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Faculté des Sciences
Département de Biologie des Organismes
Laboratoire de Biologie Marine

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Laure Moulin

Thèse présentée en vue de l'obtention du titre de Docteur en Sciences

Septembre 2014

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Lei Chou (ULB)
Chantal De Ridder (ULB)
Patrick Flammang (UMONS)
Frédéric Gazeau (Observatoire Océanologique de Villefranche-sur-Mer)

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RÉSUMÉ

Depuis le début de la période industrielle, les activités humaines ont généré une augmentation importante de la concentration atmosphérique en CO_2. Une partie de ce CO_2 s'accumule dans l'atmosphère, entraînant une augmentation de l'effet de serre naturel et de la température à la surface du globe. Ce processus est plus connu sous le terme réchauffement climatique ou global. De plus, environ 25 % du CO_2 produit sont absorbés par les océans. La dissolution du CO_2 dans l'eau de mer, entraîne une augmentation de la concentration en protons et en ions bicarbonates (HCO_3^-) et une diminution de la concentration en ions carbonates (CO_3^{2-}). Il en résulte une diminution du pH et du taux de saturation de l'eau de mer vis-à-vis du carbonate de calcium. L'ensemble de ces processus est appelé acidification des océans (AO). Le pH des eaux de surface océaniques a déjà diminué de 0,1 unité depuis le début de l'ère industrielle. Ce phénomène devrait s'intensifier au cours du siècle. Selon les prévisions moyennes d'émissions futures de gaz à effet de serre de l'IPCC, la température moyenne des eaux de surface devrait augmenter de 2 à 4 °C et son pH devrait diminuer de 0,3 à 0,4 unité d'ici 2100.

Au cours des deux dernières décennies, de nombreuses études ont mis en évidence l'impact négatif de l'AO sur les organismes marins. Les premières études ont été menées principalement en milieu artificiel et ont mis en évidence des conséquences majeures sur la physiologie des organismes, principalement au niveau individuel. Cependant, les dernières études menées dans le domaine ont souligné l'importance de mettre en place des expériences à long terme, à l'échelle de l'écosystème, et dans des conditions plus proches du milieu naturel. Ce type d'étude permet de prendre en compte les interactions écosystémiques et les processus d'acclimatation afin de mieux prévoir les effets directs mais aussi indirects de la diminution du pH dans les océans.

L'existence des récifs coralliens tropicaux dépend de la vitesse de formation du socle récifal qui les façonnent (principalement via la calcification des coraux hermatypiques) qui doit rester supérieure à sa (bio)érosion. D'une part, plusieurs études ont montré que le taux de calcification des coraux hermatypiques diminue lorsque la pCO_2 augmente. D'autre part, les oursins sont d'importants bioérodeurs des récifs et contribuent donc à la perte de masse calcaire récifale. Cependant, les oursins empêchent également, par leur broutage, le recouvrement des coraux par les algues favorisées par l'AO. Dès lors l'effet de l'élévation de la pCO_2 sur les oursins et leur capacité bioérosive peut être déterminant pour l'avenir des récifs coralliens tropicaux au cours du siècle, particulièrement ceux où la densité de ces bioérodeurs est importante. Une telle prédiction

est d'autant plus complexe si l'on prend en compte la possible acclimatation des différents acteurs à long terme.

Dès lors, le but du présent travail fut d'évaluer l'effet à long terme de l'élévation de la pCO_2 prévue en 2100 sur la physiologie et l'activité érosive d'un oursin clé de certains récifs coralliens, *Echinometra mathaei*, dans un dispositif artificiel reproduisant l'écosystème corallien.

La première étape a été la mise en place un outil expérimental permettant de maintenir à long terme un écosystème de récifs coralliens simplifié en condition contrôle et au pH prévu en 2100 tout en maintenant les autres paramètres physico-chimiques identiques et proches du milieu naturel (y compris dans leurs variations journalières). Le système mis en place est composé de scléractiniaires hermatypiques comme constructeurs de récif, d'oursins (*E. mathaei*) comme bioérodeurs et brouteurs et un substrat calcaire de récif avec ses communautés d'algues, bactéries, archae, champignons et méiofaune. Les variations journalières de pH et de température reproduisent celles mesurées *in situ* dans le site de La Saline, Ile de La Réunion, d'où proviennent une partie des organismes. Le pH moyen des aquariums contrôles a été maintenu avec succès à une moyenne de 8,09 ± 0,04, celui des aquariums à pCO_2 élevée à 7,63 ± 0,02. L'alcalinité totale du système a pu être maintenue entre 2350 et 2450 µmol.kg-1.

L'impact de l'AO prévue en 2100 (pH 7,7) sur la physiologie d'*E. mathaei* été étudié à court terme (sept semaines). La principale source de nourriture des oursins fut la communauté algale se développant sur le substrat, comme en conditions naturelles. Cette étude a permis de mettre en évidence, à court terme, la capacité de résistance de cet oursin à une AO modérée. En effet, la croissance et le métabolisme ne furent pas affectés significativement. Ces observations ont été associées au maintien de la balance acide-base du fluide extracellulaire, le liquide cœlomique, par accumulation de bicarbonates dans celui-ci.

Une même expérience a ensuite été réalisée à long terme. La diminution du pH a été induite progressivement durant six mois jusqu'à atteindre un pH moyen de 7,65 qui fut ensuite maintenu à cette valeur pendant sept mois supplémentaires. La capacité de régulation de la balance acide-base du liquide cœlomique et la résistance d'*E. mathaei* à l'AO a été confirmée à long terme. Tant la croissance que le métabolisme et les propriétés mécaniques du squelette ne furent pas affectés. Cette résistance apparaît liée aux capacités de régulation acide-base d'*E. mathaei*, un trait apparemment d'origine génétique. Cette résistance pourrait également dépendre de la quantité et de la qualité de la nourriture disponible (calcaire ou non). Il est suggéré que les ions bicarbonates impliqués dans la régulation acide-base proviendraient en partie de la nourriture.

Parallèlement à ces mesures physiologiques, l'activité érosive d'*E. mathaei* a été mesurée. Les résultats indiquent que le taux de bioérosion triple en conditions acidifiées (pH 7,65). Cette augmentation serait liée à l'augmentation de l'activité de broutage des oursins et à la dissolution biologique du substrat, les propriétés mécaniques des dents des oursins et du squelette des coraux ne semblant pas affectés significativement. Nous suggérons que cette activité érosive accrue pourrait avoir un impact sur l'équilibre dynamique entre bioerosion et bioaccrétion des coraux et pourrait déterminer l'avenir des récifs coralliens où *E. mathaei* est le principal bioérodeur. Il faut toutefois noter que l'activité érosive de cet oursin est liée à une consommation accrue des macro-algues en compétition avec les coraux et algues corallines, favorisant ainsi ces derniers.

Les résultats obtenus, associés à ceux provenant de la littérature, indiquent que les changements globaux pourraient provoquer un changement profond des écosystèmes coralliens tropicaux. En effet, l'ensemble des bioérodeurs principaux étudiés jusqu'à présent semblent résistants aux changements climatiques globaux et montrent une augmentation de leur activité érosive. Dans le cas des récifs ayant déjà à l'heure actuelle une faible calcification nette, l'augmentation de la bioérosion pourrait mener à l'érosion nette et à la réduction puis à la disparition du récif. La prédiction du devenir des récifs coralliens tropicaux à l'échelle planétaire doit toutefois prendre en compte de nombreux paramètres : acclimatation, résistance/sensibilité et interactions des différents acteurs des récifs. D'autres études comparables à celles menées dans le présent travail devraient être mises en place afin de tester ces différents facteurs. Les données obtenues pourraient dès lors être utilisées dans la construction d'un modèle mécanistique permettant de mettre en place localement des mesures de conservation du récif, en complément de l'indispensable réduction massive de l'émission de CO_2 atmosphérique à l'échelle mondiale.

ABSTRACT

Since the beginning of the industrial era, human activities have resulted in a significant increase in the concentration of atmospheric CO_2. A part of this CO_2 accumulates in the atmosphere, resulting in an increase of the natural greenhouse effect and of the temperature on the Earth's surface. This process is known as the climate change or global warming. Moreover, about 25 % of CO_2 produced is absorbed by oceans. The dissolution of CO_2 in seawater increases the concentration of protons and bicarbonate ions (HCO_3^-) and decreases the concentration of carbonate ions (CO_3^{2-}). This results in a decrease in pH and in the calcium carbonate saturation state. All of these processes are called ocean acidification (OA). The pH of surface ocean waters has already fallen by 0.1 pH units since the beginning of the industrial era. This phenomenon is expected to intensify over the next century. According to average IPCC emission scenarios of future emissions of greenhouse gases, the average temperature of the surface ocean water is expected to increase by 2 to 4 °C and pH should decrease by 0.3 to 0.4 units by 2100.

Over the past two decades, many studies have highlighted the negative impact of OA on marine organisms. The first studies were mostly conducted in artificial environments and highlighted major impact on the physiology of organisms, mainly at the individual level. However, recent studies in the field have stressed the importance to conduct long-term experiments at the ecosystem scale, and in conditions closer to the natural environment. This kind of study can take into account ecosystem interactions and acclimation processes to better predict the direct, but also indirect, effects of the decrease of the pH of the ocean waters.

The persistence of tropical coral reefs is dependent on the rate of constructive processes (mainly through the reef-building coral calcification) which must exceed its (bio) erosion. On one hand, several studies have shown that the rate of reef-building coral calcification decreases with increasing pCO_2. On the other hand, sea urchins are important bioeroders of coral reefs and thus contribute to the loss of reef material. However, sea urchins also prevent, by their grazing, the overgrowth of corals by algae which are favored by OA Therefore, the effect of elevated pCO_2 on sea urchins and their bioerosive ability can be decisive for the future of tropical coral reefs during the century, especially where the density

of these bioeroders is important. Such a prediction is even more complex if one takes into account possible acclimation of different contributors at the long-term.

The aim of this work was therefore to evaluate the long-term effect of the increase of pCO_2 expected in 2100 on the physiology and bioerosive activity of a key sea urchin in some coral reefs, *Echinometra mathaei,* using an artificial device which reproduces the coral reef ecosystem.

The first step was to set up an experimental tool which allows the maintenance at long-term (more than one year) of a simplified coral reef ecosystem in control condition and at the pH expected in 2100 while keeping other physico-chemical parameters identical and close to natural conditions (including in their daily variations). The developed system is composed of scleractinian hermatypic corals as reef builders, sea urchins (*E. mathaei*) as bioeroders and grazers and coral reef substrate with its diverse communities of algae, bacteria, archae, fungi and meiofauna. Daily variations in pH and temperature reproduce those measured *in situ* in the lagoon of La Saline, Reunion Island, from where some of the organisms originate. The average pH of control aquaria was successfully maintained at a mean of 8.09 ± 0.04, and the high pCO_2 aquaria at 7.63 ± 0.02. The total alkalinity of the system was maintained between 2350 and 2450 µmol kg^{-1}.

The impact of the OA expected in 2100 (pH 7.7) on the physiology of *E. mathaei* was studied at short-term (seven weeks). Sea urchins fed principally on algae that grow on the reef calcareous substrate, as in natural conditions. This study highlighted the resistance of this sea urchin to a moderate OA at short-term. Indeed, the growth and the metabolism were not significantly affected. This was associated to the ability of sea urchins to regulate the acid-base balance of their extracellular fluid, the coelomic fluid, through bicarbonate compensation.

The same experiment was then performed at long-term. The decrease in pH was induced gradually during six months until reaching a mean pH of 7.65, which was then maintained at this value during seven more months. The ability to regulate the acid-base balance of the coelomic fluid and the resistance of *E. mathaei* to OA was confirmed at long-term. Growth, metabolism and mechanical properties of the skeleton were not affected. This resistance appears to be related to the capabilities of acid-base regulation of *E. mathaei*, an apparently genetic trait. This resistance may also depend on the quantity and quality of the available

food (calcareous or not). We suggest that the bicarbonate ions involved in acid-base regulation are partly mediated by food.

Simultaneously to physiological measurements, the erosive activity of *E. mathaei* was measured. Results indicate that the rate of bioerosion triples under acidified conditions (pH 7.65). This increase is mediated by the increased activity of grazing of sea urchins and biological dissolution of the substrate, as the mechanical properties of the teeth of the sea urchins and coral skeletons do not appear significantly affected. We suggest that this increased bioerosion could have an impact on the dynamic balance between bioerosion and bioaccretion of corals and could determine the future of coral reefs where *E. mathaei* is the main bioeroder. It should be noted that the erosive activity of the sea urchin is associated with increased consumption of macroalgae that compete with corals and coralline algae, favoring the latter.

The results, according to those compiled from the literature, indicate that global change may cause a profound change in tropical reef ecosystems. Indeed, all major bioeroders studied so far seem to be resistant to global climate change and show an increase in erosive activity. For corals that currently show a low net ecosystem calcification, increased bioerosion could lead to net erosion and to the reduction and disappearance of the reef. However, prediction of the future of tropical coral reefs globally must take into account many parameters: acclimatization, tolerance/sensitivity and interactions of the various actors of the reefs. Other comparable studies to those carried out in the present work should be conducted in order to test these factors. The data obtained could then be used in the construction of a mechanistic model to implement, locally, reef conservation measures, in addition to the required massive reduction of atmospheric CO_2 emission worldwide.

TABLE DES MATIÈRES

ANNEXE 1

Moulin L., Catarino A., Claessens T. & Dubois P (2011) Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck 1816). *Marine Pollution Bulletin*, **62**, 48-54.

ANNEXE 2

Collard M., Laitat K., Moulin L., Catarino A.I., Grosjean Ph. & Dubois Ph (2013) Buffer capacity of the coelomic fluid in echinoderms. *Comparative Biochemistry and Physiology Part A Molecular Integrative Physiology*, **166(1)**, 199-206.

1. L'acidification des océans

1.1. L'augmentation du CO_2 atmosphérique

Au cours des 800 000 années précédant la révolution industrielle, la concentration en dioxyde de carbone atmosphérique a varié entre 172 et 300 ppmv (parties par million en volume) (Lüthi *et al.*, 2008). Cette concentration a augmenté au cours de la révolution industrielle jusqu'à dépasser 390 ppmv aujourd'hui (Fig. 1). Cette augmentation est indéniablement liée aux activités humaines (utilisation de combustibles fossiles, production de ciment, agriculture, déforestation,...). De plus, cette augmentation se fait à un taux de 0,5 % par an, ce qui est environ 100 fois plus rapide que tous les changements observés durant les 650 000 dernières années (Siegenthaler *et al.*, 2005).

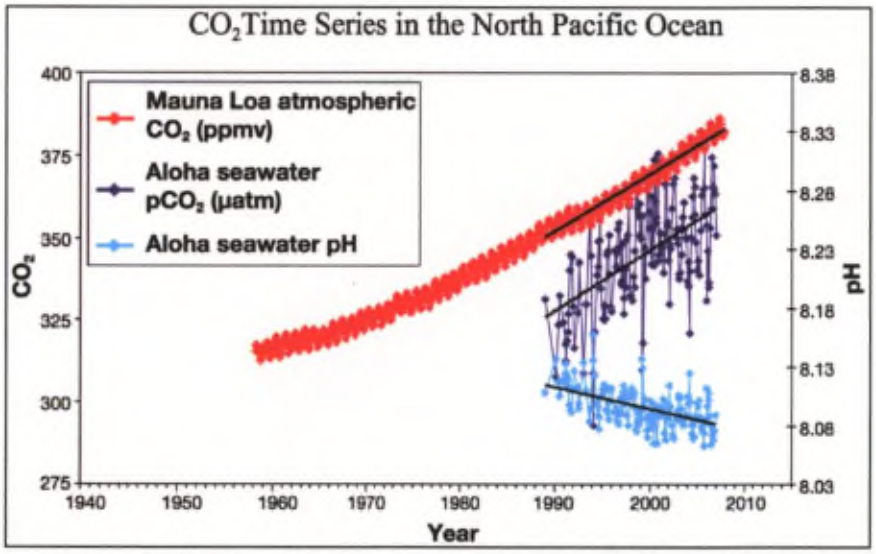

Figure 1: Séries chronologiques du CO_2 atmosphérique (en ppmv) à Mauna Loa (série rouge), de la pCO_2 (µatm) et du pH à la surface de l'océan à la station marine Aloha dans l'océan Nord Pacifique subtropical (d'après Feely *et al.*, ,2009).

La moitié du CO_2 anthropogénique reste dans l'atmosphère alors que le reste est absorbé par la biosphère terrestre et par les océans (Le Quéré *et al.*, 2009). L'accumulation de CO_2 dans l'atmosphère augmente l'effet de serre naturel, entraînant le réchauffement de l'atmosphère et des couches superficielles des océans, un phénomène connu sous le nom de réchauffement climatique ou global. Cette augmentation de température entraîne notamment la fonte des neiges et glaces, l'augmentation subséquente du niveau de la mer, l'augmentation de la stratification des océans et la perturbation de la circulation thermohaline (IPCC, 2013).

Approximativement 25 % du CO_2 anthropogénique produit depuis 1800 ont été absorbés par les océans (Sabine *et al.*, 2004). Bien que cette absorption du CO_2 atmosphérique par les océans diminue l'ampleur du réchauffement global (Feely *et al.*, 2008), le CO_2 dissout affecte également la chimie des océans.

1.2. Le système des carbonates dans l'eau de mer et l'augmentation de CO_2

L'absorption du CO_2 par les océans dépend de la différence de pression partielle en CO_2 (pCO_2) entre l'atmosphère et la surface des océans (Fig. 2). Le système tend vers un équilibre suivant la loi de Henry.

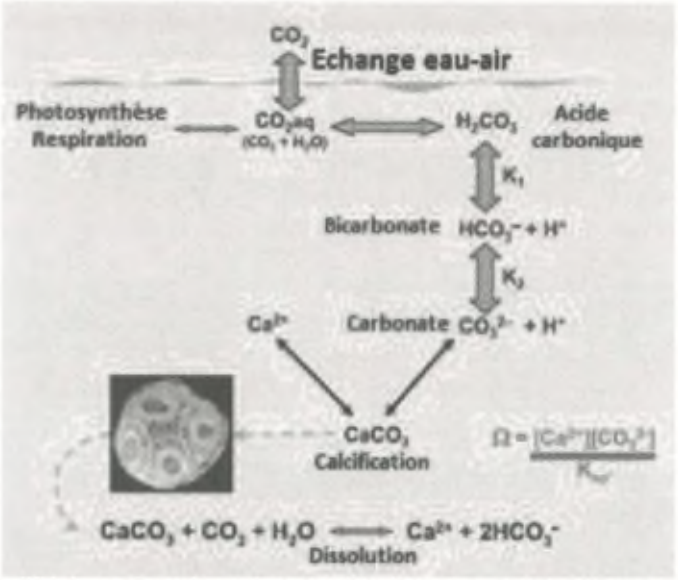

Figure 2: Diagramme simplifié du système des carbonates dans l'eau de mer. K1 et K2 représentent les constantes de dissociation pour H_2CO_3 et HCO_3^- respectivement. Ω représente de taux de saturation de l'eau de mer vis-à-vis du carbonate de calcium $CaCO_3$. $K_{sp'}$ est le produit de solubilité stœchiométrique apparent du $CaCO_3$ (modifié d'après Kleypas *et al.*, 2006).

