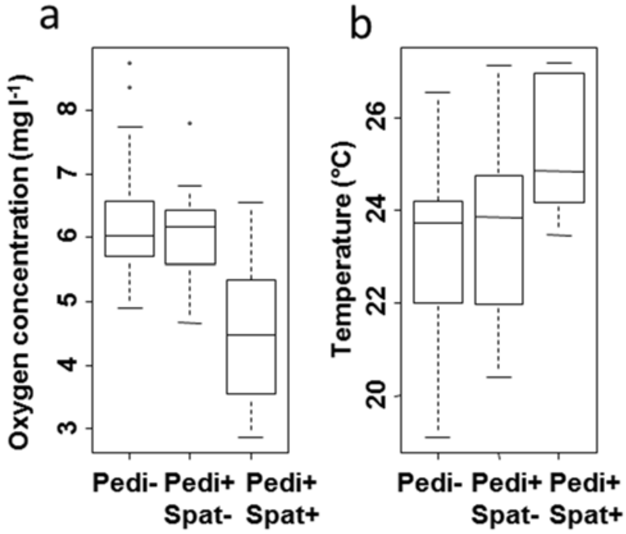

Figure 26: Environmental variables that had a significant effect on oyster recruitment according to oxygen concentration ($n_{Pedi-Spat-}=27$, $n_{Pedi-Spat+}=18$, $n_{Pedi+Spat+}=6$) and temperature ($n_{Pedi-Spat-}=46$, $n_{Pedi-Spat+}=38$, $n_{Pedi+Spat+}=12$). See Figure 24 for definitions.

Kruskall-Wallis tests based on these new categories (favorable *vs.* unfavorable windows) revealed a significant difference (p-value < 0.05) for 13 of the 46 environmental variables: the three hydrological variables (temperature, oxygen and salinity) and the 10 transformed planktonic variables.

Temporal windows that favor recruitment are associated with lower oxygen concentrations, higher temperatures and higher salinity, higher chlorophyll *a,* pico- and nanophytoplankton biomass, higher abundances of heterotrophic flagellates and trophic competitors, and lower abundances of ciliates and dinoflagellates (Figure 27). The effects of unfavorable or favorable windows were similar for picocyanobacteria, picoeukaryote and picophytoplankton abundances. Only picophytoplankton results are presented here.

In unfavorable periods, ciliates were mainly represented by higher abundances of naked ciliates like Scuticociliates (1100 cells l^{-1} including Uronematidae, Philasteridae, Balanion), *Laboea spp., Favella_erhenbergii* and tintinnid ciliates like *Eutintinus spp.* and *Tintinnopsis spp*. In the same period, dinoflagellates were represented by non-toxic species such as *Gyrodinium spirale* and *Prorocentrum micans* (both around 300 cell l^{-1}), *Protoperidinium bipes* (around 40 cells l^{1}).

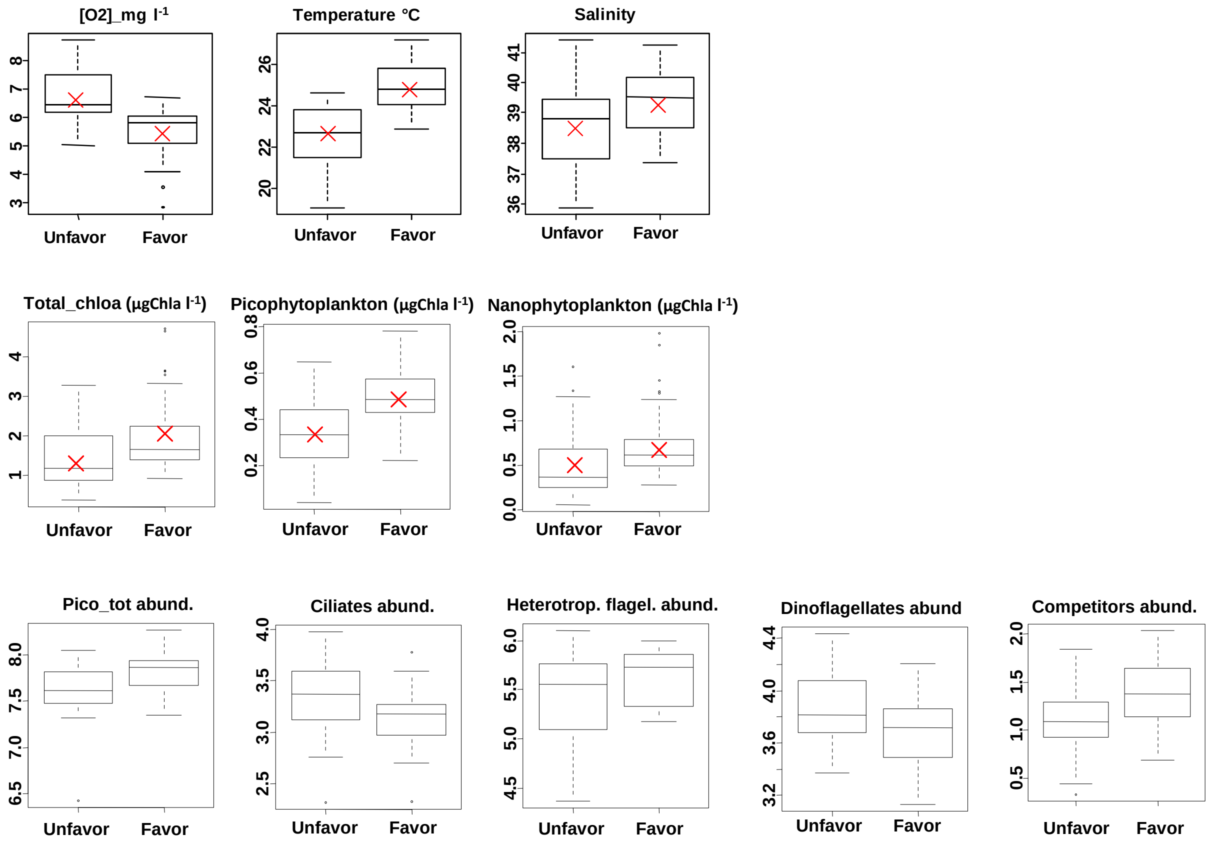

Figure 27: Environmental and planktonic abundances that showed a significant difference ($p < 0.05$) in favorable ($n = 36$) vs. unfavorable ($n = 36$) temporal recruitment windows for Pacific oyster. Total_chloa, pico- and nanophytoplankton are biomass expressed in µg Chla l^{-1}; pico_tot (picoeukaryotes+ cyanophycae) abundance is in 10^6 cells l^{-1}; ciliates, heterotrophic flagellates and dinoflagellates in $\log_{10}$ cells l^{-1}; competitors in $\log_{10}$ ind. m^{-3}. Mid-line: median; box: 25th and 75th percentiles, whiskers: 1.5× the interquartile range; circles: outliers; red crosses: means.

Discussion

Our general objective was to study recruitment by monitoring the different larval stages and exploring the effects of lagoon environmental conditions on successful larval development and recruitment. The present study showed that there was no statistical difference in spat abundance at an inter-annual scale but that there was a strong temporal variability at an intra-annual scale, with clear favorable versus unfavorable windows. The unfavorable windows (June-July) showed good supplies of larvae (more than 20 individuals per plate) but metamorphosis failure, suggesting a possible biological obstacle at this step. Early summer months are usually characterized by rising temperatures, and increasing metabolism of poikilotherm filter feeders. Consequently, only small quantities of nano- and microplankton are available during that period since nano- and microplankton are mainly consumed by filter feeders (Dupuy et al. 2000). Indeed, the unfavorable recruitment windows in early summer were characterized by low pico-, nano- and microphytoplankton biomass. We therefore suggest that a top-down control of primary production by filter feeders to explain the observed unfavorable recruitment period in Thau lagoon.

Picoplankton also support grazing by ciliate and flagellate protists (Bec et al. 2005; Lam-Hoai et al. 1997). A few authors have reported a negative impact of ciliates on the development of mollusk bivalve spat (inhibition of settlement) (Shimeta et al. 2012; Elston et al. 1999; Plunket & Hidu 1978). Our results support this hypothesis since a negative correlation was observed between the abundance of ciliates and the metamorphosis success of *C. gigas*.

Dinoflagellate and diatom population dynamics are known to be asynchronous in lagoons with respect to nutrient inputs (Collos et al. 2008). In the present study, the unfavorable recruitment window was characterized by high abundances of mixotrophic dinoflagellates during the favorable period even though summer is not the best ecological window for dinoflagellates (Collos et al. 2008). We suggest that high concentrations of chemical contaminants, such as herbicide used in antifouling boat paints or in agriculture, favor these mixotrophic and heterotrophic organisms. Dinoflagellates are part of the diet of both oyster adults and spat (Baldwin 1991; Baldwin & Newell 1995) but can inhibit the larval development and recruitment of bivalve mollusks (Mizuno et al. 2015; Mu & Li 2013).

The unfavorable recruitment window preceded a favorable window that systematically lasted from late July to early September. This favorable window is characterized by the availability of trophic variables for oyster larvae like the abundance of nanophytoplankton and *Chaetoceros spp*. Larval food sources of *Crassostrea spp.* in the natural environment are multiple (types and particle sizes) and their consumption varies with larval stages: bacteria, phytoplankton, particulate and dissolved organic matter, protozoa (Douillet 1993; Raby et al. 1997; Sommer et al. 2000). At the beginning of August, warmer conditions (temperature > 25 °C) could cause a shift in the regeneration of organic matter and in remineralization favoring fast growing diatoms (Collos et al. 2003), most of which are *Chaetoceros spp.* (Collos 1986; Collos et al. 1997). As a result, the concentrations of nano- and microphytoplankton including diatoms are high and favor successful *C. gigas* recruitment with, this time, a bottom-up control of phytoplankton on larvae. The relationship between various pelagic cues and the improvement of benthic invertebrate recruitment is well known (Miron et al. 1995; Pineda et al. 2010).

Particularly, the “trophic settlement trigger” concept could indirectly affects recruitment by strongly improving the settlement rate of pediveliger larvae (Toupoint, Gilmore-Solomon, et al. 2012; Toupoint, Mohit, et al. 2012). In addition to the match/mismatch theory (Cushing 1990), this concept show the benefit of pelagic cues in favor of settlement success. Without augured benefit for *Chaetoceros* on pelagic larval development, our results appears to support the trophic settlement trigger hypothesis induced with the significant relationship between this diatom genus and pediveliger abundance of *Crassostrea gigas*.

At spatial scale, our results showed extreme heterogeneity of oyster spat collection between the sampling sites. The ecological conditions within the Thau lagoon are known to be highly contrasted (Troussellier & Deslous-Paoli 2001) and the spatial organization of oyster recruitment will consequently need to be studied more precisely. First, the exchange of water with the open sea and the circulation of water within the lagoon (Guelorget et al. 1994; Lam-Hoai et al. 1997) define the habitat and its ecological niches depending on the proportion of marine and lagoon water volumes (Fiandrino et al. 2017). In addition, the variability of the plankton communities in space and over time is significantly influenced by the hydrodynamic conditions, which in turn, depend on the wind (Millet & Cecchi 1992; Troussellier et al. 1993). Hydrodynamic circulation patterns are also one of the main factors that influence larval dispersal and spat distribution (Larsson et al. 2016; Hubbard & Reidenbach 2015; North et al. 2008). The use of hydrodynamic models, such as Mars 3D model (Lazure & Dumas 2008; Fiandrino et al. 2017) would prove useful to quantify larval dispersal and connectivity once adapted for lagoon ecosystems in semi-closed nanotidal regimes. On the other hand, intensive shellfish farmed communities structure the interactions between species and the supply of mollusk larvae (Borsa & Millet 1992). The Pacific oyster is an ecosystem engineer and keystone species, and has a significant impact on its own ecosystem, particularly at high density in an enclosed system (Mazouni, Gaertner, et al. 1998; Souchu et al. 2001; Bec et al. 2005). Significant chlorophyll *a* and particulate organic carbon depletion were measured in the shellfish farming zone (Souchu et al. 2001) along with a reduction in microzooplankton biomass compared with that in the middle of the lagoon (Lam-Hoai et al. 1997). Oyster larvae take place in the microzooplankton compartment and a full study of the effect of the breeding zone on the structure of oyster recruitment is now needed after this first ecological approach.

Mass mortalities in oyster juveniles raised concerns about cultivation practices in Mediterranean lagoons, based on the dependency of the Thau basin on supplies of oyster juveniles from the Atlantic and from hatcheries. For this reason, the reproduction features (Ubertini et al. 2017) and natural recruitment of *Crassostrea gigas* (Lagarde, Fiandrino, et al. 2015) was observed for the first time in the Mediterranean Thau lagoon through this three-year study (2012 to 2014). This appears as an important breakthrough because the discovery of spat-producing areas at a sampling site outside the shellfish farm areas points to the possibility of new spat collection activities in Mediterranean lagoons, which, until now, were restricted to shellfish farming zones and were not financially profitable.

In these specific ecosystem, the reproduction windows stretches from the beginning of June to the end of September (Ubertini et al. 2017). Phytoplankton concentrations and assemblages affected gametogenesis with diatoms having a positive effect. No spawning event was observed below 22 °C. The temperature of 23 °C appears as a temperature threshold for the occurrence of significant spawning events. According to Ubertini et al (2017), the full and dark moon in combination with high temperature may enhance spawning events. When recruitment occurred, spat collection levels in the Thau lagoon were comparable with those in other French traditional breeding basins. From an economic perspective, spatfall events were indeed classified in terms of recruitment from low (up to 20 individuals per plate) to medium (up to 200 individuals per plate) according to the classification proposed by the French Oyster larvae monitoring network in the VELYGER project (Pouvreau, Petton, et al. 2013; Pouvreau et al. 2016). Such spatfall events may therefore be profitable for the local industry (Lagarde, Gervasoni, et al. 2015). In addition, there are other benefits in using native oysters. Native oysters may, for instance, have a better resistance to OsHv1-*µvar* (Petton, Bruto, et al. 2015). The use of native oysters may also be considered a more eco-friendly practice for spat supply.

From an industrial point of view, the discovery of spat producing areas at a sampling site outside shellfish farming area means that new spat collection activities in Mediterranean lagoons may be possible. Up to now, spat collection has been restricted to shellfish farming zones but were not profitable. Further technical experiments are necessary, at an industrial scale, to improve the yield of this emergent shellfish farming practice.

Coastal lagoons are particularly sensitive to inputs of nutrients of anthropogenic origin (Knoppers B. 1994; Cloern 2001; Kennish & Paerl 2010) due to restricted exchanges with the sea and intrinsic hydrodynamic patterns (Boesch 2002; Glibert et al. 2011). In French Mediterranean lagoons, anthropogenic nutrient inputs have been reduced leading to an oligotrophication dynamic (Leruste et al. 2016). Some authors suggest that the combination of global warming and reduced nutrient loads will reduce phytoplankton biomass, which would benefit picocyanobacteria (Collos et al. 2009). In the Thau lagoon, the expected shift in the ecological community structure would presumably change the newly defined optimum recruitment window for the Pacific oyster. Results from the present study showed that recruitment success or failure, in a Mediterranean lagoon, is related to nursery function of summer biocoenosis trajectories of a Mediterranean lagoon. Upcoming experiments will define the *in situ* tolerance limits of *Crassostrea gigas*, in a strongly fluctuating ecosystem, at the frontiers of its ecological niche.

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Chapitre 3 : Influence de l'environnement trophique sur la taille à la métamorphose et les performances de recrutement de l'huître creuse

Ce troisième chapitre a été accepté pour publication dans la revue *Marine Ecology Progress Series* le jeudi 14 juin 2018.

Résumé de la publication en français

La reproduction et le recrutement des invertébrés benthiques sont influencés par le climat et par les structures écologiques des écosystèmes marins eux-mêmes sous influence des pressions anthropiques locales telles que l'eutrophisation ou l'oligotrophisation.

En utilisant l'huître creuse *Crassostrea gigas* comme modèle biologique, nous avons testé l'hypothèse que la variabilité de la taille des coquilles de prodissoconque II (la taille de PII se réfère à la taille à la métamorphose) dépend du fonctionnement écologique de l'écosystème. La fixation des larves et le recrutement de naissain ont été évalués sur cinq sites d'échantillonnage de la lagune de Thau, en Méditerranée, pendant les trois principaux mois de recrutement, au cours de trois années (2012-2014). Des analyses hydrobiologiques et planctoniques ont été effectuées sur trois sites d'échantillonnage. Nos résultats ont montré que le recrutement était extrêmement hétérogène, allant de 0 à 260 ± 27 ind. dm^{-2} dans l'ensemble de l'écosystème et qu'il était lié à la variabilité de la taille des PII (180 à 296 µm). Le patron temporel annuel des tailles de coquilles de PII a été influencé par la température pendant la période de fixation de larves, alors que le patron spatial dépendait de la biomasse du phytoplancton et du fonctionnement trophique de l'écosystème. Les plus petites tailles de PII étaient significativement corrélées avec la biomasse de phytoplancton la plus élevée, tandis que les plus grandes tailles étaient positivement corrélées avec l'abondance de cryptophytes mixotrophes. Nous avons trouvé une relation inverse entre la taille de PII et la survie après la métamorphose, ce qui montre que le succès du recrutement est associé à des tailles de PII plus petites. Les conditions climatiques régionales et le fonctionnement trophique local semblent être des facteurs clés dans la métamorphose et contribuent par conséquent à l'hétérogénéité spatio-temporelle du recrutement. D'autres études pourraient être réalisées dans d'autres écosystèmes suivant une trajectoire d'oligotrophisation pour généraliser ce résultat.

Trophic environments influence size at metamorphosis and recruitment performance of the Pacific oyster

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Introduction

While the impacts of eutrophication were a preoccupation in the 2000s (Nixon 1995; De Jonge & Elliott 2001; Smith & Schindler 2008), increased demand for the recovery of ecosystem services (Bullock et al. 2011) and good environmental status (Vethaak et al. 2017) have raised new questions about the repercussions of oligotrophication on community structure (Lie et al. 2011; Saeck et al. 2013) and about the function of some coastal ecosystems (Jeppesen et al. 1998; Jeppesen et al. 2005; Duarte et al. 2009; Yanagi 2015). French Mediterranean lagoons (Collos et al. 2009; Leruste et al. 2016), like some Japanese shellfish farmed estuaries and bays (Yanagi 2015), are subject to oligotrophication processes caused by wastewater management. A decrease in nutrient inputs into Mediterranean lagoons led to a decrease in phytoplankton biomass and hence to a shift in the structure of phytoplankton community from diatoms, cryptophytes and green algae to mixotrophic dinophytes (Leruste et al. 2016; Gowen et al. 2015), toxic dinoflagellates and picocyanobacteria (Collos et al. 2009). Changes in the ecological structure or ecological function of phytoplankton communities may have consequences for the food web (Cloern 2001; Alvarez-Cobelas & Rojo 2000). A reduction in phytoplankton biomass could cause major problems and could jeopardize the productive carrying capacities of coastal ecosystems that are exploited for shellfish farming, like in the Thau lagoon and hence their sustainability (Dame 2012).

We suggest that these impacts may be accentuated in the first development stages of bivalves, as larvae and newly settled juveniles have been widely shown to be the most susceptible to stressful conditions (Jenewein & Gosselin 2013; Qiu et al. 2002; Rayssac et al. 2010). Their susceptibility can be expressed in many ways, but is usually reflected in growth and survival. In the Pacific oyster *Crassostrea gigas*, newly referred to as *Magallana gigas*, a planktotrophic stage corresponds to a pelagic free-swimming period during which the veliger larvae feed, grow and disperse via water currents generally for a period of between two and four weeks (Kennish et al. 1995; Bhaud 2000). Near the end of its planktonic period, the advanced veliger develop competent characteristics, like feet for territorial prospecting behavior (pediveliger stage) and become able to settle (Pechenik & Heyman 1987; Hadfield et al. 2001) followed by metamorphosis, when they are transformed into post larval benthic mollusks (Zardus & Martel 2002; Bishop et al. 2006; Pechenik 2006). In marine invertebrates, metamorphosis integrated early ontogenic factors and environmental selection (Bishop et al. 2006) and is generally achieved in oysters within 1^{-3} days (Baker & Mann 1998). In bivalves, metamorphosis is defined as the morphological change associated with the attachment of the individual to a substrate and the secretion of a dissoconch shell (Bayne 1976). Metamorphosis involves four stages, 1) attachment to the substrate by the foot, 2) a change in the particle collecting structure from velum to completely developed heterorhabdic gills (Cannuel & Beninger 2006), 3) shell growth beyond the prodissoconch 4) loss of all larval organs to complete metamorphosis into a fully developed juvenile (Baker & Mann 1994). Metamorphosis is triggered by chemical and/or physical cues when conditions allowing the morphological transformation are favorable, e.g. a suitable habitat, thermal and hyaline requirements, the quantity and quality of available food (Bishop et al. 2006; Toupoint, Gilmore-Solomon, et al. 2012; Pechenik 1990). The lack of settlement/metamorphosis cues may delay metamorphosis, thereby prolonging the larval stage until a suitable environment is found (Pechenik 2006; Coon et al. 1990). Thus, size at metamorphosis estimated by the prodissoconch II (PII) measurements can be highly variable (Martel et al. 2014; Martel et al. 1995; Coon et al. 1990). Although significant research has been conducted on *Mytilus edulis* and suggests that trophic factors affect settlement/size at metamorphosis (Martel et al. 2014), to date, no study has been conducted on oysters, one of the most economically important groups of invertebrates. The purpose of our study was thus to test the hypothesis that the size of *C. gigas* at metamorphosis is linked to specific environmental/trophic conditions that may affect the success of subsequent recruitment. The Thau lagoon was selected to test this hypothesis because of its high temporal and spatial ecological heterogeneity that affect oyster recruitment (Lagarde et al. 2017).

The specific objective of this study was to characterize the spatial and temporal variability of oyster spat recruitment to (i) test the existence of variation of PII size in the Thau lagoon, (ii) evaluate the relationship between this variation and environmental and/or trophic conditions and (iii*)* assess its impact on recruitment success. The originality of this study is the simultaneous monitoring of larvae, juveniles (abundance and survival), size at metamorphosis, hydrobiological conditions and planktonic characteristics in the same species in three different years (2012 to 2014) at five different sampling sites.

Materials and Methods

Thau lagoon and sampling sites

The Thau lagoon is the largest nanotidal lagoon in the Occitanie region (formerly known as Languedoc-Roussillon) in southern France. It covers an area of 7,500 ha (19 km × 4.5 km) orientated along a north-east / southwest axis and has a mean depth of 3.5 m. Seawater from the Mediterranean Sea enters through narrow channels. Five sampling sites were monitored to assess pre-settled oyster larvae and post-settled spat abundances in pelagic and benthic habitats: two sites with collectors suspended inside shellfish farming structures (Bouzigues and Marseillan) and three with collectors suspended outside structures anchored on specially designed mooring systems (Listel, Meze_osfz and Balaruc) (Figure 28). Hydrological and planktonic data were monitored at the three sampling sites Bouzigues, Marseillan and Listel.

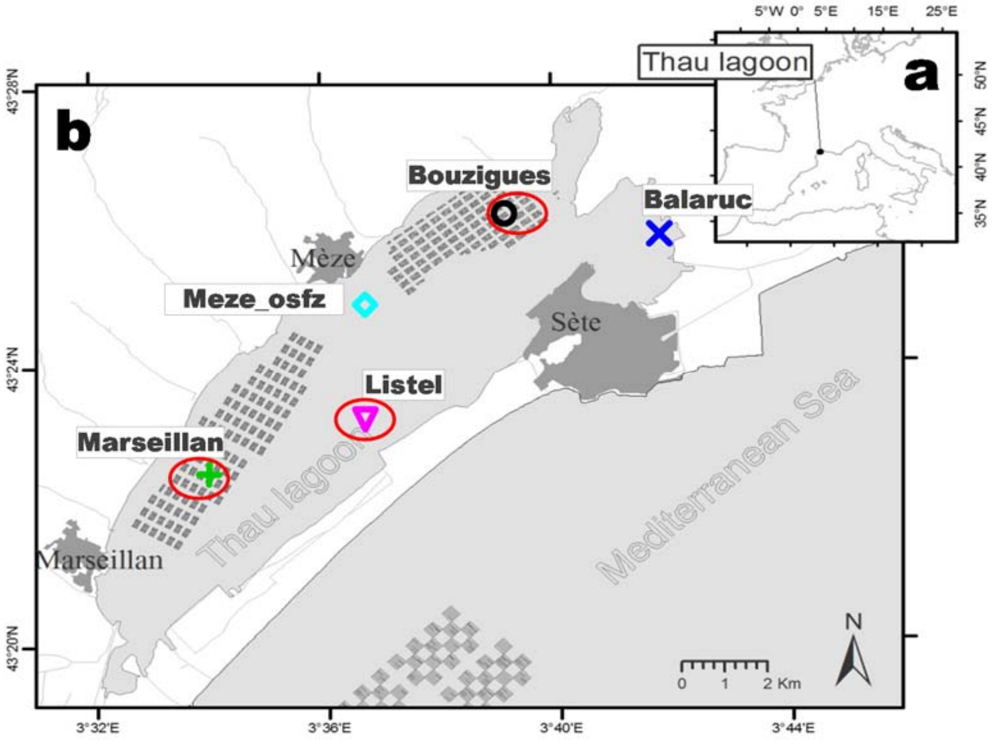

Figure 28: (a) The Mediterranean Thau lagoon in France and (b) sampling sites in Thau lagoon. The five sampling sites where benthic Pacific oyster larvae and juvenile abundances were monitored are (Marseillan, green cross; Meze_osfz, cyan diamond; Bouzigues, black circle and Listel, blue cross). Red circles show the location of the hydrological and plankton sampling sites. Grey boxes indicate the location of shellfish farms.