Le dioxyde de carbone dissout dans l'eau de mer réagit en premier lieu avec une molécule d'eau pour former de l'acide carbonique H_2CO_3 (Fig. 2) :

$$CO_2\ (g) + H_2O \Leftrightarrow H_2CO_3^*\quad (éq.\ 1)\quad \text{avec } K0^* = [H_2CO_3^*]/pCO_2\ (g)$$

Où K0* est la constante de dissociation apparente exprimée en terme de concentration, $[H_2CO_3^*]$ la concentration en $H_2CO_3^*$ et pCO_2 la pression partielle en CO_2. $H_2CO_3^*$ est noté avec un astérisque car quand le CO_2 se dissout dans l'eau de mer, il forme aussi du CO_2 aqueux. $H_2CO_3^*$ représente donc la somme de H_2CO_3 et CO_2(aq).

Au pH de l'eau de mer, $H_2CO_3^*$ se dissocie en un proton et un ion bicarbonate HCO_3^-, ce qui induit une diminution de pH (Fig. 2) :

$$H_2CO_3^* \Leftrightarrow H^+ + HCO_3^-\quad (éq.\ 2)\quad \text{avec } K1' = [H^+][HCO_3^-]/[H_2CO_3^*]$$

où K1' est la première constante de dissociation de l'acide carbonique. Elle est influencée par la température, la salinité et la pression hydrostatique.

Les protons libérés peuvent réagir avec les ions carbonates de la colonne d'eau et former un ion bicarbonate :

$$H^+ + CO_3^{2-} \Leftrightarrow HCO_3^-\ (éq.\ 3)\qquad \text{avec } K2' = [H^+][CO_3^{2-}]/[HCO_3^-]$$

où K2' est la deuxième constante de dissociation de l'acide carbonique. Elle est également influencée par la température, la salinité et la pression hydrostatique.

Les carbonates ne sont toutefois pas présents en assez grande quantité pour tamponner tous les protons libérés. Ainsi, l'effet net de la dissolution du CO_2 dans l'eau de mer est d'augmenter les concentrations en H^+ et HCO_3^- tout en diminuant la concentration en CO_3^{2-} et le pH de l'eau de mer, un phénomène connu sous le nom d'acidification des océans (AO) (Fig. 3).

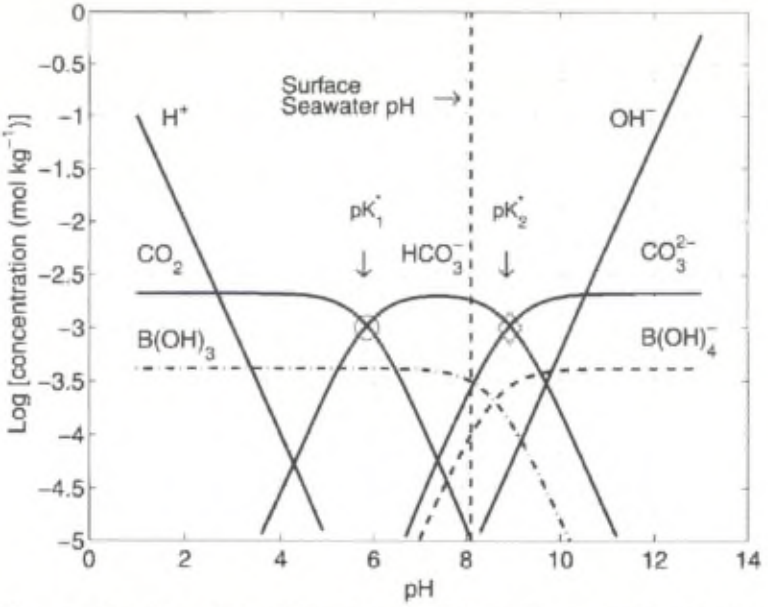

Figure 3: Système des carbonates dans l'eau de mer (CID = 2,1 mmol kg-1, S = 35, T = 25 °C). Notez que la proportion relative de CO_2, HCO_3^- et CO_3^{2-} contrôle le pH (d'après Zeebe & Wolf-Gladrow, 2001)

Lorsque l'on somme les équations (1), (2) et (3), on obtient :

$$CO_2\,(aq) + H_2O + CO_3^{2-} \Leftrightarrow 2\,HCO_3^- \text{ (éq. 4)}$$

Les vitesses de réaction entre les espèces dissoutes étant très rapides (de l'ordre du centième de seconde), l'eau de mer constitue un milieu dans lequel H_2CO_3*, HCO_3^- et CO_3^{2-} sont considérés comme étant en permanence à l'équilibre (Fig. 3). Ces 3 formes inorganiques dissoutes de carbone sont regroupées sous le nom de carbone inorganique dissout (CID) :

$$[CID] = [H_2CO_3^*] + [HCO_3^-] + [CO_3^{2-}] \text{ (éq. 5)}$$

La précipitation/dissolution des carbonates de calcium est représentée par :

$$CaCO_3\downarrow \Leftrightarrow Ca^{2+} + CO_3^{2-} \text{ (éq. 6)}$$

Lors de la dissolution du CO_2 dans l'eau de mer, la concentration en ions CO_3^{2-} diminue (équation 3). La réaction (éq. 6) se déplace alors vers la droite défavorisant la formation du carbonate de calcium inorganique et favorisant la dissolution de cristaux de carbonate de calcium. Les ions carbonates CO_3^{2-} peuvent réagir avec les protons (éq. 3). Ce mécanisme réduit le processus d'AO. Toutefois, ce processus consomme des ions carbonates et le pouvoir tampon de l'eau de mer diminue à mesure que la concentration en CO_2 augmente (éq. 3).

Il existe différentes formes de carbonate de calcium : la calcite ($CaCO_3$ comme chez les coccolithophores et foraminifères), l'aragonite ($CaCO_3$, forme principalement produite par les coraux hermatypiques) et la calcite magnésienne ($Ca_{1-x}Mg_xCO_3$ où x est la proportion molaire de carbonate de magnésium $MgCO_3$, forme produite, entre autres, par les échinodermes et algues corallines). La calcite est la forme la moins soluble, vient ensuite l'aragonite ou la calcite magnésienne en fonction de la concentration en ions Mg^{2+} dans cette dernière. La calcite et l'aragonite ont la même formule générale mais diffèrent par la structure de leur réseau cristallin : la calcite cristallise dans le système rhomboédrique alors que l'aragonite cristallise dans le système orthorhombique. Dans la calcite magnésienne, des ions Ca^{2+} sont substitués par des ions Mg^{2+}, plus petits, causant des variations dans la structure cristalline et la rendant plus soluble. Plus la fraction d'ions Mg^{2+} dans le réseau cristallin est grande, plus la calcite magnésienne est soluble. La calcite magnésienne a la même solubilité que l'aragonite pour un pourcentage molaire en magnésium allant de 8 à 12 mol% en fonction de la courbe de solubilité expérimentale adoptée (Andersson *et al.*, 2008).

Le degré de saturation des carbonates dans l'eau de mer, omega (Ω) s'exprime par la fonction :

$$\Omega = [Ca^{2+}][CO_3^{2-}]/K'sp \quad (éq.\ 7)$$

où K'sp est le produit de solubilité stœchiométrique apparent de la forme considérée de carbonate de calcium à la pression hysdrostatique, la salinité et la température considérées.

K'sp augmente avec la pression et diminue avec la température. Cette constante va donc augmenter avec la profondeur et, partant, Ω va diminuer. Elle est plus élevée pour l'aragonite et la calcite magnésienne que pour la calcite. D'autre part, si la concentration en ions Ca^{2+} est à peu près constante dans l'eau de mer, à une salinité donnée, la concentration en ions CO_3^{2-}, pour sa part, va également influencer Ω. La dissolution de CO_2 dans l'eau, qui réduit la concentration en ions CO_3^{2-}, va donc entraîner une diminution de Ω. Du point de vue purement chimique, quand Ω est supérieur à 1, l'eau de mer est sursaturée vis-à-vis de la forme de carbonate de calcium considérée et le minéral pourra précipiter. Quand Ω est inférieur à 1, l'eau est sous-saturée et le carbonate de calcium a tendance à se dissoudre. La profondeur à laquelle Ω est égal à 1 est appelée l'horizon de saturation. L'apport de CO_2 anthropique qui diminue la concentration en ions carbonates a donc pour conséquence de faire remonter l'horizon de saturation à des profondeurs moindres.

Une autre notion importante utilisée dans la description du système des carbonates est l'alcalinité totale (A_T). L'alcalinité totale est définie comme le nombre de moles d'ions hydrogène équivalent à l'excès d'accepteurs de protons (bases formées à partir d'acides forts avec une constante de dissociation $K \leq 10^{-4.5}$ à 25 °C et à force ionique nulle) par rapport aux donneurs de protons (acides avec $K > 10^{-4.5}$) dans un kilogramme d'eau de mer (Dickson, 1981).

$$A_T = [HCO_3^-] + 2\,[CO_3^{2-}] + [B(OH)_4^-] + [OH^-] + [HPO_4^{2-}] + 2[PO_4^{3-}] + [SiO(OH)_3^-] + [NH3] + [HS] - [H^+]_{libre} - [HSO_4^-] - [HF] - [H_3PO_4] - \ldots \quad \text{(éq. 8)}$$

où l'ellipse représente les bases et acides mineurs non identifiés ou présents en faible quantité, considérés comme négligeables. $[H^+]_{libre}$ est la concentration d'ions hydrogènes libres.

En termes de bases majeures, dans l'eau de mer, ce sont principalement les carbonates (C) et les borates (B) qui ont cette capacité.

Contrairement à Ω et à la concentration en CID, l'A_T ne varie pas avec la concentration océanique en CO_2. En effet, lorsque le CO_2 se dissout dans l'eau de mer, il réagit avec un ion CO_3^{2-} pour former deux ions HCO_3^- (éq. 4). L'A_T est donc inchangée puisque deux protons peuvent toujours être neutralisés. Celle-ci varie donc majoritairement avec les processus de précipitation/dissolution du carbonate de calcium (respectivement, diminution/augmentation de l'A_T) et de manière moins importante avec la photosynthèse/respiration (respectivement, augmentation/diminution de l'A_T via la variation de la concentration en nutriments, voir équation 9 et Fig. 4).

$$106\,CO_2 + 16\,NO_3^- + PO_4^{3-} + 122\,H2O + 18\,H^+ \Leftrightarrow (CH_2O)_{106}(NH_3)_{16}(H_3PO_4) + 138\,O_2 \quad \text{(éq. 9)}$$

A partir d'un couple des paramètres du système des carbonates (pCO_2, pH, [CID] et A_T), de la température, salinité et pression (qui déterminent les constantes de dissociation) et des équations décrites précédemment, on peut calculer l'ensemble des paramètres du système des carbonates dans l'eau de mer.

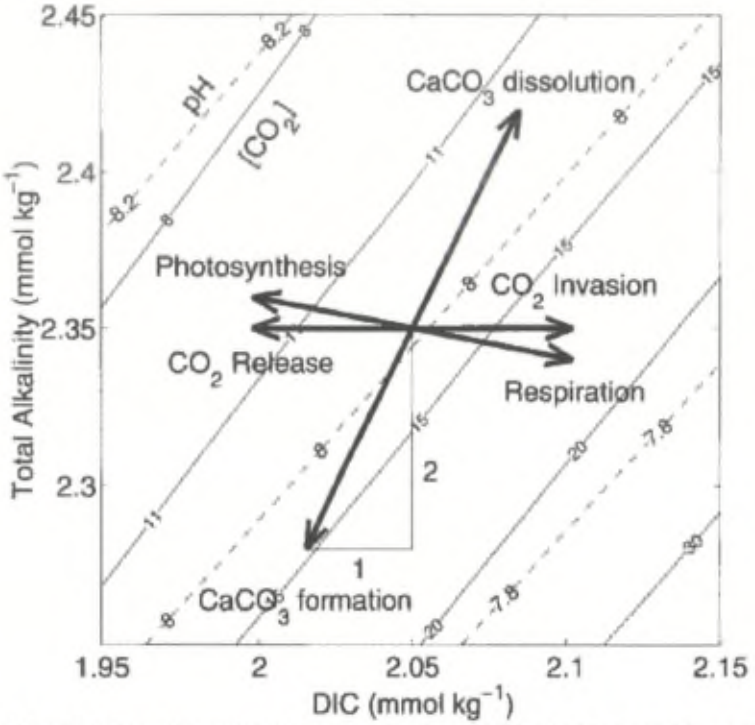

Figure 4: Effets (flèches) de différents processus sur la concentration en CID (ici DIC en anglais) et l'alcalinité totale (A_T). Les lignes pleines et pointillées représentent, respectivement, la concentration en CO_2 (en µmol kg^{-1}) et le pH en fonction de la [CID] et de l'A_T. La formation de $CaCO_3$, par exemple, réduit la [CID] et A_T, augmente la concentration en CO_2 et diminue le pH. La dissolution du CO_2 dans l'eau de mer augmente la [CID] mais ne modifie pas A_T (modifié d'après Zeebe & Wolf-Gladrow, 2001).

Il est important de noter que plusieurs échelles de pH existent. Le pH est défini comme :

$$pH = - \log_{10} a_{H^+} \quad \text{(éq. 10)}$$

où a_{H^+} représente l'activité des protons H^+ dans la solution.

Cependant, il est difficile de mesurer le pH en respectant cette définition. En effet, l'activité d'un seul ion dans une solution ne peut être déterminée expérimentalement puisque la concentration de celui-ci ne peut varier indépendamment à cause de l'électroneutralité (Klotz & Rosenberg, 2000). Ainsi, dans la pratique, on définit le pH suivant l'échelle NBS/NIST ($pH_{NBS/NIST}$, National Bureau of Standards maintenant connu comme National Institute of Standards and Technology) à l'aide de solutions tampons standards pour lesquelles on a mesuré des valeurs de pH proches de la meilleure estimation de $- \log_{10} a_{H^+}$. On peut par exemple utiliser une électrode de pH et un voltmètre à très haute impédance (ici appelé pH-mètre) qui affiche la différence de potentiel (ΔE) se développant entre l'électrode de pH et la solution tampon standard. Ainsi, à chaque solution tampon est assignée une valeur de ΔE propre à l'électrode de pH utilisée et une valeur correspondante de pH connue. Pour l'eau de mer, on utilise en général des tampons NBS de pH ≈ 4,01, 6,87 et 9,18. Une fois l'appareil étalonné (droite de calibration reliant ΔE au pH), la différence de potentiel mesuré dans la solution étudiée sera transformée en valeur de pH par simple interpolation puisque la ΔE évolue proportionnellement à la valeur du pH.

Cependant, ces tampons NBS/NIST ont une force ionique très faible (≈ 0,1) alors que dans l'eau de mer, la force ionique est beaucoup plus grande (≈ 0,7). Cette différence de force ionique entre les tampons utilisés pour calibrer la mesure du pH et l'eau de mer entraîne dès lors une surestimation du pH. Ainsi, lorsque l'on travaille en milieu marin, il est conseillé d'utiliser une autre échelle de pH, l'échelle de pH total (pH_T) qui se base sur des tampons préparés dans de l'eau de mer artificielle et qui ont donc des forces ioniques proches des échantillons à analyser (Hansson, 1973). De plus, dans l'eau de mer, les protons réagissent avec les ions sulfate SO_4^{2-}. Ainsi, la concentration en protons est définie comme :

$$[H+] = [H+]_{libre} + [HSO_4^-] \quad (éq.\ 11)$$

L'échelle totale permet de prendre en considération l'effet des ions sulfates puisque les tampons utilisés en contiennent également. Enfin, si la concentration en ions fluorure est élevée dans les échantillons à analyser, il convient d'utiliser l'échelle « seawater scale » (SWS) où :

$$[H^+] = [H^+]_{libre} + [HSO_4^-] + [HF] \quad (éq.\ 12)$$

Cependant, dans de l'eau de mer à une salinité donnée, la concentration en HF est généralement négligeable.

Le pH mesuré avec l'échelle NBS/NIST et l'échelle totale peut différer jusqu'à 0,15 unité (Zeebe & Wolf-Gladrow, 2001.). Il est donc important de spécifier quelle échelle est utilisée et, dans la mesure du possible, d'utiliser une échelle commune permettant une comparaison plus aisée entre les différentes études. Même si l'usage de l'échelle totale de pH est fortement conseillé, de nombreuses études dans le domaine de l'AO expriment encore à l'heure actuelle le $pH_{NBS/NIST}$.

1.3. Les prédictions en 2100-2300

Depuis le début de la période industrielle, le pH des océans a déjà diminué en moyenne de 0,1 unité (Caldeira & Wickett, 2003 ; IPCC, 2013). Afin de prédire la diminution de pH au cours des siècles à venir, plusieurs scénarios d'émissions futures de gaz à effet de serre ont été formulés en tenant compte notamment des changements démographiques, socio-économiques et technologiques à venir. Le GIEC a présenté en 1992 un ensemble de scénarios d'émissions qui lui ont servi à établir des projections climatiques. Ces scénarios d'émissions ont été appelés scénarios IS92. Le scénario IS92a, utilisé comme référence dans de nombreuses études traitant des effets de l'AO, est plus connu sous le nom de scénario « buisness-as-usual » (IPCC, 2007). Celui-ci prend en compte une population mondiale qui s'élèverait à 11,3 milliards en 2100, une croissance

économique moyenne et un mélange de sources d'énergie conventionnelles et renouvelables. Il prédit une diminution de pH de 0,3-0,5 unité pour 2100 et 0,7-0,8 unité pour 2300 (Caldeira & Wickett, 2005 ; IPCC, 2007). L'augmentation de température serait de 2 °C.

Dans le rapport spécial du GIEC consacré aux scénarios d'émissions (Nakicenovic & Swart, 2000), de nouveaux scénarios d'émissions, appelés « scénarios SRES », ont été publiés (Table 1). D'après ceux-ci, l'augmentation moyenne de température varierait entre 1,8 et 4 °C en 2100. Ces différents scénarios ont permis de calculer les évolutions possibles du pH et de la profondeur des horizons de saturation de l'aragonite et de la calcite dans les océans pour 2100 et 2300 (Caldeira & Wickett 2005, Fig. 5). Cette étude prédit une diminution du pH d'environ 0,4 unité pour 2100 à la surface des océans (Fig. 5A) et une réduction de la profondeur des horizons de saturation pour la calcite et l'aragonite (Fig. 5B). Cependant, seul l'océan austral serait sous-saturé par rapport à l'aragonite en 2100. Pour 2300, les prévisions moyennes indiquent une diminution de pH de l'ordre de 0,8 unité (Fig. 5C) et une sous-saturation par rapport à la calcite et l'aragonite s'étendant à de nombreux océans bien que l'océan tropical reste sursaturé (Fig. 5D).

Table 1 : Caractéristiques principales des différents scénarios formulés par le GIEC (d'après http://www.grida.no/publications/other/ipcc_sr/?src=/Climate/ipcc/emission/091.html)

Scénario	A1B	A2	B1	B2
Croissance économique	très rapide	moyenne	rapide	moyenne
Croissance de la population	pic vers 2050 et diminution ensuite	forte	pic vers 2050 et diminution ensuite	moyenne
Utilisation des énergies	très forte	forte	faible	moyenne
Changement de l'utilisation des terres	faible	faible/fort	fort	moyen
Disponibilité des ressources	moyenne	faible	faible	moyenne
Changements technologiques	Rapide et efficace	lent	moyen	moyen
Energie utilisée	équilibre fossile – non fossile	régionale	efficacité et dématérialisation	équilibre fossile – non fossile

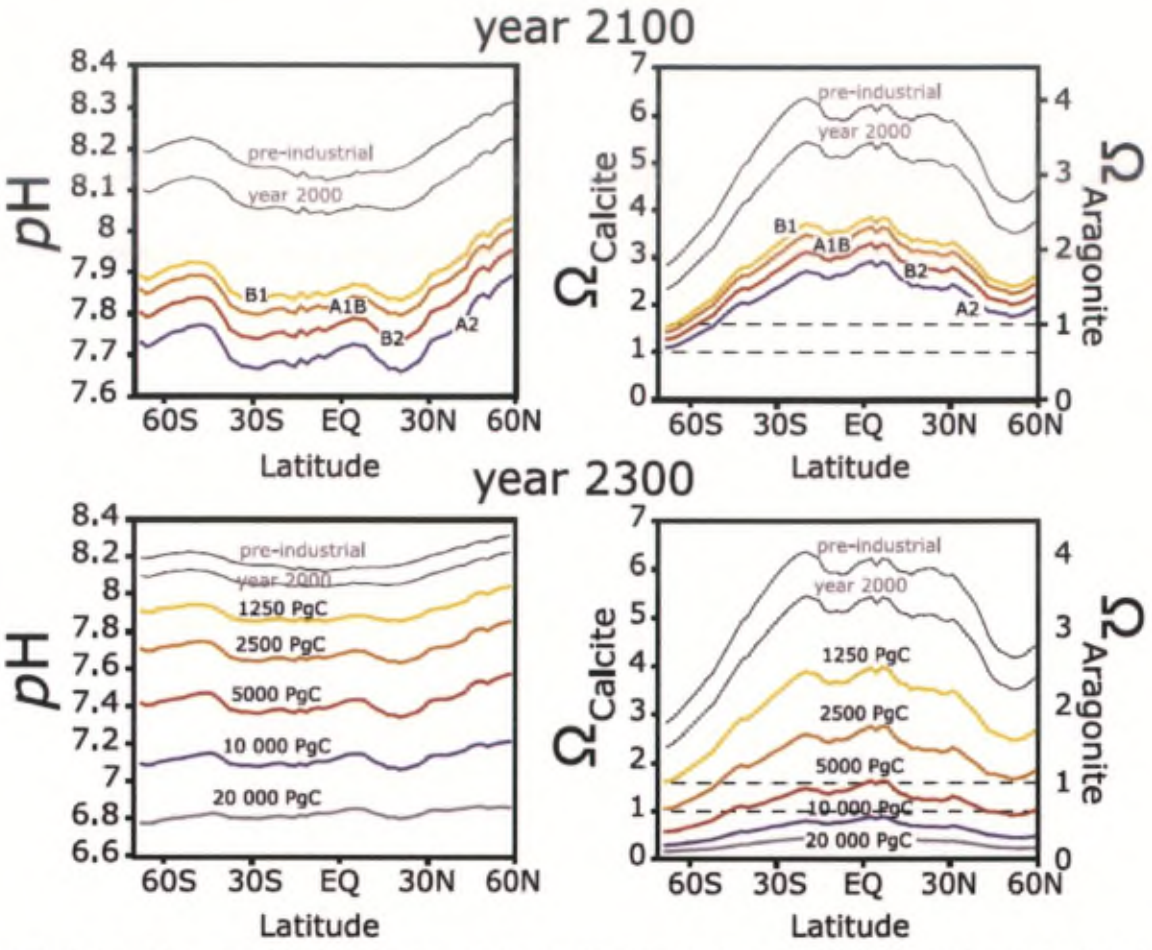

Figure 5: Evolution prédite du pH de la surface des océans et de l'état de saturation de l'eau de mer vis-à-vis de la calcite ($\Omega_{calcite}$) et de l'aragonite ($\Omega_{aragonite}$) selon les scénarios d'émission SRES en 2100 et selon le scénario d'émission suivant une courbe logistique en 2300. Les lignes discontinues représentent la saturation de la calcite et de l'aragonite (d'après Caldeira & Wickett, 2005).

En 2013, pour établir le cinquième Rapport d'évaluation du GIEC (IPCC, 2013), la communauté scientifique a défini un ensemble de quatre nouveaux scénarios appelés RCP (pour « Representative Concentration Pathway » en anglais). Ces scénarios sont identifiés par leur forçage radiatif total approximatif pour l'année 2100 par rapport à 1750. Le forçage radiatif mesure l'impact de certains facteurs affectant le climat sur l'équilibre énergétique du système couplé Terre/atmosphère (Fig. 6). Un forçage radiatif causé par un ou plusieurs facteurs est dit positif lorsqu'il entraîne un accroissement de l'énergie du système Terre/atmosphère et donc le réchauffement du système. Le forçage radiatif est estimé à 2,6 W m^{-2} pour le RCP2,6 ; 4,5 W m^{-2} pour le RCP4,5 ; 6,0 W m^{-2} pour le RCP6,0 et 8,5 W m^{-2} pour le RCP8,5.

Contrairement aux scénarios SRES, ces quatre RCP prennent en compte la mise en place d'une politique climatique : un scénario d'atténuation conduisant à un niveau de forçage très bas (RCP2,6), deux scénarios de stabilisation (RCP4,5 et RCP6,0) et un scénario aux émissions de gaz à effet de serre très élevées (RCP8,5). Les concentrations en CO_2 prévues par ces scénarios sont estimées à environ 421 ppm (RCP2,6), 538 ppm (RCP4,5), 670 ppm (RCP6,0) et 936 ppm (RCP8,5).

La diminution du pH de l'eau de mer associée varie entre -0,05 et -0,35 unité alors que l'augmentation de température prédite se situe entre +1 et +4 °C pour 2100.

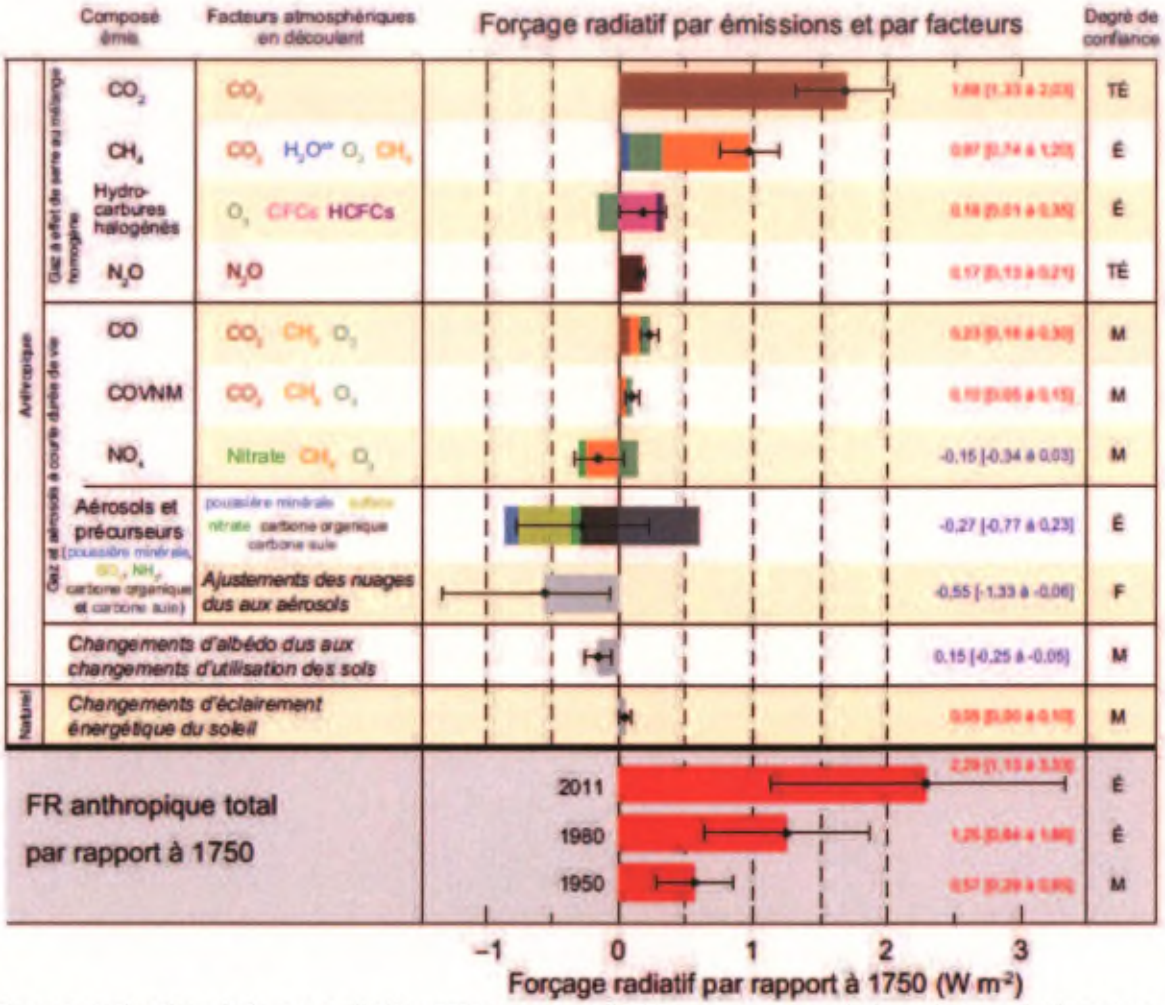

Figure 6 : Estimations du forçage radiatif en 2011 par rapport à 1750 concernant les principaux facteurs du changement climatique. Les valeurs sont des moyennes du forçage radiatif global, réparties selon les composés émis ou les processus qui aboutissent à une combinaison de facteurs. Les meilleures estimations du forçage radiatif net sont présentées sous la forme d'un losange noir avec les intervalles d'incertitude correspondants ; les valeurs numériques sont fournies sur la droite de la figure de même que le degré de confiance (TÉ – très élevé, É - élevé, M - moyen, F - faible, TF – très faible). Le forçage radiatif anthropique total est indiqué pour trois années différentes par rapport à 1750 (d'après IPCC, 2013).

Le niveau actuel d'AO est irréversible à l'échelle d'une vie humaine. La circulation thermohaline mondiale des océans permettra à long terme la réaction entre les eaux de surface chargées en CO_2 et les sédiments riches en $CaCO_3$. Ce processus pourrait théoriquement tamponner les effets de l'acidification. En effet, dans le passé, les augmentations de la concentration atmosphérique en CO_2 se sont faites sur des périodes suffisamment longues pour qu'il y ait un mélange des eaux de surface avec les eaux de fond en contact avec les sédiments. Cependant, l'accroissement anthropique actuel se fait à une échelle beaucoup plus courte (siècle) alors qu'une molécule d'eau effectue le circuit de circulation thermohaline mondiale des océans en environ 1000 ans (Ross 1995). Ainsi, ce processus est trop lent par rapport au phénomène d'acidification actuel et ne pourrait produire ses effets, au mieux, que dans plusieurs centaines d'années (Andersson *et al.*,

2003). La pompe biologique de carbone inorganique est également à prendre en compte. En effet, la fixation photosynthétique de CO_2 par le phytoplancton, le dépôt de carbone organique particulaire dans les océans profonds et son oxydation entraînent le CID en profondeur, réduisant la concentration en CO_2 à la surface des océans. Cependant, Hofmann & Schellnhuber (2009) ont simulé cet effet et ont montré que ce processus ne suffirait pas à tamponner l'AO. Dès lors, la seule solution est un abattement important de la production de CO_2 atmosphérique de façon à ralentir le processus.

1.4. Les dispositifs expérimentaux et l'étude de l'acidification des océans en milieu benthique

Afin de comprendre et de prédire les conséquences de l'AO sur notre environnement, différentes méthodes ont été utilisées par la communauté scientifique. Cette section reprend une brève synthèse des méthodes utilisées en milieu benthique (pour une synthèse plus générale, voir Widdicombe *et al.*, 2010 ; Stewart *et al.*, 2013).

Une première approche est l'observation de terrain. En effet, on retrouve à l'heure actuelle des environnements où le pH de l'eau de mer est naturellement bas comme les mares intertidales (Moulin *et al.*, 2011 ; Truchot & Duhamel-Jouve, 1980), les évents à CO_2 (Cigliano *et al.*, 2010 ; Fabricius *et al.*, 2011 ; Hall-Spencer *et al.*, 2008 ; Johnson *et al.*, 2012 ; Martin *et al.*, 2008 ; Suggett *et al.*, 2012), les zones d'upwelling (Feely *et al.*, 2008) et l'océan profond (Park, 1966). Les avantages et inconvénients des études *in situ*, ainsi que ceux des autres méthodes, sont repris dans la Table 2.

Une autre approche est l'expérimentation en aquariums. Afin de démontrer une relation directe de cause à effet, on peut réaliser des expériences dans lesquelles on va modifier le pH tout en maintenant les autres paramètres constants. On peut ainsi mener des études sur des individus appartenant généralement à une seule espèce ou à un assemblage écosystèmique simple. On peut également étudier, en plus du pH, l'effet d'autres variables (température, nutriments, UV, ...) et de leurs interactions.

Enfin, on peut utiliser des mésocosmes. On peut soit prélever un échantillon de l'écosystème (assemblage naturel) ou en recréer un artificiellement (limitation des interactions et donc des facteurs confondants, Fig. 7). La méthode consiste en un changement du paramètre étudié, une diminution de pH ici, tout en maintenant les autres paramètres proches de leur valeur naturelle et en incluant des interactions écosystèmiques. Ce design permet de se rapprocher des conditions naturelles tout en restant dans des conditions expérimentales semi-contrôlées. Cette méthode

représente un compromis entre les études de terrain et les expériences de laboratoire étudiant une seule espèce ou un assemblage d'espèces simple.

L'interaction du milieu benthique et du milieu pélagique est généralement difficilement applicable pour les expériences en mésocosmes réalisées en laboratoire. Une nouvelle génération de mésocosmes benthiques placés *in situ*, les FOCE (« Free Ocean CO_2 Enrichment ») pourrait prendre en compte celles-ci. En effet, les FOCE sont définis comme une « technologie facilitant l'étude des effets de l'AO sur les organismes marins et les communautés qui permet un contrôle précis de l'enrichissement en CO_2 *in situ* de parcelles expérimentales partiellement ouvertes » (Gattuso *et al.*, 2014). Le concept semble idéal et prometteur. Cependant, les aspects logistiques et techniques de ces outils sont difficiles à mettre en œuvre et les coûts associés élevés. A l'heure actuelle, la plupart des FOCE déployés ou en cours de mise en place considère uniquement l'impact de l'élévation de la pCO_2 (Kilne *et al.*, 2013, Gattuso *et al.*, 2014). En effet, l'étude de l'interaction de la diminution du pH et d'une augmentation de la température semble techniquement impossible à cause du coût énergétique énorme nécessaire pour modifier la températures de masses d'eaux importantes (Gattuso *et al.*, 2014).

Ainsi, de nombreux paramètres sont à prendre en compte : stress expérimental, cycle naturel de l'organisme, variations cycliques naturelles du paramètre étudié, influence de l'origine de la population, variabilité interindividuelle, importance des interactions biologiques, durée de vie des organismes *vs.* durée de l'expérience, acclimatation (aux conditions expérimentales et au stress étudié), échange entre le milieu benthique et pélagique, ... Les moyens mis en œuvre et la méthode choisie lors de la mise en place d'une expérience dédiée à l'étude des effets de l'AO et du changement global en général, dépendent essentiellement de la question à laquelle on veut répondre et des différents paramètres précités.

Table 2 : Avantages et inconvénients des différentes méthodes utilisées dans l'étude de l'impact de l'acidification des océans en milieu benthique

Méthode	Avantages	Inconvénients
Observations *in situ*	• Cycle journalier et/ou saisonnier naturel • Interactions écosystèmiques • Interaction milieu benthique/pélagique • Adaptation des organismes aux conditions du milieu	• Pas de contrôle des paramètres physico-chimiques • Nombreux facteurs confondants (température, concentration en oligo-éléments et nutriments, pression hydrostatique,...) • Peu de réplicats → faible puissance statistique • Échange possible d'organismes avec l'environnement extérieur au site d'étude → résultats difficilement interprétables • Étude des interactions avec d'autres facteurs généralement impossible
Expérimentation en aquariums en laboratoire	• Contrôle fin des paramètres physico-chimiques • Pas de facteurs confondants • Possibilité de tester les interactions avec d'autres facteurs de manière contrôlée • Réplication importante → grande puissance statistique • Acclimatation si étude à long terme	• Généralement, cycle journalier et/ou saisonnier naturel (pH, intensité lumineuse, pression de prédation,...) pas ou peu reproduits • Pas ou peu d'interactions écosystèmiques • Pas d'interaction milieu benthique/pélagique → Non pertinence des résultats dans le milieu naturel
Mésocosmes	• Possibilité d'inclure des cycles journaliers/saisonniers • Interactions écosystèmiques • Si mésocosme ouvert, interaction milieu benthique/pélagique • Acclimatation possible si étude à long terme • Contrôle des paramètres physico-chimiques • Limitation des facteurs confondants • Possibilité de tester les interactions avec d'autres facteurs de manière contrôlée • Réplication possible → augmentation de la puissance statistique	• Prudence quant à l'interprétation en milieu naturel en fonction du degré de complexité de l'écosystème reproduit

Figure 7 : Mésocosme artificiel de récif tropical du laboratoire ECONUM à l'Université de Mons (photographie prise par Ph. Grosjean).

2. Les récifs coralliens tropicaux et le changement global

2.1. Biologie des récifs coralliens tropicaux

Les récifs coralliens tropicaux sont des écosystèmes marins peu profonds consistant en un récif composé de carbonate de calcium principalement secrété par les coraux Scléractiniaires hermatypiques. Ces coraux constructeurs de récifs vivent en colonies où chaque individu est nommé « polype ». Chaque polype abrite des algues dinoflagellés communément appelées zooxanthelles (*Symbiodinium sp.)* (Fig. 8).

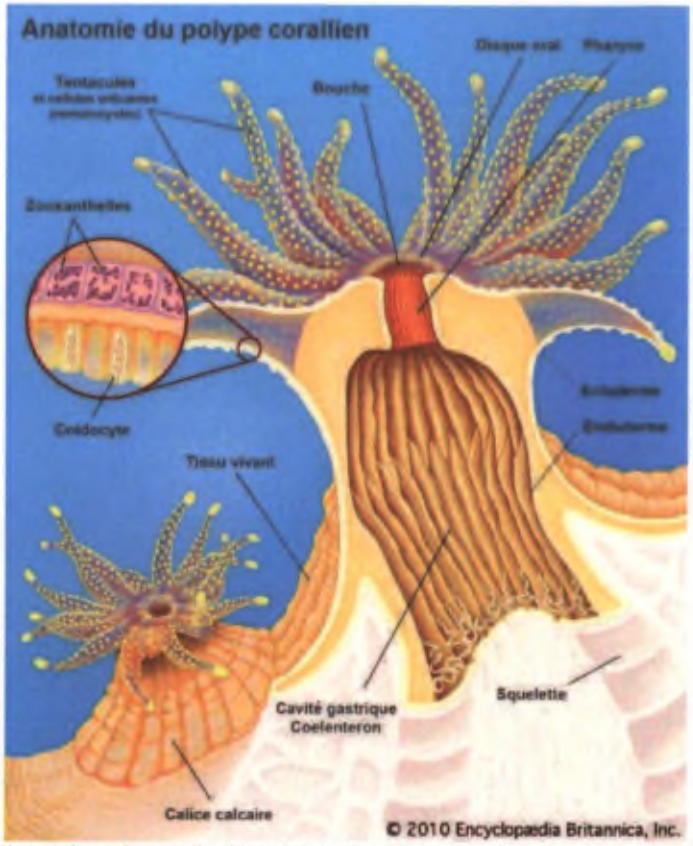

Figure 8 : Anatomie d'un polype de corail Scléractiniaire hermatypique (d'après Encyclopaedia Britannica, 2010)

Le squelette de corail Scléractiniaire hermatypiques est extracellulaire et situé du côté aboral du polype (Fig. 8). Les parois du polype sont constituées de deux couches cellulaires (épiderme et gastroderme) séparés par un réseau de collagène, la mésoglée. Le gastroderme délimite une cavité, le cœlentéron, avec une seule ouverture, la bouche, entourée de tentacules. Cette cavité sert en même temps de cavité gastrique et de système circulatoire entre les polypes, et est donc également appelée cavité gastro-vasculaire.