Abundance of young settlers and survival rate at metamorphosis

As reported by Lagarde et al. (2017), young oyster abundances were estimated every two weeks at three different settler stages: pre-settled larvae (pediveliger), young postlarvae and newly settled juveniles (Arakawa 1990b) from June to September in 2012, 2013 and 2014. An original method with a temporal overlap of collector deployment was used (Hughes et al. 2000; Arnold & Steneck 2011; Lagarde et al. 2017). Pediveligers showed prodissoconch 2 (PII) shell sizes ranging from 180 to 300 µm, young postlarvae were strictly benthic and cemented with a dissoconch shell ranging from 300 to 1,000 µm and newly settled juveniles (maximum four weeks old) measured from 1 to 8 mm. To collect these settler stages, the sites were equipped with three replicated sets of two collectors each measuring 110 cm composed of 44 white plates (15 cm diameter; surface area 250 cm^2, Figure 29).

The collectors were vertically submerged 2 m below the surface, suspended inside shellfish farming structures, or outside farming structures (see Figure 29). The systems were designed to support two sets of collectors immersed for two or four weeks. Pediveliger and post larvae abundance was assessed on the 2-week collectors and oyster juvenile abundance was assessed on the 4-week collectors. After being sampled, the 4-week collector was replaced with a new one, i.e. a replacement every two weeks throughout the summer. Each collector was sampled at three vertical levels: close to the top (39th plate), in the middle (22th plate) and at the bottom (5th plate). Both faces of the plates (above and below) were examined under a binocular microscope to assess the mean abundance of pediveliger, post-larvae and newly settled juveniles, or macroscopically when possible. On each occasion, counting was evaluated on subunits, i.e. from one to four basic subunits (15 to 60 cm^2), replicated three times per face (Figure 29b). The abundance of each stage per plate was averaged from subunit counts and converted into total individual abundances per plate, and then expressed in relation to the sampling surface per dm^2. Survival rates at metamorphosis corresponded to the ratio of the abundance of oyster juvenile survivors on the collectors immersed for four weeks divided by the pediveliger abundance on the collectors immersed for two weeks.

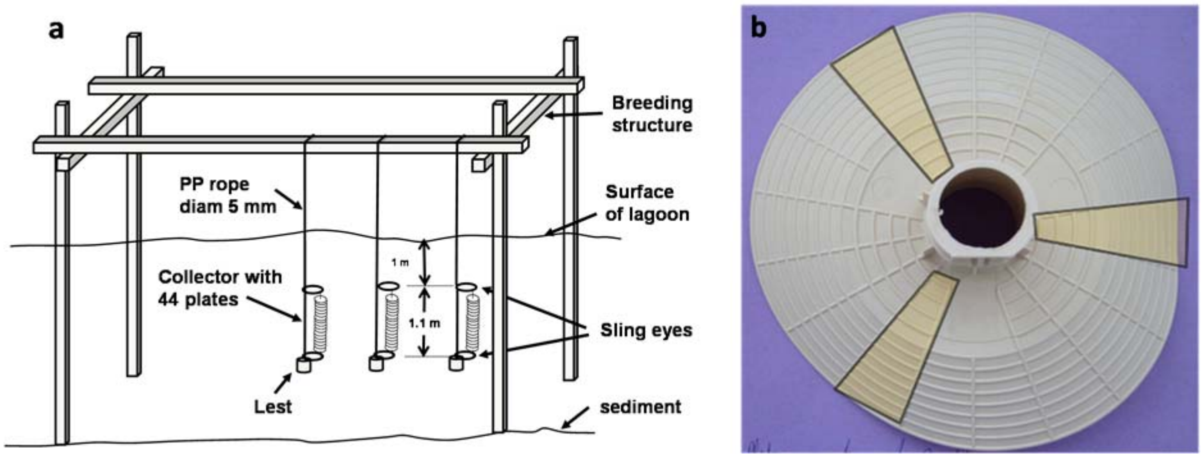

Figure 29: The breeding structure carried a set of 3 replicate collectors within the shellfish farming zone. Outside the shellfish farming zone, a mooring system as described by Lagarde et al. (2017) was used. (b) Top view of a collector plate with counting subunits in yellow, in this case: 14 cm2.

Prodissoconch II size

To assess prodissoconch II (PII) size, 10 postlarvae per plate were removed from three plates (at the top, middle and bottom of the collectors) and placed side by side on the microscope blade to obtain an optimal quantity of 30 measurements per site and per year for the main recruitment event in 2012, 2013 and 2014. Postlarvae were individually detached from their plate, placed and oriented on the plasticine flange of a holding blade (Martel et al. 2014) of an Olympus SZ61 binocular microscope. A digital microscope Keyence, VHX 2000 (200-300× magnification and set in high dynamic range mode with light shifts) was used to measure PII sizes. Based on a lateral view, PII heights were measured in accordance with a maximum dorsoventral dimension from the umbo to the most distant part of the clear demarcation formed by a growth line at the boundary layer between PII and dissoconch (Figure 30).

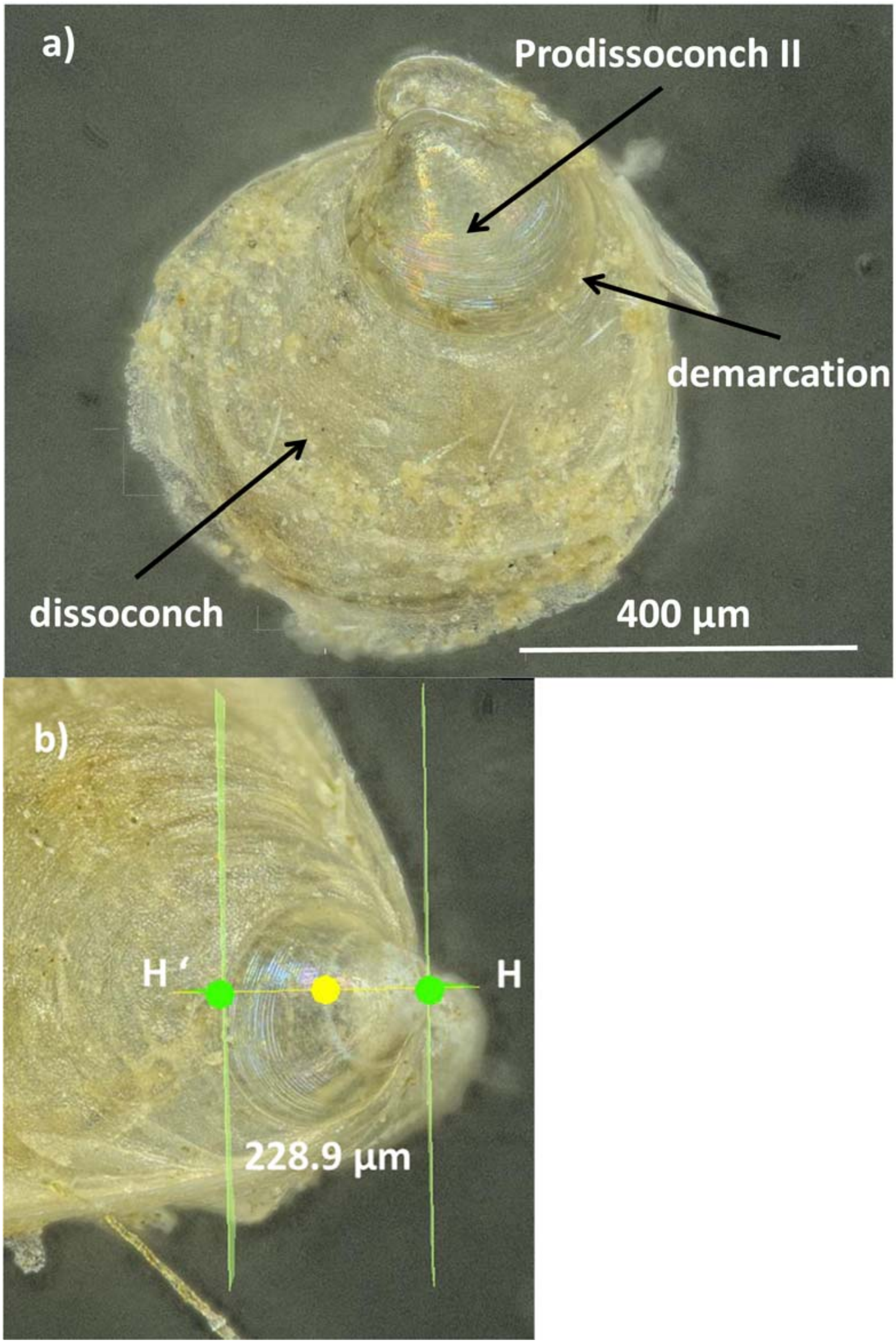

Figure 30: Measurement of maximum shell height along maximal (H-H’) dorsoventral axis of larvae and juvenile oysters (Bayne 2017) in Thau lagoon. (a) Prodissoconch II and dissoconch shells showing demarcation of the growth line delimiting metamorphosis. (b) Prodissoconch II size (H-H’= 228.9 µm, 2012 Bouzigues, Replicate 1, middle plate, measurement 8).

Environmental measurements

Hydrological and plankton samples were collected every Monday morning from June 1 to September 30 in 2012, 2013 and 2014. Samples were collected at Listel and Mèze sites.

Bouzigues and Marseillan sites were located less than 1 km from where environmental measurements are permanently monitored (REPHY – French Observation and Monitoring program for Phytoplankton and Hydrology 2017). For Bouzigues and Marseillan, hydrological and plankton samples associated with juvenile sampling sites were in biocoenosis, characterized by the same phytoplankton, zooplankton and benthic populations (Jouffre & Amanieu 1991; Guelorget et al. 1994; Jarry et al. 1990).

Temperature and salinity were measured twice a week with WTW® probes positioned between 1 and 1.5 m below the surface. Oxygen concentrations were measured once a week at the bottom of the water column at the Bouzigues site.

The three sampling campaigns provided a total of 135 observations of plankton characteristics: 3 sampling sites x 15 weeks x 3 years. The Bouzigues, Meze_osfz and Marseillan sites were sampled in 2012 and the Bouzigues, Listel and Marseillan sites were sampled in 2013 and 2014. Each phytoplankton and protozooplankton sample was collected at each sampling site and date as subsamples of a 4 liter sample. Samples were collected weekly using a Standard Water Sampler acc. to Ruttner (Hydro-Bios Apparatebau GmbH) without analytical replicates or sampling replicates.

For total chlorophyll a [Total Chl. a] measurements, seawater samples (200 mL) were filtered (Bec et al. 2005; Bec et al. 2011) under vacuum (<10 cm Hg) on Whatman GF/F membranes (0.7 μm porosity) and stored in glass tubes at –20 °C. The filters were ground in 90% acetone and extracted for 24 h at 4 °C in the dark. Chlorophyll a biomass was also determined after size fractioning (200 ml for picophytoplankton and 200 ml for nanophytoplankton from the 4 l sample) through Nuclepore membranes (3 and 20 μm, respectively) to determine the contribution of picophytoplankton (PICO, < 3 μm), nanophytoplankton (NANO, 3-20 μm) and micro-phytoplankton (MICRO, > 20 μm) to total phytoplankton biomass. The pigment content (μgChla l-1) was measured using a spectrofluorometer (Perkin-Elmer LS50b)(Neveux & Lantoine 1993b).

The abundances of picocyanobacteria (CYAN, < 1 μm), autotrophic picoeukaryotes (PEUK, < 3 μm) and nanophytoplankton (NANO TOT, 3-20 μm) were measured on the basis of a sampled volume of 1 ml with a Becton Dickinson FACSCalibur flow cytometer (Bec et al. 2011). Total picophytoplankton (PICO TOT) abundances were estimated as the sum of the CYAN and PEUK abundances. Among nanophytoplankton, Cryptophytes (CRYPTO) were distinguishable from other photosynthetic organisms (NANO) by their strong orange fluorescence associated with phycoerythrin pigment and their size. The abundances are expressed as 10^6 cells l^{-1}.

To measure bacterial abundance, samples (1 ml taken from the 4 l sample) were fixed with prefiltered (0.2 µm) buffered formaldehyde (final concentration 2%) and stored in liquid nitrogen. The abundances were determined using the Becton Dickinson method with a FACSCalibur flow cytometer (Marie et al. 1997). The procedure was slightly modified as higher concentrations of fluorochrome (SYBR Green I) were used (Bouvy et al. 2016). One milliliter of fixed samples was incubated with SYBR Green I (Molecular Probes) at a final concentration of 1/375 for 15 min at 4 °C in the dark. Stained bacterial cells excited at 488 nm were determined according to their side-scattered light (SSC) and green fluorescence (FL1) and collected using a 530/30 nm filter. Fluorescent beads (0.94 µm, Polysciences Inc.) were added to each sample.

The taxonomic composition of protozooplankton was estimated (heterotrophic flagellates in a 30 ml sample and ciliates in a 100 ml sample) and phytoplankton (in a 10 ml sample) using the standard Utermohl method NF-EN-152014, 2006. For the phytoplankton, two main classes, “Diatoms” (aggregating 52 taxa) and “Dinoflagellates” (aggregating 38 taxa) were first used to explore the dataset. Taxonomic composition is expressed as the number of individuals per liter. The taxonomic composition of phytoplankton was not monitored in 2012 at the “Meze_osfz” sampling site. For analysis of heterotrophic flagellates (HF), the 30 ml samples were preserved in an 8% formaldehyde solution and stored in a cold room at 4 °C in the dark until analysis. A 10 ml subsample was stained using 4’,6-diamidino-2-phenylindole (DAPI) at a final concentration of 2.5 µg ml-1. The HF counts were performed using an epifluorescence microscope (Olympus AX70) with UV illumination (Sherr et al. 1993). For the determination of naked ciliates and tintinnids, 100 ml samples were preserved in 2% Lugol’s iodine solution and kept in a cold room at 4 °C in the dark until analysis. Naked ciliates and tintinnids were identified, measured and counted with an inverted microscope (Olympus IX70) after a 100 ml sample was left to settle in an Utermohl chamber for 24 h (Utermöhl 1931).

Data analysis

Data analyses and graphics were performed with R statistical software (R Core Team 2015). Decimal logarithm transformations were used to tend towards linearity of response variables and to linearize the relationship with explanatory variables (Tableau 8). The environmental data from our three annual hydrobiological and plankton survey were averaged over a 14 day period before the retrieval of the collector during the main recruitment event.

The main recruitment events were identified each year by considering the highest oyster juvenile abundances observed at the different sampling sites. A non-parametric one way ANOVA (Kruskall test, ‘pgirmess’ package) was performed using data pooled for each date and associated with a multiple comparison test of Siegel and Castellan (Siegel & Castellan 1988) to target the best sampling period for oyster recruitment at each sampling site each year. Oyster juvenile abundance was graphically described using comparison of means with standard error intervals.

A two-way cross PermANOVA (PermANOVA-Primer-E7.0.12 PermANOVA Plus; Primer-E Ltd, Plymouth, UK) was performed to compare PII size measured during the main oyster recruitment events among sampling sites (5 fixed levels: Marseillan, Listel, Meze_osfz, Bouzigues or Balaruc), year (3 fixed levels: 2012, 2013 and 2014) and their interaction (Site x Year). Resemblance matrixes were calculated on Bray-Curtis dissimilarities. PermANOVA was used rather than ANOVA because of non-orthogonality of our larval settler databases. This approach using similarities, like ANOVA, is more accurate than non-parametric analysis, because it uses the permutation method (9999 permutations). Homoscedasticity was verified by using the Permdisp test (Anderson et al. 2008).

A graphical representation of PII measurements and environmental variables was made with a principal component analysis (PCA) with the factor ‘year and sampling site’ (Lê et al. 2008). This PCA was used to explore and illustrate the heterogeneity of the three sampling sites according to the year and interactions between the PII sizes and their environment. The means of PII sizes were calculated using individuals from the collector replicates (1 collector is represented by 3 replicates of plates, each replicate is intended to optimally represent about 30 PII measurements). Each average PII size was compared with the averages of the environmental data acquired over the two weeks prior to the retrieval of the collector at the site. The broken stick model and Kaiser criterion were used to compare the eigenvalues and performed to interpret axes whose eigenvalues were higher than mean of all eigenvalues (Borcard et al. 2011; Oksanen et al. 2015). Linear models were used to fit regression and single stratum analysis of variance was performed following assumptions of random sampling, homoscedasticity (tested and confirmed with the Fligner-Killeen test) and normality (verified with Shapiro–Wilk tests), respectively to test the influence of year, temperature and survival at metamorphosis on the means of PII sizes.

Tableau 8: Variables characterizing interactions between the environment and larvae. Each environmental variable was averaged over a 2-week period preceding the retrieval of the collectors to characterize the environment for metamorphosis.

Variables	Description	Unity	Abbreviation
Target variables			
prodissoconch II height	measure	µm	P2 size
oyster spat	abundance	ind. dm^{-2}	oyster spat
pediveligers	abundance	ind. dm^{-2}	pedi
Environmental variables			
spat / pediveliger survival	ratio of oyster spat to pediveliger abundance	-	metamorphosis survival
oxygen concentration	daily average	mg l^{-1}	oxygen
temperature	daily average	°C	temperature
salinity	daily average	No unit	salinity
bacteria	abundance	10^6 cell. l^{-1}	bacteria[A]
total picoeukaryotes	abundance	10^6 cell. l^{-1}	peuk[A]
total picocyanophyceae	abundance	10^6 cell. l^{-1}	cyan[A]
nanophytoplankton	abundance	10^6 cell. l^{-1}	nano[A]
cryptophytes	abundance	10^6 cell. l^{-1}	crypto[A]
heterotrophic flagellates	abundance	cell l^{-1}	hf[A]
ciliates	abundance	cell l^{-1}	ciliates[A]
tintinnids	abundance	cell l^{-1}	tintinnids[A]
diatoms	abundance	cell l^{-1}	diatoms[A]
dinoflagellates	abundance	cell l^{-1}	dinoflagellates[A]
total chlorophyll *a*	biomass	µgChla l^{-1}	Total_chlo_*a*[B]
picophytoplankton	biomass	µgChla l^{-1}	pico[B]
nanophytoplankton	biomass	µgChla l^{-1}	nano[B]
micro-phytoplankton larger than 20 µm	biomass	µg l^{-1}	microphyto[B]

Results

Oyster spat abundance and survival after metamorphosis

In 2012, two significant recruitment events were characterized (Figure 31). The first harvest date (August 13) was characterized by a juvenile abundance of 45 ± 4 ind. dm^{-2} at Listel and of 8 ± 1 ind. dm^{-2} at Bouzigues. The second significant recruitment event (September 25) was characterized by 14 ± 2 ind. dm^{-2} at Bouzigues, 12 ± 2 ind. dm^{-2} at Balaruc and 7 ± 1 ind. dm^{-2} at Listel. In 2013, the highest oyster juvenile abundances were recorded on August 13 and August 28 (Figure 31) highlighting excellent recruitment at the Meze_osfz sampling site with a density of 68 ± 17 ind. dm^{-2} and 92 ± 17 ind. dm^{-2}. On August 28, the Listel site had an average juvenile oyster abundance of 32 ± 5 ind. dm^{-2} harvested, and a high juvenile abundance was also recorded at Meze_osfz on October 9, 2013 (172 ± 19 ind. dm^{-2}). The 2014 profile revealed that the recruitment period lasted two months (Figure 31) with four consecutive recruitment events (August 13 and 27 and September 10 and 24). The Meze_osfz site showed remarkable juvenile abundances (75 ± 6 ind. dm^{-2}, 260 ± 27 ind. dm^{-2}, 73 ± 18 ind. dm^{-2}, 30 ± 6 ind. dm^{-2}) during these four consecutive events. The Listel site showed the same range of juvenile abundance as in 2012 and 2013 with 24 ± 6 ind. dm^{-2} on August 27 and 39 ± 11 ind. dm^{-2} on September 10. In 2014, it should be noted that experimental stations in the shellfish aquaculture zones (Bouzigues, Meze_osfz and Marseillan) had, on average, extremely low recruitment levels of between 0 and 2 ind. dm^{-2}. Taken together, these results showed that the main oyster recruitment events occurred on August 13 in 2012, August 28 in 2013 and August 27 in 2014 (Figure 31).

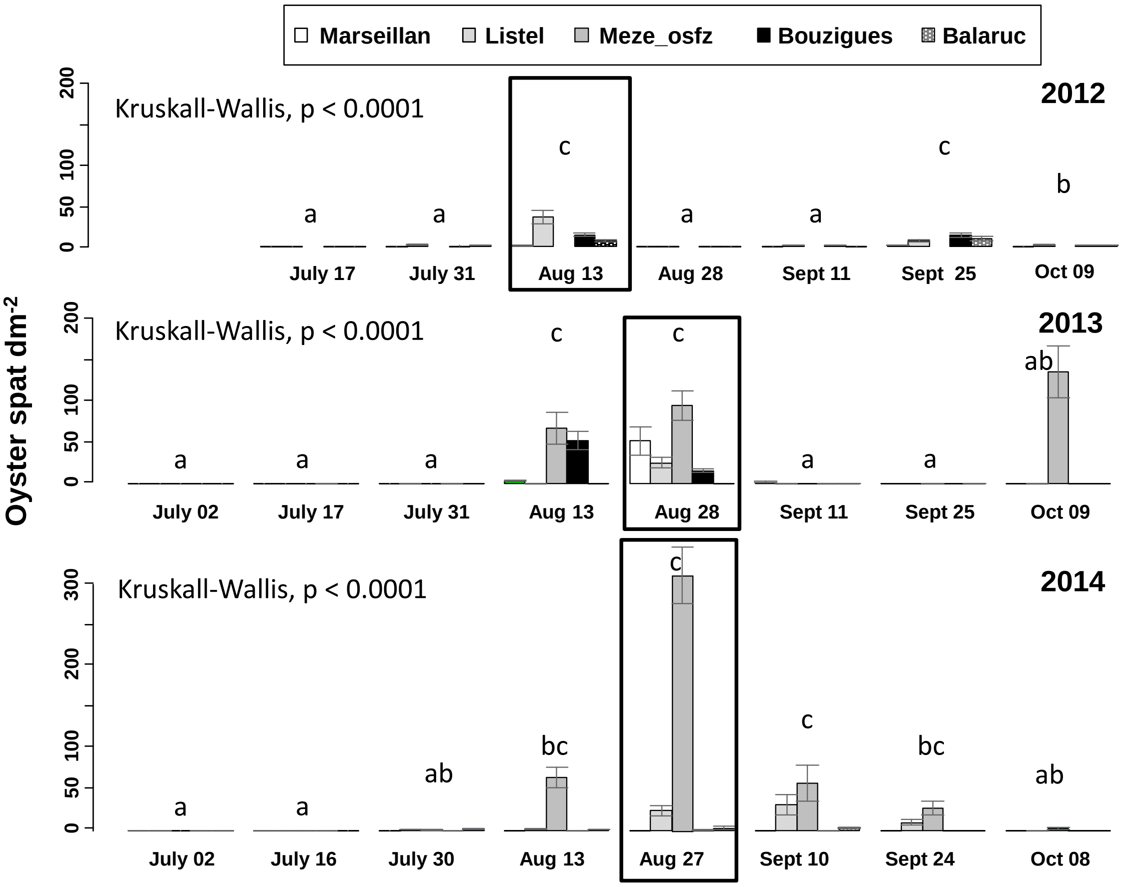

Figure 31: Means (± standard error) Pacific oyster spat abundance per dm2 observed at the 5 sampling sites on a west-east gradient (Marseillan, Listel, Meze_osfz, Bouzigues and Balaruc), at two-week intervals throughout the summer in 2012, 2013, and 2014. Juvenile abundances were estimated after four weeks of immersion (n=27 per date & sampling site). Letters indicate significant groups resulting from the Siegel and Castellan multiple comparisons test ($p \leq 0.05$). The squares indicate the main annual recruitment event used to characterize variations in prodissoconch II size.

Prodissoconch II size

PII size was determined during the main oyster recruitment event each year, i.e. on August 13, 2012, August 28, 2013 and August 27, 2014 (Figure 31). PermANOVA revealed a significant year and site interaction effect ($df^{year \times site} = 7$, $df^{total} = 404$, Pseudo-F = 3.99, $p < 0.001$). In 2012, the pairwise PermANOVA showed significant differences in PII size depending on the site, with larger PII size (mean ± se) at the Marseillan site ($p < 0.03$; 247.0 ± 5.2 µm, n=12) and smaller PII size at Balaruc ($p < 0.02$; 213.7 ± 4.1 µm, n=31). In 2013, four size categories were observed (Figure 32), the largest PII size at Marseillan ($p < 0.003$; 270.7 µm ± 3.3 µm, n=35) and the smallest at Meze_osfz ($p < 0.01$; 222.1 ± 3.4 µm, n=35). In 2014, the PII size was categorized in two groups with larger sizes ($p < 0.001$) at the Marseillan site (273.2 ± 5.2 µm, n=11), Bouzigues (265.9 ± 3.8 µm, n=24) and Balaruc (275.0 ± 2.6 µm, n=15) and smaller ($p < 0.001$) in Listel (254.8 ± 4.5 µm, n=30) and Meze_osfz (243.8 ± 4.3 µm, n=35).

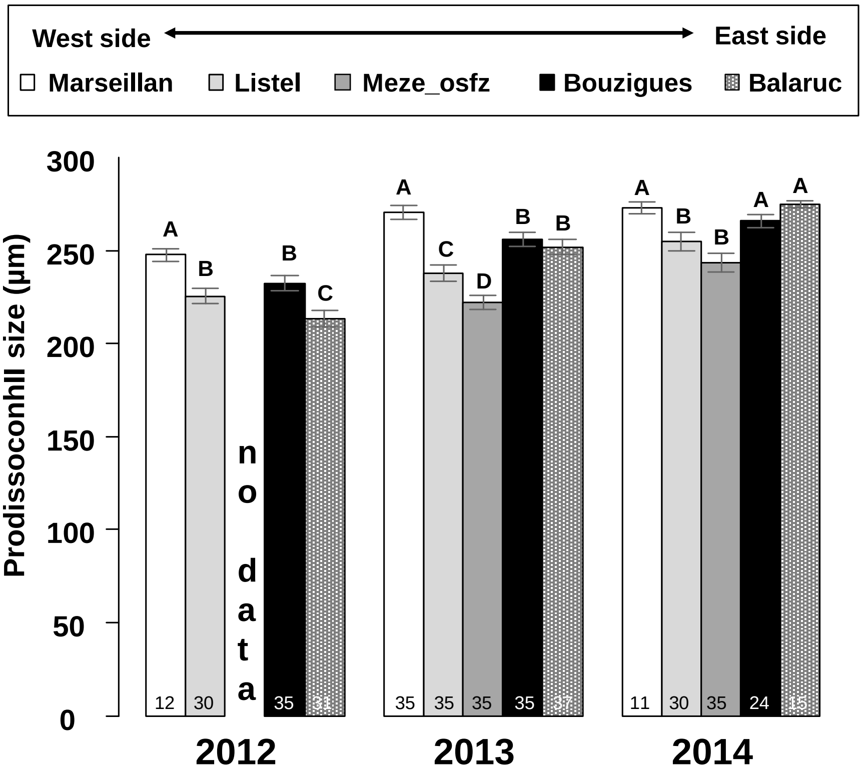

Figure 32: Mean (± standard error) of prodissoconch II size as a function of the sampling site on a west-east gradient (Marseillan, Listel, Meze_osfz, Bouzigues and Balaruc) and of the year (2012, 2013 and 2014) during the main annual recruitment events with the associated strength. Letters indicate significant differences according to the two-way-cross permutation ANOVA, pair-wise tests 'Year x Sampling site' for pairs of levels of the factor 'Sampling site'. Numbers at the bottom of the histograms indicate the number of samples per year and per sampling site.

Relations between PII size, hydrology and plankton data

A principal component analysis was conducted on the hydrological measurements, plankton data and PII sizes to explore their relationship and to characterize structured ecological gradients and spatial patterns (Figure 33). Applying the “broken stick” model and Kaiser criterion, four axes were mainly explanatory but only the first three axes are shown because they each individually represent more than 10% of the total inertia (Figure 33a). On the axis1/axis2 plot (Figure 33b), the first axis opposed autotrophic organism (bottom right; biomass and abundance of nanophytoplankton and biomass of total chlorophyll *a* with hetero/mixotrophic organisms (top left; fh^A, tintinnids^A, ciliates^A and crypto^A). The second axis was mainly driven by abundances of picoplanktonic organisms such as ciliates^A, peuk^A and crypto^A. The first two axes accounted for 64.0% of variance. Although weakly represented on the factorial plane of axes 1 and 2, the PII size appeared to be positively correlated with hetero/mixotrophic organisms such as Crypto^A and Ciliates^A and anti-correlated with the variables representing autotrophic organisms (biomass and abundance of nanophytoplankton and total chlorophyll *a* biomass). The low representation of the PII size variable on these first two axes reveals the marked influence of ecological heterogeneity and gradients in the ecosystem in response to the observed biological effect. The axis1/axis3 plot (Figure 33c) shows that in planes with axis 1 and 3, the right part of plot showed high and positive contributions of autotrophic organisms (diatoms^{A}, nano^{A}, nano^{B}, total chlorophyll a^{B} and cyan^{A}) and the left part showed negative contributions by fh^{A}, crypto^{A} and ciliates^{A}. The Dim2-Dim3 plot (Figure 33d) shows the correlations between the variables on axes 2 and 3 with axis 3 (12.6%) opposing PII size and Tintinnids^{A}, fh^{A}, temperature and bacteria^{A}. The distribution of sampling sites in factorial planes 1-2 indicates that the sites expressed their heterogeneity differently between years with respect to the autotrophic or hetero/mixotrophic regime (Figure 33e, f, g). The sites Marseillan 2012, Marseillan 2014, and Bouzigues 2014 had the biggest PII, the highest heterotrophic abundances and the lowest phytoplankton biomass and abundance. The Listel site had the smallest PII size. Overall, the right to left progression from autotrophic to heterotrophic is clear on the first two axes whereas the temperature has a structuring effect and is related to the sizes of PII on the first two axes of this PCA.

Annual averages (mean ± se) of size at metamorphosis increased significantly (ANOVA, F value = 48.5, $p < 0.0001$, Figure 34a) from 2012 (226.7 ± 23.3 µm, n = 113) to 2013 (248.0 ± 25.99 µm, n = 177) and 2014 (258.2 ± 24.50 µm, n=115).

Water temperature (mean ± se) during the main oyster recruitment events was on average 25.7 ± 0.1 °C in 2012, 24.8 ± 0.1 °C in 2013, 23.3 ± 0.1 °C in 2014 (Figure 34b) with a significant anticorrelation with PII size (Spearman’s correlation coefficient = -0.74 and $p < 0.001$) (Figure 34c). Figure 35 shows six trophic parameters significantly correlated with PII size ($p < 5\%$); Cryptophyte abundances were positively correlated (Corr. Coef. =0.69 and $p < 0.001$) and anticorrelated with total chlorophyll-*a* biomass (Corr. Coef. =-0.51 and $p < 0.01$), micro-phytoplankton biomass (Corr. Coef. =-0.77 and $p < 0.001$), nanophytoplankton biomass (Corr. Coef. =-0.57 and $p < 0.05$), picocyanobacteria (corr. Coef. =-0.62 and $p < 0.05$) and tintinnid abundances (Corr. Coef. = -0.41 and $p < 0.01$).

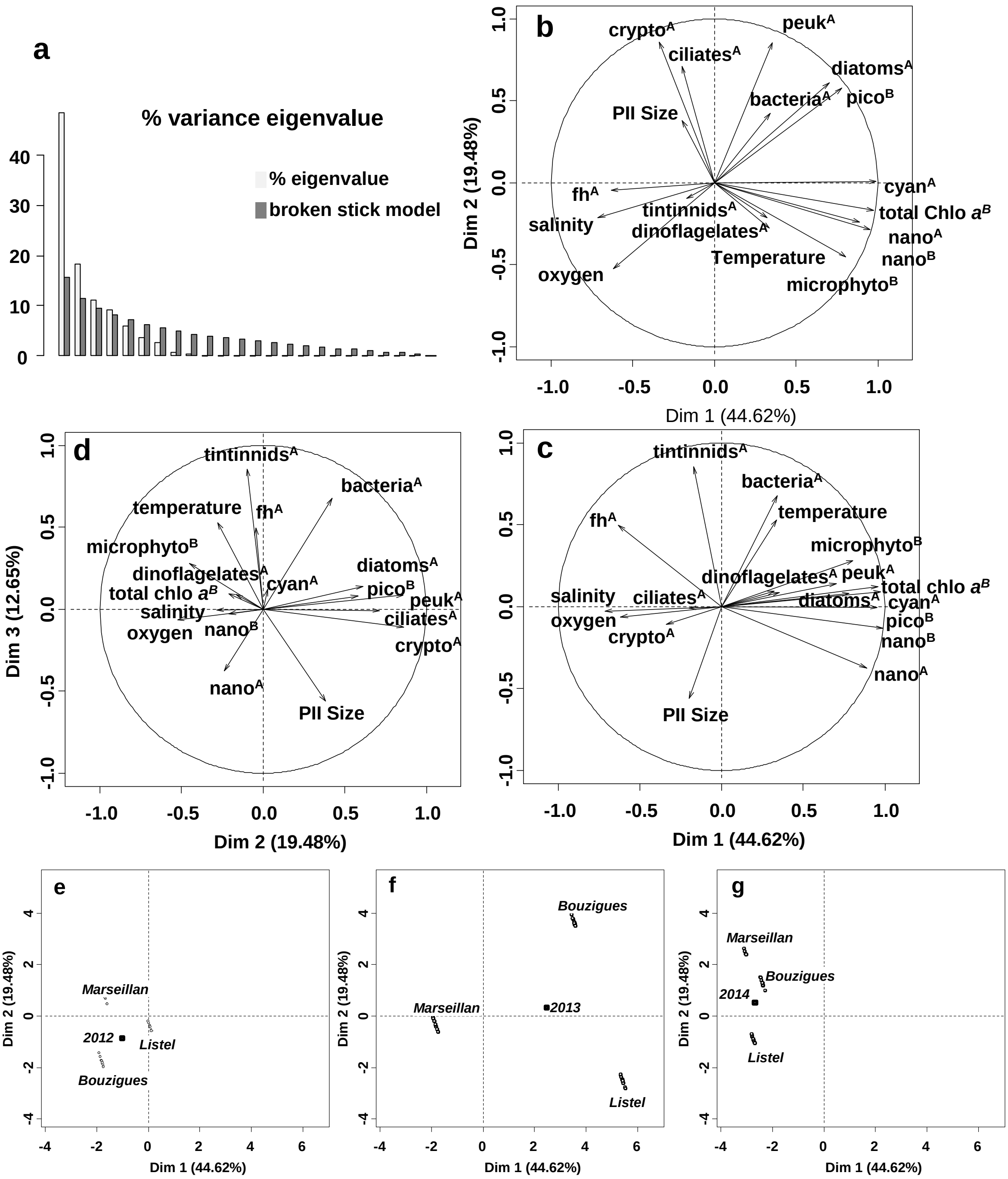

Figure 33: Principal component analysis (a) Comparison of the broken stick model and eigenvalues, (b) Biplots with correlation circle axis 1 / axis 2 of averaged environmental data for a 14 day period preceding the retrieval of the collector (superscript A: abundance, B: biomass), (c) Correlation circle Dim 1 / Dim 3, (d) Correlation circle axis 2 / axis 3 and the Site factor per year (e) 2012, (f) 2013, (g) 2014.

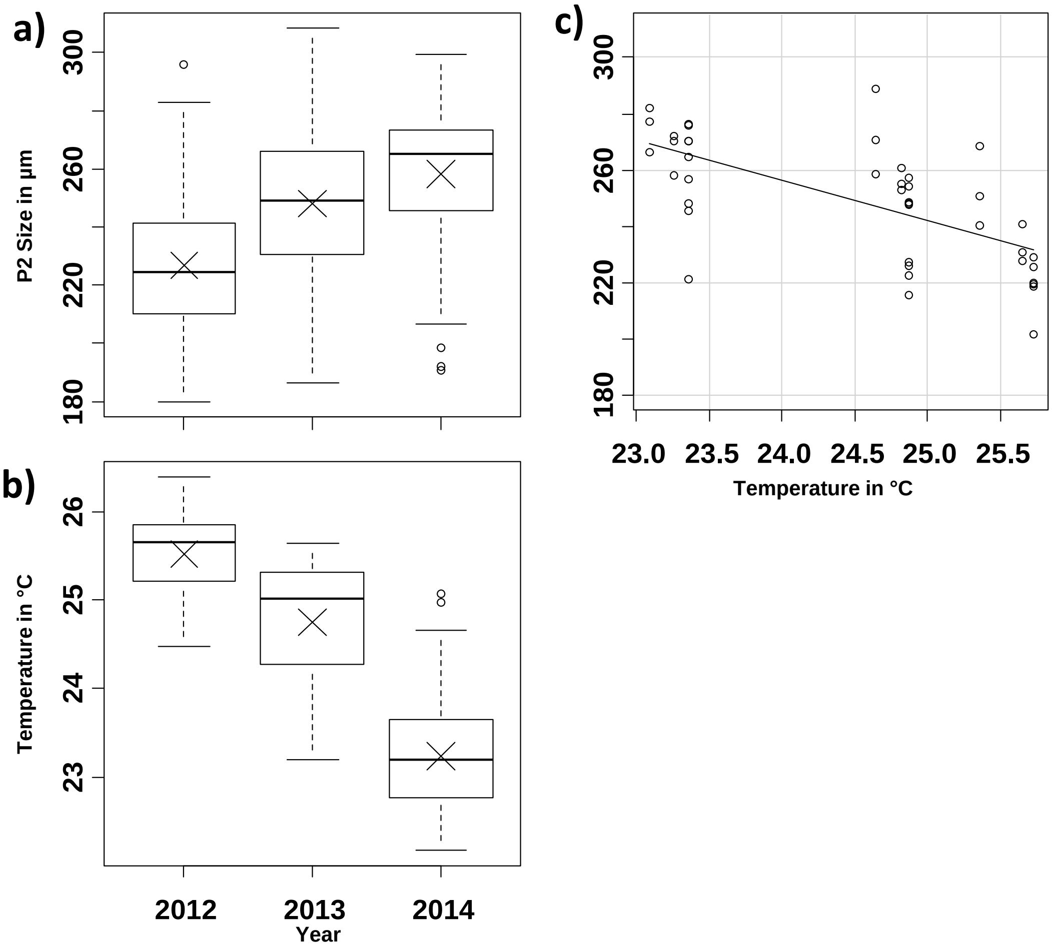

Figure 34: (a) Increasing trend in PII size versus year, (b) Daily temperatures during the 2 week metamorphosis period preceding retrieval of the collector (August 13, 2012, August 28, 2013, and August 27, 2014), (c) Significant relationship between PII size and temperature means over the duration of the metamorphosis period. Mid-line: median; box: 25th and 75th percentiles, whiskers: 1.5× the interquartile range; circle: outliers; line: least square regression; crosses: means.

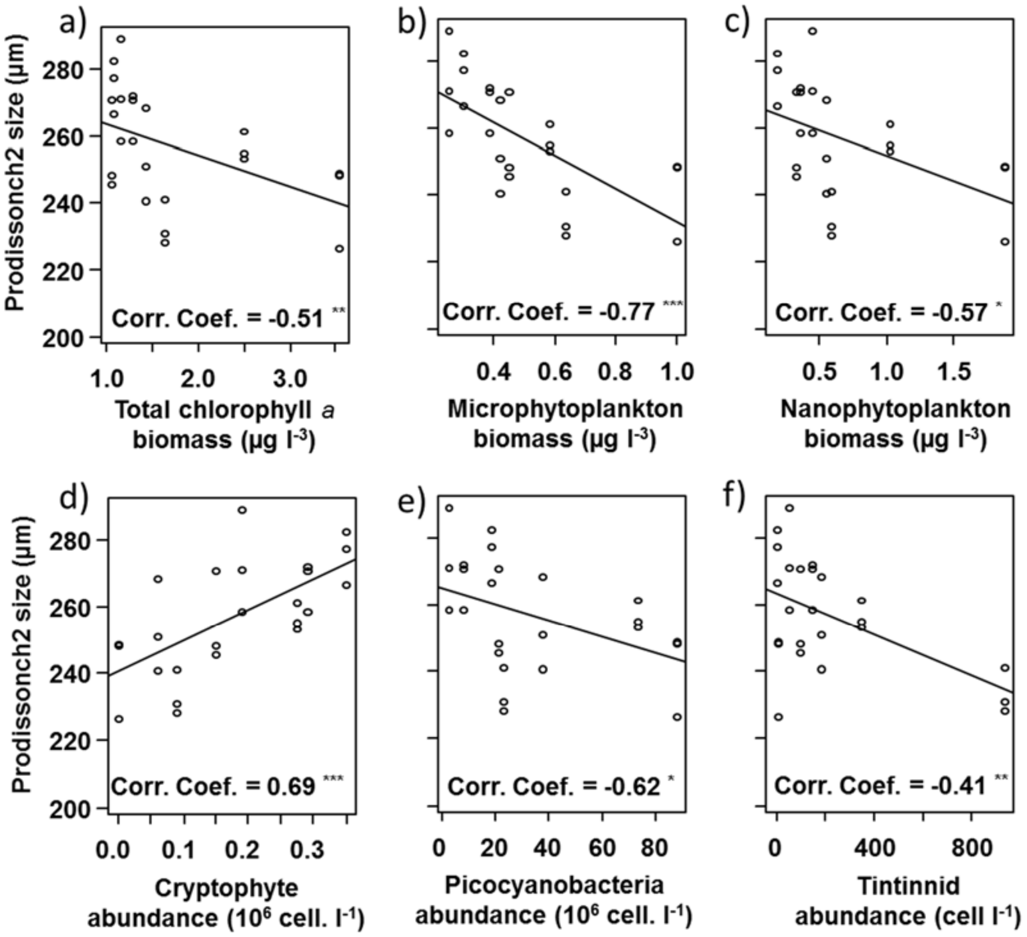

Figure 35: Relationship between PII size (mean of 10 measurements per circle, 3 replicated plates per collector) with plankton during the metamorphosis period, (a) total chlorophyll-a biomass, (b) micro-phytoplankton biomass, (c) nanophytoplankton biomass, (d) cryptophyte abundance (e) picocyanobacteria abundance (f) tintinnid abundance. Significant values * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Relation between PII size and survival after metamorphosis

Figure 36 shows a negative linear relation between PII sizes and survival after metamorphosis (cor. Coef = - 40.8%, df. = 379, $p < 0.0001$). The significant negative slope (b = -40.9, $p < 0.0001$) shows that juvenile survival decreased with an increase in PII size (Tableau 9). The value of 254.8 µm ($p < 0.0001$) at the intercept for PII size indicates low survival compared to 213.9 µm for high juvenile survival. The proportion of variance explained by the regression is relatively low (multiple R-squared = 0.167) compared to the total dispersion because of the great variability of the PII sizes measured.

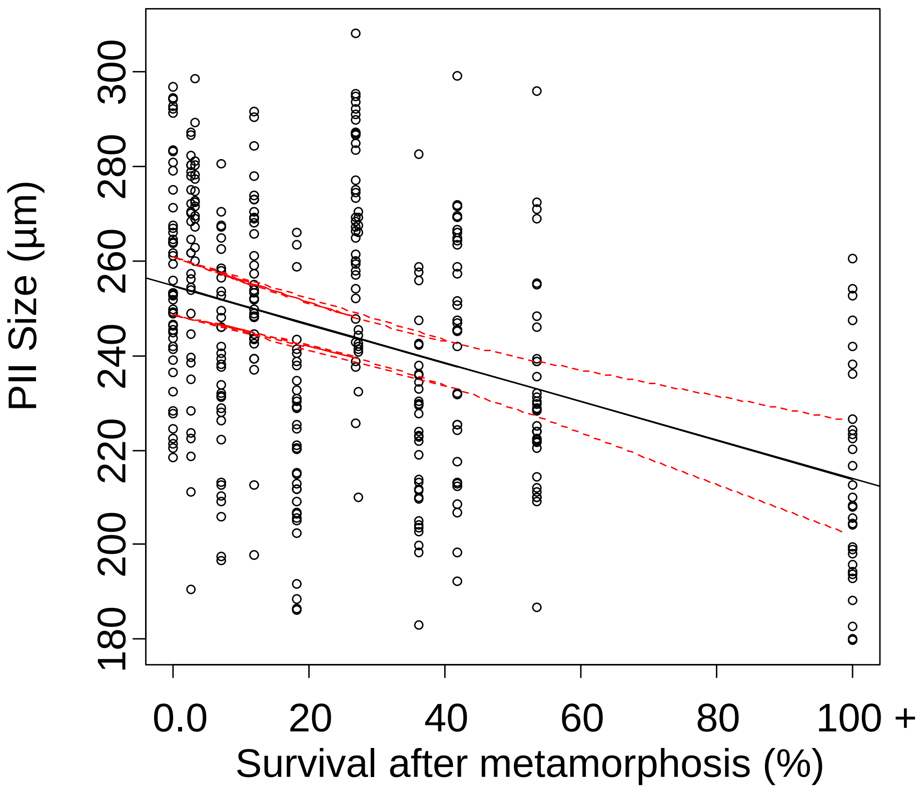

Figure 36: Prodissoconch II size (ANOVA p < 0.001) as a function of metamorphosis survival expressed in percent (n = 333, censured data 100+ for over 100% survival); black line: regression line, red dotted line: ± 95% confidence interval.

Tableau 9: Linear regression between the survival after metamorphosis and prodissoconch II size. Significant values are in bold.

ANOVA					
Response: PII size	Df	Sum Sq	Mean Sq	F	p
Survival after metamorphosis	1	47975	47975	75.9	< **0.0001**
Residuals	379	239600	632		

Linear model: P2 size ~ Survival after metamorphosis				
Coefficients	Estimate	Std. Error	T	p
Intercept	254.7	1.817	140.237	<**0.0001**
Survival after metamorphosis	-40.947	4.700	-8.71	<**0.0001**
Residual standard error	25.14 on 379 degrees of freedom			
Multiple R-squared: **0.1668**	Adjusted R-squared: 0.1646			
F: 75.89 on 1 and 379 DF	p < 0.0001			

Discussion

In a new context of oligotrophication, our overall objective was to explore variability of recruitment of *Crassostrea gigas*, the most highly exploited bivalve species in the world (FAO 2012). This is the first time variability of prodissoconch II size has been assessed during the main oyster recruitment events in *C. gigas* in a semi-enclosed nanotidal ecosystem, Thau lagoon in southern France. Likewise, the hypothesis that, in *C. gigas*, larger size at metamorphosis is linked to lower juvenile survival rates was confirmed. The retention of competent larvae in the water column was mainly related to temperature and environmental/trophic conditions. Contrariwise, rapid settlement and metamorphosis at a smaller size increased the survival probability of Pacific oyster juveniles and had a positive impact on recruitment success.

The recruitment variability of benthic invertebrates has already been studied to ensure the sustainability of the established populations (Pechenik 2006; Barbier et al. 2017; St-Onge et al. 2015). In our case, monitoring in 2012, 2013 and 2014 revealed spatial and temporal patterns that had an environmental influence on different stages, i.e. spawning, settlement and metamorphosis (Lagarde et al. 2017; Ubertini et al. 2017). This shows that the intrinsic ecological functioning of the lagoon combined with heterotrophic/mixotrophic vs. autotrophic regimes (Leruste et al. 2016) can influence gametogenesis, spawning behavior, settlement of larvae, and hence recruitment success. As already described for lakes, these changes in functioning affect the trophic chain through a cascade of interactions extending from bacteria to fish (Ozkan et al. 2016; Jeppesen et al. 1998; Jeppesen et al. 2005). The concept of a time window for recruitment, as described by Pineda *et al.* (2006), is fully applicable to the recruitment abundances in our study context. The ecological specificities of Thau lagoon are expressed in the heterogeneous oyster recruitment within the narrow recruitment windows defined by specific spatial and temporal patterns (Lagarde et al. 2017). The temporal component of Pacific oyster recruitment in the lagoon according to three determinants: (i) The recruitment determinant is the temperature of the ecosystem that results in different ecological functioning (hypoxic / normoxic, autotrophic / heterotrophic); (ii) the determinant of pediveligers abundance is the abundance of diatoms *Chaetoceros spp*; (iii) the determinant of metamorphosis survival is the abundance of nanophytoplankton. It is known that Thau lagoon is spatially heterogeneous due to its topogeography, currents, confinement and its intensive exploitation for shellfish farming. The effects of shellfish farming on the ecosystem are well known, particularly significant depletions of biomass and abundance of plankton in shellfish growing areas due to bivalve filtration (Deslous-Paoli et al. 1993; Lam-Hoai et al. 1997; Mazouni, Deslous-Paoli, et al. 1998). Lagarde et al. (2017) showed that plankton depletion, including *Chaetoceros spp* and nanophytoplankton, are not favorable for a larval cycle up to recruitment, particularly in periods of top-down trophic control of shellfish areas by filter feeders during recruitment windows.

The larval stage and particularly the steps of metamorphosis may be delayed depending on environmental functioning (Pechenik 2006; Pechenik 1999; Pechenik 1990; Lutz & Jablonski 1978). Martel *et al.* (2014) highlighted the relation between a longer delay before metamorphosis, an increase in the PII size of the blue mussel *Mytilus edulis* and a decrease in settlement and recruitment success. In the present study, *in situ* spatial and temporal patterns of prodissoconch II sizes were identified for the first time in *C. gigas* based on environmental cues. Prodissoconch II heights were measured during the best recruitment windows each year as representative of the functioning scenarios of the entire Thau basin exploited for shellfish farming. The variation in size was on average 20% per sampling site with individual maxima of up to 40%, i.e., from 180 to 296 µm. Our results suggest that the yearly temporal pattern is mainly driven by temperature, while the higher spatial variability of PII size is related to the quantity and quality of available food (autotrophic vs. heterotrophic regime) for larvae and juveniles (Lagarde et al. 2017; Ubertini et al. 2017). Further study is needed on the impacts of delayed metamorphosis on the duration of larval pelagic stage, changes in PII sizes in terms of physiological states or indicators such as energy reserves, total lipids and essential fatty acids.

Temperature controls activities that affect the kinetics of the biological and ecological processes (Frontier et al. 2008). It is known that temperature determines the patterns of distribution of organisms as a function of gradients and discontinuities (Viña 2002). At an individual scale, the growth rates of poikilotherm organisms are governed by temperature. Temperature has a direct effect on the physiology of oyster larvae by enabling high metabolism rates (Rico-Villa et al. 2010) thereby accelerating development, but temperature may not affect total metabolic demand (Hoegh-Guldberg et al. 1991). In the present study, the annual means of PII size were negatively correlated with water temperature (2012: 25.7 °C, 2013: 24.8 °C, 2014: 23.3 °C), which contradicts the physiological rules and disagrees with the results obtained by Arakawa (1990), who studied the eutrophicated Hiroshima Bay in the 1990s. However, this negative correlation between seawater temperature and PII size has already been reported in *Mya arenaria* (Lutz & Jablonski 1978), *Mytilus edulis* (Bayne 1965) and *Crassostrea gigas*

(Flores-Vergara et al. 2004). This relationship could be due to the longer time available for feeding and growth at lower temperatures. At high temperatures, the transition between feeding stages, such as velum resorption, is shortened, and could result in smaller PII sizes (Lutz and Jablonski, 1978).