Les polypes sont reliés entre eux par le coenosarque, composé d'un tissu oral (face à l'eau de mer) et d'un tissu aboral (face au squelette). Chacun d'eux comporte une couche de cellules épidermiques et une couche de cellules gastrodermiques (Fig. 9). La plupart des zooxanthelles sont situés dans le gastroderme oral alors que l'épiderme oral est riche en cnidocytes (cellule

urticante propre au phylum des cnidaires). L'épiderme calicoblastique est responsable de la formation du squelette.

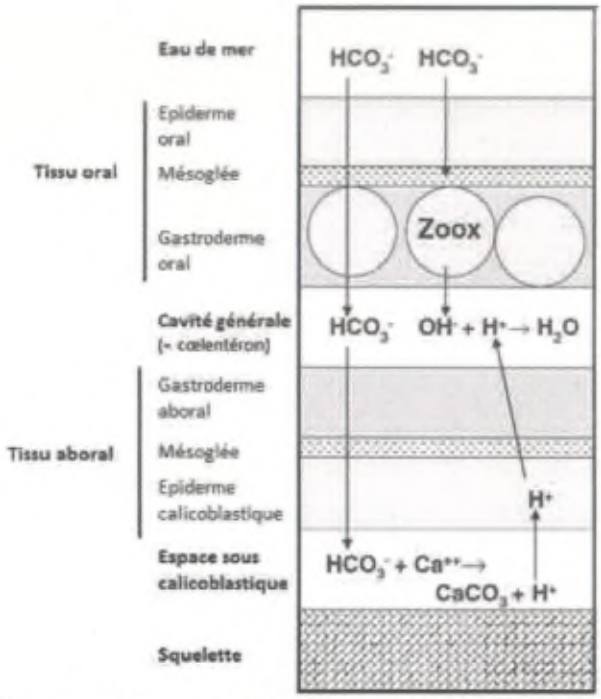

Figure 9 : Coupe transversale du coenosarque montrant l'organisation tissulaire et la relation entre la photosynthèse des zooxanthelles (=Zoox) et la calcification chez les coraux d'après Allemand *et al.* (2004) (modifié d'après Jokiel, 2011).

Les coraux et leurs zooxanthelles forment une symbiose mutualiste : les coraux reçoivent des photosynthétats (sucres et acides aminés) alors que leurs symbiontes, en plus d'un milieu protégé, bénéficient des nutriments provenant des déchets métaboliques de leur hôte (Trench, 1979). De plus, on observe un taux de calcification supérieur de jour chez les coraux (Kawaguti & Sakumoto, 1948). Ainsi la calcification est favorisée par la photosynthèse des zooxanthelles. A l'heure actuelle, le mécanisme expliquant cette observation est encore controversé. Les OH^- produits par la photosynthèse permettraient de tamponner les protons produits durant la calcification (Allemand *et al.*, 2004 ; Comeau *et al.*, 2013a) (Fig. 9). Cependant, chez certains coraux branchus, le taux de calcification est maximum au niveau des extrémités des branches de coraux (zone de calcification rapide) qui pourtant ne comportent pas de zooxanthelles (par exemple, *Pocillopora damicornis* dans Brown *et al.*, 1983 ; *Acropora cervicornis* dans Gladfelter, 1982). Récemment, Jokiel (2011) a proposé un autre modèle : il y aurait séparation entre les zones de photosynthèse rapide et les zones de calcification rapide. Cela réduirait la compétition pour HCO_3^- utilisé par ces 2 processus. Les protons produits par la calcification dans la zone de photosynthèse rapide seraient consommés par la photosynthèse. Le couplage zone de photosynthèse rapide/zone de calcification rapide se ferait par une translocation efficace des photosynthétats qui fournissent l'énergie nécessaire à l'efflux des protons dans les zones de calcification rapide.

2.2. Le changement global en milieu récifal tropical

Les récifs coralliens constituent un des écosystèmes les plus productifs et les plus diversifiés de la planète, abritant 25 % des espèces marines. Outre cet intérêt écologique, les récifs coralliens jouent un rôle social, économique et culturel majeur. Environ 275 millions d'individus vivent à moins de 30 km des récifs coralliens qui leur apportent de nombreux services (pêche, protection côtière, tourisme, produits pharmaceutiques, ...) (Burke *et al.*, 2011).

Les récifs coralliens sont des écosystèmes fragiles. La croissance des coraux est intimement liée aux conditions environnementales, dont la température, la lumière, la concentration en nutriment, la salinité, la turbidité et la force des vagues. Jusque dans les années 80, la plupart des perturbations anthropiques étaient principalement locales (développement côtier, eutrophisation, pollution, surpêche, pêche à la dynamite, ...). Au cours des 30 dernières années, des évènements massifs de blanchiment du corail, résultant de la perte des zooxanthelles, ont coïncidé avec une augmentation de la température (Kleypas *et al.*, 2006). L'observation de ces épisodes de blanchiment massif a permis d'estimer la limite de tolérance thermique des coraux, et donc, des récifs coralliens et de prédire notamment, dans de nombreuses régions, le blanchiment massif observé en 1998 (programme « Hotspot », Goreau & Hayes, 1994). Si on combine cela avec la prédiction de l'augmentation de la température des eaux de surfaces au cours des siècles à venir, ces évènements de blanchiment massif pourraient devenir de plus en plus fréquents (Hoegh-Guldberg, 1999).

Parallèlement à l'augmentation de la température, l'AO menace également les récifs coralliens. En effet, de par la diminution de l'état de saturation de l'aragonite dans l'eau de mer ($\Omega_{aragonite}$), la calcification des coraux pourrait être réduite. Plusieurs études ont montré une diminution linéaire du taux de calcification liée à une diminution de $\Omega_{aragonite}$ (Fig. 10, Kleypas *et al.*, 2006 ; Langdon & Atkinson, 2005 ; Orr *et al.*, 2005 ; Schneider & Erez, 2006).

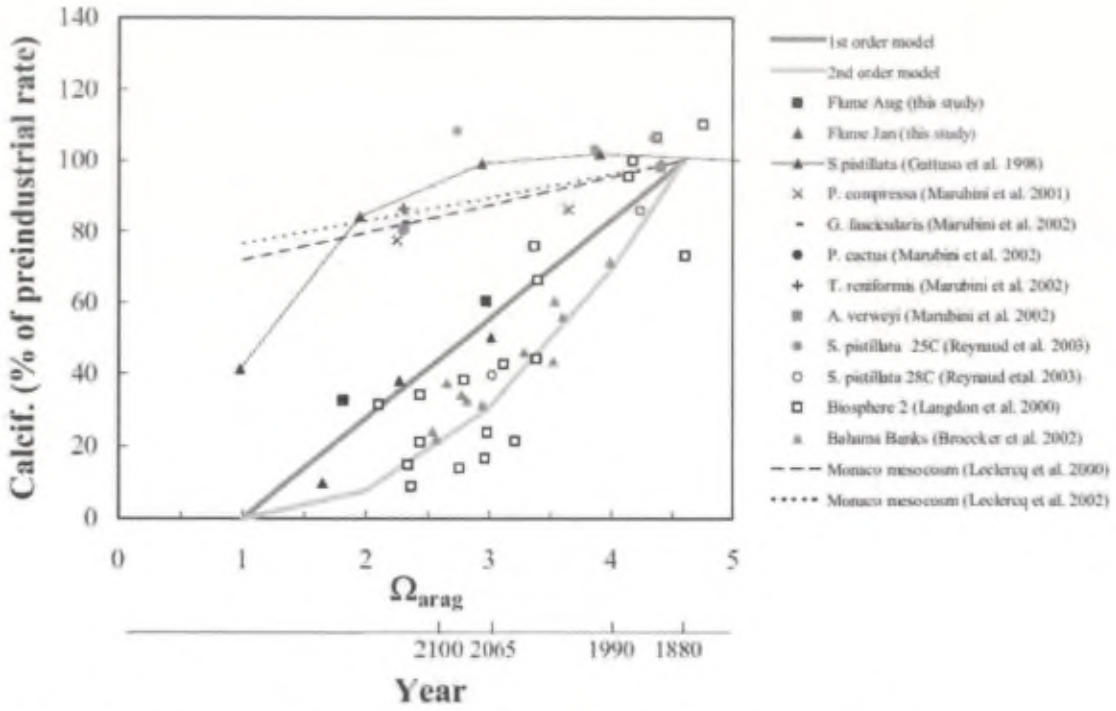

Figure 10 : Effet de la diminution du taux de saturation de l'eau mer vis-à-vis de l'aragonite (Ω_{arag}) sur la calcification des coraux. Les modèles présentés (courbes pleines grises et noires) ont été calculés sur l'ensemble des données (d'après Langdon & Atkinson 2005).

La diminution du taux de calcification ne serait cependant pas causée directement par une diminution de la concentration en CO_3^{2-} dans l'eau de mer puisque la calcification utilise les ions HCO_3^- (Allemand *et al.*, 2004 ; Comeau *et al.*, 2013a ; Jokiel, 2011). De plus, les coraux forment leur squelette en produisant des cristaux d'aragonite dans l'espace sous-calicoblastique dans lequel la composition ionique et le pH sont contrôlés biologiquement par l'épiderme calicoblastique (Fig. 9, Allemand *et al.*, 2004). Ce milieu et le squelette sont séparés de l'eau de mer par des cellules (Fig. 9). La diminution du taux de calcification irait de pair avec une diminution du ratio $[CID]/[H^+]$, celui-ci étant fortement corrélé à Ω (Jokiel, 2013). La diminution de la calcification chez les coraux soumis à l'AO serait en fait le résultat d'une augmentation des coûts métaboliques associés à l'efflux des protons. En effet, le maintien du pH dans l'espace sous-calicoblastique serait d'autant plus couteux à mesure que le pH de l'eau de mer diminue, réduisant dès lors l'énergie disponible pour la calcification (Holcomb *et al.*, 2014).

Cependant, il faut prendre en compte l'acclimatation à long terme et la plasticité phénotypique des coraux (Edmunds & Gates, 2008 ; Gates & Edmunds, 1999). Par exemple, le blanchiment du corail pourrait être un mécanisme adaptatif. Après quelques semaines ou quelques mois, la symbiose serait rétablie entre le corail blanchi et un clade différent de zooxanthelles plus résistant aux changements de l'environnement (Buddemeier *et al.*, 2004). D'autres études ont mis en évidence une réponse spécifique chez les coraux puisque chez certaines espèces, la calcification

n'est pas affectée par une élévation de la pCO_2 (par exemple, *Pocillopora damicornis* dans Comeau *et al.*, 2013c ; *Porites spp.* dans Edmunds 2011 ; *Stylophora pistillata* dans Reynaud *et al.*, 2003 ; Venn *et al.*, 2012 ; *Cladocora caespitosa* dans Rodolfo-Metalpa *et al.*, 2009). A l'échelle du récif, la calcification globale pourrait donc être moins affectée que précédemment prédit et résulter plutôt en un changement des espèces dominantes. Par exemple, Fabricius *et al.* (2011) ont montré, à proximité d'évents volcaniques, qu'une réduction du pH allant jusqu'à 7,7 entraînait une perte de diversité mais pas de la couverture corallienne. Les colonies massives de *Porites* devenaient alors dominantes par rapport aux autres espèces.

Cependant, les effets indirects sont également à prendre en compte. Dans les récifs, il y a une compétition perpétuelle pour la lumière et l'espace entre les coraux et les algues (McCook *et al.*, 2001). Hoegh-Guldberg *et al.* (2007) ont simulé une réduction de croissance de 20 % chez des coraux du genre *Porites* sur une durée de 50 ans en prenant en compte différents taux de broutage des algues benthiques. Ils ont ainsi montré que les récifs coralliens ayant un faible taux de calcification ont une plus grande tendance à basculer vers un écosystème dominé par les algues et cela d'autant plus si le taux de broutage est faible (Fig. 11). Ainsi, une augmentation de la compétitivité des algues face aux coraux et/ou une réduction du taux de broutage des algues peuvent influencer la résistance du récif face aux changements climatiques globaux. Ce risque est d'autant plus grand que les macroalgues non calcifiantes sont favorisées par l'augmentation de la pCO_2 (Kock *et al.*, 2013 ; Porzio *et al.*, 2011).

Enfin, le changement globaux pourrait également affecter la reproduction, le développement larvaire et le recrutement des coraux (Albright & Langdon, 2011 ; Edmunds, 2007 ; Kroeker *et al.*, 2010 ; Nakamura *et al.*, 2011 ; Suwa *et al.*, 2010).

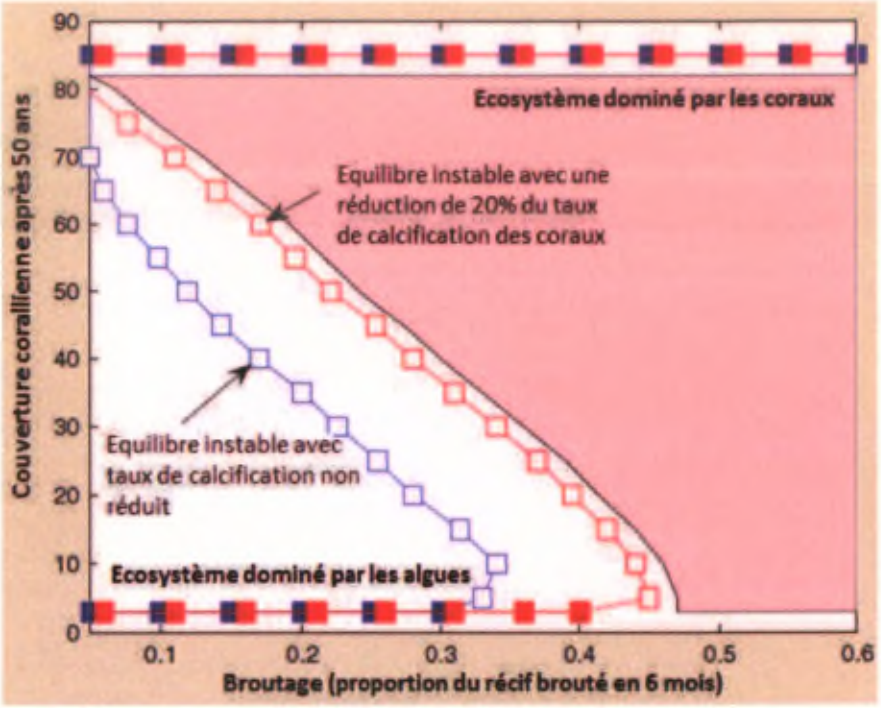

Figure 11 : Réduction de la résilience d'un récif attribuée à une diminution du taux de croissance des coraux. Les courbes représentent l'équilibre entre un écosystème dominé par les algues (zone blanche) et un écosystème dominé par les coraux (zone rose) pour un taux de calcification non affecté (courbe bleu) et réduit de 20 % (courbe rouge). On observe que la zone rose est réduite lorsque le taux de calcification des coraux est diminué de 20 %. Pour maintenir une même couverture corallienne dans ces conditions, le taux de broutage doit augmenter de 25 % (modifié d'après Hoegh-Guldberg *et al.*, 2007).

Les algues calcifiées participent également à la production de $CaCO_3$ dans les récifs coralliens (Goreau, 1963). Le terme « algues calcifiées » reprend à la fois des algues de la classe Rhodophyta (dont les algues corallines) et de la classe Chlorophyta (par exemple *Halimeda*). De manière générale, l'AO défavorise les algues calcifiées puisqu'on observe chez celles-ci une diminution du taux de photosynthèse, du taux de calcification et de leur abondance lorsque le pH diminue (Kroeker *et al.*, 2013). Parmi les algues corallines, on retrouve des espèces encroûtantes qui ont un rôle important dans les récifs coralliens puisqu'elles servent de ciment et consolident la structure du récif. De plus, elles libèrent des composés chimiques dans la colonne d'eau qui facilitent le recrutement du corail (Doropoulos *et al.*, 2012 ; Heyward & Negri, 1999 ; Webster *et al.*, 2013). Celles-ci sont sensibles à l'AO (Johnson & Carpenter, 2012 ; Kuffner *et al.*, 2008). L'interaction algue coralline-corail semble donc également menacée.

3. La (bio)érosion en milieu récifal tropical

Le taux de calcification nette des récifs coralliens dépend à la fois de la formation du $CaCO_3$ (la calcification des coraux et algues calcifiées) mais également de la perte de $CaCO_3$ (érosion). L'érosion et la dégradation des récifs coralliens sont régies par des procédés interdépendants physiques, biologiques et chimiques (Andersson & Gledhill, 2013).

Les courants et vagues érodent constamment et déterminent la structure des récifs sur de longues périodes. En revanche, les grandes vagues et tempêtes majeures peuvent rapidement causer de graves dommages structurels aux récifs coralliens et l'exportation de grandes quantités de $CaCO_3$ en eaux plus profondes. La dégradation physique des récifs coralliens pourrait augmenter à cause des changements globaux car les tempêtes et ouragans pourraient être plus fréquents et plus intenses au cours des 2 prochains siècles (IPCC, 2007).

La dissolution chimique peut être divisée en 2 catégories : la dissolution environnementale, se produisant lorsque $\Omega < 1$ (section 1.2.) et la dissolution métabolique. Cette dernière se produit dans les microenvironnements du récif où la respiration de la communauté microbienne, notamment, entraîne une sous-saturation vis-à-vis du $CaCO_3$ et sa dissolution. L'augmentation de la dissolution environnementale est peu probable au cours des 2 prochains siècles puisque, d'après les prédictions, Ω devrait rester supérieur à 1 dans les eaux de surfaces tropicales (section 1.3). La dissolution métabolique pourrait par contre augmenter si le métabolisme des organismes responsables de celle-ci augmente bien que cette hypothèse doive encore être vérifiée (Andersson & Gledhill, 2013).

De nombreux organismes récifaux contribuent à la dégradation du récif. On s'y réfère comme l'érosion biologique ou bioérosion. Celle-ci se fait soit par le biais d'abrasion mécanique, soit par pénétration active du substrat via la libération de CO_2 ou d'autres acides métaboliques qui dissolvent le $CaCO_3$. La bioérosion est généralement l'œuvre des brouteurs (poissons, oursins, mollusques et crustacés), des macroorganismes endolithes (macrobioérosion - polychète, siponcles, mollusques bivalves et éponges perforantes) et des microorganismes endolithes (microbioérosion - principalement les cyanobactéries et chlorophytes) (Fig. 12, pour une synthèse : Glynn, 1997; Hutchings, 1986 ; Tribollet & Golubic, 2005).