The temperature of the sea water has known effects on the activation of spat oyster pathogens such as the Ostreid herpes virus OsHV-1 and bacteria of the group *Vibrio splendidus* (Pernet, Barret, et al. 2012; Petton, Boudry, et al. 2015; Petton, Bruto, et al. 2015). OsHV-1 mortality in France started in early May and lasted until late September, with an interruption in July and August when seawater temperatures increased from 16 °C to more than 23 °C (Pernet, Lagarde, Jeanné, et al. 2014). In our study context, reproduction and recruitment processes were mainly studied in a temperature window ranging from 23 °C to 27 °C. We thus presume that pathogen agent OsHV-1 was not active during our recruitment window.

Temperatures drastically affect the whole ecological functioning of the Mediterranean lagoon in summer (Chapelle et al. 2000; Chapelle et al. 2001). In the case of high summer temperatures, diatoms, cryptophytes and chlorophyta are known to represent_phytoplankton communities in this mesotrophic Mediterranean lagoon (Leruste et al. 2016; Collos et al. 2009; Bec et al. 2011). Moreover, a spatial gradient in ecological functioning was revealed with smaller PII sizes in the middle of the ecosystem (Listel and Meze_osfz) and larger sizes at the eastern (Marseillan) and western (Balaruc) ends. Analysis of environmental variables showed that the smaller PII sizes associated with better survival were driven by higher biomass of autotrophic organisms such as picocyanobacteria, nano and micro-phytoplankton and total chlorophyll *a* biomass. In contrast, larger PII sizes with lower survival rates were associated with lower autotrophic biomass. The significant negative correlation between PII size and nanophytoplankton and micro-phytoplankton biomass highlights the importance of the planktotrophic relationship during settlement and metamorphosis (Toupoint, Gilmore-Solomon, et al. 2012). Our results are in agreement with those of Toupoint *et al.* (2012), who showed in the blue mussel that high cyanobacteria, pico- and nano-eukaryote biomasses were correlated with better recruitment. Considering a context of heterogeneity caused, on the one hand, by the effects of depletion due to shellfish farming areas and on the other hand, by geographical gradients, we hypothesize that pico-organisms (cyanobacteria eukaryotes), nano- and micro-phytoplankton biomasses act as a trigger for trophic settlement, as already demonstrated in mussels (Toupoint, Gilmore-Solomon, et al. 2012; Martel et al. 2014), and given their impact on PII size, probably also trigger metamorphosis.

Our results show that high cryptophyte abundances correspond to large PII sizes. Cryptophytes are mixotrophic nano-flagellates that play a role in the remineralization of organic matter and in predation on picoplankton such as the *Synechococcus sp.* picocyanobacteria (Du Yoo et al. 2017) and bacteria (Klaveness 1989; Pastoureaud et al. 2003). The appearance of picocyanobacteria (mostly *Synechococcus sp.*) has been reported to be related to a reduction in nutrient loading and to an increase in water temperature (Collos et al. 2009). Cryptophyte abundance and biomass respond rapidly to both nutrient loads and picocyanobacteria prey abundance due to their high growth rate (Du Yoo et al. 2017). These mixotrophic features (Cloern & Dufford 2005) make cryptophytes revealers of a transitional state during oligotrophication. What is more, thanks to their high nutritional values, digestibility and nanophytoplankton size, cryptophytes are considered as good prey for filter feeders like adults oysters (Klaveness 1989; Pastoureaud et al. 2003). However, cryptophytes have ejectile organelles named trichocysts/ejectosoms (Klaveness 1989) that may play a defensive role against predation by protozoa (Yamagishi et al. 2012). It has also been reported that, in the case of Raphidophyceae in lake ecosystems, trichocysts may also act as repellents to grazers (Lebret et al. 2012). Here, we hypothesize that the anatomy of cryptophytes with trichocysts/ejectosoms would be inappropriate for larval development and would thus be metamorphosis inhibitors/delayers. Of course, this hypothesis needs to be tested in experimental studies. Tintinnids are heterotroph organisms, predators of phytoplankton, specifically of nano-phytoplankton, including cryptophytes, COD and bacteria (Sitran et al. 2009; Dolan 2000). Tintinnid abundance probably reflects picocyanobacteria populations and cryptophyte dynamics and acts as a booster of settlement or metamorphosis or perhaps just as a neutral trophic competitor of oyster larvae, revealing mixo/heterotrophic traits of oligotrophication process. These results fill some of the many gaps in our knowledge of the interactions between cryptophytes, ciliates and oyster larvae.

Bacteria have been shown to facilitate the settlement of many invertebrate larvae, including oysters (Fitt et al. 1990; Fitt et al. 1989; Coon et al. 1990). In the present study, we show that high picocyanobacteria abundance corresponds to small PII size. We hypothesize that picocyanobacteria abundance boosts settlement or metamorphosis in the presence of an efficient trophic chain.

The functional approach (heterotrophic vs. autotrophic) has been used to understand how environmental changes or gradients drive phytoplankton community structure (Leruste et al. 2016; Litchman et al. 2010). Some morphological and physiological traits, in particular cell size, maximum growth rate and trophic regime reflect how phytoplankton adapt to nutrient availability (Litchman et al. 2007; Litchman et al. 2010). During oligotrophication, the reduction in nutrient inputs could thus favor small cells, which compete more effectively for nutrient uptake and have high growth rates (Chisholm 1992; Kamenir & Morabito 2009; Litchman et al. 2010), and mixotrophic species, which have some advantages over strictly autotrophic cells (Anneville & Pelletier 2000). In our case, an ecosystem with heterotrophic functioning indeed resulted in sizes at metamorphosis ranging from 250 to 300 µm, while autotrophic functioning resulted in sizes ranging from 200 to 250 µm. This ecological heterogeneity expresses a specificity of Thau lagoon with regard to PII size, i.e., an west-east gradient that tended to be linear in 2012 (with the largest sizes in the west and the smallest in the east) and another gradient as a reverse parabolic curve in 2013 and 2014 that recalls the optimal production curves of Shelford's law of tolerance (Begon et al. 2007). The central zone of the lagoon represented by the "Meze_osfz" site appears to be the zone of ecological preference (with small PII size, high survival and high recruitment rates) and, at both west and east ends of the lagoon, tolerance limits of the species with high PII sizes, lower survival and recruitment rates.

Concerning the impact of a shellfish exploitation zone, the influence of filter feeders (oysters and their epibiota) on the spatial distribution of particulates and dissolved compounds determines the biogeochemical processes both in the water column (Chapelle et al. 2001; Mazouni, Gaertner, et al. 1998; Chapelle et al. 2000) and in the sediment (Gilbert et al. 1997; Mazouni et al. 1996). The biocoenosis caused by shellfish farming results in specific ecological functioning and in a tenfold reduction in nanophytoplankton, and micro-phytoplankton biomass (Chapelle et al. 2001; Tournier & Pichot 1987; Jarry et al. 1990) as well as in microzooplankton included tintinnids, rotifers, anthozoan larvae and crustacean and mollusk larvae compared to values measured in the middle of the lagoon (Lam-Hoai et al. 1997). This ecological heterogeneity, as exemplified by the Thau basin, should affect some marine invertebrate larvae (Borsa & Millet 1992) and affect oyster recruitment at biocoenosis scale.

Our results showed that the best metamorphosis survival rate was associated with small PII size. Our results are in agreement with those of Martel et al. (2014) on *Mytilus edulis* and support the hypothesis that inter-annual variations in the adult population of most benthic invertebrates are not only related to variability in larval supply, but even more to post-settlement processes (Gosselin & Qian 1997; Fuchs et al. 2013; Hunt & Scheibling 1998).

In Hiroshima bay, the first industrial spatfall basin of *C. gigas* in Japan, the ratio of the nano- and pico-phytoplankton (>10-20 micro m) to total chl*a* in 2010 clearly decreased when the lack of natural oyster recruitment became serious (Hori, *com. pers.*). In comparison with the 1990s, when recruitment was successful, the ratio of nano- and pico-phytoplankton to total chlorophyll *a* was more than 90% from May to September (Sik Lee et al. 1996) whereas in 2017, the ratio was less than 30-60% (Matsubara, unpublished data). In France, temperature related to nanophytoplankton have been shown to have a significant influence on the success of recruitment in both the Arcachon basin and Thau lagoon (Lagarde et al. 2017; Auby & Maurer 2004). These results in France and Japan reinforce the hypothesis that the origin of the variability in the success of larval development, metamorphosis and recruitment in natural spatfall basins is mainly driven by a combination of temperature and the quality and quantity of phytoplankton. Other studies conducted in Atlantic coastal ecosystems also support this hypothesis (Pouvreau 2016).

Conclusion

In the presence of ecological heterogeneity, plasticity responses in larval traits can affect size at metamorphosis, juvenile development, and recruitment survival. Ecological structuring offers different kinds of pelagic and benthic habitats that correspond to recruitment windows (Pineda et al. 2006; Buckley et al. 2010) some of which may be optimal (Cury & Roy 1989; Lagarde et al. 2017; Pineda et al. 2006). Pechenik (1990) emphasized the absolute need for field studies to assess variability of PII size, delays in metamorphosis and their impact on juvenile survival and development. Our work helps characterize interactions between planktonic larvae and trophic and environmental conditions, including the impact of filter-feeders on the recruitment of *C. gigas.* This study highlights for the first time the existence of variability in prodissoconch II size in *C. gigas* during major oyster recruitment events in the French Mediterranean Thau lagoon in 2012, 2013 and 2014. PII size varied with the temperature and with trophic conditions and was smaller under high water temperature conditions combined with high nano and micro-phytoplankton biomass, and with tintinnid and picocyanobacterial abundances. Inversely, when cryptophytes were more abundant and water temperatures were lower, the larvae that settled and metamorphosed were bigger. We also demonstrated that the survival rate of smaller prodissoconch II shells was higher while the larger PII sizes had lower survival rates. Under oligotrophication, we hypothesize that in a favorable autotrophic context, it is better for larvae survival to metamorphose early and to be smaller whereas in unfavorable heterotrophic/mixotrophic conditions, the larvae were bigger with potential metamorphosis delays. Links with trophic conditions need to be confirmed by studying the relation between the energetic values of nano-seston and postlarvae during major recruitment events. Further laboratory experiments are recommended to test the link between size at metamorphosis, energetic reserves of larvae and the delay in metamorphosis in different trophic conditions. The negative influence of cryptophytes on metamorphosis also needs to be confirmed in lab experiments. These innovative results lead us to hypothesize that the shift from an autotrophic to a heterotrophic system could increase the variability of *C. gigas* recruitment and possibly jeopardize the sustainability of shellfish farming in coastal ecosystems undergoing oligotrophication.

Acknowledgements

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Chapitre 4 : Effet de l'approvisionnement trophique et de la connectivité hydrodynamique sur les patrons spatiaux du recrutement de l'huître creuse

Ce quatrième chapitre a été soumis pour publication dans la revue *Marine Ecology Progress Series.*

There is a tide in the affairs of men.
Which, taken at the flood, leads on to fortune;
Omitted, all the voyage of their life
Is bound in shallows and in miseries.
On such a full sea are we now afloat,
And we must take the current when it serves,
Or lose our ventures.
William Shakespeare. Julius Caesar Act 4, scene 3, 218–224

Résumé de la publication en français

La découverte récente de gisement de naissain d'huîtres dans une lagune méditerranéenne exploitée de manière intensive par la conchyliculture a révélé d'importants contrastes dans les schémas spatiaux de recrutement. Notre objectif est ici de comprendre cette variabilité en comparant le recrutement observé, la connectivité hydrodynamique simulée et les variables écologiques. Nous avons émis l'hypothèse que la variabilité spatiale du recrutement dépend d'une part de la connectivité hydrodynamique et d'autre part de l'écologie larvaire en termes d'approvisionnement larvaire, fixation, survie à la métamorphose et enfin des paramètres environnementaux. Nous avons évalué le recrutement par échantillonnage larvaire et collecte de naissain à l'intérieur et à l'extérieur des zones ostréicoles et selon un gradient de confinement Est-Ouest de 2012 à 2014. La connectivité hydrodynamique a été simulée à l'aide d'un modèle numérique de transport et évaluée par un indicateur eulérien.

L'approvisionnement en larves umbonées n'est pas apparu significativement différent à l'intérieur des zones ostréicoles et à l'extérieur alors que les abondances de pédivéligères approvisionnant les sites à l'extérieur des élevages conchylicoles étaient structurées en fonction de la connectivité hydrodynamique. À l'intérieur des zones d'élevage, les conditions trophiques défavorables mettent en péril la fixation des larves sur les collecteurs confirmant l'hypothèse d'un contrôle trophique top-down par les huîtres dans les zones conchylicoles. Les connaissances sur la dispersion, la connectivité et le recrutement des huîtres dans les lagunes côtières contribuent à l'échelle locale au développement d’une nouvelle pratique culturale durable de collecte de naissains sauvages. À l'échelle globale, la compréhension des patrons spatiaux de recrutement d’huîtres contribuera à une meilleure gestion de l'industrie ostréicole basée sur la collecte de naissains natifs dans un contexte d'exploitation durable et de restauration écologique des écosystèmes conchylicoles.

Duality of trophic supply and hydrodynamic connectivity drives spatial patterns of Pacific oyster recruitment

Franck Lagarde, Annie Fiandrino, Martin Ubertini, Emmanuelle Roque d’orbcastel, Serge Mortreux, Claude Chiantella, Béatrice Bec, Delphine Bonnet, Cécile Roques, Ismaël Bernard, Marion Richard, Thomas Guyondet, Stéphane Pouvreau, Christophe Lett

Introduction

Additional knowledge on reproduction and recruitment is needed to improve the understanding of marine population dynamics and fishery stock management, both of which are facing changing conditions (Hunt & Scheibling 1997b; Pérez-Ruzafa et al. 2018; Cowen et al. 2007). The early life of most marine benthic invertebrates includes spawning, larval development, dispersal (Todd 1998; Cowen & Sponaugle 2009), settlement (Gaines et al. 1985), and ultimately recruitment of juveniles into the host ecosystem (Keough & Downes 1982). These different processes are encompassed into the concept of marine population connectivity between spawning and recruitment areas (Pineda et al. 2007) and define the rate of transfer of organisms between locations (Bryan-Brown et al. 2017). More specifically, hydrodynamic connectivity is defined as a landscape metric used to compare the role of different hydrodynamic basins and to individualize dispersal, concentration or cumulated flux of tracer per volume or area (Ghezzo et al. 2015; Thomas, Dumas, et al. 2016; Lagarde, Fiandrino, et al. 2015) allowing characterization of systems at different spatial and temporal scales. The number of studies to improve connectivity knowledge has increased markedly over the last 30 years (Bryan-Brown et al. 2017). However, Ghezzo et al. (2015) pointed at the lack of connectivity studies mixing modelling and field work with direct data of larval development and environmental factors. Here, we wish to fill this gap studying the transition from pelagic to benthic life of the Pacific oyster (*Crassostrea gigas*) in a coastal, nanotidal, and semi-enclosed Mediterranean lagoon.

For most marine larvae, the transition between pelagic development and benthic phase is induced by metamorphosis competence allowing settlement. Settlement is initially driven by the hydrodynamics of the system (Wing et al. 2003; Todd 1998; Archambault & Bourget 1999), but the biological properties of the organisms also play an important role in terms of physiological (trophic diet, food limitation or energy depletion), ethological (migration, trophic settlement trigger) and ecological (predation, competition) aspects of the development phase (Hunt & Scheibling 1997b).

Despite the species' economic importance, the early benthic settlement and post settlement period have been poorly studied *in situ* for oysters (Lagarde et al. 2018). The origins of recruitment variability are still vastly unknown because of species and habitats specificities and the complexity of ecosystem dynamics that prevent the derivation of generalities (Roughgarden et al. 1988). This complexity calls for the study of local structures and for checking the general functioning of recruitment and production by species (Hori et al. 2017).

The recent discovery of oyster spatfields in the Mediterranean Thau lagoon highlighted some strong temporal biological and ecological processes influencing recruitment (Lagarde et al. 2017). Temporal windows of oyster recruitment in the Mediterranean Thau lagoon were shown to depend on autotrophic *vs.* heterotrophic conditions related to water temperature and to the global ecological functioning of the ecosystem. Such results are best obtained in studies examining several larval stages, in order to determine the relative importance of each stage and a potential ecological lock in larval development (Hunt & Scheibling 1997a; Ghezzo et al. 2015; Pouvreau 2018). In addition to the temporal component, spatial patterns in settlement or early post-settlement were found to influence the distribution and abundance of benthic marine invertebrate juveniles and adults (Hunt & Scheibling 1997b; Thomas, Dumas, et al. 2016). A similar trend should be expected in coastal lagoons such as Thau, as it is heavily exploited by shellfish industries in specific biocoenosis (Lam-Hoai et al. 1997; Mazouni, Deslous-Paoli, et al. 1998; Souchu et al. 2001) and its confinement level interferes with benthic assemblages (Borsa & Millet 1992; Ghezzo et al. 2015; Pérez-Ruzafa et al. 2018).

Through the connectivity approach, we studied the spatial variability of oyster recruitment including recruitment limitation in the oligotrophication context of the Thau lagoon. The population connectivity of oysters should result in societal gain locally by informing the development of a virtuous practice of natural spatfall collection. At the global level, the described processes will help manage areas intensely exploited by shellfish farming and for which the conciliation between ecological restoration, oligotrophication and sustainable exploitation must be adjusted. For that, we tested the hypothesis that the spatial variability of recruitment depends on hydrodynamic connectivity and on larval ecology in terms of supply, settlement and recruitment (Souchu et al. 2010; Collos et al. 2009; Lagarde et al. 2017). We aimed to explain the spatial pattern of recruitment of *C. gigas* with data produced by both field observations and hydrodynamic modeling in order to establish relationships between larval supply, connectivity and ecological functioning. Our objective was to disentangle and evaluate the role of connectivity compared to biotic factors.

Materials and methods

Study site

The study site is the French shellfish-exploited Mediterranean Thau Lagoon located on the northern coast of the Mediterranean Sea along the Gulf of Lion (Figure 37). This restricted nanotidal semi-enclosed hydrosystem (Kjerfve 1994) covers an area of 7500 ha (19 × 4.5 km) on a Northeast-Southwest axis with a mean depth of 3.5 m (Amanieu et al. 1989). The lagoon is influenced by seawater inputs from the Mediterranean Sea by 2 artificial inlets, i.e. the Sète channel in the North supplying 90% of seawater exchanges and the channel de Pisse-Saumes inlet in the South. Wild oyster stocks are negligible compared to oyster farming. Twenty percent of the total Thau Lagoon area is dedicated to intense cultivation of mussels and oysters, whereas other areas are used for fishing and are devoid of shellfish culture. Eight spatfall sites of the Thau lagoon (Figure 37) were monitored to assess pre-settled oyster larvae and post-settled spat abundances in pelagic and benthic compartments: three inside shellfish-farming zones (Marseillan_ISFZ, Meze_ISFZ and Bouzigues_ISFZ) and five outside (Marseillan_OSFZ, Listel_OSFZ, Meze_OSFZ, Bouzigues_OSFZ and Balaruc_OSFZ).

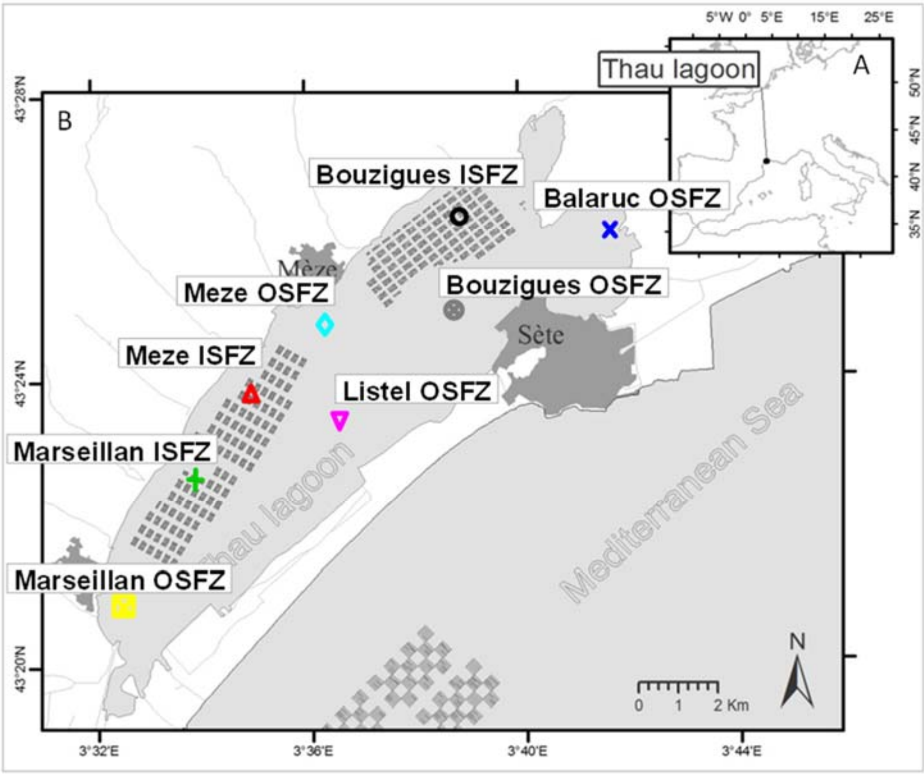

Figure 37 : (A) The Mediterranean Thau lagoon in South of France and (B) sampling sites within the Thau lagoon. ISFZ: Inside Shell Fish Farming Zone (monitoring took place under farmed structures); OSFZ: Outside the shellfish farmed zone (monitoring took place at specially designed mooring systems, see Lagarde et al 2017). Labels are the 8 sampling sites with three ISFZ as Marseillan_ISFZ (green plus), Meze_ISFZ (red triangle point up) and Bouzigues_ISFZ (black open circle) and five sites OSFZ with Marseillan_OSFZ (yellow square cross), Listel_OSFZ (magenta triangle point down), Meze_OSFZ (cyan diamond), Bouzigues_OSFZ (grey circle cross) and Balaruc_OSFZ (blue cross) where pelagic larvae and benthic Pacific oyster larvae, spat abundances, hydrological and plankton data were monitored. Grey boxes: shellfish farms, dark grey areas: towns

Larval and recruitment abundance

As detailed by Lagarde et al. (2017), *C. gigas* pelagic and benthic larval abundances were assessed from June to September 2012, 2013 and 2014. Pelagic larvae assessments were carried out twice a week using a standard protocol provided by the Oyster Larvae Monitoring French Network (Pouvreau, Bellec, et al. 2013; Pouvreau 2016). A sampling volume of 1.5 m^3 was pumped and filtered through a 40 µm plankton net to assess the abundance of oyster larvae ranging from ‘D-larvae’ (size between 60 and 100 µm) to ‘large umbo larvae’ (180 to 300 µm) (Pouvreau et al. 2016). Benthic oyster abundances were estimated on plate collectors every 2 weeks at 3 different settler stages: pre-settled larvae as pediveligers, size ranging from 190 to 300 µm, young post-larvae after metamorphosis from 300 to 1000 µm (Coon et al. 1990; Pechenik 2006) and newly settled spat from 1 to 8 mm (Arakawa 1990a).

Our definition of the recruitment of benthic marine invertebrates includes attainment of a specific stage of development with settlement and metamorphosis and survival to a period of 4 weeks of collector immersion (Booth & Brosnan 1995).

Environmental measurements

As detailed by Lagarde et al. (2017), environmental parameters (hydrological and plankton samples) were recorded every week from June to September 2012, 2013 and 2014 nearby spatfall sites Listel_OSFZ, Bouzigues_ISFZ and Marseillan_ISFZ. Temperature and salinity were measured twice a week and weekly averaged with WTW® probes positioned between 1 and 1.5 m below the surface. The planktonic community (bacteria (<1 µm), pico- (< 3 µm), nano- (3µm to 20µm) and microphytoplankton (>20 µm), protozooplankton and mesozooplankton) was monitored for its biomass and abundance (Tableau 10) every week from June to September 2012, 2013 and 2014 nearby spatfall sites Bouzigues_ISFZ, Marseillan_ISFZ and Listel_OSFZ (except Listel_OSFZ in 2012) (Lagarde et al. 2017; REPHY – French Observation and Monitoring program for Phytoplankton and Hydrology 2017). Abundances of potential predators and trophic competitors of *C. gigas* larvae were estimated by taxonomic identification using a stereoscopic microscope (Rose 1933). The 'trophic competitors' group was determined as the sum of copepod nauplii, annelids and barnacle, ascidia and gastropod larvae. 'Potential predators' were assessed as the sum of cladocerans (*Penilia avirostris, Podon spp., and Evadne spp.*), decapod larvae, mysids and hydrozoa (*Obelia spp.*).

The wind velocity and direction were measured at Marseillan_ISFZ station with a Campbell Scientific datalogger (CR1000) and Ultra Sonic Wind Sensor (Windsonic Inc.). These data were acquired at a frequency of 1 minute and averaged over 10 minutes. Wind speeds and directions are considered as spatially homogeneous over the lagoon (Fiandrino et al. 2017).

Tableau 10: Variables characterizing the interactions between the environment and Pacific oyster larvae from the sampling sites Bouzigues_ISFZ, Marseillan_ISFZ and Listel_OSFZ monitored from June to the end of September 2012, 2013 and 2014 (except Listel_OSFZ in 2012).

Variables	Description	Unity
Target variables		
oyster spat	abundance	ind. plate^{-1}
postlarvae	abundance	ind. plate^{-1}
pediveligers	abundance	ind. plate^{-1}
Environmental variables		
hydrodynamic connectivity	Daily ratio	% d^{-1}
D larvae	D larvae abundance	ind. m^{-3}
Umbo larvae	Umbo larvae abundance	ind. m^{-3}
pediveliger/spat survival	ratio of pediveliger abundance on oyster spat	-
temperature	daily average	°C
salinity	daily average	*No unit*
bacteria	abundance	10^{6} cell. l^{-1}
autotrophic picoeukaryotes	abundance	10^{6} cell. l^{-1}
picocyanobacteria	abundance	10^{6} cell. l^{-1}
picophytoplankton	abundance	10^{6} cell. l^{-1}
nanophytoplankton	abundance	10^{6} cell. l^{-1}
cryptophycae	abundance	10^{6} cell. l^{-1}
nanophytoplankton + cryptophycae	abundance	10^{6} cell. l^{-1}
heterotrophic flagellates	abundance	cell l^{-1}
naked ciliates	abundance	cell l^{-1}
tintinnidae	abundance	cell l^{-1}
diatoms	abundance	cell l^{-1}
dinoflagellates	abundance	cell l^{-1}
Chaetoceros	abundance	cell l^{-1}
total chlorophyll *a*	biomass	µgChla l^{-1}
picophytoplankton	biomass	µgChla l^{-1}
nanophytoplankton	biomass	µgChla l^{-1}
picophytoplankton+ nanophytoplankton	biomass	µgChla l^{-1}
microphytoplankton larger than 20µm	biomass	µg l^{-1}
competitors	abundance	ind. m^{-3}
predators	abundance	ind. m^{-3}

Hydrodynamic connectivity index

We used an Eulerian indicator to simplify as much as possible the complexity of the processes involved. We defined the hydrodynamic connectivity (Ghezzo et al. 2015) between a finite emission volume (V_E) and a finite destination volume (V_D) as the cumulative amount of a passive conservative tracer released from V_E and entering into V_D over a given period of time (ΔT). The intrinsic movement of larvae (horizontal swimming and vertical migration) and their mortality are not taken into account as the study focuses strictly on the hydrodynamic aspect of connectivity.

To assess hydrodynamic connectivity for *C. gigas* larvae in Thau, we used the 3-dimensional hydrodynamic Model for Application at Regional Scale (MARS-3D). The MARS-3D model (Lazure & Dumas 2008) was already implemented and validated to simulate water exchange between the Thau lagoon and the Mediterranean sea (Fiandrino et al. 2017). The model grid has a spatial resolution of 100 m and bathymetry was obtained from the 2010 survey by the "Cellule de qualité des Eaux Littorales" of Région Occitanie/Languedoc-Roussillon. On the vertical axis, 10 sigma layers are distributed to represent both the bottom and the surface boundary layers.

A passive conservative tracer was released from the seashell farming zones at the beginning of the simulation and used to materialize the water bodies circulation associated to larval transport. The amount of tracer $Q_D(i,j,T_{Integ})$ entering the volume of each cell of the horizontal 2D-grid (integrated over the whole water column) was cumulated over $T_{Integ} = 18$ days, corresponding to the potential larval settlement duration (Lagarde, Fiandrino, et al. 2015; Pouvreau 2015). Moreover, the integration started 4 days after tracer release to reflect the minimal pelagic larval duration (T_{PLD}) observed for *C. gigas* (Pouvreau 2018).

The local hydrodynamic connectivity index $CI(i,j,T_{Integ})$ is then defined as the ratio of $Q_D(i,j)$ to the total amount of tracer released from shellfish farming areas $Q_E = [C_{PCT}].V_E$ where V_E is the combined volume of all shellfish farming areas and $[C_{PCT}]$ is vertical concentration in all cells of the grid corresponding to shellfish farming areas (n=566).

$$CI(i,j,T_{Integ}) = \frac{Q_D(i,j)}{QE} \Big/ T_{Integ}$$

To compare observed recruitment and simulated hydrodynamic connectivity, CI values were calculated for the dates of each collectors harvest used to estimate the abundance of pediveligers, i.e. every 2 weeks from June 1st to October 1st for years 2012-2014.

Simulation setup, forcing and open boundary conditions of the MARS-3D model

The passive conservative tracer is released at the beginning of the simulation with a uniform horizontal concentration in the shellfish farming areas. A preliminary simulation was done to calculate and record the daily hydrodynamic states of the system from the first of April until the first of October for each studied year. The relevant recorded daily system states were used as initial conditions for each simulation of hydrodynamic connectivity indicator.

A detailed description of atmospheric forcing and open boundary conditions for the Thau lagoon is provided by Fiandrino et al (2017). Some meteorological data described the state of the atmospheric boundary layer: the wind speed measured at 10 m above sea level, and the air pressure at the sea level. Chronologies of wind speed and direction were available directly above the Thau lagoon and are considered as homogeneous for the total surface of the lagoon. At the open boundary the model is forced by the tide gauge data acquired in Sète channel (Holgate et al. 2013) with 2016 data from Permanent Service for Mean Sea Level. At the open boundary temperature and salinity are prescribed according to temperature and salinity monitored fortnightly within the scope of the French National Phytoplankton and Phycotoxin Monitoring Network (REPHY – French Observation and Monitoring program for Phytoplankton and Hydrology 2017) at the Sète-Mer station, located just off the Sète channel inlet.

Data analysis

Statistical analyses were performed with R statistical software (Core Team 2015). Oyster spat recruitment was displayed graphically using comparison of means with 95% confidence intervals. Box and whisker plots were used to discriminate ISFZ *vs* OSFZ biocoenosis effect on larval abundance for the four different stages of development (D-Larvae, Umbo Larvae, pediveliger and spat). We tested the hypothesis that larval abundances did not vary according to ISFZ-OSFZ biocoenosis (under $\log_{10}$ transformation) with a parametric analysis of variance if prerequisites of normality, homoscedasticity and independence were respected; otherwise, we used a non-parametric Kruskall-Wallis test.

To establish yearly maps of averaged hydrodynamic connectivity on the three studied years, we performed 50 simulations started every 3 days from June the 1st to September the 29th. The mean of hydrodynamic connectivity index was averaged with the 50 values of indicators calculated over periods of 18 days corresponding to the maximal potential settlement larval duration.

Simulated connectivity values for the eight experimental sampling sites were presented with box and whiskers plots with additional Tukey Contrasts Multiple Comparisons of Means (‘multcomp’ package) differentiating the connectivity levels of the different sites.

The relationship between simulated connectivity and abundances of the different larval stages was assessed by mean and standard error bars on scatterplot in both the x and y directions, and regression lines.

Results

Spatial variability of oyster spat recruitment

We found significant differences of oyster spat recruitment among the sampling sites in the Thau lagoon (Figure 38). In 2012, the first significant event of spat collection was recorded on the collector harvest of August 13th with 126 and 47 spat ind. plate^{-1} at Listel_OSFZ and Bouzigues_ISFZ, respectively (Figure 38). The second important spat harvest occurred on September 24th at the same sites, and was characterized by lower abundances (45 and 21 ind. plate^{-1} at Bouzigues_ISFZ and Listel_OSFZ, respectively). We also collected 34 ind. plate^{-1} at Balaruc_OSFZ at that time. In 2013, there was one main spatfall event lasting for two consecutive harvests in August with 244 (August 13th) and 341 (August 28th) ind. plate^{-1} at Meze_OSFZ, 92 ind. plate^{-1} at Listel_OSFZ (August 28th), and 188 (August 13th) and 56 (August 28th) ind. plate^{-1} at Bouzigues_ISFZ. There was a second, even stronger spatfall event (488 ind. plate^{-1}) that was only detected at Meze_OSFZ during the October 9th harvest. In 2014, we obtained the most contrasting results between ISFZ and OSFZ sites, with spatfall occurring on all OSFZ sites at the exception of Marseillan OSFZ during 2 months. This period was characterized by 4 consecutive harvest dates between August 13th and September 24th, versus no recruitment at all ISFZ sites. Meze_OSFZ showed the most remarkable spat abundances, peaking at 1112 ind. plate^{-1} on August 27th.

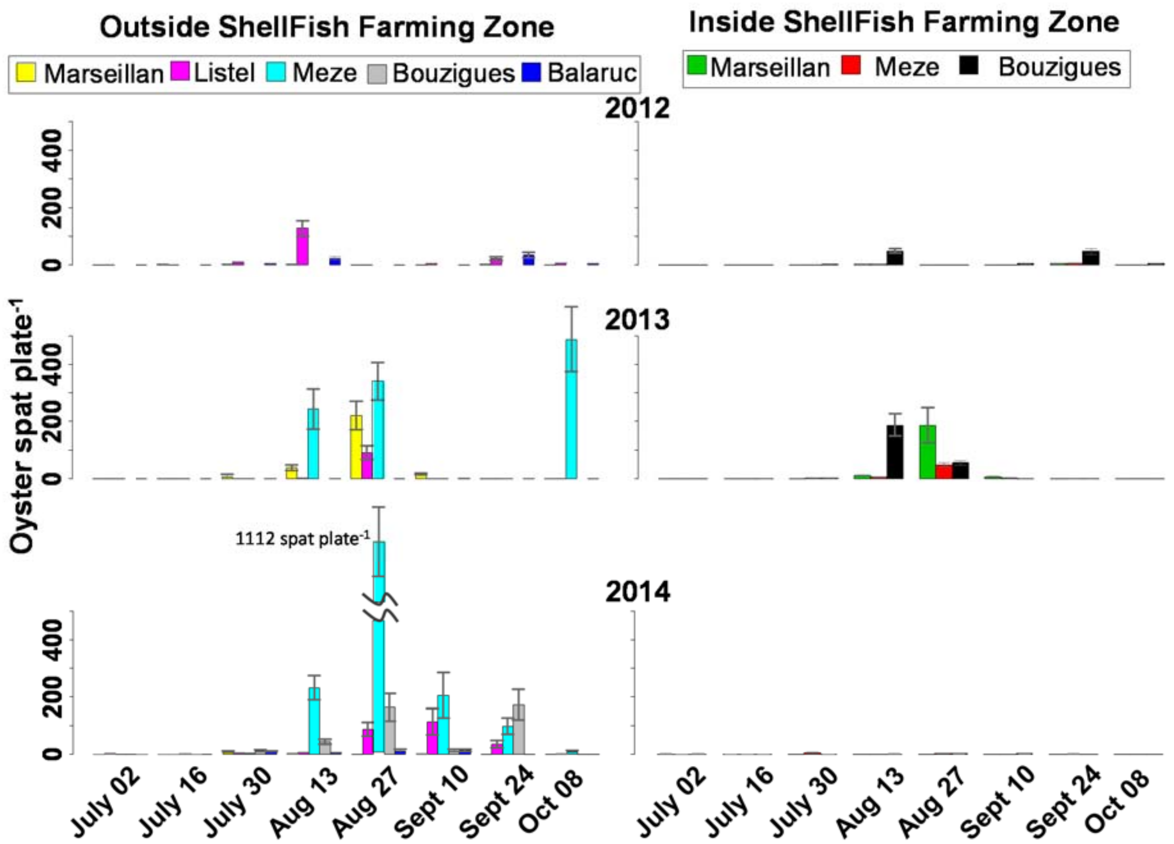

Figure 38 : Mean (±95% CI) oyster spat abundance per plate observed at eight sampling sites of the Thau lagoon, three located inside shellfish farming zones (ISFZ) (Marseillan_ISFZ -green-, Meze_ISFZ -red- and Bouzigues_ISFZ -black-) and five outside (OSFZ) (Marseillan_OSFZ -yellow-, Listel_OSFZ -magenta-, Meze_OSFZ -cyan-, Bouzigues_OSFZ -grey- and Balaruc_OSFZ -blue-). Observations were done at 2-week intervals throughout summers 2012, 2013, and 2014. Spat abundances were estimated after 4 weeks of collector immersion (n = 54 per date and sampling site).

Spatial variability of oyster larvae abundances

The mean abundances of D larvae (over all sampling dates and all three year) were significantly different ($p < 0.0001$) and higher ISFZ (1.58×10^4 ind. m^{-3}, n = 46) than OSFZ ($1.58\ 10^3$ ind. m^{-3}, n = 68) (Figure 39a). By contrast, the mean abundances of umbo larvae were not statistically different ($p > 0.05$) between ISFZ (170 ind. m^{-3}, n = 66) and OSFZ (209 ind. m^{-3}, n = 79), and median values were also the same (~100 ind. m^{-3}) (Figure 39b).

Mean abundances of pediveligers were significantly different ($p < 0.0001$) between ISFZ and OSFZ (Figure 39c), but this time with lower abundances ISFZ (21 ind. $plate^{-1}$, n = 46) than OSFZ (182 ind. $plate^{-1}$, n =68). This holds true for mean oyster spat abundances, with significantly lower abundances ISFZ ($p < 0.0001$, 2 ind. $plate^{-1}$, n = 72) than OSFZ (5 ind. $plate^{-1}$, n = 96) (Figure 39d).

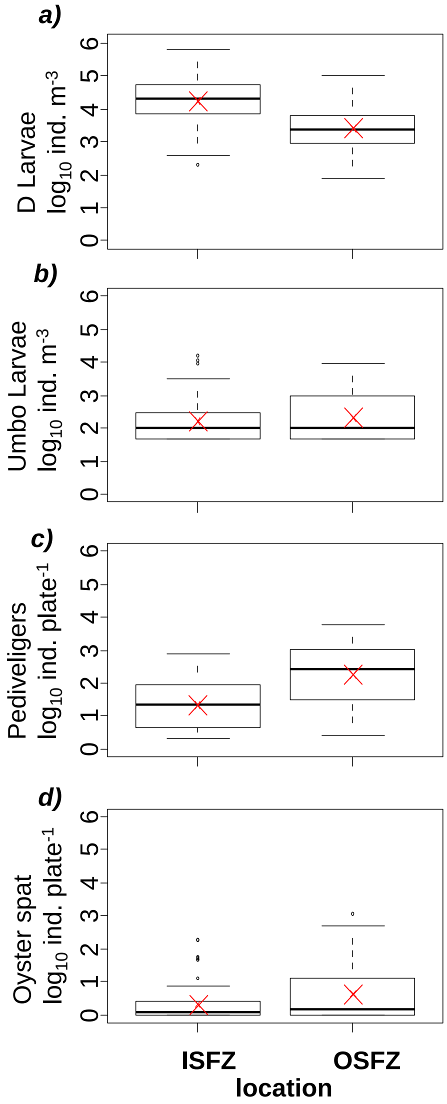

Figure 39: Distributions of oyster D larvae ($n_{ISFZ} = 46$; $n_{OSFZ} = 68$), umbo larvae ($n_{ISFZ} = 66$; $n_{OSFZ} = 79$), pediveligers larvae ($n_{ISFZ} = 46$; $n_{OSFZ} = 68$) and oyster spat ($n_{ISFZ} = 72$; $n_{OSFZ} = 96$) inside (ISFZ) and outside (OSFZ) shellfish farming zones of the Thau lagoon. Mid-line: median; box: 25th and 75th percentiles; whiskers: 1.5 × the interquartile range; circles: outliers. Red crosses: means.

Spatial patterns of simulated connectivity

The spatial distribution of simulated hydrodynamic connectivity values over the Thau lagoon (Figure 40) for passive tracers released over the oyster farming zones showed low values all along the periphery of the lagoon and two gradients of increasing values i) from ISFZ to OSFZ and ii) from West to East elsewhere, with weak interannual variability.

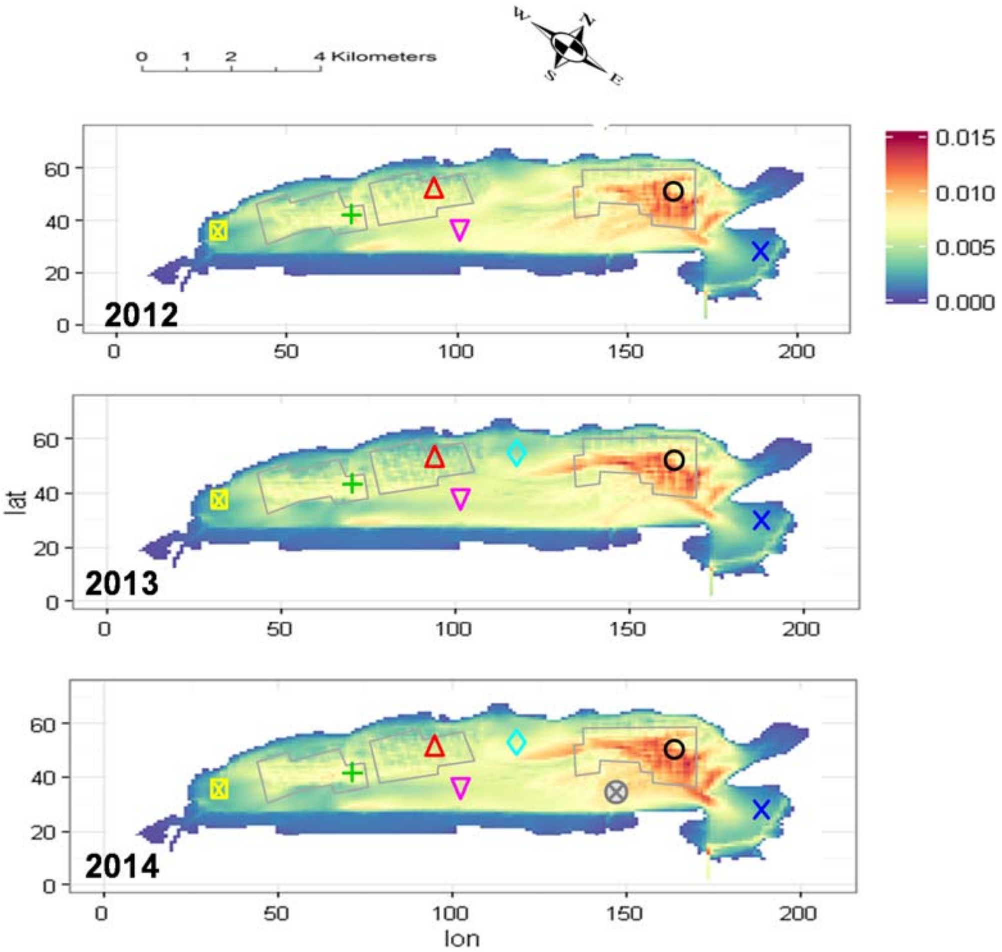

Figure 40: Spatial distribution of simulated hydrodynamic connectivity values over the Thau lagoon. The values (in % .d^{-1}) represent the percentage of passive tracers concentrations released over the oyster farming zones (see Fig. 36) that reach the represented locations after 18 days of transport (see text), averaged for the summer atmospheric conditions of years 2012 to 2014. Labels are the 8 sampling sites with three ISFZ as Marseillan_ISFZ (green plus), Meze_ISFZ (red triangle point up) and Bouzigues_ISFZ (black open circle) and five sites OSFZ with Marseillan_OSFZ (yellow square cross), Listel_OSFZ (magenta triangle point down), Meze_OSFZ (cyan diamond), Bouzigues_OSFZ (grey circle cross) and Balaruc_OSFZ (blue cross) where pelagic larvae and benthic Pacific oyster larvae, spat abundances, hydrological and plankton data were monitored. Grey boxes: shellfish farms.

We found groups of sampling sites significantly different regarding their levels of connectivity ($p < 0.0001$; Tukey Contrasts Multiple Comparison Tests)(Figure 41). The lowest mean connectivities (CI) were obtained for the two most peripheral sites, Balaruc_OSFZ (CI = 0.003 d^{-1}, n=17) and Marseillan_OSFZ (CI = 0.002 d^{-1}, n=17). By contrast, Bouzigues_ISFZ (CI = 0.008 d^{-1}, n = 16) and Bouzigues_OSFZ (CI = 0.009 d^{-1}, n = 6) had the highest connectivity values. The four remaining sites had intermediate levels of connectivity, ranging from 0.004 to 0.007, and were grouped differently (b, c, d groups) by the multiple comparison test depending either on their location along the east-west axis of the lagoon or according to their ISFZ/OSFZ location relative to the shellfish farming zones.

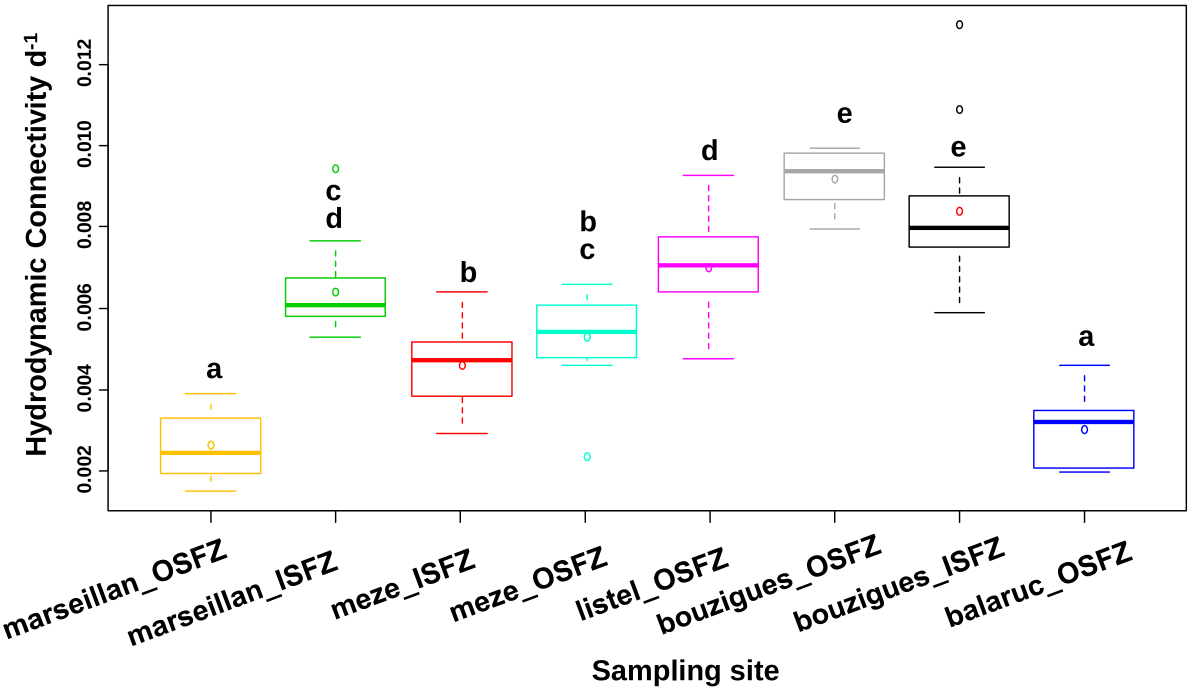

Figure 41: Simulated connectivity (n_{total}=132) averaged over the experimental sampling sites and dates. Letter above represent groups defined by Tukey Contrasts Multiple Comparisons of Means.

Connectivity and larval supply

Putting observed larval abundances and simulated connectivity values in correspondence for ISFZ and OSFZ biocoenosis, we found a non-significant relationship ($p > 0.05$) of umbo larvae abundance with connectivity in both types of biocoenosis (Figure 42a). The two eastern and western sides, Marseillan_OSFZ and Balaruc_OSFZ, had the lowest connectivity and recorded also the lowest abundances of umbo larvae. The abundances of umbo larvae were non-significantly different regarding biocoenosis ($p > 0.05$) with a very low depletion ISFZ compared to OSFZ. In contrast, the interaction "simulated connectivity: biocoenosis" was significant ($p < 0.005$) with a significant positive relationship between connectivity and pediveligers abundance was observed with OSFZ sites ($p < 0.0001$) while in ISFZ sites this relationship was not significant (Figure 42b). The Bouzigues_OSFZ site was defined by high values of connectivity and by high mean abundances of pediveligers (group e, 2291 ind. plate^{-1}). With a similar level of connectivity, the Bouzigues_ISFZ site had a mean abundance of pediveligers about seventy times lower (group ac, Bouzigues_ISFZ= 28 ind. plate^{-1}). The western sites Marseillan_ISFZ and Marseillan_OSFZ were characterized by low abundances of pediveligers (respectively, group a and ab, 17 ind. plate^{-1} and 28 ind. plate^{-1}). In the central part of the lagoon, the pediveliger abundances were approximately thirty times higher at Listel_OSFZ than at Meze_ISFZ (group ab, 22.4 ind. plate^{-1}). A ratio of 70 is found between the pediveligers abundances of the site with the highest connectivity (Bouzigues_ISFZ) and the site with the lowest connectivity (Marseillan OSFZ). Regarding intercepts of fitted regression models, sites in the ISFZ such as Bouzigues_ISFZ, Meze_ISFZ and Marseillan_ISFZ had significantly lower pediveliger abundances (20 ind. plate^{-1}, $p < 0.05$) than in the OSFZ sites (204 ind. plate^{-1}, $p < 0.001$). Concerning Ancova results, there was a significant effect of the connectivity ($p < 0.05$) on pediveliger supply, on the biocoenosis type ISFZ or OSFZ ($p < 0.0001$) and on the "Connectivity : biocoenosis type" interaction ($p < 0.01$).