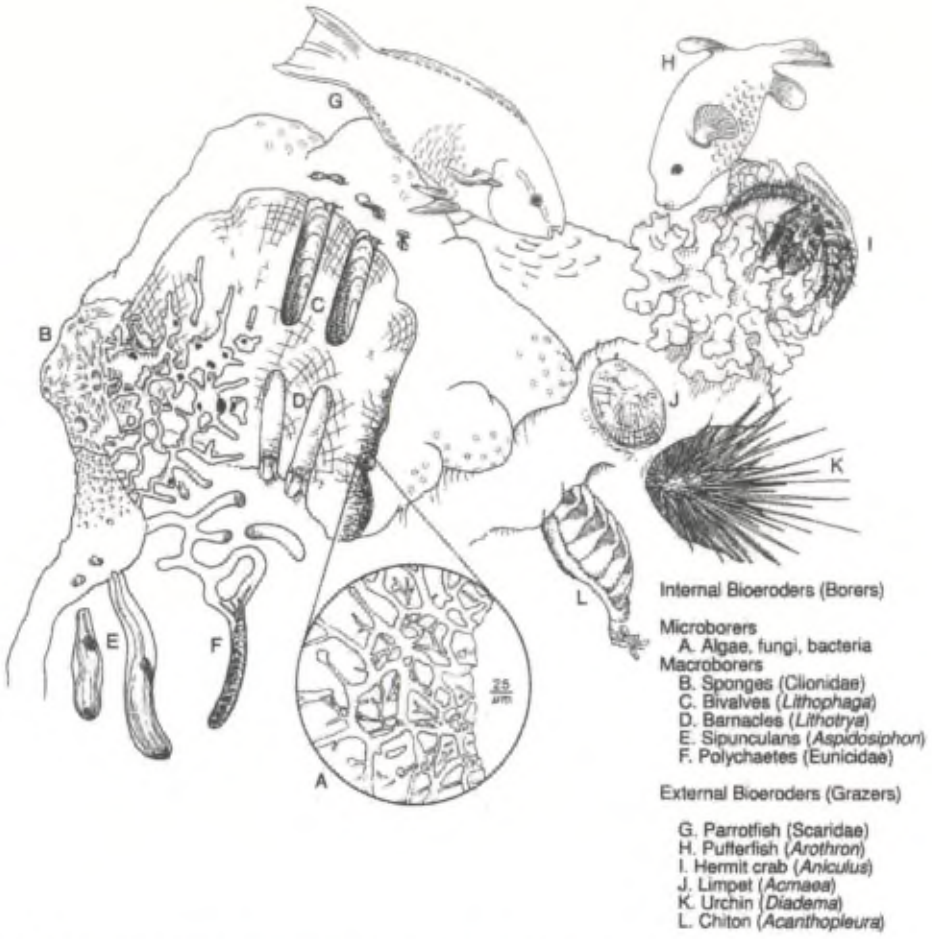

Figure 12 : les différents organismes responsables de la bioérosion des récifs coralliens (d'après Glynn 1997).

La dominance et le taux de bioérosion de chacun des différents groupes bioérodeurs varient en fonction du récif considéré (Table 3).

Table 3 : Exemples de taux de microbioérosion, macrobioérosion et bioérosion effectués par les brouteurs (kg $CaCO_3$ m^{-2} an^{-1}) dans différents récifs coralliens. Dans toutes ces études, les oursins et poissons étaient les brouteurs principaux.

Location	Microbioérosion	Macrobioérosion	Brouteurs	Source
Polynésie française	0,2-0,6	<0,1	0,4-2,3	Chazottes *et al.*, 1995
Galapagos	2,6*		22,8	Reaka-Kudla *et al.*, 1996
Grande Barrière de corail	0,2-1,3	0,1-1,2	0,2-5,4	Tribollet & Golubic, 2005
Ile de la Réunion	<0,1	<0,1	1,5-4,3	Chazottes *et al.*, 2002

* dans cette étude, la micro- et la macrobioérosion n'ont pas été différenciées

L'existence de chaque récif corallien dépend donc du taux de calcification qui doit rester supérieur au taux d'érosion. Le taux de calcification net des récifs varie en fonction des espèces dominantes qui le forment, de la localisation du récif, de la saison et donc des conditions environnementales : température, concentration en nutriments, lumière, régime hydrographique, chimie de l'eau de mer, ... (Buddemeier & Kinzie, 1976, voir exemple Fig. 13).

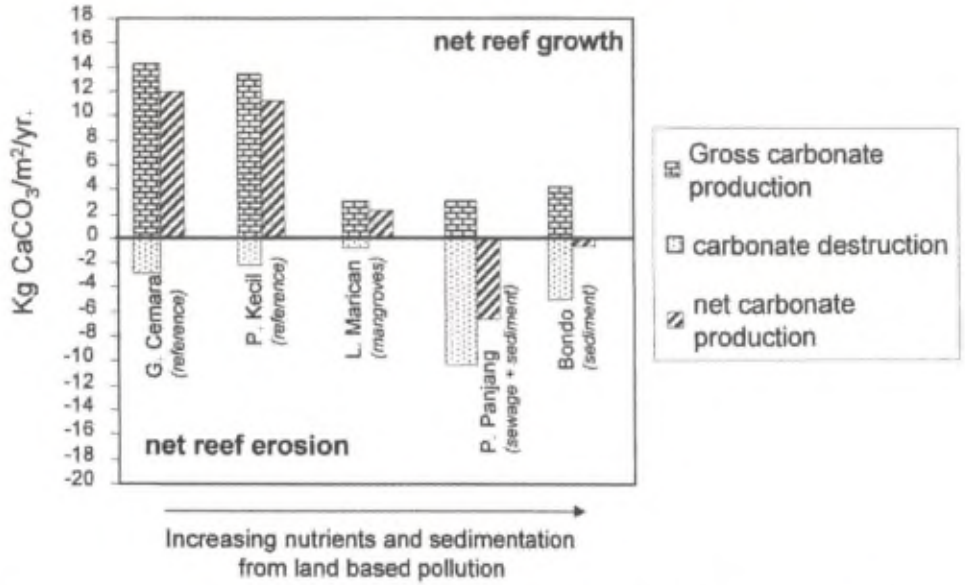

Figure 13 : Calcification brute (symboles en briques), érosion (symboles pointillés) et calcification nette (symboles hachurés) de carbonate de calcium (en kg m^{-2} an^{-1}) dans différents récifs situés dans la mer de Java. Ces récifs diffèrent entre eux notamment par l'apport de nutriments et de sédiment provenant des terres (augmentation de ceux-ci en allant vers la droite dans le graphique) (d'après Edinger *et al.*, 2000).

Ainsi, une réduction modérée du taux de calcification et/ou une augmentation du taux de bioérosion peut faire basculer l'écosystème d'un état de production nette vers un état d'érosion du récif et à sa destruction (Andersson *et al.*, 2005).

Jusqu'à présent, peu d'études ont examiné les effets indirects de l'AO sur la résilience des récifs coralliens due à une augmentation de la bioérosion (Andersson & Gledhill 2013). Andersson *et al.* (2009) ont mesuré le taux de calcification net de communautés coralliennes subtropicales incubées dans des mésocosmes à flux continu. Le taux de calcification net à pH 8,0 était d'environ +2,3 kg CaCO3 m^{-2} an^{-1} alors qu'à pH 7,76, il devenait négatif (-0,3 kg CaCO3 m^{-2} an^{-1}). Cette diminution a été attribuée à une réduction de 24 % du taux de calcification et à une augmentation de 138 % de l'érosion. Cette dernière était causée par la dissolution métabolique et la bioérosion. Similairement, la microbioérosion par l'algue endolithe *Ostreobium* augmente parallèlement à l'augmentation de la pCO_2 de l'eau de mer (Reyes-Nivia *et al.*, 2013 ; Tribollet *et al.*, 2009). Ces résultats sont cohérents avec des études menées sur l'éponge perforante *Cliona orientalis* qui montrent également une capacité bioérosive plus importante dés pH 7,9 et en dessous (Fang *et*

al., 2013, 2014 ; Wisshak *et al.*, 2012, 2013 , 2014). Cependant, jusqu'à maintenant, aucune information n'est disponible quant à l'impact de l'AO sur d'autres bioérodeurs des récifs, comme les poissons et les échinides qui peuvent éroder respectivement jusqu'à 9,1 kg (Kiene, 1988) et 22,3 kg $CaCO_3$ m^{-2} an^{-1} (Glynn, 1988).

De plus, l'érosion biologique et l'érosion chimique facilitent les dégradations physiques. Inversement, ces dernières entraînent un fractionnement du substrat en particules de plus petites tailles qui favorisent la dissolution chimique et biologique (augmentation du rapport surface/volume). Ainsi une augmentation de la dissolution et/ou de la bioerosion du récif favorisa une balance accrétion-érosion négative du $CaCO_3$ et rendant les récifs plus fragiles face aux détériorations physiques et inversement.

4. Echinometra mathaei

4.1. Taxonomie

Echinometra mathaei (Blainville 1825) est un oursin vivant en eau généralement peu profonde en milieu récifal. Cette espèce est largement distribuée dans la zone intertropicale Indopacifique. Initialement, elle a été décrite comme une seule espèce montrant une grande variété de couleurs et de formes du test (Mortensen 1943, Clark & Rowe 1971). Des études génétiques, morphologiques, biochimiques, écologiques et reproductives ont montré qu'il y aurait 4 espèces sympatriques d'*Echinometra* dans l'océan Indopacifique auxquelles on se réfère comme *Echinometra* espèce A, B, C et D (Fig. 14, Arakaki *et al.*, 1998). La spéciation de ces espèces serait récente, moins de 3 millions d'années (Palumbi, 1996). L'espèce B est reconnue comme *Echinometra mathaei* (Arakaki *et al.*, 1998) alors que l'espèce D correspond à *Echinometra oblonga* (Blainville 1825). Les espèces A et C n'ont pas été décrites et dénommées différemment jusqu'à présent. On continue dès lors à s'y référer comme *Echinometra mathaei sp. A* et *Echinometra mathaei sp. C* (Arakaki *et al.*, 1998). A l'ile Maurice, on retrouve 3 types d'*Echinometra* : des oursins de couleurs diverses (majoritairement verts) dénommés « B-like », des oursins de couleur violette (couleur absente à Okinawa) et des oursins de couleur noire (Fig. 14). Les 2 premiers types correspondraient à l'espèce B et donc à *Echinometra mathaei*. En effet, les gamètes de ces 2 types et de l'espèce B d'Okinawa sont hautement compatibles (taux de fécondation > 98 %) (Arakaki *et al.*, 1998).

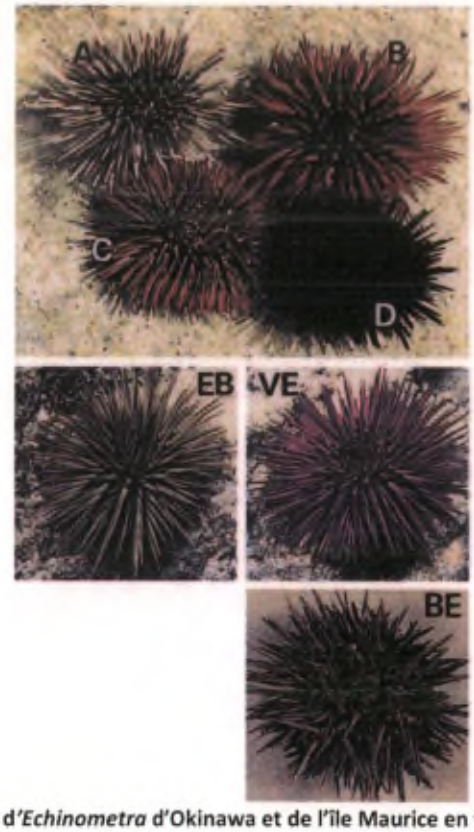

Figure 14: Les différentes espèces d'*Echinometra* d'Okinawa et de l'île Maurice en vue aborale. Les lettres A, B, C et D représentent respectivement Echinometra sp. A, B, C et D à Okinawa. Les lettres EB, VE et BE représentent respectivement Echinometra « B-like », violet et noir à l'île Maurice (d'après Arakaki *et al.*, 1998).

4.2. Morphologie - Anatomie

Echinometra mathaei, comme tous les échinides, possèdent un squelette de calcite magnésienne. Ce dernier est composé du test, de l'appareil masticateur (appelé lanterne d'Aristote) et d'appendices externes (les piquants et pédicellaires) (Fig. 15).

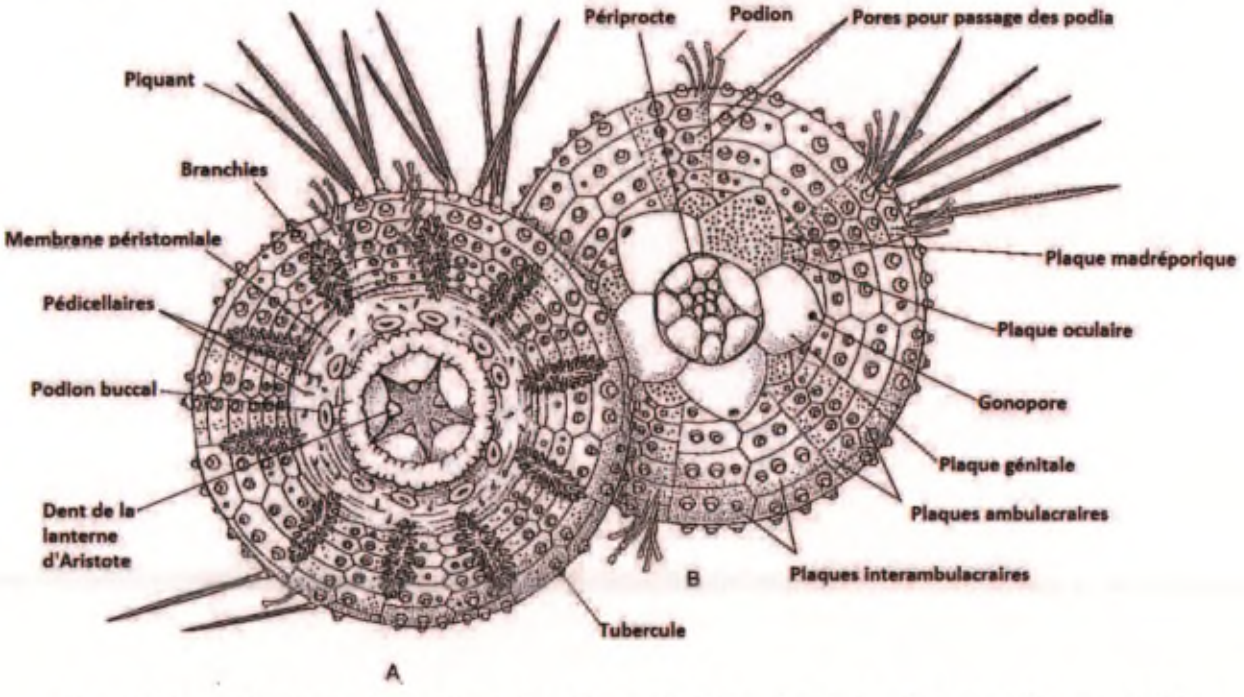

Figure 15: Anatomie externe d'un oursin régulier. A, face orale. B, face aborale (d'après Rupperts & Barnes, 1994).

Seul le test et la lanterne d'Aristote (principalement la dent) seront décrits ici puisqu'ils ont fait l'objet d'études dans le présent travail.

Le test peut être comparé à une sphère aplatie selon un axe oral-aboral. Il est divisé en 10 régions méridiennes, chacune formée d'une double série de plaques : 5 régions ambulacraires alternent avec 5 régions interambulacraires (Fig. 15). Seules les plaques ambulacraires sont percées de pores par où passent les podia. Les plaques sont assemblées entre elles via des fibres musculaires et conjonctives. L'anus se trouve au sommet de la face aborale (périprocte). La bouche, située au centre du péristome, fait face au substrat. Le test, d'origine mésodermique, est situé dans le derme (endosquelette) et est recouvert d'un épiderme (qui recouvre également les piquants). Un épithélium cilié, appelé épithélium péritonéal, tapisse la face interne du test et délimite la cavité générale (Fig. 16).

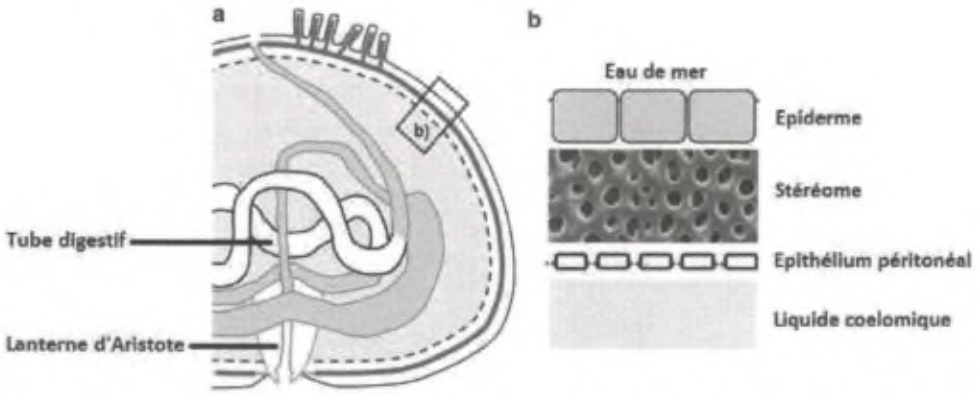

Figure 16 : Anatomie générale simplifiée d'un oursin régulier (a) et détail histologique de la paroi du corps (b) (modifié d'après Holtmann *et al.*, 2013).

On retrouve différents systèmes chez les échinides comme le système hémal (système circulatoire rudimentaire), le système nerveux, le système aquifère, le système digestif et le système reproducteur (les gonades) (Figs. 16 et 17). La cavité générale est remplie du liquide cœlomique (LC). Le LC joue un rôle important dans la distribution des nutriments, dans l'excrétion de déchets métaboliques solubles et dans le transport des gaz (Ferguson, 1982). La ciliature de l'épithélium péritonéal assure la circulation constante du LC. La composition inorganique du celui-ci est proche de celle de l'eau de mer environnante (Farmanfarmaian, 1966). Le pH du liquide cœlomique des oursins est naturellement plus bas de 0,5 à 1,5 unité que celui de l'eau de mer et varie entre 6,5 et 7.5 en fonction de l'espèce considérée (Calosi *et al.*, 2013 ; Cole, 1940 ; Collard *et al.*, 2013 ; Farmanfarmaian, 1966). Ainsi, l'élimination du CO_2 dépend du gradient de diffusion existant entre le liquide cœlomique et l'eau de mer (Farmanfarmaian, 1966). Le LC assure dès lors le principal moyen d'échange gazeux avec le système aquifère. Il contient également des composés organiques (acides aminés, sucres réduits, protéines, lipides et déchets azotés)

(Ferguson, 1982). On y trouve également des cellules circulantes, les cœlomocytes, qui sont notamment impliquées dans la réponse immunitaire et la coagulation en cas de lésion (Endean, 1966).