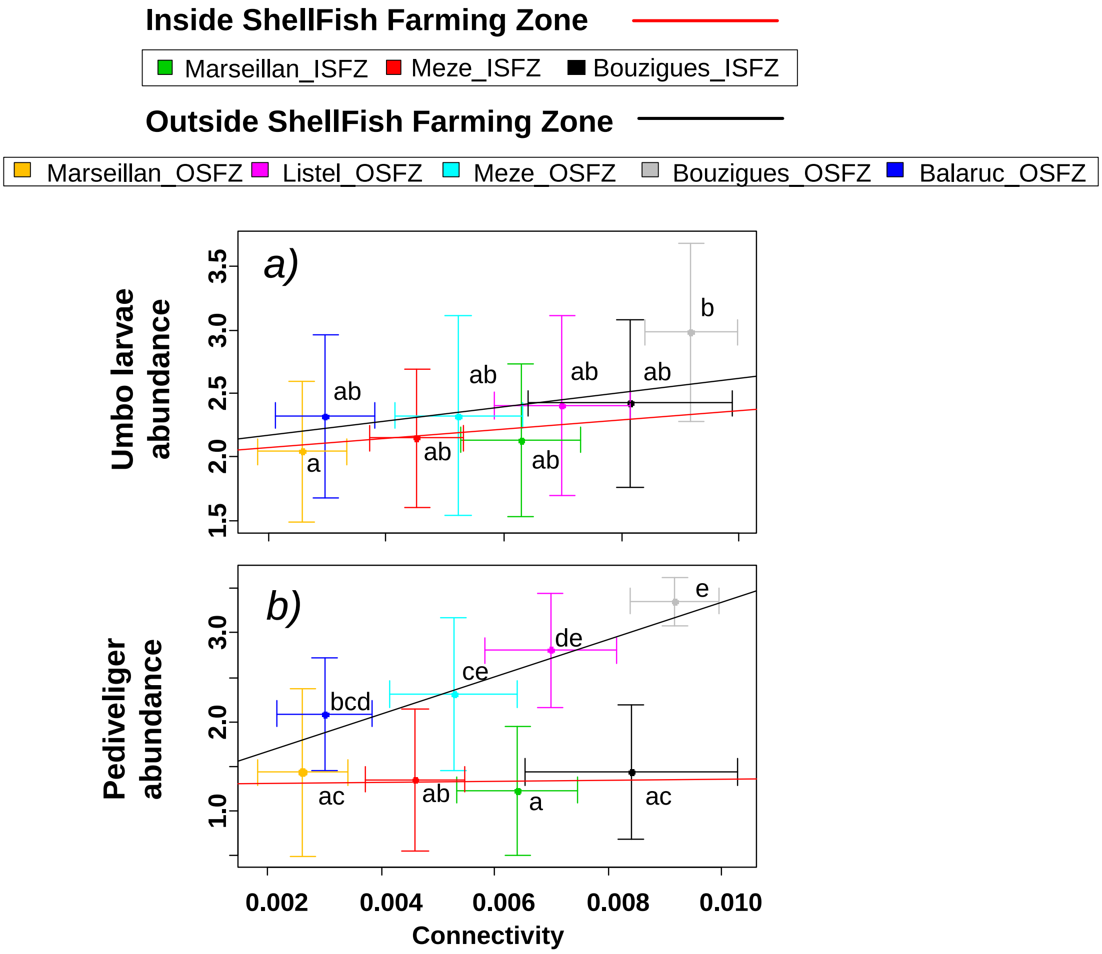

Figure 42: Relationship between connectivity (mean ± SE) and abundance of a) Umbo larvae ($\log_{10}$ m^{-3}, n_{ISFZ}=66, n_{OSFZ}=79), b) pediveligers ($\log_{10}$ plate^{-1}, n_{ISFZ}=46, n_{OSFZ}=68) for the eight sampling sites. Letters differentiate the level of group according to the Tukey Contrasts Multiple Comparisons of Means. Regression lines: Red for Inside Shellfish Farming Zone, Black for Outside Shellfish Farming Zone.

Spatial variability of ecological interactions

Eight of the twenty-six monitored environmental variables (Tableau 11) were significantly different (Kruskall-Wallis tests, $p < 0.05$) among three sampling stations (Marseillan_ISFZ, Bouzigues_ISFZ and Listel_OSFZ): biomasses of pico + nanophytoplankton (< 20 µm), microphytoplankton (> 20 µm) and nanophytoplankton (3-20 µm) and abundances of autotrophic picoeukaryotes, nanophytoplankton, *Chaetoceros spp,* mesozooplankton competitors and predators (Figure 43). Generally, abundances and biomasses were lowest at Marseillan_ISFZ, intermediate at Bouzigues_ISFZ and highest at Listel_OSFZ, except for the picoeukaryote abundances for which Listel_OSFZ had the lowest value. Between the two ISFZ sites, significant differences occurred for 5 of the 26 variables (pico + nanophytoplankton biomass, nanophytoplankton biomass, microphytoplankton biomass, *Chaetoceros spp* abundance, and predators abundance), with lower values obtained at Marseillan than at Bouzigues (Figure 43). Concerning pico + nanophytoplankton, the averaged biomass at Marseillan_ISFZ was weaker (0.87 ± 0.33 µgChla l^{-1}) than Bouzigues_ISFZ (1.14 ± 0.45 µgChla l^{-1}) like for $\text{Nanophytoplankton}_{\text{Marseillan\_ISFZ}}$ (0.44 ± 0.27 µgChla l^{-1}) and $\text{Nanophytoplankton}_{\text{Bouzigues\_ISFZ}}$ (0.71 ± 0.37 µgChla l^{-1}) and $\text{Microphytoplankton}_{\text{Marseillan\_ISFZ}}$ (0.29 ± 0.25 µgChla l^{-1}) and $\text{Microphytoplankton}_{\text{Bouzigues\_ISFZ}}$ (0.82 ± 0.51 µgChla l^{-1}). The abundances of *Chaetoceros spp* between Marseillan_ISFZ and Bouzigues_ISFZ were different with respectively $4.53 \times 10^4 \pm 9.87 \times 10^4$ cell l^{-1} and $2.13 \times 10^5 \pm 3.22 \times 10^5$ cell l^{-1}. The ISFZ and OSFZ contrasts was mainly expressed by the abundances of Picoeukaryote, *Chaetoceros spp*, Nanophytoplankton, Competitors and Predators. Concerning the picoeukaryote, the similar abundances of Marseillan_ISFZ ($3.6 \times 10^7 \pm 2.1 \times 10^7$ cell l^{-1}) and Bouzigues_ISFZ ($3.4 \times 10^7 \pm 1.7 \times 10^7$ cell l^{-1}) was practically twice higher than at the Listel_OSFZ site ($1.8 \times 10^7 \pm 0.9 \times 10^7$ cell l^{-1}). *Chaetoceros spp* revealed a high cell abundance of $4.2 \times 10^5 \pm 5.0 \times 10^5$ cell l^{-1} at Listel_OSFZ, corresponding to an abundance two and nine fold higher than one recorded at Bouzigues_ISFZ and at Marseillan_ISFZ respectively. Regarding nanophytoplankton, there was 30% more abundance at Listel_OSFZ ($4.0 \times 10^6 \pm 2.36 \times 10^6$ cell l^{-1}) than at Bouzigues_ISFZ sites ($3.2\ 10^6 \pm 2.66 \times 10^6$ cell l^{-1}). Competitors' abundances were on average twice as high OSFZ ($\text{competitors}_{\text{Listel\_OSFZ}} = 36.1 \times 10^3 \pm 28.5 \times 10^3$ ind. m^{-3}) than ISFZ ($\text{competitors}_{\text{Bouzigues\_ISFZ}} = 18.6 \times 10^3 \pm 12.8 \times 10^3$ ind. m^{-3} and $\text{competitors}_{\text{Marseillan\_ISFZ}} = 20.3 \times 10^3 \pm 17.0 \times 10^3$ ind. m^{-3}). The predators abundance was lower ISFZ ($\text{predators}_{\text{Marseillan\_ISFZ}} = 0.05 \pm 0.06$ ind. m^{-3}; $\text{predators}_{\text{Bouzigues\_ISFZ}} = 0.19 \pm 0.22$ ind. m^{-3}) compared to OSFZ ($\text{predators}_{\text{Listel\_OSFZ}} = 0.46 \pm 0.44$ ind. m^{-3}).

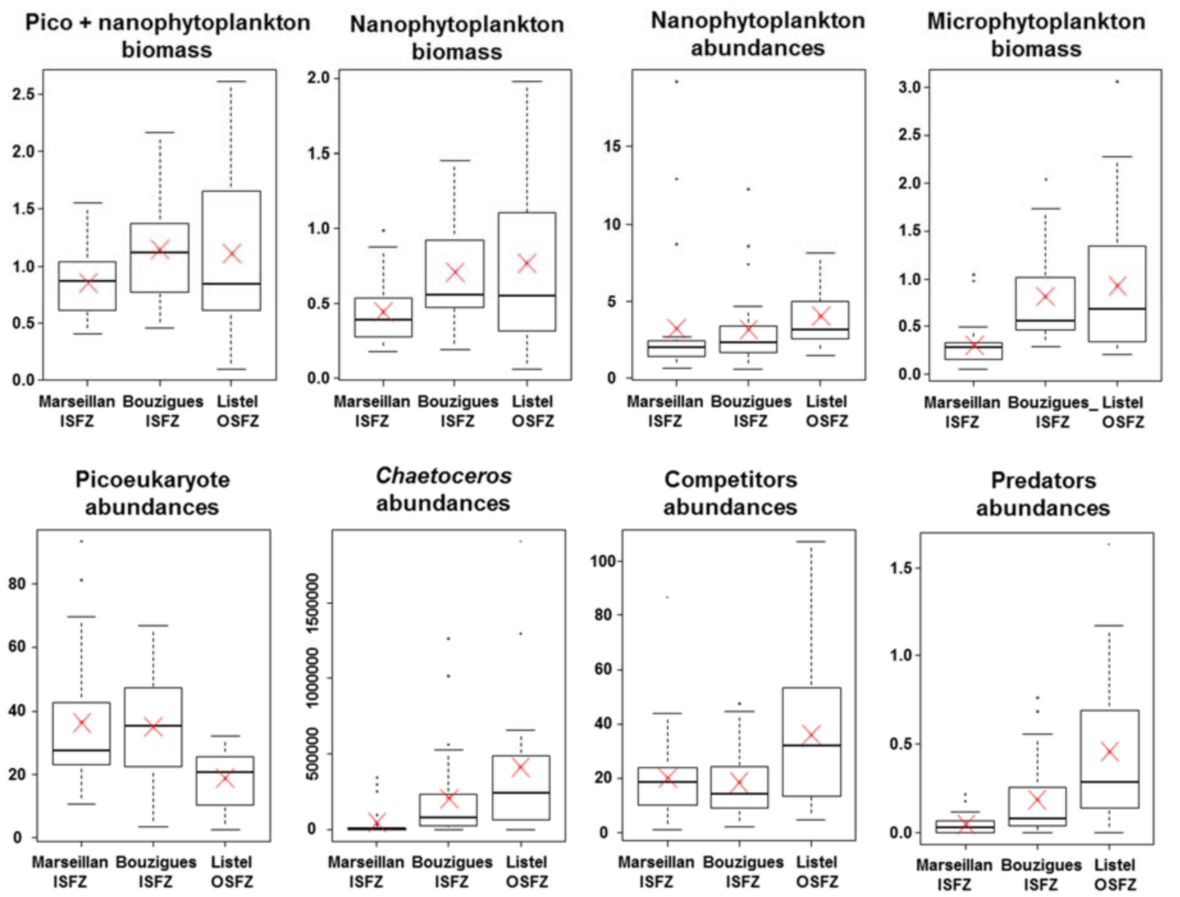

Figure 43: Planktonic abundances that showed a significant difference ($p<0.05$) between sampling sites Marseillan_ISFZ ($n_{Marseillan\_ISFZ} = 25$), Bouzigues_ISFZ ($n_{Bouzigues\_ISFZ} = 25$) and Listel_OSFZ ($n_{Listel\_OSFZ} = 17$). Red Xs indicate means. Nanophytoplankton and picoeukaryote abundances in 10^6 cell l^{-1}, *Chaetoceros spp* in cell l^{-1}, competitors in 10^3 individuals per cubic meter and predators in log_{10} individuals per cubic meter.

Tableau 11 : Spatial contrasts of environmental measurements regarding sampling sites Bouzigues_ISFZ, Marseillan_ISFZ and Listel_OSFZ in Thau lagoon during larval durations.

Variables	Sampling sites	Mean ± Standard deviation
picophytoplankton+ nanophytoplankton biomass	Marseillan_ISFZ	0.87 ± 0.33 µgChla l^{-1}
	Bouzigues_ISFZ	1.14 ± 0.45 µgChla l^{-1}
	Listel_OSFZ	1.11 ± 0.74 µgChla l^{-1}
autotrophic picoeukaryotes abundances	Marseillan_ISFZ	$3.6 \times 10^7 \pm 2.1 \times 10^7$ cell l^{-1}
	Bouzigues_ISFZ	$3.4 \times 10^7 \pm 1.7 \times 10^7$ cell l^{-1}
	Listel_OSFZ	$1.8 \times 10^7 \pm 0.9 \times 10^7$ cell l^{-1}
nanophytoplankton biomass	Marseillan_ISFZ	0.44 ± 0.27 µgChla l^{-1}
	Bouzigues _ISFZ	0.71 ± 0.37 µgChla l^{-1}
	Listel_OSFZ	0.77 ± 0.59 µgChla l^{-1}
nanophytoplankton abundance	Marseillan_ISFZ	$3.0 \times 10^6 \pm 4.3 \times 10^6$ cell l^{-1}
	Bouzigues _ISFZ	$3.2 \times 10^6 \pm 2.7 \times 10^6$ cell l^{-1}
	Listel_OSFZ	$3.9 \times 10^6 \pm 2.4 \times 10^6$ cell l^{-1}
microphytoplankton biomass	Marseillan_ISFZ	0.29 ± 0.25 µgChla l^{-1}
	Bouzigues_ISFZ	0.82 ± 0.51 µgChla l^{-1}
	Listel_OSFZ	0.93 ± 0.80 µgChla l^{-1}
Chaetoceros spp abundance	Marseillan_ISFZ	$4.53 \times 10^4 \pm 9.87 \times 10^4$ cell l^{-1}
	Bouzigues_ISFZ	$2.13 \times 10^5 \pm 3.22 \times 10^5$ cell l^{-1}
	Listel_OSFZ	$4.2 \times 10^5 \pm 5.0 \times 10^5$ cell l^{-1}
Competitors abundance	Marseillan_ISFZ	$20.3 \times 10^3 \pm 17.0 \times 10^3$ ind. m^{-3}
	Bouzigues_ISFZ	$18.6 \times 10^3 \pm 12.8 \times 10^3$ ind. m^{-3}
	Listel_OSFZ	$36.1 \times 10^3 \pm 28.5 \times 10^3$ ind. m^{-3}
Predators abundance	Marseillan_ISFZ	0.05 ± 0.06 ind. m^{-3}
	Bouzigues_ISFZ	0.19 ± 0.22 ind. m^{-3}
	Listel_OSFZ	0.46 ± 0.44 ind. m^{-3}

Discussion

The present work describes the spatial patterns of Pacific oyster larval supply, settlement and recruitment in a Mediterranean nanotidal semi-enclosed coastal lagoon during a 3-year study.

Results show a strong variability of observed oyster recruitment at a lagoon scale including high and low spat collections. This variability reflects the ecological heterogeneity observed in the Thau lagoon. Lagarde et al. (2017, 2018) showed that the temporal variability of recruitment was related to lagoon functioning driven by temperature and trophic inputs (abundances of nanophytoplankton and *Chaetoceros spp*). Here, the observed spatial heterogeneity suggests that different ecological processes occurred at the same time for East and West sampling sites and for inside/ outside shellfish farming zones. The East and West sides of the lagoon showed the lowest recruitment while the most favorable recruitment spatial windows appeared to be in the center of the lagoon outside the shellfish farming zone.

There is negligible wild oyster broodstock in the coastal Thau lagoon, thus the collected larvae came mainly from reared oyster stock inside shellfish farming zones. The gamete emissions and the development of larval cohorts in the water column are the prerequisite for the recruitment of benthic invertebrates. We hypothesize that the nanotidal lagoons promote larval retention by the enclosed characteristics and limited effects of nanotidal regime. In this context, the emergence of D larvae cohorts after adult spawning events defines the larval supply to the system. The assessed abundances of D larvae in this study were higher inside shellfish farming zones than outside, confirming that the D larvae were from the oyster rearing areas. Further, abundances of umbo larvae were homogeneous for both ISFZ and OSFZ biocoenosis, likely due to the combined effects of high larval retention and hydrodynamic currents, driving forces of dispersion and connectivity in the Thau Lagoon. The abundances of umbo larvae in the water column ranged from 30 to 660 larvae per cubic meter between 2012 and 2014 which correspond to high values compared to other French oyster basins (Pouvreau 2018; Ubertini et al. 2017). This supply of pelagic larvae was guaranteed by the high density of oyster broodstock and self-recruitment ability of the system. Considering this homogeneous and high abundances of pelagic umbo larvae, we hypothesize that the abundance of pelagic larvae is not a limiting factor for oyster recruitment in the Thau lagoon.

The relative homogeneity of umbo larvae abundances in ISFZ and OSFZ suggests that there is no intense larviphagy (Troost, Veldhuizen, et al. 2008; Troost, Kamermans, et al. 2008; Lehane & Davenport 2004) in the biogeographic context. The phenomena of larviphagy in the experimental context - although probably existing - had a minor effect on the variability of recruitment.

However, at the following larval stage, we observed lower abundances of settled pediveligers larvae ISFZ than OSFZ. Lagarde et al. (2017) showed that the abundance of pediveligers was driven by the abundance of specific trophic plankton such as *Chaetoceros spp* in this ecosystem, supporting the hypothesis of the trophic settlement trigger (Toupoint, Gilmore-Solomon, et al. 2012). Here, significant differences of phytoplankton abundances and biomasses were detected between shellfish farming sites Bouzigues_ISFZ in the East of the lagoon and Marseillan_ISFZ in the West. This East-West gradient was due to nutrients inputs (main inputs came from perennial rivers in the east of the lagoon) and was amplified by the trophic depletion of phytoplankton biomass, with 1.7 times more nanophytoplankton and 2.8 times more microphytoplankton at the eastern site of Bouzigues_ISFZ than at the western site of Marseillan_ISFZ. In the same way, regarding phytoplankton abundance, there are almost 5 times more *Chaetoceros spp* in abundance at Bouzigues_ISFZ than at Marseillan_ISFZ. The ISFZ *vs.* OSFZ contrasts were expressed with in abundance twice as many picoeukaryote at ISFZ than at OSFZ. Observations of *Chaetoceros spp* reveal abundances at Listel_OSFZ, twice that of Bouzigues_ISFZ and 9 times that of Marseillan_ISFZ. Regarding nanophytoplankton, there is 30% more abundance at Listel_OSFZ than at ISFZ sites. We hypothesize that the loss of pediveliger settlement competence inside oyster rearing zones is linked to a depletion of larval trophic cues i.e. nanophytoplankton and *Chaetoceros spp*, leading to a lower effect of the trophic settlement trigger. At the next larval stage, a decrease in the abundance of spat both inside and outside shellfish farmed zones compared to the pediveliger abundances was observed, suggesting a potential low survival at metamorphosis in both biocoenosis. Lagarde et al. (2017) showed that survival at metamorphosis was positively correlated with nanophytoplankton abundances. It was previously showed that the presence of shellfish results in a decrease in zooplankton (Lam-Hoai et al. 1997) and phytoplankton (Souchu et al. 2001), biomasses, with deficits of about 30 and 40% respectively (Bec et al. 2005). In line with these results, here we observed that nanophytoplankton abundance is lower ISFZ than OSFZ. This depletion is due to trophic filtration (top-down control inside shellfish farming zones) of adult shellfish, including oysters (Deslous-Paoli et al. 1993; Pernet, Malet, et al. 2012; Lagarde et al. 2017), with an indirect impact on pediveligers abundances, metamorphosis survival and spat abundances. Therefore, we believe that feeding constraints for sensitive stages such as settlement and metamorphosis were responsible for the heterogeneous observed recruitment at the lagoon scale.

The larval dispersal with hydrodynamic circulation is considered as an important process in the marine invertebrates recruitment (Le Corre 2013; Ghezzo et al. 2015; Levin 2006). The hydrodynamic circulation in coastal lagoons is strongly constrained by the borders, the bathymetry and the wind direction and intensity (Fiandrino et al. 2017; Ghezzo et al. 2015). Here, we used an indicator of hydrodynamic connectivity based on accumulated flows of Eulerian tracers in a given volume of seawater according to the potential duration of the larval settlement phase (in our case, maximum duration). Preliminary simulation results showed that, similarly to pearl oyster in a Pacific lagoon (Thomas et al., 2014), the distribution of the last pelagic larval stage tends to be spatially homogeneous in the Thau lagoon (not shown).

However, cumulative fluxes of tracers revealed significant differences in hydrodynamic connectivity between sites, depending on their bathymetry and location within the lagoon. Similarly to Perez-Ruzafa et al. (2018), our results show that in coastal lagoons, the effects of hydrodynamic connectivity, as the effects of biotic environmental gradients, are fundamental in biological and community spatial structuring. Low hydrodynamic connectivity is usually found for sites with high confinement (Guelorget & Perthuisot 1992; Guelorget et al. 1987) like the western part of Thau lagoon, or areas under the influence of the sea like the eastern Thau lagoon. Indeed, the westernmost (Marseillan_OSFZ) and easternmost (Balaruc_OSFZ) sampling sites had the lowest values of simulated connectivity. Conversely, the two sampling sites characterized by the highest connectivity, Bouzigues_ISFZ and Bouzigues_OSFZ, are not only influenced by the main oyster zone of Bouzigues, but also located in the main, deepest part of the basin.

Comparing simulated hydrodynamic connectivity with observed recruitment allows one to specify and discriminate the role of hydrodynamics compared to ecological factors that operate during the recruitment process. Overall, our results suggest that the larval supply at the umbo stage is structured initially by the presence of shellfish growing areas nearby, and then by hydrodynamics with a relative homogeneity in the middle of the system (Lagarde et al. 2017; Lagarde et al. 2018). Then, in the ISFZ biocoenosis, decrease in recruitment comes from a loss of settlement competence at pediveliger stage, leading to a dramatic average loss of 10 times of pediveligers abundance per plate.

The heterogeneity of the pediveliger supply is partly explained by the connectivity for the OSFZ sites and by the biocoenosis effect (ISFZ vs. OSFZ) of the sampling sites under the assumption of ecological disturbances (metamorphosis delays, energy deficiency / physiological stress induced by trophic competition with adults). We hypothesized that the destructuring of the supply of pediveligers is due to a food depletion (trophic competition, energy deficiency) leading to an energy deficiency of the larvae.

Competitors and predators probably followed the population dynamics of autotrophic prey without impacting significantly and negatively the oyster recruitment. In the case of competitors, the abundances are low ISFZ compared to OSFZ because the filtration pressure of the adult oysters exerts a top-down control of the trophic chain.

In the case of picoeukaryotes, our results sustained anterior ones with higher abundances at the two sites inside shellfish farming zones, Marseillan_ISFZ and Bouzigues_ISFZ than outside at Listel_OSFZ. This is an indication that while filter feeders (such as oysters and mussels in breeding) can affect the biomass and abundance of nano and microplankton, they have much less impact on picoplankton, as they are not able to directly retain these smaller particles (Lefebvre et al. 2000; Vaquer et al. 1996; Dupuy et al. 2000). Non-retention of picoeukaryotes by oyster filtration induces increased picoplankton abundances (Bec et al. 2005) and evidence of top-down trophic control in ISFZ biocoenosis (Lagarde et al. 2017).

Our hypothesis that the ISFZ biocoenosis of the Thau lagoon could be a case of food limitation for oyster larvae modulates statement that food limitation is seldom important for invertebrate larvae (Olson & Olson 1989), as for herbivorous insects (Strong et al. 1984). These authors argued that food limitation is more common in the open ocean than in near shore waters. Our results suggest that oyster recruitment in coastal lagoons could be negatively impacted by trophic competition during summer trophic top down control, particularly in case of highly exploited shellfish farming.

This study of the spatial patterns of recruitment and connectivity completes the explanations related to the recent discovery of oyster spatfields in the Thau lagoon (Lagarde et al. 2017; Lagarde et al. 2018). Confirmation of spatfields outside shellfish growing areas with levels of collection never measured to date, opens up new avenues for nursery of native oysters' juveniles. These scientific inputs should prove useful in supporting the blue economic development of a sector through a virtuous practice of natural oyster collection according to sustainable uses in an ecosystem currently undergoing ecological restoration.

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Discussion générale, perspectives et conclusion

"Le remplacement d'une pensée dogmatique par une réflexion qui s'appuie sur le doute, l'esprit critique et la raison nous mènera d'une organisation théocratique et autoritaire de la société à une organisation démocratique." La vie de Galilée selon Bertolt Brecht

Les connaissances acquises au cours de ce travail de thèse ont permis de décrire les principaux mécanismes de la reproduction de l'huître creuse en lagune méditerranéenne. Ces descriptions améliorent, dans ce milieu particulier, la compréhension des relations entre chaque phase du processus reproductif et permettent d'expliquer la variabilité spatiale et temporelle du recrutement de naissain. Sur le plan pratique, elles nous permettent de proposer un schéma synthétique expliquant la variabilité du captage naturel en lagune de Thau (Figure 44).