Le système aquifère est un réseau de canaux rempli d'un liquide d'une composition très similaire à l'eau de mer. Il s'ouvre vers l'extérieur par la plaque madréporique et se prolonge jusqu'aux podia. Les podia permettent de se fixer au substrat mais sont également impliqués dans la fonction respiratoire (De Ridder & Lawrence, 1982). En effet, des échanges gazeux entre les podia et l'eau de mer s'effectuent au niveau de la paroi des podia. Ainsi, les podia servent à la fois à la fixation au substrat, au déplacement, à la manipulation de la nourriture et aux échanges gazeux avec le milieu extérieur (Johansen & Vadas, 1967).

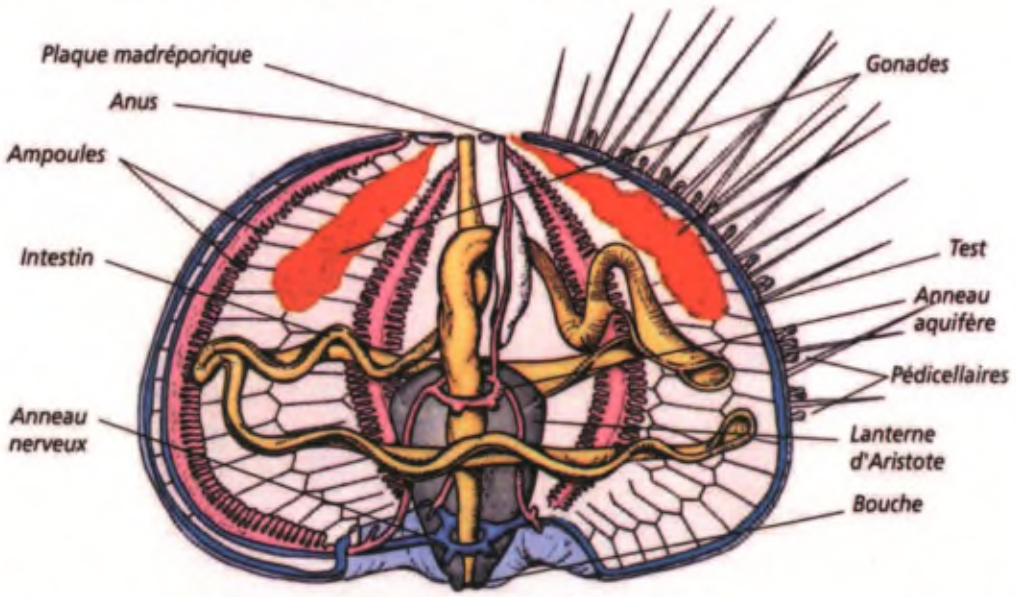

Figure 17 : Anatomie interne d'un oursin régulier montrant le système aquifère (en rose), le système reproducteur (les gonades, en orange), le système digestif (en jaune), le système nerveux (en bleu) (d'après http://pageconcept.org/invertebres/echinos/oursins/oursin.jpg).

Chaque plaque du test consiste en un réseau tridimensionnel de trabécules, le stéréome, qui délimite un réseau interne et complémentaire de tissu conjonctif, le stroma. (Fig. 16). Le stroma est constitué d'une matrice extracellulaire, de fibres, et de différents types de cellules (cellules immunitaires, sclérocytes et fibroblastes) (Dubois & Chen, 1989). Le stéréome est composé en majeure partie de calcite magnésienne (99.9 % du poids sec, la phase minérale) associée à des protéines, pigments et carbohydrates (la phase organique) (Swift *et al.*, 1986 ; Weiner, 1985).

Le squelette des individus post-métamorphiques est formé par les sclérocytes localisés dans le stroma (Fig. 18). Ces cellules développent des processus cellulaires qui fusionnent en un syncytium au sein duquel est formée une vacuole, qui constitue le site de calcification (Ameye *et al.*, 1998 ;

Märkel, 1990 ; Märkel *et al.*, 1986). L'extension du site de calcification réduit progressivement l'épaisseur des processus cellulaires qui l'entourent entraînant la rupture du manchon cytoplasmique et résultant en la formation de processus distaux qui entourent l'ossicule alors extracellulaire (Dubois & Chen, 1989). Les sclérocytes impliqués dans la croissance préservent localement de tels processus sur les trabécules les plus extérieures de l'ossicule, permettant ainsi une éventuelle reprise de sa croissance (Märkel *et al.*, 1986).

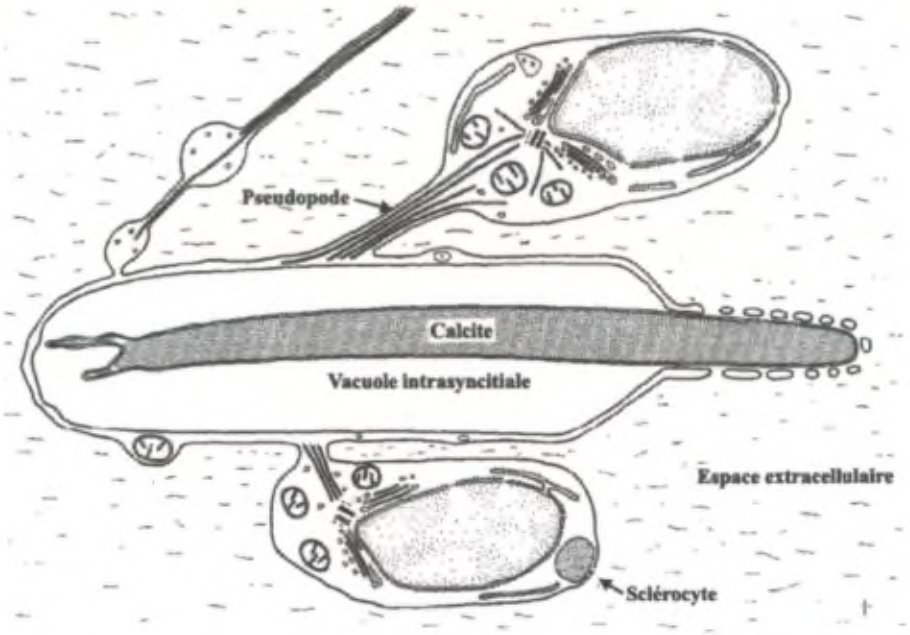

Figure 18: Ossicule d'échinides en cours de formation et complètement formé (respectivement à gauche et à droite dans schéma) (d'après Märkel *et al.*, 1986).

Une fois formé, le squelette grandit par croissance périphérique des ossicules préexistants (en latitude et en longitude), mais aussi par addition de nouveaux ossicules du côté adoral au niveau du système apical (Fig. 19).

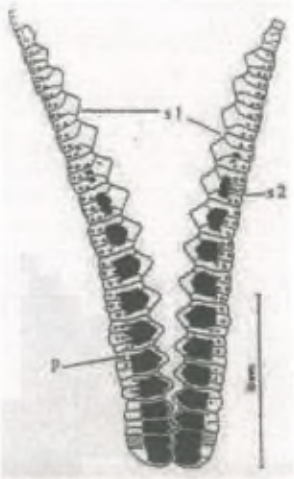

Figure 19: Croissance latitudinale et longitudinale des plaques ambulacraires. Les plaques apicales sont situées en haut dans la figure. Les zones noires correspondent à la taille des plaques avant croissance (marquage par la tétracycline) alors que les zones blanches correspondent au squelette nouvellement formé (après marquage par la tétracycline et croissance en aquarium). p : pore ambulacraire, s1 : suture entre plaques ambulacraires, s2 : suture entre plaques ambulacraires et plaques interambulacraires (d'après Märkel, 1981).

La lanterne d'Aristote est centrée au niveau du péristome dans la cavité péripharyngienne qui est isolée de la cavité générale par une fine membrane (Figs. 15, 16 et 17). Elle est constituée par 5 ensembles complexes, les mâchoires, portant chacune une dent (Fig. 20). Chaque mâchoire est constituée de deux hémi-pyramides. Les mâchoires sont liées entre elles et rattachées au test par des ligaments et des muscles. Le jeu de ces muscles assure les mouvements des mâchoires et de l'ensemble de la lanterne. Les dents font saillie à l'extérieur au niveau de la membrane péristomiale.

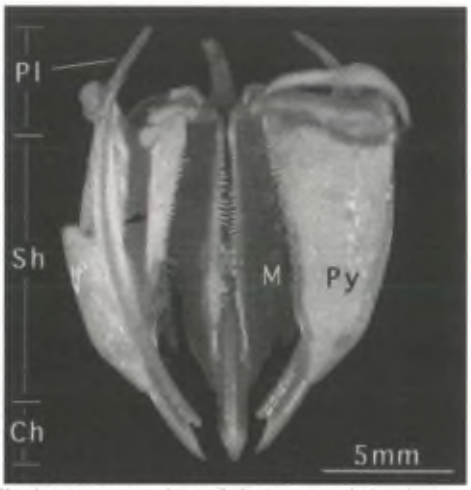

Figure 20: Photographie d'une lanterne d'Aristote. Deux des mâchoires ont été enlevées afin de rendre les dents visibles (flèche). Ch : partie de la dent qui sert à racler le substrat, Sh : hampe de la dent, Pl : plumula de la dent, Py : machoire, M : muscle (d'après Wang *et al.*, 1997).

Chaque dent peut être divisée en trois parties le long de son axe: la partie servant à racler qui fait saillie vers le substrat, la hampe, et la zone aborale faiblement calcifiée en croissance appelée plumula (Fig. 20). La plupart des oursins se nourrissent en raclant les algues à la surface du substrat dur. Les dents se déplacent simultanément de manière centripète, et la pointe de mastication de chaque dent est usée en biseau en permanence. La dent est continuellement renouvelée par la croissance au niveau de la plumula. La formation de la dent se fait de manière analogue aux plaques du test mais les cellules formatrices sont appelées odontoblastes. La partie mature de la dent se compose de trois zones principales avec des structures et des fonctions différentes (Fig. 21): la zone de plaques primaires, la carène et la partie dure (« Stone part » en anglais). Cette dernière est la zone la plus dure, qui érode le substrat (Fig. 22 ; Märkel & Gorny, 1973 ; Wang *et al.*, 1997).

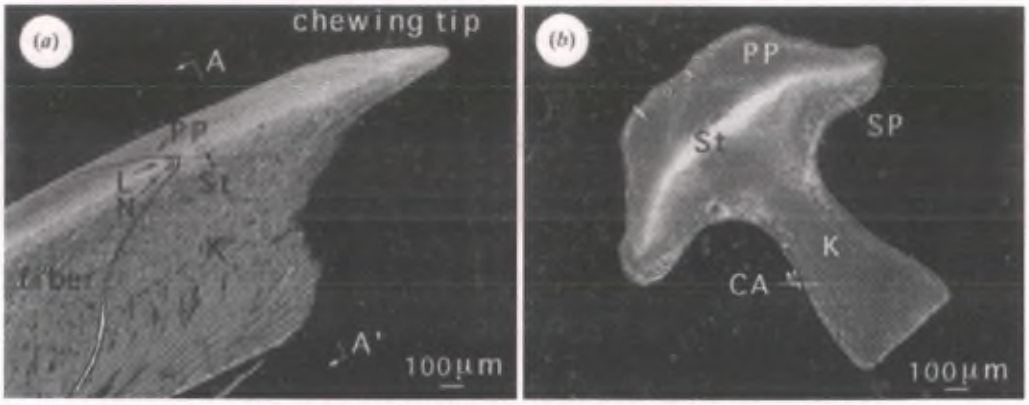

Figure 21: section longitudinale (a) et transversale (b) de la partie mature de la dent d'un oursin. PP : zone de plaques primaires, St : partie dure, K : carène, SP : zone de plaques secondaires, CA : appendices en forme de carène des plaques secondaires, L : lamelle, N : aiguilles (d'après Wang *et al.*, 1997).

Figure 22: dureté de la dent (microdureté de Vickers en kg mm^{-2}) dans les différentes zones d'une section transversale de la dent (d'après Wang *et al.*, 1997).

La zone de plaques primaires a une structure stratifiée, qui peut résister à l'effort de pression subi au cours du raclage du substrat (Fig. 23b ; Märkel *et al.*, 1973). La carène et la partie dure ont été décrites comme un matériau composite de disques calcaires qui renforcent un réseau parallèle de fibres (Fig. 23a). Ce type de structure a une haute résistance à la force de traction (Brear & Currey, 1976 ; Märkel *et al.*, 1977). Les dents sont également composées de calcite magnésienne et d'environ 0,2 ± 0,25 % en poids sec de macromolécules organiques, dont la plupart sont des protéines.

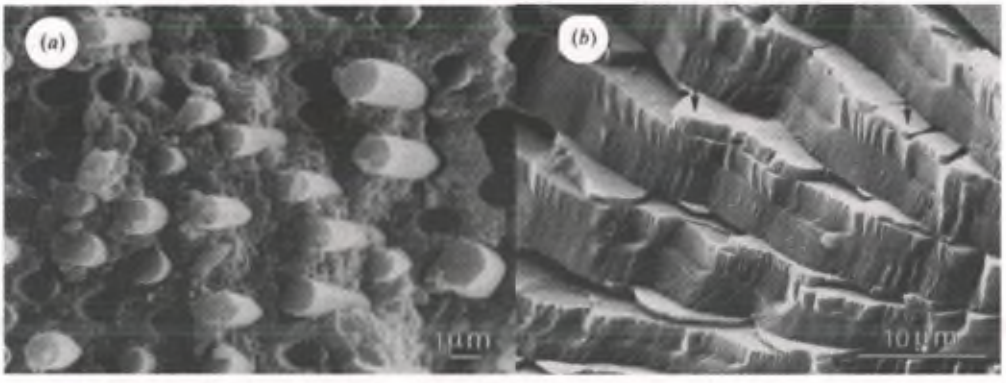

Figure 23: fracture dans la partie dure représentant les fibres entourées de disques calcaires (a) et dans la zone de plaques primaires (b) (d'après Wang *et al.*, 1997).

La teneur en magnésium de la calcite magnésienne chez les oursins dépend de divers facteurs dont des facteurs génétiques et environnementaux (Andersson *et al.*, 2008). En effet, la teneur en magnésium du squelette varie entre différentes espèces d'échinides vivant dans la même localité et serait donc contrôlée par des facteurs génétiques. La concentration en magnésium varie également au sein même de l'individu en fonction l'élément squelettique considéré. Elle est située généralement entre 5 et 15 % mais peut atteindre 43.5 % au niveau de la partie dure de la dent (Märkel *et al.*, 1977 ; Schroeder *et al.*, 1969 ; Weber, 1969, 1973). Plusieurs facteurs environnementaux influencent la concentration en Mg du squelette. En effet, les espèces vivant dans des eaux plus chaudes montrent une plus grande concentration en magnésium dans leur squelette que ceux vivant en eaux froides (Chave, 1954 ; Clarke *et al.*, 1922 ; Hermans *et al.*, 2010 ; Mackenzie *et al.*, 1983 ; Ries, 2004, 2009 ; Weber, 1973). La salinité et le rapport Mg/Ca de l'eau de mer affecteraient également le rapport Mg/Ca du squelette d'oursins adultes (Hermans *et al.*, 2011 ; Ries 2004, 2009), respectivement au travers de la régulation sélective du calcium dans les fluides extracellulaires et la diffusion des ions à travers les structures biologiques (Hermans *et al.*, 2011). L'état de saturation de l'eau de mer par rapport à la calcite magnésienne n'a pas d'effet sur la concentration en magnésium incorporée dans le squelette d'*Eucidaris tribuloides* (Ries, 2011).

4.3. Physiologie et écologie en milieu récifal

E. mathaei se nourrit principalement d'algues épibenthiques et dérivantes (De Ridder & Lawrence, 1982). Son régime alimentaire dépend essentiellement de conditions environnementales comme la pression de prédation et l'hydrodynamisme. En effet, *E. mathaei* peut creuser des logettes dans le substrat calcaire pour échapper à ses prédateurs ou lorsque l'hydrodynamisme est trop important. Dans ces conditions, il se nourrit principalement d'algues dérivantes (Johansson *et al.*, 2010 ; McClanahan & Muthiga, 1988). Sa croissance, comme la plupart des oursins, est asymptotique se rapprochant de zéro pour un diamètre d'environ 40 mm, qui correspondrait à un âge de 5 à 7 ans (Ebert *et al.*, 2008).

Il a de faibles besoins énergétiques et, comme tous les échinides, son métabolisme est faible. McClanahan & Kurtis (1991) ont mesuré un taux de respiration d'environ 0,9 µmol O2 g^{-1} poids frais h^{-1} chez des individus nourris à satiété. Lorsque ceux-ci étaient à jeun pendant 30 jours, le taux de respiration diminuait jusqu'à 0,3 µmol O2 g^{-1} poids frais h^{-1}. Comparativement, les poissons de récifs ont un taux de respiration trois à six fois supérieur (McClanahan, 1992). *E. mathaei* peut survivre jusqu'à 170 jours sans nourriture (Lawrence & Bazhin, 1998). McClanahan & Kurtis (1991) ont manipulé expérimentalement la densité d'*E. mathaei* en milieu naturel. Dans les

récifs où la densité de population était importante, les individus montraient une réduction de la croissance, du contenu digestif, du métabolisme et de la taille des gonades alors que le taux de mortalité n'était pas modifié. Par contre, d'avantage d'énergie était allouée à la croissance de la lanterne d'Aristote puisqu'une mâchoire plus large (et donc une pression de broutage supérieure) permet de compenser la réduction de la disponibilité en nourriture (Black *et al.*, 1982, 1984 ; Ebert, 1980). Les auteurs ont également testé les effets d'une absence de nourriture associée à une faible densité de population en aquariums et ont observé une même réponse des individus, concluant que la réponse observé en milieu naturelle était due à une diminution de la disponibilité en nourriture et non à des comportements agonistiques. Ainsi, chez E. mathaei, la régulation de population se fait au niveau de l'organisme, permettant de fortes densités de population en diminuant l'énergie disponible pour tous les individus. Ainsi, dans certains récifs où la densité de poissons prédateurs d'oursins est faible, la densité d'*E. mathaei* est très importante, supérieure à 30 individu m^{-2}, et ceux-ci supplantent les poissons herbivores (McClanahan, 1992 ; McClanahan & Kurtis, 1991). Ceci serait expliqué par ses faibles besoins énergétiques qui en feraient une espèce à croissance lente, relativement tolérante à l'absence de nourriture et à la compétition. Comparativement, les autres herbivores, notamment les poissons nécessitent 3 à 5 fois plus de nourriture (par exemple, *E. mathaei* : 0,5 g d'algues en poids sec ind^{-1} j^{-1} – *Plectroglyphidodon lacrymatus* 1,6 g d'algues en poids sec ind^{-1} j^{-1} ; McClanahan, 1992). Ainsi, une reproduction et un recrutement élevés chez *E. mathaei* lui permettent de coloniser rapidement des zones où ses prédateurs sont peu nombreux. Cependant, une croissance lente, en comparaison de celle des poissons herbivores, augmente le temps nécessaire pour atteindre le maximum de biomasse. Dès lors, ses caractéristiques énergétiques permettent à *E. mathaei* de devenir dominant dans des conditions stressantes où il y a une faible pression de prédation. Cependant, un stress intense, des maladies et une augmentation importante de la pression de prédation peuvent entraîner une diminution rapide de la population nécessitant un longue période pour revenir à l'état initial (McClanahan & Muthiga, 1988).