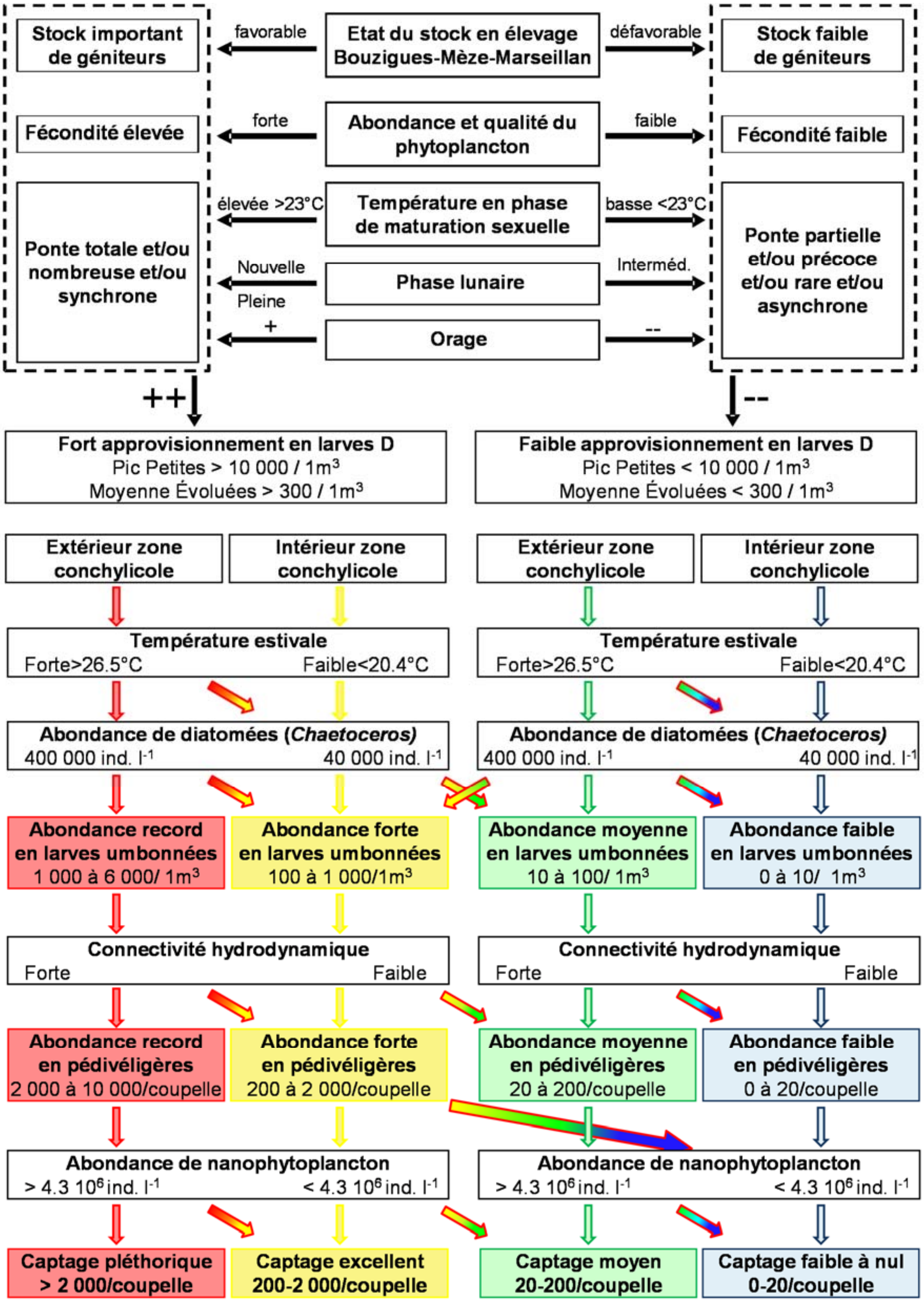

Figure 44 : Schéma synthétique adapté de Pouvreau et al. (2015) illustrant la variabilité du captage de l'huître creuse en lagune de Thau, sur la base des analyses effectuées dans le cadre de cette thèse, des observations acquises de 2012 à 2014.

Synthèse des principaux résultats et perspectives

Le premier chapitre de cette thèse décrit d'un point de vue écophysiologique**, l'originalité des processus expliquant la gamétogénèse et les pontes de l'huître creuse en lagune méditerranéenne**. Il a ainsi été montré que les communautés phytoplanctoniques autotrophes de type diatomées ont des effets positifs sur la gamétogénèse, contrairement aux assemblages planctoniques mixo-hétérotrophes tels que les dinoflagellés. En ce qui concerne les émissions de gamètes, une spécificité a été montrée avec un seuil thermique critique à 23°C pour le déclenchement des évènements de ponte de fortes intensités (> à 10 000 larves m^{-3}). Ce seuil est supérieur à celui connu pour l'huître en milieu intertidal (autour de 18°C, e.g. Pouvreau et al., 2006). Durant nos suivis, les pontes sont apparues synchronisées avec les phases lunaires (nouvelle lune et pleine lune) en particulier lorsqu'elles étaient combinées à des élévations de température. Il a aussi été montré que l'intensité des pontes était amplifiée par les épisodes orageux. Cette première partie expose donc les particularités de la reproduction en lagune de Thau, sa dépendance à l'environnement et surtout au mode de fonctionnements écologiques, autotrophe ou hétérotrophe, de la lagune. Les productions autotrophes sont contrôlées par les apports en nutriments limités par la réglementation européenne, dans l'objectif de l'atteinte du bon état écologique des masses d'eau établi par la Directive Cadre sur l'Eau (DCE). Des réductions d'apports en nutriment et de biomasse phytoplanctonique sont constatées ces dernières décennies sur la lagune (Collos et al. 2009; Gowen et al. 2015) posant la question de leur impact sur la fonction de reproduction du cheptel d'huître. Utiliser des scénarios issus de modèle de simulation permettrait d'évaluer l'impact de ces réductions sur la mise en place de la gamétogénèse et du déclenchement des pontes d'huîtres en élevage. Par exemple, un modèle biogéochimique de bilans de matières tel que l'outil de simulation pour la Gestion des Aménagement des Milieux Eutrophisés avec l'application aux Lagunes exploitées par la conchyliculture (GAMELag-Conch) pourrait être utilisé pour évaluer les effets de différents niveaux d'apports en azote et en phosphore (contexte de bon état écologique ou d'oligotrophisation) ou de l'augmentation de température (Pete et al. 2018).

Le deuxième chapitre de cette thèse démontre les fortes capacités de recrutement de l'huître dans la lagune de Thau et explique le rôle de l'environnement dans la variabilité de ce recrutement. Les abondances de jeunes naissains et les paramètres environnementaux enregistrés sur plusieurs sites d'échantillonnage pendant trois ans (2012-2014) ont permis de révéler des niveaux de recrutement comparables à ceux des autres bassins ostréicoles naisseurs français, remettant en cause le paradigme d'absence de reproduction et de recrutement de l'huître creuse en lagune méditerranéenne. Les conditions favorables de la fenêtre temporelle du recrutement sont liées à la concomitance des températures élevées de l'eau et à une richesse trophique associant *Chaetoceros spp* (production autotrophe) et nanophytoplancton. Sur le plan conceptuel, cette démonstration se rapproche en partie des hypothèses développées dans la théorie du "*Match-Mismatch*" (Cushing 1990). Inversement, lorsque la lagune de Thau passe dans un mode de fonctionnement hétérotrophe avec production de bactéries, ciliés et flagellés hétérotrophes, les conditions deviennent défavorables au recrutement. Des expériences complémentaires en milieu expérimental pourraient être envisagées pour mieux comprendre le type d'interaction entre certaines familles de ciliés nus tels que les scuticociliés (*Uronema spp*, les Philasteridae et *Balanion spp*) et les larves d'huîtres tout au long de leur développement jusqu'à leur métamorphose. L'objectif serait de caractériser les interactions qui peuvent être trophiques (prédateur-proie) ou infectieuses (hôte-pathogène). Par ailleurs, une étude des assemblages benthiques au moment de la fixation et de la métamorphose des larves sur les collecteurs permettrait de caractériser les interactions entre espèces sur ces collecteurs (prédation, compétition trophique et/ou territoriale). L'utilisation de modèles probabilistes de co-occurrence d'espèces (Veech 2013; Griffith et al. 2016) permettrait de caractériser pour chaque site le type d'interactions interspécifiques dans l'espace et dans le temps. La connaissance de ces interactions pourrait être utile pour caractériser non seulement le fonctionnement de l'écosystème dans le contexte de fluctuations environnementales induites par l'oligotrophisation et le changement climatique mais aussi pour mieux qualifier la fonction écologique "nurserie de bivalves exploités" du point de vue des assemblages benthiques.

Le troisième chapitre de la thèse a permis de caractériser plus finement le lien entre l'étape ultime de la métamorphose, l'hétérogénéité du recrutement et le fonctionnement écologique de la production (autotrophe vs. hétérotrophe) de l'écosystème. Nous avons montré que la distribution temporelle des tailles de coquilles prodissoconque II (taille à la métamorphose) est contrôlée par la température alors que la distribution spatiale est structurée par la qualité et la quantité de biomasse phytoplanctonique. En outre, il s'avère aussi que les meilleures survies à la métamorphose sont associées aux plus petites tailles de coquilles de PII. En complément, nous avons montré que les plus petites tailles de PII sont associées aux fortes biomasses chlorophylliennes de micro- et nanophytoplancton alors que les abondances de cryptophytes ont un effet inverse sur cette taille. Ces résultats soutiennent, pour un nouveau modèle biologique (ici l'huître creuse), l'hypothèse du déclencheur trophique de fixation mise en évidence pour la moule bleue (Toupoint, Gilmore-Solomon, et al. 2012; Martel et al. 2014) et soulèvent l'existence d'un potentiel retard de métamorphose des larves d'huître dans la lagune, pouvant être à l'origine d'un déficit potentiel de recrutement selon les sites et/ou périodes. Des expériences dédiées à l'étude de ce "retard à la métamorphose" permettraient de mieux préciser ce phénomène. Ainsi, une étude écologique globale incluant les analyses isotopiques et lipidiques (lipides totaux et acides gras) de la matière organique particulaire (POM) et des larves au stade pédivéligère, des mesures de tailles de coquilles PII et des durées de vie larvaire pourrait être réalisée pour mieux caractériser ces délais de métamorphose. Cette étude devrait intégrer une stratégie spatiale avec des sites contrastés écologiquement (axe Est / Ouest, intérieur / extérieur des zones d'élevage, avec / sans herbier à zostères) permettant de maximiser l'expression des délais de métamorphose et de définir le rôle des composantes du plancton au sein des réseaux trophiques dans des contextes écologiques différents (Morimoto et al. 2017; Perez et al. 2013; Gaillard et al. 2017; Hori et al. 2017). Cette caractérisation permettrait aussi de préciser le fonctionnement de la chaîne trophique dans le contexte de restauration écologique de la lagune, du retour des herbiers et des changements associés.

Enfin, le quatrième chapitre permet de mieux comprendre les rôles respectifs des facteurs biotiques et abiotiques dans la structuration des patrons spatiaux d'abondance larvaire et de recrutement d'huître. Sur la base des relations établies entre abondance larvaire, recrutement observé, connectivité hydrodynamique et facteurs écologiques, les résultats montrent que l'approvisionnement en larves pédivéligères est structuré par la connectivité à l'extérieur des zones conchylicoles, alors qu'à l'intérieur des zones d'élevage, cet approvisionnement est très faible et sans structure particulière. A l'intérieur des zones d'élevage, les abondances trophiques sont diminuées jusqu'à 10 fois par rapport à l'extérieur des zones d'élevage. On suggère que la déplétion trophique provoquée par la compétition trophique limite l'alimentation des stades larvaires à l'intérieur des zones d'élevage (contrôle top-down des filtreurs suspensivores en élevage). Ainsi, il est confirmé que la structure des biocœnoses et des habitats est aussi d'une importance majeure dans le succès ou l'échec du recrutement de l'huître creuse en lagune.

Les apports trophiques comme variable-clé du recrutement en lagune

Nos résultats concernant le recrutement de l'huître creuse, les apports trophiques et les mécanismes de colonisation / fixation / métamorphose corroborent ceux obtenus à différentes latitudes dans d’autres milieux. L'examen de coquilles de prodissoconque II de moules bleues (*Mytilus edulis*) ayant un développement planctotrophique en lagune côtière oligotrophique subarctique a démontré le rôle du déclencheur trophique de fixation dans la vie benthique précoce (Toupoint, Gilmore-Solomon, et al. 2012). Ainsi, dans la lagune subarctique de l’Ile de la Madeleine (Québec, Canada), les plus fortes abondances pendant la colonisation des moules sont associées aux plus petites tailles à la métamorphose (Martel et al. 2014). En Manche, dasn l’archipel de Chausey, la qualité et la quantité de différents groupes de producteurs primaires et d'hétérotrophes expliquent les différences interannuelles du succès du recrutement de différents bivalves (Barbier et al. 2017; Barbier 2016). La mise en évidence du rôle la ressource trophique pélagique sur le déclenchement de la phase de fixation et de la métamorphose est ici encore un point commun permettant de soutenir l'hypothèse du déclencheur trophique de colonisation. La structuration temporelle du recrutement répond aux mêmes types de forçages généraux qu’en lagune méditerranéenne pour les huîtres à la nuance près que, en lagune, l’hétérogénéité est plus spatiale que temporelle lors des évènements de recrutement. A des latitudes plus proches de celle de la lagune de Thau, le bassin d'Arcachon est un hautlieu du captage de l'huître creuse en France. Les études menées entre 1985 et 2004 ont toujours souligné l'importance majeure du fonctionnement trophique de la lagune avec une variable déterminante pour le succès du recrutement: le nanophytoplancton (His & Robert 1985; Auby & Maurer 2004). Il s’agira donc d'en savoir plus sur les détails de ce compartiment "nanophytoplancton" pour connaître les taxons associés aux apports énergétiques déterminants le succès du recrutement. Dans le contexte du changement global et de la variabilité du climat associé, la compréhension des réponses des écosystèmes par l’évolution de leur structure et leur fonctionnement demeure une relative inconnue. Les pressions environnementales anthropiques locales et globales induisent parfois des effets conjugués ou antagonistes dans les écosystèmes. Cependant, l’utilisation d’indicateurs tels que les analyses de réseaux écologiques (ecological network analysis - ENA) doit pouvoir aider au diagnostic de la structure et du fonctionnement des réseaux trophiques selon les niveaux de pressions anthropiques (Saint-Béat et al. 2015; Fath et al. 2007). Le rôle fonctionnel des micro-organismes doit être mieux défini, qu’ils soient autotrophes ou hétérotrophes, pour qualifier et quantifier les flux de matière et d’énergie au sein des cycles biogéochimiques (Mostajir 2014; Mostajir et al. 2015). Les analyses taxonomiques de la biodiversité utilisant l’analyse de séquence d’ADN par métabarcoding renforceront les diagnostiques écologiques dans l’avenir (Taberlet et al. 2012; Hebert et al. 2003). Le réseau trophique microbien est un compartiment clé-de-voute du fonctionnement des écosystèmes aquatiques exploités par la conchyliculture ; il apparaît nécessaire d'en avoir un diagnostic précis.

Le volet contaminant chimique comme limite de notre étude

Parmi les facteurs abiotiques pouvant structurer le recrutement larvaire, les contaminants chimiques constituent une piste qui semble importante à explorer. En effet, des travaux historiques ont montré que les contaminants chimiques (*e.g.* le tri-butyl-étain TBT) peuvent avoir un impact sur les larves avec un recrutement nul ou faible ou sur le nanoplancton induisant des perturbations dans le régime trophique des larves (His & Robert 1985). Bien qu'aucune étude sur ce sujet n'ait été conduite en lagune de Thau, on peut supposer que le potentiel reproducteur de l'huître ait été inhibé jusque dans les années 2000 du fait de ces contaminations chimiques au TBT (Héral et al. 1989; Alzieu et al. 1990; Ifremer 2002). Il conviendrait de porter attention à ce risque chimique dans la mesure où des travaux récents ont montré l'existence de périodes à risque liées à la présence de mélanges de pesticides dans les lagunes méditerranéennes françaises (Munaron et al. 2017). Bien que Thau ne soit pas la lagune la plus exposée, il existe un risque lié aux effets "cocktail" de contaminants pour cet écosystème. Mais à ce jour, bien qu'une littérature fournie existe sur la caractérisation des effets de biocides sur des larves d'huîtres en laboratoire (His & Seaman 1993; Mai et al. 2013; Sussarellu et al. 2018; Behrens et al. 2016) , l'impact direct ou indirect de ces mélanges de polluants sur le développement larvaire ou la dynamique du phytoplancton *in situ* n'est que peu étudié (Vázquez-Boucard et al. 2014; Mai et al. 2013; Auby & Maurer 2004). Les correspondances entre période à risque d'exposition aux pesticides sur Thau (juin-juillet) et les périodes d'échec du recrutement (juin-juillet) relevées dans le deuxième chapitre de cette thèse posent la question d'un éventuel lien qu'il conviendrait d'explorer dans une étude à venir, prenant en compte l'ensemble des facteurs environnementaux capables d'influencer ce recrutement comme par exemple le régime trophique associé *in-situ*.

De l'importance de définir la fonction écologique "nurserie d'huître"

Nos résultats permettent de mettre en avant une fonction écologique "nurserie d'huîtres" qui apparaît comme nouvelle au sein de la lagune de Thau. La notion de fonction écologique est aujourd'hui définie comme l'ensemble des processus biologiques de fonctionnement et de maintien des écosystèmes (de Groot et al. 2009; Maurel et al. 2010). La fonction écologique intègre une dimension spatiale, temporelle et écologique des structures (Jax 2005). Chaque fonction est le résultat d'interactions complexes entre les composantes biotiques (organismes vivants) et abiotiques (chimiques et physiques) des écosystèmes à travers les forces motrices universelles de la matière et de l'énergie (Ouisse et al. 2016). Les échelles d'intégration écologique traduisent différents niveaux d'interactions des individus jusqu'aux populations (Bremner et al. 2003). La dynamique des processus physiques (courantologie, sédimentaire, bathymétrique) et la distribution spatiale ou temporelle des compartiments biologiques (phytoplancton, macrophytes, macrofaune endogée) sont renseignés pour cette lagune par des diagnostics pluriannuels depuis les années 1980 (programmes PNEC, PNOC, Ecothau, OxyThau, RSL et DCE). Mais le concept de fonction "nurserie des habitats benthiques" est peu abordé par la recherche et les parties-prenantes jusqu'à maintenant (Barbier et al. 2017). Cependant, la fonction écologique "nurserie de poisson" commence à être définie comme une zone permettant 1) une nutrition adaptée, (2) un habitat favorable à l'installation des postlarves et qui les protège des prédateurs et des pressions pendant toute leur période juvénile jusqu'à la taille refuge, (3) un environnement dans lequel les juvéniles grandissent plus vite et ont un meilleur taux de survie que dans tous les autres habitats, (4) une localisation qui permet un déplacement vers les habitats adultes (Beck et al. 2001; Gillanders et al. 2003; Lenfant et al. 2015). Sur la base des nouvelles connaissances issues de ce projet doctoral, il conviendra de définir dans l'avenir les contours géographiques de cette fonction "nurserie d'huître" afin d'optimiser la mise en place du nouveau service écosystémique de production lié au captage naturel en lagune. L'utilisation de la démarche scientifique pour définir les facteurs structurant l'habitat et la fonction nurserie permettra de caractériser le périmètre géographique pour le développement de ce service écosystémique. Une approche empirique entreprise par les professionnels permettrait également d'aboutir à la définition de ce périmètre mais probablement à une échelle de temps plus longue. La caractérisation du périmètre devra prendre en compte les contraintes réglementaires et la conciliation des différents usages en place dans la lagune (*e.g.* navigation, pêche).

Vers une pratique locale du captage naturel en lagune méditerranéenne

Les travaux de cette thèse ont permis d'apporter les réponses sur le potentiel écologique d'une pratique de captage naturel sur la lagune de Thau à l'échelle expérimentale. Aujourd'hui, le captage relevé chez les ostréiculteurs est toujours variable avec des valeurs moyennes comprises, entre 4 et 141 naissains par coupelle depuis 2010 (Figure 45, Pouvreau et al. 2018).

Au delà des aspects liés au milieu (phytoplancton, connectivité hydrodynamique, situation géographique), les performances obtenues dépendent des techniques mises en œuvre par les professionnels (Figure 46). L'optimisation de ces pratiques dans l'avenir devrait permettre d'obtenir de meilleurs résultats. Ainsi, en 2017 et 2018, certains ostréiculteurs ont réussi à collecter leur naissain jusqu'à deux fois par été (Deslous Paoli, pers. obs.) les obligeant à développer des tables de captage et de nurserie en simultané (Figure 46).

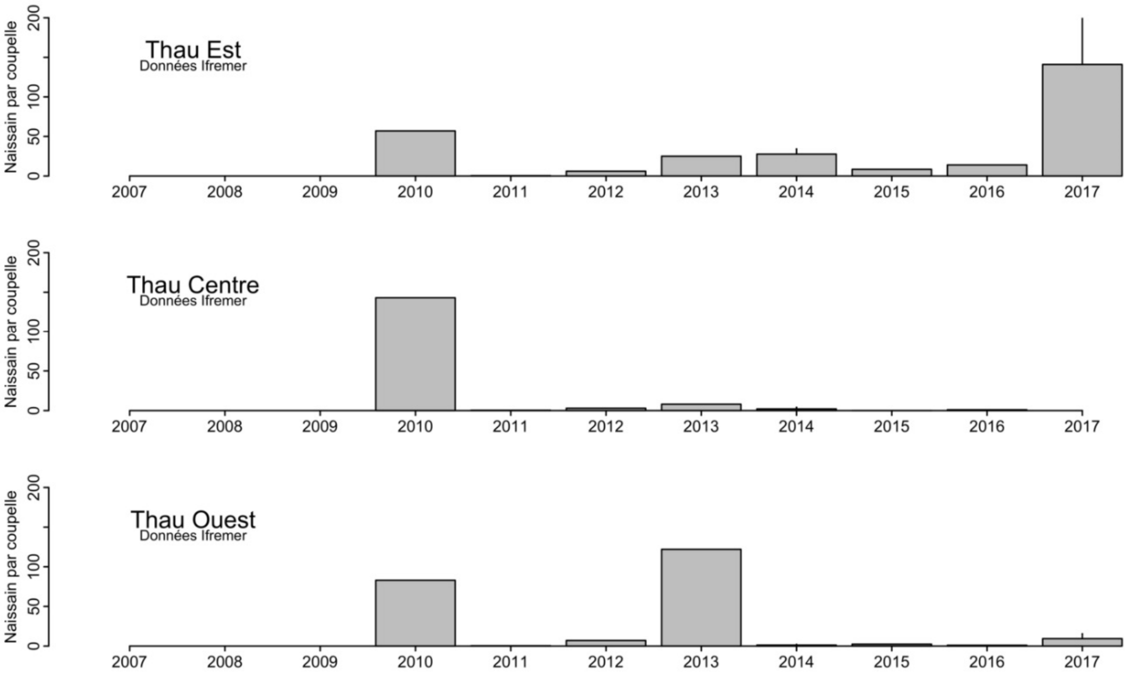

Figure 45 : Abondance moyenne du captage obtenu chez les ostréiculteurs en lagune de Thau : nombre moyen de naissains par coupelle (vivants et morts). Les données sont acquises en automne dans les établissements ostréicoles au moment du détroquage (Pouvreau et al 2018).

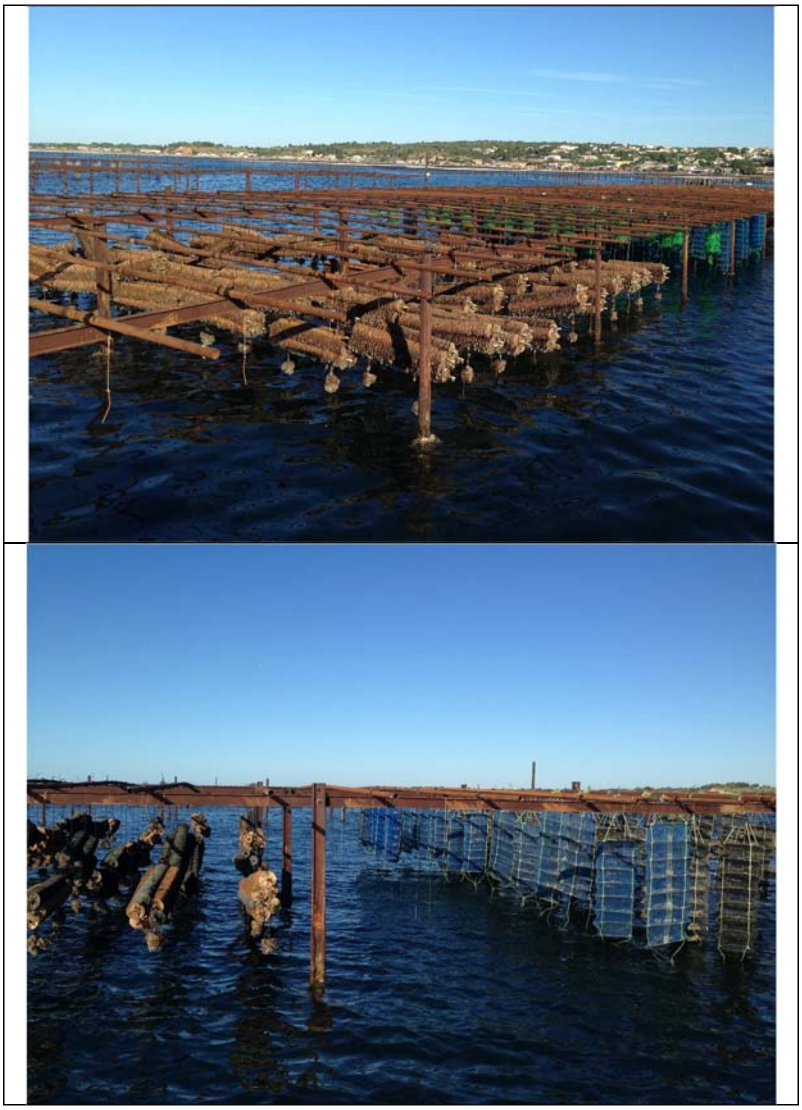

Figure 46 : Table à perche tournante pour captage sur collecteurs à coupelle et prégrossissement en lanterne du naissain natif de la lagune de Thau, zone de Bouzigues, Août 2017.