La production algale benthique nette des récifs coralliens varie entre 0,8 et 3 g de carbone organique m^{-2} j^{-1} en fonction de la location du récif, de la zone du récif et de la saison (Klumpp & McKinnon, 1989 ; Paddack *et al.*, 2006 ; Russ, 2003 ; Russ & McCook, 1999) ou ~20 g de matière organique humide m^{-2} j^{-1} (Atkinson & Grigg, 1984). Un oursin consomme entre 0,05 et 0,24 g de matière organique humide j^{-1} (Bronstein & Loya, 2014 ; Carreiro-Silva & McClanahan, 2001). Là où *E. mathaei* atteint localement des densités de population très élevées, il peut donc facilement

consommer une grande partie de la production primaire benthique des macroalgues et algues encroûtantes. Ainsi son influence sur l'écosystème benthique récifal peut être importante.

Plusieurs études ont montré qu'une réduction expérimentale de la densité de la population d'*E. mathaei* en milieu naturel entraînait une augmentation de l'abondance des macroalgues et une diminution de l'abondance de coraux (McClanahan *et al.*, 1996 ; Prince, 1995). De même, une faible densité d'oursins et des poissons herbivores dans le Récif de La Saline à la Réunion, ainsi qu'un enrichissement en nutriments provenant de la côte, ont favorisé le basculement du récif vers un écosystème dominé par les algues (Cuet *et al.*, 1988). En effet, le broutage des macroalgues par les herbivores, dont les oursins, empêche le recouvrement des coraux par celles-ci et favorise donc la croissance et le recrutement des coraux (Dart, 1972 ; Hughes, 1994 ; Hughes *et al.*, 1987 ; Lirman 2001).

Cependant, une densité trop élevée d'*E. mathaei* résulte également en une bioérosion importante du récif, et finalement en une diminution de la couverture corallienne, de la complexité topographique du récif et de la diversité des autres espèces (McClanahan & Kurtis, 1991; McClanahan & Muthiga, 1988). De même, une densité population très importante d'*E. mathaei* dans des récifs non protégés contre la pèche a été associée à la disparition de leur principaux prédateurs, les poissons balistes. La couverture corallienne et la complexité topographique était alors négativement corrélées à la densité d'oursins (McClanahan & Shafir, 1990). Le taux de bioérosion moyen par *E. mathaei* varie entre 0,1 et 0,9 g $CaCO_3$ $individu^{-1}$ j^{-1} (Downing & El-Zahr, 198 7 ; McClanahan & Kurtis, 1991 ; McClanahan & Muthiga, 1988 ; Mills *et al.* 2000 ; Mokady *et al.*, 1996 ; Russo, 1980). Cette variation s'explique par le type de nourriture ingérée (algues majoritairement encroûtantes ou en épaves par exemple), la densité de la population (consommation plus faible si la compétition interindividuelle est élevée) et la taille des oursins (Bak 1990, 1994). Ainsi, une forte densité de population est responsable d'une importante bioérosion (Fig. 24). Par exemple, E. mathaei (densité de 74 individus m^{-2}) érode jusque 8,3 kg CaCO3 m^{-2} an^{-1} à la Réunion (Conand *et al.*, 1997). Un tel niveau de bioerosion est plus important que l'accrétion de certains récifs coralliens qui varie entre 0,5 à 22 kg CaCO3 m^{-2} an^{-1} (Andersson & Gledhill, 2013 ; Bak, 1990 ; Bak, 1994).

Ainsi la balance entre les effets positifs du broutage des algues et les effets négatifs de la bioérosion sur le devenir des récifs coralliens dépend de la densité de la population des oursins et donc d'un équilibre subtil entre pression de prédation et croissance de la population.

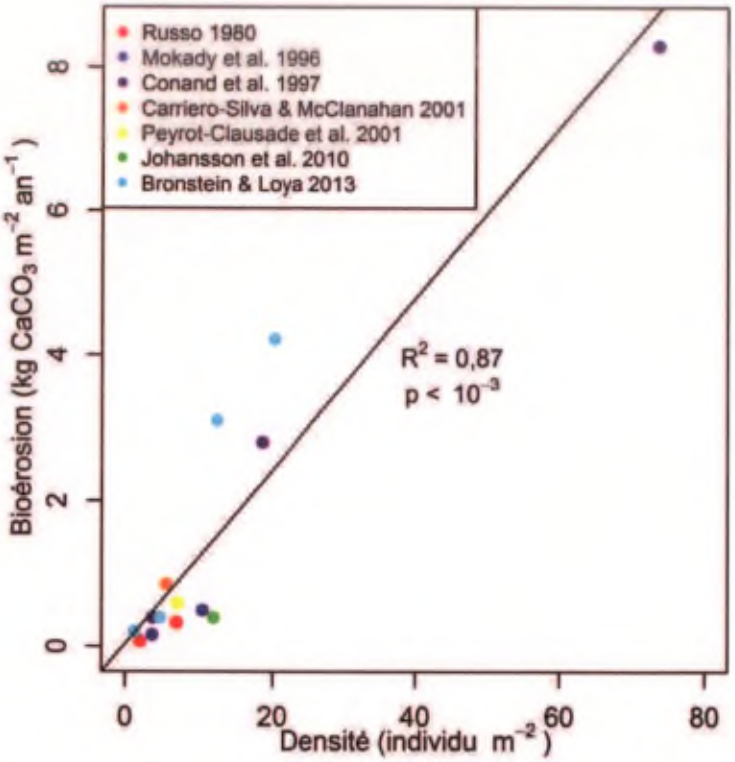

Figure 24 : estimation du taux de bioérosion en fonction de la densité d'*Echinometra mathaei* d'après plusieurs études. Un modèle linéaire reprenant l'ensemble des données des différentes études a été utilisé pour prédire le taux de bioérosion en fonction de la densité d'oursin. Le coefficient de détermination (R^2) et le niveau de significativité (p) du modèle sont inscrits sur le graphique.

5. Impact de l'acidification des océans sur les métazoaires marins

5.1. Hypercapnie, acidose et mécanismes de compensation

Chez les organismes marins pour lesquels la balance acide-base a été étudiée, une augmentation de la pCO_2 de l'eau de mer entraîne directement une élévation de la pCO_2 des fluides extracellulaires. Cette augmentation se poursuit jusqu'à ce que le gradient de diffusion de ce gaz entre les fluides extracellulaires et l'eau de mer soit suffisant pour rétablir la diffusion du CO_2 vers le milieu extérieur (Seibel & Walsh, 2003). En effet, l'élimination du CO_2 issu de la respiration (et éventuellement de la calcification) dépend de ce gradient (Heisler, 1986 ; Melzner *et al.*, 2009).

Les organismes marins doivent naturellement contrer l'augmentation des produits métaboliques dont le CO_2 (on parle d'hypercapnie lorsque la pCO_2 devient anormalement élevée dans les fluides corporels) et des protons. Ces derniers résultent de la dissociation de H_2CO_3 formé à partir du CO_2 métabolique, mais également d'autres processus comme la calcification (un pH anormalement bas dans les fluides corporels est qualifié d'acidose). Le transport des produits métaboliques est assuré par les fluides internes extracellulaires (Heisler, 1989). Deux systèmes tampons existent pour contrer l'hypercapnie et l'acidose résultante: le système CO_2-HCO_3^- (système bicarbonate) et le système non carbonate (Melzner *et al.*, 2009). Le système bicarbonate est limité puisque l'excès de protons entraîne une augmentation de CO_2 dans les fluides extracellulaires qui doit à son tour être éliminé (voir équation 2, section 1.2.). Le système non carbonate est principalement assuré par l'association des protons aux chaînes latérales des acides aminés (le plus souvent l'histidine et la cystéine aux valeurs de pH physiologique), au groupement N-terminal des protéines ou aux groupements phosphates organiques/inorganiques. Cependant, ce système ne fait que masquer transitoirement les protons et les protons excédentaires doivent être éliminés pour rétablir le pH d'origine du liquide. Cela ne peut être réalisé que par le transport actif d'ions à travers les épithéliums spécialisés comme les branchies, les tissus rénaux ou digestifs (Fig. 25 ; Melzner *et al.*, 2009).

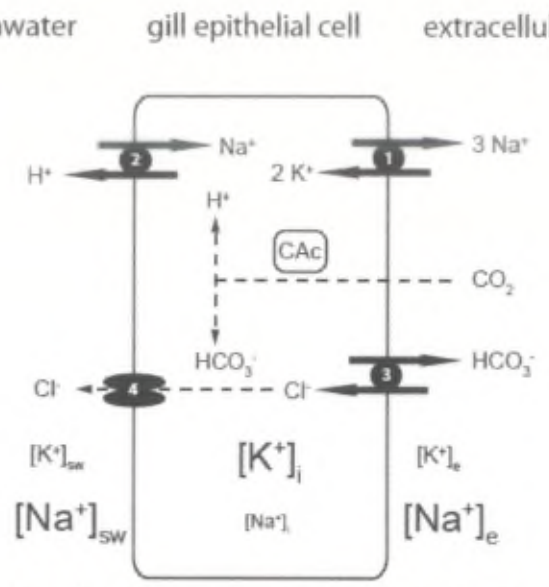

Figure 25: Représentation schématique simplifiée d'une cellule épithéliale des branchies d'un poisson téléostéen. Les crustacés décapodes et les céphalopodes possèdent des protéines similaires. 1 = Na+/K+ ATPase, 2 = échangeurs Na+/H+, 3 = échangeurs Cl-/HCO_3-, 4 = canal Cl-, Cac = anhydrase carbonique cytoplasmique. Les Na+/K+ ATPase permettent de maintenir une concentration intracellulaire faible en Na+ et haute en K+. Les transporteurs actifs secondaires comme les échangeurs Na+/H+ utilisent le gradient de Na+ pour exporter les H+. Ceux-ci sont produits lorsque le CO_2 hydraté (H_2CO_3) est dissocié en H+ et HCO_3-. Cette réaction est accélérée par l'anhydrase carbonique. Les HCO_3- résultants peuvent être transférés dans les fluides extracellulaires alors que les ions Cl- sont exportés vers l'eau de mer grâce à des canaux Cl- afin de maintenir l'électroneutralité (d'après Melzner et al., 2009).

Les premières études concernant l'impact de l'AO se sont principalement concentrées sur les organismes calcifiants, en particulier les organismes produisant les formes les plus solubles du carbonate de calcium, l'aragonite et les calcites magnésiennes (Fabry *et al.*, 2008 ; Orr *et al.*, 2005 ; Royal Society, 2005). En effet, de par la diminution de la concentration en ions carbonates, la réaction chimique de précipitation du carbonate de calcium est défavorisée (voir section 1.2.). Cependant, seul l'océan austral et l'océan Arctique seraient sous-saturés par rapport à l'aragonite en 2100. De plus, la calcification chez les organismes marins est biologiquement contrôlée et se déroule généralement dans un site confiné où un état sursaturé par rapport à la forme de carbonate de calcium précipitée est maintenu par l'intermédiaire de transporteurs ioniques (notamment chez les coraux et les oursins, voir respectivement section 2 et 4.3.). De plus, dans la majorité des cas, l'espèce transportée est le CO_2 ou HCO_3^- et non CO_3^{2-} (Weiner & Dove, 2003). Ainsi, l'impact de l'AO sur les structures calcifiées n'est donc en général pas direct mais se ferait par l'intermédiaire d'autres processus physiologiques immédiatement impactés (Pörtner, 2008). Plusieurs études montrent que l'équilibre acide-base des fluides extracellulaires (sang, liquide cœlomique, hémolymphe) est la première caractéristique physiologique directement altérée lorsque la chimie de l'eau de mer est modifiée (voir Pörtner, 2008 pour une synthèse). Le pH de ces fluides apparaît comme la variable clé à la base des effets métaboliques de l'acidification. En effet, l'acidose extracellulaire entraîne notamment une diminution du métabolisme, une réduction

des échanges ioniques, une réduction de la synthèse des protéines et affecte le comportement (Fig. 26, Langenbuch *et al.*, 2006 ; Melzner *et al.*, 2009 ; Pörtner, 2008 ; Pörtner *et al.*, 1998 ; Pörtner *et al.*, 2004 ; Reipschläger *et al.*, 1997). La croissance (somatique et squelettique) serait affectée en retour par la diminution du métabolisme ou l'augmentation des coûts énergétiques liés à l'élimination des protons (Hofmann &Todgham, 2010 ; Pörtner, 2008).

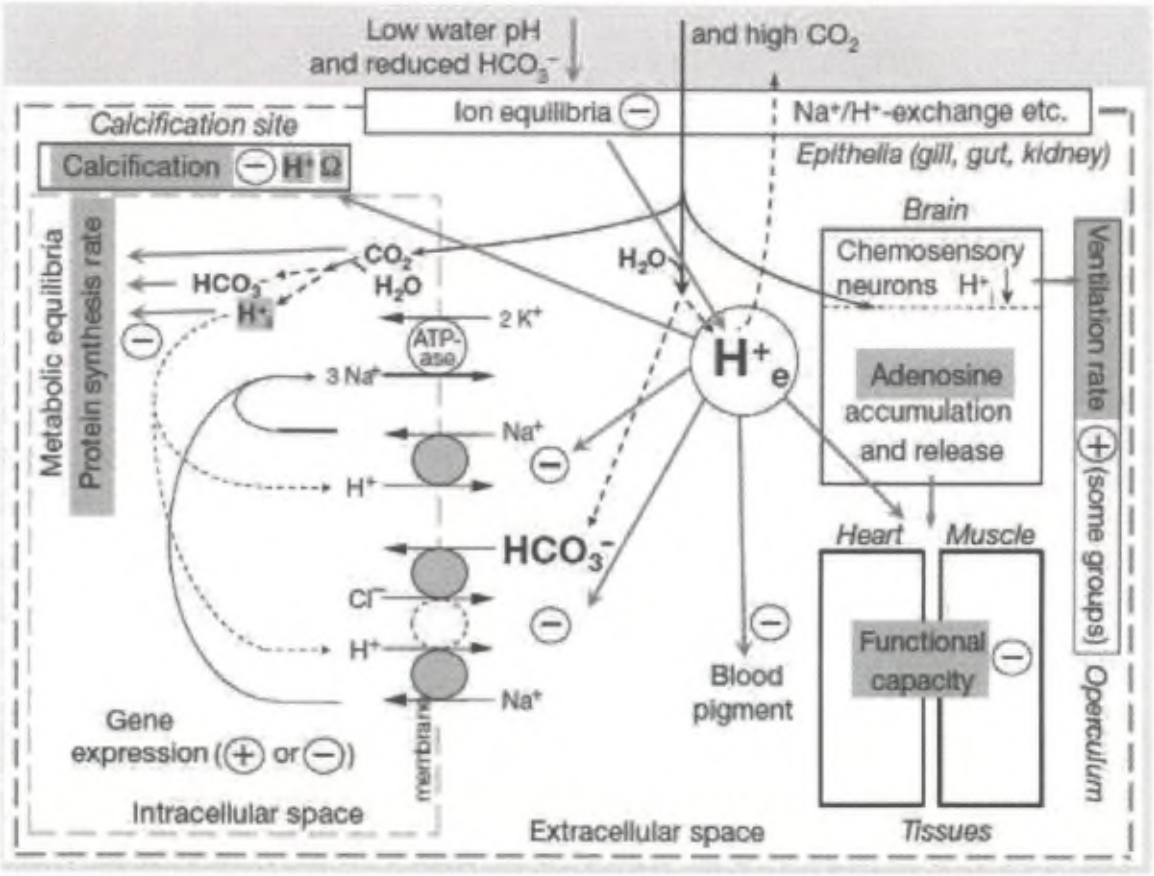

Figure 26: Vue d'ensemble des processus et mécanismes affectés par l'acidification/élévation en CO_2 des océans chez un organisme marin, démontrant l'importance du maintien du pH extracellulaire. Les signes (+) et (-) représentent respectivement les effets positifs et négatifs. Les zones ombrées indiquent les processus impliqués dans un changement du budget énergétique (d'après Pörtner, 2008).

Ainsi la capacité de résistance à l'AO apparait, en partie, liée à la capacité de régulation de la balance acide-base des fluides extracellulaires. Les organismes possédant des mécanismes de compensation efficaces comme des structures d'osmorégulation et d'excrétion spécialisées, notamment les taxa caractérisés par un haut métabolisme seraient plus tolérants à l'AO (Melzner *et al.*, 2009 ; Pörtner *et al.*, 2004 ; Pörtner, 2008).

5.2. Le stade post-métamorphique des échinides

5.2.1. La régulation acide base

Les oursins possèdent un métabolisme bas, une faible capacité de régulation osmotique et ionique et ne disposent pas d'organe excréteur spécialisé (section 4.2. ; Jangoux, 1982 ; Shick, 1983 ; Stickle & Diehl, 1987). Les échanges gazeux, dont l'élimination du CO_2, sont limités par l'absence de pigments respiratoires et de structures spécialisées associées à un mécanisme respiratoire actif (Farmanfarmaian, 1966 ; Shick, 1983). Ainsi, l'élimination du CO_2 dépendrait essentiellement du gradient de diffusion existant entre le liquide cœlomique (LC) et le milieu environnant (Farmanfarmaian, 1966).

Plusieurs auteurs ont étudié l'effet de l'augmentation de la pCO_2 et la capacité de régulation acide-base chez les oursins réguliers. En fonction de l'espèce, la compensation du pH du LC des oursins (pH_{LC}) réguliers est absente, partielle ou complète et se ferait principalement par une accumulation de bicarbonate dans le LC (Table 4). Une des premières hypothèses formulée concernant l'origine de l'augmentation du bicarbonate dans le LC comme mécanisme de compensation acide-base était la dissolution passive du squelette. En effet, Spicer *et al.* (1988) ont observé une augmentation de la concentration en ions Mg^{2+} et Ca^{2+} dans le LC lorsque *S. droebachiensis* était soumis à une acidose respiratoire causée par une émersion durant 24h. Cependant, Miles *et al.* (2007) ont suggéré que la contribution du test serait minime et transitoire puisque les oursins immergés ont accès au pool d'ions bicarbonates de l'eau de mer. Cette hypothèse a ensuite été soutenue par Holtmann *et al.* (2013). En effet, ces auteurs ont montré, chez l'espèce tempérée *Strongylocentrotus purpuratus*, que l'épithélium péritonéal qui tapisse la face interne du test est perméable aux ions et favorise l'exposition du stéréome aux conditions du LC. Cependant, ils ont analysé la microstructure du stéréome et ont montré que la dissolution des ossicules était faible. Ainsi, ce mécanisme de compensation n'est que transitoire et se fait lors d'une diminution brève du pH de l'eau de mer (pH_{EM}). A plus long terme, des transporteurs ioniques localisés au niveau de l'épithélium intestinal apporteraient des HCO_3^- du tube digestif vers le LC. Au contraire, les ions HCO_3^- présents dans le LC ne peuvent pas traverser l'épithélium intestinal qui contribue dès lors à l'accumulation des bicarbonates dans le LC. Les auteurs font également l'hypothèse que ces ions pourraient provenir de l'hydratation du CO_2 métabolique puis de sa dissociation par l'anhydrase carbonique au niveau des cellules de l'épithélium intestinal. Les protons formés seraient dès lors sécrétés dans le tube digestif. Ainsi, des transporteurs HCO_3^-, l'anhydrase carbonique et des pompes à protons seraient impliqués dans les mécanismes de

régulation du pH extracellulaire. Collard (2014) a récemment fait l'hypothèse qu'un tel mécanisme pourrait également avoir lieu au niveau de la membrane des podia (surface importante d'échanges), permettant un apport rapide d'ions HCO_3^- dans le LC. En effet, des résultats préliminaires ont montré chez *Paracentrotus lividus* que la signature isotopique de carbone $\delta^{13}C$ du LC suivait de près celle de l'eau de mer, impliquant dès lors un échange important entre ces 2 compartiments.