Malgré ces démonstrations de faisabilité, la pratique du captage concerne actuellement un nombre restreint d'ostréiculteurs (moins d'une dizaine) de la lagune de Thau. Et par conséquent, la filière ostréicole méditerranéenne repose toujours sur un approvisionnement en naissain provenant d'écloserie et de captage naturel issu de bassins ostréicoles atlantiques (principalement le bassin d'Arcachon et les Pertuis Charentais). Le coût financier et la fragilisation de la filière conchylicole induite par sa dépendance aux approvisionnements extérieurs s'ajoutent au risque de transfert de pathogènes lors de ces approvisionnements. En 2016-2017, les achats de naissain de taille 6 à 10mm représentent environ 295 millions d'individus (73% triploïdes, 22% diploïdes et 5% sauvages) à un prix moyen estimé à 11€ pour 1000 individus (Gervasoni & Giffon 2016), soit un total autour de 3 250 000€ par an. Les achats d'huîtres prégrossies concernent 83% des entreprises pour 157 millions d'individus (29%

triploïdes, 21% diploïdes et 50 % sauvages), à un prix moyen en 2016 de 34€ pour 1000 individus, soit un total d'environ 5 338 000€ par an. L'enquête menée par le Cepralmar révèle que, en 2016, 64% des ostréiculteurs régionaux souhaiteraient pouvoir s'approvisionner localement en naissains d'huîtres et 33% s'intéressent au développement du captage naturel dans les lagunes méditerranéennes et souhaiteraient pouvoir réaliser leur propre captage, soit potentiellement une centaine d'entreprises. A l'heure actuelle, encore 59% des enquêtés considèrent que la lagune de Thau ne permet pas la pratique du captage naturel alors que 27% considèrent manquer de connaissances en la matière. 24% des exploitants considèrent que la pratique du captage naturel représente trop de travail, trop d'investissement ou une concurrence trop rude des autres bassins. Un travail de communication pour le transfert de nos résultats et des nouvelles connaissances est donc nécessaire à ce stade vers une profession qui semble prête à les recevoir. A ces arguments écologiques et économiques, s'ajoute un argument zoosanitaire fort puisqu'il a été montré que le naissain issu de captage naturel en lagune de Thau présente une meilleure résistance à l'Ostreid Herpes Virus avec une meilleure survie (taux de survie allant de 70 à 80%) pendant la phase de grossissement (Petton, Bruto, et al. 2015; Lagarde, Gervasoni, et al. 2015).

A l'aube d'un changement …

La production alimentaire en général a un coût environnemental (Hilborn et al. 2018). Hilborn *et al.* (2018) précisent que les impacts les plus faibles viennent des espèces qui se nourrissent naturellement dans les zones côtières et qui peuvent être récoltées avec de faible besoin en carburant. L'impact environnemental de l'ostréiculture en lagune de Thau s'inscrit dans ce schéma et peut encore être amélioré par la relocalisation d'une partie des approvisionnements en naissain grâce à la pratique du captage naturel. Le potentiel économique du captage naturel en lagune de Thau est avéré (Lagarde, Fiandrino, et al. 2015) mais des défis importants sont à relever, en particulier le développement de la compétence des ostréiculteurs pour la pratique du captage naturel et la définition des zones de nurserie à l'échelle de la lagune. Le développement d'une telle pratique peut alors donner deux opportunités. La première est de pouvoir profiter de l'amélioration de la compréhension de l'habitat en tant que nurserie d'espèces marines pour améliorer la gestion environnementale de l'écosystème tel que proposé par Beck et al. (2001). Relevant plus de la psychologie sociale, la deuxième opportunité est de pouvoir créer une nouvelle relation entre les conchyliculteurs et leur écosystème en leur offrant la possibilité de développer une sensibilité au micro et nanobiome et en particulier à la vie larvaire des huîtres. Cette sensibilisation devra néanmoins passer par la formation des ostréiculteurs et par l'éducation des étudiants au niveau local. Cette relation passera par une phase d'apprivoisement avec ses nécessités, son engagement, ses rites, ses aléas et ses succès.

L'organisation des Nations Unies pour l'Alimentation et l'Agriculture (FAO) propose de construire une vision commune pour une aquaculture et une alimentation durables[8]. La vision est de gérer les ressources naturelles de manière à préserver les écosystèmes et leur aptitude à répondre aux besoins actuels et futurs. Cette vision globale de l'aquaculture durable tient compte des forces externes majeures qui la façonnent en tenant compte de la macro-économie et du changement climatique. Pour cela une approche a été choisie : l'agro-écologie[9]. "*Cette approche est basée sur l'utilisation des principes et concepts de l'écologie, dans le but d'optimiser les interactions entre les plantes, animaux, humains et l'environnement, tout en prenant en compte les services écosystémiques et les aspects sociaux indispensables à la durabilité et à l'équité des systèmes alimentaires. Pour la FAO, dix indicateurs permettent de caractériser l'agro-écologie : la diversité des espèces et la biodiversité, la co-création et le partage de connaissance entre acteurs, la valorisation des synergies entre les différentes activités du système de production et avec l'environnement, l'efficience de l'utilisation des ressources et leur recyclage, la résilience des communautés et des écosystèmes (notamment face aux changements climatiques), les valeurs sociales et humaines, les traditions culturelles et culinaires, la bonne gouvernance et une économie circulaire et solidaire*". La mise en œuvre de cette vision de durabilité de la production alimentaire est envisagée en s'appuyant sur les meilleures données scientifiques disponibles. Cette amélioration nécessitera conviction, engagement, mobilisation des connaissances et des acteurs ainsi que l'appropriation de la démarche et de ses objectifs par l'ensemble de cette collectivité.

Les travaux présentés dans cette thèse concernent le captage naturel d'huître en lagune méditerranéenne et s'inscrivent pleinement dans cette dynamique agroécologique. Les nouvelles connaissances acquises ici à l'échelle expérimentale réfutent des paradigmes historiques et ouvrent un nouveau domaine des possibles. Ces connaissances correspondent à la période de l'étude, c'est-à-dire la décennie 2010-2020. Mais le contexte du changement climatique à l'horizon 2020-2030 et au-delà va probablement induire une période d'instabilité écologique dont il est difficile, voir impossible de prédire la déclinaison des effets globaux à

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[8] http://www.fao.org/sustainability/fr/

[9] http://www.fao.org/agroecology/fr/

l’échelle locale. L’avenir dira si les stratégies, institutions, mobilisations, appropriations permettent de faire un pas de plus vers l’économie bleue locale avec une exploitation sobre et durable de la lagune de Thau, intégrant la pratique du captage naturel d’huîtres natives.

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Annexes

Valorisation par la communication des travaux de thèse, année 2015

Conférence internationale, présentation orale	**F. Lagarde**, M. Ubertini, S. Mortreux, A. Perignon, A. Leurion, P. Le Gall, C. Chiantella, S. Meddah, J. L. Guillou, B. Bec, C. Roques, D. Bonnet, H. Cochet, I. Bernard, E. Gervasoni, M. Richard, G. Miron, A. Fiandrino, S. Pouvreau, E. Roque d'orbcastel (2015.11.19) New knowledges about determinism of pacific oysters (*Crassostrea gigas*) larval recruitment in the Mediterranean Thau lagoon. Promotion of cooperative research on fisheries/oceanography between France and Japan. The sea under human and natural impacts: Challenge of oceanography to the future earth. 16th Japanese-French Oceanography Symposia. Shiogama (Tohoku) Japon.
	F. Lagarde (2015.11.21) Cooperation between France and Japan on Oyster culture and future prospects. 16th Japanese-French Oceanography Symposia. The hall of Nichifutsu Kaikan (La Maison Franco-Japonaise). Tokyo/Japon.
Présentation en groupe de travail au niveau international	**F. Lagarde,** M. Ubertini, S. Mortreux, A. Perignon, A. Leurion, P. Le Gall, C. Chiantella, S. Meddah, J. L. Guillou, B. Bec, C. Roques, D. Bonnet, H. Cochet, I. Bernard, E. Gervasoni, M. Richard, G. Miron, A. Fiandrino, S. Pouvreau, E. Roque d'orbcastel (2015.10.11) New knowledges about determinism of pacific oysters (*Crassostrea gigas*) larval recruitment in the Mediterranean Thau lagoon. Canada, NB, Moncton - MPO/Université et (2015.10.13). Canada, NB, Moncton - Ministère provincial de l'Agriculture, Aquaculture et Pêche de la Province du Nouveau-Brunswick.
Présentation en groupe de travail au niveau régional	**F. Lagarde,** M. Ubertini, S. Mortreux, A. Perignon, A. Leurion, P. Le Gall, C. Chiantella, S. Meddah, J. L. Guillou, B. Bec, C. Roques, D. Bonnet, H. Cochet, I. Bernard, E. Gervasoni, M. Richard, G. Miron, A. Fiandrino, S. Pouvreau, E. Roque d'orbcastel (2015.04) New knowledges about determinism of pacific oysters (*Crassostrea gigas*) larval recruitment in the Mediterranean Thau lagoon. Pôle Mer Languedoc-Roussillon.
Rapport Scientifique et technique	**F. Lagarde**, A. Fiandrino, M. Richard, I. Bernard, S. Mortreux, M. Ubertini, C. Chiantella, M. Boj, S. Meddah, A. Leurion, S. Soriano, M. Fuhrmann, P. Le Gall, C. Chiera, T. Berteaux, J.-L. Guillou, E. Roque D'Orbcastel, A. Perignon, D. Bonnet, C. Roques, Bec B., H. Cochet, E. Gervasoni, G. Miron, S. Pouvreau (2015). Déterminisme du recrutement larvaire de l'huître creuse (*Crassostrea gigas*) dans la lagune de Thau. http://archimer.ifremer.fr/doc/00279/39054/
	F. Lagarde, A. Perignon, E. Gervasoni, S. Mortreux, S. Meddah, A. Leurion, P. Le Gall, J.-L. Guillou, M. Boj, M. Fuhrmann, M. Richard, M. Ubertini, A. Fiandrino, E. Roque D'Orbcastel, S. Pouvreau (2015). Recherche de gisements de captage naturel de *Crassostrea gigas* en lagune de Thau. Répartition spatiotemporelle du naissain, aspects culturaux et économiques. http://doi.org/10.13155/37103
Participation à Reportage/documentaire télévisuel	Ora Maritima (2015) Une journée sur la lagune de Thau. Vidéo documentaire de présentation de l'évènement. Syndicat Mixte du Bassin de Thau, 10 minutes. https://www.youtube.com/watch?v=LZAmMPqVi5k

Valorisation par la communication des travaux de thèse, année 2016

Conférence internationale, présentation orale	**F. Lagarde**, S. Mortreux, M. Ubertini, C. Chiantella, B. Bec, C. Roques, D. Bonnet, I. Bernard, H. Cochet, G. Messiaen, P. Le Gall, M. Richard, A. Fiandrino, S. Pouvreau, E. Roque d'Orbcastel (2016.09.23). How oyster (*Crassostrea gigas*) benthic larval supply and metamorphosis stage condition recruitment in a Mediterranean shellfish farmed ecosystem? AE2016 - Aquaculture Europe 2016, Mollusc session, Edinburgh, Scotland (UK).
Jeux de donnée	Pouvreau Stephane, Maurer Daniele, Auby Isabelle**, Lagarde Franck**, Le Gall Patrik, Cochet Hélène, Bouquet Anne-Lise, Geay Amélie, Mille Dominique (2016). VELYGER Database: The Oyster Larvae Monitoring French Project. SEANOE. http://doi.org/10.17882/41888
Présentation en groupe de travail au niveau international	**F. Lagarde** , M. Ubertini, S. Mortreux, A. Perignon, A. Leurion, P. Le Gall, C. Chiantella, S. Meddah, J. L. Guillou, B. Bec, C. Roques, D. Bonnet, H. Cochet, I. Bernard, E. Gervasoni, M. Richard, G. Miron, A. Fiandrino, S. Pouvreau, E. Roque d'Orbcastel (2016.06.12) New knowledges about determinism of pacific oysters (*Crassostrea gigas*) larval recruitment in the mediterranean Thau lagoon. Workshop JFREA-Ifremer Sète.
Présentation en colloque au niveau national	**F. Lagarde**, M. Ubertini, S. Mortreux, A. Perignon, A. Leurion, P. Le Gall, C. Chiantella, S. Meddah, J. L. Guillou, B. Bec, C. Roques, D. Bonnet, H. Cochet, I. Bernard, E. Gervasoni, M. Richard, G. Miron, A. Fiandrino, S. Pouvreau, E. Roque d'orbcastel·(2016.12.09) Production de Naissain d'Huître creuse *Crassostrea gigas* en Méditerranée, caractérisation de la variabilité du recrutement dans un écosystème lagunaire méditerranéen. GDRI RECHAGLO-UQAR.
	F. Lagarde , M. Ubertini, S. Mortreux, A. Perignon, A. Leurion, P. Le Gall, C. Chiantella, S. Meddah, J. L. Guillou, B. Bec, C. Roques, D. Bonnet, H. Cochet, I. Bernard, E. Gervasoni, M. Richard, G. Miron, A. Fiandrino, S. Pouvreau, E. Roque D'orbcastel (2016.06.14) New knowledges about determinism of pacific oysters (*Crassostrea gigas*) larval recruitment in the mediterranean Thau lagoon. Groupe de travail MoU Fishery Research and Education Agency du Japon-Ifremer.

Valorisation par la communication des travaux de thèse, année 2017

Conférence internationale, présentation orale	**F. Lagarde**, E. Roque D'Orbcastel, S. Mortreux, I. Bernard, C. Chiantella, B. Bec, C. Roques, A. Correia-Martins, J.-B. Nadalini, S. Pouvreau, R. Tremblay (2017.09.18-21). Unexpected in situ relationship between metamorphosis size and recruitment success of *Crassostrea gigas*. Physiomar - The physiology of aquatic invertebrates in a changing world, Cambridge, UK.
	F. Lagarde *et al*. (2017.10.08) Temporal recruitment windows of *Crassostrea gigas* in Mediterranean lagoon under oligotrophication. Symposium Coast CNRS. Bordeaux, France.
	F. Lagarde and M. Hori (2017.10.08) Dynamics of shellfish-exploited ecosystems under oligotrophication, Objectives of the research exchange project between France and Japan, Meeting Symposium Franco-Japonais d'océanographie. Bordeaux, France.
présentation en groupe de travail au niveau international	**F. Lagarde,** M. Ubertini, S. Mortreux, A. Perignon, A. Leurion, P. Le Gall, C. Chiantella, S. Meddah, J. L. Guillou, B. Bec, C. Roques, D. Bonnet, H. Cochet, I. Bernard, E. Gervasoni, M. Richard, G. Miron, A. Fiandrino, S. Pouvreau, E. Roque d'Orbcastel (2017.11.17) Production de Naissain d'Huître creuse *Crassostrea gigas* en Méditerranée, caractérisation de la variabilité du recrutement dans un écosystème lagunaire méditerranéen. Université du Québec à Rimouski.
	Lagarde F. (2017.10.12) workshop FRA/Ifremer, SAKURA project, thematic: Benthic larval supply and metamorphosis stage condition *Crassostrea gigas* recruitment in a shellfish farmed ecosystem. Hiroshima/Japan.
	M. Hori & **F. Lagard**e (2017.10.16.) Dynamics of shellfish-exploited ecosystems under oligotrophication. Objectives of the research exchange project between France and Japan. NOAA/FRA United States Japan Natural Resources Panel On Aquaculture, Hiroshima/Japan.
	F. Lagarde (2017.10.12) Workshop Exploring the variability of larval recruitment of Japanese oyster , Preliminary results on experiment 2017 ecological function "food for oyster"., FREA, Hiroshima/Japan.
	F. Lagarde et al. (2017.10.13) Detection of metamorphosis delays on *Crassostrea gigas* in Thau Lagoon et Presentation de l'UMR MARBEC et IFREMER, Seminaire, FREA, Hiroshima/Japan.
	M. Hori & **F. Lagarde (**2017.10.16.) Dynamics of shellfish-exploited ecosystems under oligotrophication. Objectives of the research exchange project between France and Japan. NOAA/FRA United States Japan Natural Resources Panel On Aquaculture, Hiroshima/Japan.
Présentation en groupe de travail au niveau régional	**F. Lagarde**, S. Mortreux, M. Ubertini, C. Chiantella, B. Bec, C. Roques, D. Bonnet, I. Bernard, H. Cochet, G. Messiaen, P. Le Gall, M. Richard, A. Fiandrino, S. Pouvreau, E. Roque D'orbcastel (2016.05.20) Comment l'approvisionnement larvaire et la métamorphose conditionnent le recrutement en écosystème lagunaire. Captage d'huître creuse en lagune méditerranéenne, définition des actions du CRCM, du Cépralmar et de l'Ifremer pour 2016, perspective 2017 & 2018. Groupe de travail Ifremer-CRCM-Cepralmar.
	F. Lagarde (2016.11.24) Production de Naissain d'Huître creuse *Crassostrea gigas* en Méditerranée, caractérisation de la variabilité du recrutement dans un écosystème lagunaire méditerranéen. Groupe de travail Ifremer-CG34.
	F. Lagarde (2016.11.25) Production de Naissain d'Huître creuse *Crassostrea gigas* en Méditerranée, caractérisation de la variabilité du recrutement dans un écosystème lagunaire méditerranéen. Réunion de thème UMR Marbec. (2017.04.11) Société MediThau. (2017.06.10) Ifremer LER Arcachon/France.
	F. Lagarde (2017.09.29) Ora Maritima, Dynamique des écosystèmes conchylicoles en phase d'oligotrophisation : Objectifs du projet d'échanges de chercheurs entre France et Japon. Sète/France.
	2018.01.12. Bilan 2016 et 2017 du captage naturel en lagune et actions à définir CRCM et Cepralmar 2018. CRCM-Ifremer-Cepralmar. Sète/France.

Article de presse, Ouvrage Grand Public	*Chûgoku Shimbun*, Participation à 3 articles dans le quotidien régional d'Hiroshima, Japon (29/04/2017-30/04/2017, 01/05/2017)
	Midi Libre (2017.07.25) festival Voix Vives de Méditerranée, « Quand la poésie rencontre les gens de mer ». Sète/France.
	F. Lagarde, Recrutement naturel de l'huître creuse en lagune conchylicole méditerranéenne sous processus d'oligotrophisation, Revue Agropolis Occitanie Littorale et Maritime.
Intervention Grand Public	Lycée du Parc Lyon (2017.03.23) Classe préparatoire aux Grandes Ecoles. *Huître en lagune de Thau, le naturel reviendra-t-il au galop?* Caractérisation de la variabilité du recrutement dans un écosystème lagunaire méditerranéen, conférence 1h30. (220 élèves). Lyon/France.
	C. Ber (FRANCE) et **F. Lagarde**, Ingénieur Ifremer & biologiste marin, Présentation : Georges Drano. Festival Voix Vives de Méditerranée, poètes, pêcheurs, jouteurs et gens de mer. Lecture / Echanges / Discussion, durée : 1h00, lieu : terrasse panoramique, Rencontre entre ceux dont les métiers sont liés à la mer et la parole des poètes venus de toutes les rives de la Méditerranée. Sète/France.
	F. Lagarde (2017.11) Présentation du métier de scientifique, écologie et biologie marine. Enjeux et recherche pour la conchyliculture en France. Elèves Ingénieur, Agro-Paris Tech. Sète/France.
Participation à Reportage /documentaire télévisuel	Documentaire télévisuel pédagogique. Les coquillages. Xenius/ Arte. Juin 2017. Sète/France.
	Méditerraneo (2016) les reines de l'étang de Thau, 7'45.

Valorisation par la communication des travaux de thèse, année 2018

Conférence internationale, présentation orale	M. Hori, **F. Lagarde**, V. Derolez, M. Richard, M. Hamaguchi, J. Hori and M. Makino (2018.03.18-22). Coastal management using oyster seagrass interactions for sustainable aquaculture, fisheries and environment. National Shellfisheries Association (NSA) 110th Annual Meeting. Seattle, Washington, USA.
	F. Lagarde, A. Fiandrino, M. Ubertini, R. Tremblay, S. Mortreux, C. Chiantella, B. Bec, C. Roques, D. Bonnet, I. Bernard, H. Cochet, G. Messiaen, M. Richard, E. Roque D'orbcastel, S. Pouvreau, C. Lett (2018.08.25-29) Trophic interactions and connectivity structure spatial patterns of Pacific oyster recruitment in a heavily shellfish farmed mediterranean lagoon. WAS AQUA2018 Montpellier. 25-29 august 2018.
Conférence internationale, poster	A. Correia-Martins, **F. Lagarde,** N. Guenineche, G. Devique, A. Crottier, S. Pouvreau, E. Roque d'orbcastel, M. Hori, M. Hamaguchi, T. Miyajima, J.-B. Nadalini, R. Tremblay (2018.08.25-29) Recruitment succes of Pacific oysters, *Crassostrea gigas*, in relation to trophic quality, larval quality and metamrohposis delay. WAS AQUA2018 Montpellier.
Présentation en groupe de travail au niveau international	**F. Lagarde (**2018.02.11) Ostréiculture en lagune méditerranéenne, le naturel reviendra-t-il au galop ? Caractérisation de la variabilité du recrutement dans un écosystème lagunaire méditerranéen. Conférence à l'Université de Moncton, Nouveau-Brunswick, Canada. **(**2018.03.18) Conférence à l'UQAR/ISMER, Rimouski, Québec, Canada.
	F. Lagarde (2018.03.14) Monitoring oyster recruitment in France & Characterization of recruitment variability in a Mediterranean lagoon. Oyster Larval Interactions Workshop – Atlantic Veterinary College Charlottetown, Prince Edward Island, Canada.
	Assemblée générale du Comité Régionale de la Conchyliculture (2018.06.18). Captage naturel de naissains d'huîtres dans le Bassin de Thau (**Franck LAGARDE –** IFREMER / Adeline PERIGNON – CRCM), Intervention de 15 minutes. Juin 2018
Présentation en groupe de travail au niveau régional	It's sea time, De l'est à l'ouest de la lagune, le captage naturel des huîtres ; **Franck Lagarde** et équipe du Ifremer/LERLR. 3'35. Reportage documentaire interne Ifremer, pôle audiovisuel Ifremer Ambre Bodenes &Olivier Dugornay. Juillet 2018.
Jeux de données	Messiaen Gregory, Mortreux Serge, Le Gall Patrik, Crottier Anais, **Lagarde Franck** (2018). Marine environmental station database of Thau lagoon. SEANOE. http://doi.org/10.17882/52404

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Résumé :

Les lagunes méditerranéennes hébergent de nombreux usages anthropiques dont la conchyliculture depuis le début du 20ème siècle. Importée en 1970, l'huître creuse, *Crassostrea gigas*, s'y est adaptée parfaitement et a permis un développement ostréicole important jusqu'en 2008, année d'apparition des surmortalités de naissain liées à l'émergence d'un variant du virus OsHv1. La raréfaction du naissain et la spéculation sur ce produit ont modifié régionalement les pratiques culturales et les modes d'approvisionnement en juvéniles, avec notamment la volonté de développer une activité locale de captage. Mais le cycle de reproduction de l'espèce et le recrutement de juvéniles étaient mal connus en milieu lagunaire. Dans ce contexte, l'objectif de cette thèse est de caractériser la variabilité de la reproduction et du recrutement de l'huître creuse en lagune méditerranéenne. La configuration lagunaire offre des atouts de productivité pour le cheptel de géniteurs et de connectivité hydrodynamique pour les larves. Cependant, il apparaît que le fonctionnement écologique du système à la base de la production primaire joue un rôle essentiel sur les apports trophiques pour le succès de la reproduction des huîtres. Ce travail s'inscrit, en outre, dans le contexte d'un écosystème en voie de restauration écologique entamant un processus d'oligotrophisation dont nous décrivons les premiers effets sur le cycle de reproduction de l'huître.

Mots clés : huître, écologie larvaire, ressource trophique, connectivité, recrutement, lagune côtière

Ecology of the reproduction of the Pacific oyster in Mediterranean lagoon.

Abstract:

The Mediterranean lagoons host many anthropogenic uses including shellfish aquaculture since the beginning of the 20th century. Imported in 1970, the cupped oyster, *Crassostrea gigas*, has adapted perfectly and has allowed an important development of oyster's industry until 2008, the year of occurence of spat outbreak related to the emergence of an Ostreid Herpes-virus µvar. The scarcity of spat and the speculation on this product changed regionally cultural practices and the supply of juveniles, with in particular the wish of developing a local activity of spat collection. However the cycle of reproduction of the species and the recruitment of juveniles were poorly known in lagoon environments. In this context, the objective of this thesis is to characterize the variability of the reproduction and the recruitment of the cupped oyster in Mediterranean lagoon. The lagoon configuration offers the advantages of productivity for the broodstock and hydrodynamic connectivity for the larvae. However, it appears that the ecological functioning of the system at the base of the primary production plays an essential role on the trophic resources for the success of oyster reproduction. This work takes place in the context of the ecological restoration under oligotrophication, which first effects we describe on the oyster's reproductive cycle.

Keywords: oyster, larval ecology, trophic resource, connectivity, recruitment, coastal lagoon