Le pH_{LC} des oursins cidaroïdes est naturellement plus bas que celui des oursins réguliers et ne serait pas affecté par une relativement faible diminution du pH_{EM} chez *Eucidaris tribuloides* (Table 4) bien que cette observation doive encore être confirmée chez d'autres espèces.

Table 4 : effet de l'acidification des océans sur le pH et la concentration en ions bicarbonates du liquide cœlomique (pH_{LC} et $[HCO_3^-]$) chez plusieurs espèces d'échinides. Seuls les pH de l'eau de mer (pH_{EM}) ≥ 7.1 ont été pris en compte.

Espèce	Climat	Temps d'exposition	pH_{EM-T} traitement	pH_{LC} control	pH_{LC} traitement	↓ du pH_{LC} significative	↑ de $[HCO_3^-]_{LC}$ significative	Référence
Euéchinoïdes réguliers								
Arbacia lixula	tempéré	2-4 jours (b)	7,6*	6,6-6,9	6,9 – 6,8	non	non	Calosi *et al.*, 2013
		24 heures	7,6*	6,9	6,9	non	non	
Hemicentrotus pulcherrimus	tropical	9 mois	7,8*	7,6	7	oui	-	Kurihara *et al.*, 2013
Paracentrotus lividus	tempéré	19 jours	7,7 – 7.4	7,6	7,5 – 7,4	non - oui	-	Catarino *et al.*, 2012
		2-4 jours (b)	7,6*	7,2 – 7,1	7,2 – 7,3	non	oui	Calosi *et al.*, 2013
		24 heures	7,6*	7,2	7	oui	oui	
		7 jours	7,7 – 7,4	7,5	7,4 - 7,1	non - oui	oui - oui	Collard *et al.*, 2013
		22 jours	7,65 – 7,5	7,4	7,1 – 6,8	non - non	oui - oui	Collard *et al.*, 2014
Psammechinus miliaris	tempéré	8 jours	7,45*	7,6	6,9	oui	oui	Miles *et al.*, 2007
Strongylocentrotus droebachiensis	boréal	5 jours	7,35*	7,5	7,3	oui	oui	Spicer *et al.*, 2011
		3 - 5 jours	7,6*	7,6	7,4 – 7,5	oui - non	-	Dupont & Thorndyke, 2012
		10 jours	7,5 – 7,1	7,7	7,8 – 7,5	non - oui	oui - oui	Stumpp *et al.*, 2012
		45 jours	7,7 – 7,25	7,8	7,7 – 7,5	non - non	oui - oui	
			7,25 (a)		7,2	oui	non	
Strongylocentrotus fragilis	tempéré	7 - 31 jours	7,5	7,6 – 7,7	7,5 – 7,7	oui - non	non - non	Taylor *et al.*, 2014
			7,1	7,6 – 7,7	7,3 – 7,3	oui	oui	
Tripneustes ventricosus	tropical	32 jours	7,7 – 7,4	7,4	7,3 – 7,4	non - non	oui - oui	Collard *et al.*, 2014
Cidaroïde								
Eucidaris tribuloides	tropical	32 jours	7,65 – 7,5	6,5	6,4 – 6,5	non - non	oui - oui	Collard *et al.*, 2014

(a) Individus à jeun

(b) transplantation in situ sur évent volcanique à CO_2

* Afin d'uniformiser l'échelle du pH de l'eau de mer utilisée, le pH a été recalculé en échelle totale (pH_{EM-T}) à partir des paramètres du système des carbonates en utilisant le logiciel R et la fonction carb() du package Seacarb (http://www.R-project.org/). Ce calcul n'a pu être fait pour le pH_{LC} est donc exprimé en échelle NBS pour les études notées avec un astérisque.

5.2.2. La croissance

L'impact de l'AO sur la calcification des oursins semble être spécifique puisque certaines espèces sont affectées alors que d'autres non (Table 5). Cependant, de manière générale, les oursins sont toujours capables de former leur squelette malgré la diminution du pH_{EM} puisque la calcification nette reste positive. Cette diminution de croissance serait associée à une diminution de l'allocation des ressources à la croissance, l'énergie étant utilisé pour le maintien de la balance acide-base du liquide cœlomique (Holtmann *et al.*, 2013 ; Stumpp *et al.*, 2012).

Le stade juvénile est le stade le plus sensible à l'AO. En effet, une réduction de la croissance squelettique semble être générale quel que soit l'espèce considérée (Table 5). La croissance d'*E. mathaei* n'est pas affectée au stade adulte après 6 semaines d'exposition à $pH_{EM\text{-}T}$ 7,45 (Uthicke *et al.*, 2012, 2014) contrairement au stade juvénile exposé pourtant comparativement à une faible diminution du pH_{EM} ($pH_{EM\text{-}NBS}$ 7,9) mais durant une plus longue période (9 mois, Shiramaya & Thornton 2005). Byrne *et al.* (2010) ont également observé une augmentation de pourcentage de juvéniles présentant une morphologie anormale chez *Heliocidaris erythrogramma*. Cependant, l'acclimatation est également être un facteur important à considérer. En effet, la croissance de juvéniles issus de parents acclimatés durant 4 mois à $pH_{EM\text{-}T}$ 7,7 chez *S. droebacheinsis* n'est pas affectée par une même diminution du pH_{EM} contrairement aux juvéniles issus de parents non acclimatés (Dupont *et al.*, 2012). Un autre paramètre à prendre en compte est la nature de la nourriture ingérée par les oursins. En effet, Asnaghi *et al.* (2014) ont montré que la croissance d'oursins juvéniles *P. lividus* n'est pas affectée lorsque ceux-ci sont nourris d'algues corallines.

Table 5 : Effet de l'acidification des océans sur la croissance au stade adulte et au stade juvénile chez plusieurs espèces d'échinides. Seuls les pH de l'eau de mer (pH_{EM-T}) ≥ 7.1 ont été pris en compte.

Espèce	Climat	Temps d'exposition	pH_{EM-T} traitement	Effet	Référence
Stade adulte					
Arbacia punctulata			7,8* – 7,65* – 7,2*	↗ – ↗ – ↘	Ries *et al.*, 2009
Echinometra mathaei	tropical	6 semaines	7,9 – 7,6 – 7,45	= – = – =	Uthicke *et al.*, 2012
		70 jours	7,7*	=	Uthicke *et al.*, 2014
Echinometra viridis	tropical	60 jours	8,0	↘	Courtney *et al.*, 2013
Eucidaris tribuloides	tropical	60 jours	7,8* – 7,65* – 7,2*	= – = – ↘	Ries *et al.*, 2009
Heliocidaris (Anthocidaris) crasspina	tempéré	140 jours	7,7* – 7,2*	= – ↘	Wang *et al.*, 2013
Hemicentrotus pulcherrimus	tropical	9 mois	7,7*	=	Kurihara *et al.*, 2013
Strongylocentrotus droebachiensis	boréal	45 jours	7,7 – 7,25 (globale)	= – ↘	Stumpp *et al.*, 2012
			7,7 – 7,25 (au niveau des plaques du test)	↘ – ↘	Holtmann *et al.*, 2013
Strongylocentrotus fragilis	tempéré	130 jours	7,7 – 7,2	=	Taylor *et al.*, 2014
Stade juvénile					
Echinometra mathaei	tropical	6 mois	*7,9 (NBS)*	↘	Shirayama & Thornton, 2005
		9 mois			
Hemicentrotus pulcherrimus	tropical	6 mois	*7,9 (NBS)*	↘	Shirayama & Thornton, 2005
		9 mois			
Heliocidaris erythrogramma	tempéré	2 semaines	7,7* – 7,5* – 7,3*	= – = – ↘	Wolfe *et al.*, 2013
Lytechinus variegatus	tropical	3 mois	8,0 – 7,8	↘ – ↘	Albright *et al.*, 2012
		3 mois	7,7	↘	Challener *et al.*, 2014
Paracentrotus lividus	tempéré	1 mois	8,0 – 7,8 – 7,7	= – = – =	Asnaghi *et al.*, 2014
				↗ si nourris d'algues corallines	
Strongylocentrotus droebachiensis	boréal	3 mois	7,6*	=	Dupont *et al.*, 2012
				↗ si issus de parents acclimatés durant 4 mois à bas pH	

* Afin d'uniformiser l'échelle du pH de l'eau de mer utilisée, le pH a été recalculé en échelle totale (pH_{EM-T}) à partir des paramètres du système des carbonates en utilisant le logiciel R et la fonction carb() du package Seacarb (http://www.R-project.org/). Ce calcul n'a pu être fait pour certaines études par manque d'information (NBS spécifié entre parenthèses).

Très peu d'études se sont focalisées sur la lanterne d'Aristote. Outre son importance dans la nutrition, cette structure montre une grande plasticité phénotypique en fonction des conditions environnementales, notamment une allométrie de croissance positive par rapport au test lorsque la quantité de nourriture disponible est réduite (Black *et al.*, 1982 ; Ebert, 1968, 1980). Récemment, Wang *et al.* (2013) ont observé une diminution d'épaisseur des dents à pH_{EM-T} 7.7 et des anomalies morphologiques de celles-ci à pH_{EM-T} 7.2. Des traces de corrosion ont été observées au niveau de la lanterne d'Aristote par Asnaghi *et al.* (2013). Cependant, la réponse à l'AO, comme pour la croissance du test, était fonction du régime alimentaire (algue calcaire ou non) dans cette étude. Ainsi le régime alimentaire est crucial dans la capacité de résistance à l'AO.

5.2.3. Les propriétés mécaniques du test

Les propriétés mécaniques du test ne semblent pas affectées par une augmentation de la pCO_2 de l'eau de mer. Collard (2014) a étudié l'impact de l'AO sur les propriétés mécaniques de plaques apicales (en formation) et de plaques ambitales du test de l'oursin tempéré *P. lividus*. Les individus étudiés ont été exposés pendant 1 an à un pH_{EM-T} de 7,8 ou provenaient d'un évent volcanique montrant un pH_{EM-T} similaire. La résistance, la rigidité et la dureté de ces plaques n'étaient pas affectées par la diminution du pH_{EM}. Une diminution de la résistance du test entier a été mise en évidence chez cette même espèce par Asnaghi *et al.* (2013). Cependant, les tests mécaniques ont été réalisés sur des tests séchés dont les plaques sont dès lors assemblées par des ligaments déshydratés. Ces mesures reflètent plus les propriétés de ces ligaments que celles du test (Dubois, 2014 ; Ellers *et al.*, 1998). Chez une autre espèce tempérée, *S. droebachiensis*, Holtmann *et al.* (2013) n'ont pas observé d'effet sur la résistance des plaques du test. Challener *et al.* (2014) observent même une augmentation de la résistance du test à la perforation chez l'oursin tropical *Lytechinus variegatus* à pH_{EM} 7,7.

5.2.4. Le métabolisme

Peu d'études se sont intéressées à l'effet de l'AO sur le métabolisme des échinides. Dans deux d'entre elles, celui-ci restait constant malgré une diminution du pH_{EM} à une valeur comparable à celle prévue en 2100 (Catarino *et al.*, 2012 pour *P. lividus* ; Stumpp *et al.*, 2012 pour *S. droebachiensis*). Wang *et al.* (2013) ont observé une diminution du taux de respiration à pH_{EM-T} 7,7 et 7,2 mais celui-ci revenait à une valeur contrôle après, respectivement 80 et 140 jours d'exposition. Le taux de respiration d'*E. mathaei* augmente à pH_{EM-T} 7,6 et 7,9 par rapport au pH control (pH_{EM-T} 8) mais pas à pH_{EM-T} 7,5 (Uthicke *et al.*, 2012).

5.2.5. *La reproduction*

L'allocation des ressources aux gonades dépend des conditions environnementales et reflète les coûts énergétiques liés à la survie des individus lorsque le milieu est défavorable. De manière générale, un milieu stressant conduira à une diminution de l'allocation des ressources aux gonades (Byrne, 1990 ; Shpigel *et al.*, 2004 ; Siikavuopio *et al.*, 2004 ; Russel, 1998). De même, l'AO entraîne un changement du budget énergétique chez l'espèce boréale *S. droebacheinsis*. Chez celle-ci, l'AO favorise la maintenance de l'homéostasie interne (régulation acide-base) et défavorise la croissance des gonades (Stumpp *et al.*, 2012). En effet, lorsque le temps d'acclimatation à une diminution du pH_{EM} est inférieur à 4 mois, l'AO entraîne un retard du développement des gonades, une diminution de production de gamète chez les mâles ainsi qu'une diminution de leur mobilité et de leur vitesse (Table 6). Cependant, cet effet négatif disparaît lorsque l'acclimatation aux conditions de pH_{EM} plus bas est supérieure à 4 mois. En effet, Dupont *et al.* (2012) ont montré que la fécondité de *S. droebacheinsis* était affectée après 4 mois d'exposition à pH_{EM-T} 7,6 mais pas après 16 mois d'acclimatation aux mêmes conditions. De même, le taux de fécondation des gamètes d'oursins naturellement acclimatés dans une mare intertidale à fortes variations de pH_{EM} était supérieur à ceux d'oursins provenant d'une mare à faible fluctuation chez l'espèce tempérée *P. lividus* (Moulin *et al.*, 2011). Enfin, un retard de la maturation des gonades a été observé chez l'espèce tropicale *Hemicentrotus pulcherrimus* lorsque celle-ci est soumise durant 9 mois à une diminution de pH_{EM} de 0,6 unité. Cependant, ce retard n'avait pas d'impact sur le nombre d'ovules produits (Kurihara *et al.*, 2013). Ainsi une acclimatation suffisamment longue à un pH plus bas de l'eau de mer pourrait permettre aux échinides de restaurer l'énergie nécessaire à la reproduction.

Table 6 : Effets délétères de l'acidification des océans sur la reproduction chez plusieurs espèces d'échinides lorsque le temps d'exposition aux conditions de pHEM plus bas (pHEM-T traitement) est inférieur à 4 mois.

Espèce	Climat	Temps d'exposition	pH_{EM-T} traitement	Référence
Diminution de croissance /retard de développement des gonades				
Heliocidaris (Anthocidaris) crasspina	tempéré	140 jours	7,7* – 7,2*	Wang *et al.*, 2013
Hemicentrotus pulcherrimus	tropical	9 mois	7,7*	Kurihara *et al.*, 2013
Strongylocentrotus droebachiensis	boréal	45 jours	7,7 – 7,25 7,0 (NBS)	Stumpp *et al.*, 2012 Siikavuopio *et al.*, 2007
Diminution de la production de gamètes mâles				
Echinometra mathaei	tropical	6 semaines	7,9 – 7,6 – 7,45	Uthicke *et al.*, 2012
Diminution de la mobilté et de la vitesse des gamètes mâles				
Heliocidaris erythrogramma	tempéré	5 jours 5 jours	7,7 (NBS) 7,7*– 7,5*	Havenhand *et al.*, 2008 Schlegel *et al.*, 2012
Diminution du taux de fécondation				
Heliocidaris erythrogramma	tempéré	5 jours 5 jours	7,7 (NBS) 7,7*– 7,5*	Havenhand *et al.*, 2008 Schlegel *et al.*, 2012

Les premières études concernant l'impact de l'acidification des océans, liée à l'augmentation de la pCO_2, sur les organismes marins ont émis l'hypothèse d'une vulnérabilité importante des échinides face à ces changements environnementaux. Plusieurs raisons ont été avancées : la synthèse d'un squelette en calcite magnésienne, une faible capacité de régulation ionique et acide-base et un faible métabolisme. Cependant, des études récentes ont montré que certaines espèces sont capables, au moins à court terme, de réguler la balance acide-base de leur fluide cœlomique, un paramètre clé de la capacité de résistance à l'acidification des océans. Ces mêmes études ont souligné l'importance de mener des expériences à long terme à l'échelle de l'écosystème afin de mieux prévoir les effets indirects de l'élévation de la pCO_2 de l'eau de mer. Ces effets indirects reprennent notamment les interactions écosystèmiques mais également les coûts énergétiques liés aux processus d'acclimatation à long terme.

Les récifs coralliens tropicaux figurent parmi les écosystèmes les plus menacés par les changements climatiques globaux et l'acidification des océans. Leur existence dépend de calcification des coraux hermatypiques qui doit rester supérieure à l'érosion du récif. Plusieurs espèces d'oursins participent activement à l'écologie des récifs coralliens. D'une part, ils broutent les algues et empêchent donc qu'elles ne recouvrent les coraux. D'autre part, et parallèlement, ils érodent le substrat récifal entraînant une perte de la masse de carbonate de calcium du récif. Quelques études ont montré que l'érosion des récifs coralliens pourrait augmenter dans un futur acidifié à cause de l'augmentation de l'érosion physique, chimique et biologique. Cependant, jusqu'à présent, aucune étude n'a été menée sur l'impact de l'acidification des océans sur la bioérosion des récifs par les oursins, ces derniers représentant pourtant les principaux bioérodeurs dans certains récifs.

Dès lors, le but de la présente thèse est d'évaluer les effets de l'acidification des océans sur l'oursin *Echinometra mathaei*, un bioérodeur majeur dans certains récifs coralliens tropicaux. Les études ont été menées en mésocosmes récifaux artificiels où la diminution du pH de l'eau mer a été réalisée progressivement et maintenue sur plusieurs mois afin de prendre en compte les possibles processus d'acclimatation ainsi que les interactions écosystèmiques. Les aspects techniques du maintien à long terme d'un tel outil sont développés dans le chapitre 1. L'impact de la diminution du pH sur la capacité de régulation acide-base, la croissance, le métabolisme et les

propriétés mécaniques du squelette chez cet oursin a été évalué à moyen terme (chapitre 2) et à long terme (chapitre 3). Enfin, l'évolution du taux de bioérosion en fonction des conditions de pH a été étudiée afin de prévoir l'impact de l'acidification des océans sur la balance entre bioaccrétion et bioérosion des récifs coralliens tropicaux (chapitre 4).

Artificial coral reef mesocosms for ocean acidification investigations

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Abstract

The design and evaluation of replicated artificial mesocosms (which are defined here as (semi)-closed "cosms" in the laboratory with a more realistic physico-chemical environment than microcosms) are presented in the context of a thirteen month experiment on the effects of ocean acidification on tropical coral reefs. Important physico-chemical parameters (i.e. pH, pO_2, pCO_2, total alkalinity, temperature, salinity, total alkaline earth metals and nutrients availability) were successfully monitored and controlled. Daily variations of light intensity and pH were applied to approach field conditions. Results highlighted that it was possible to maintain realistic physico-chemical parameters, including daily changes, into artificial mesocosms. On the other hand, the two identical artificial mesocosms evolved differently in terms of global community oxygen budgets although the initial biological communities and physico-chemical parameters were comparable. Artificial reef mesocosms seem to leave enough degrees of freedom to the enclosed community of living organisms to organize and change along possibly diverging pathways.

1. Introduction

Over the last century, anthropogenic atmospheric carbon dioxide (CO_2) emissions have raised (Doney et al., 2009). One of the consequences is ocean acidification (OA) as CO_2 dissolves in seawater. The carbonate chemistry equilibrium is thus modified and pH is decreased. In parallel, the interest of the scientific community for OA has raised in the last decade. Several strategies were used to understand OA effects and possible acclimation or adaptation of marine organisms (Widdicombe et al., 2010).

Studies have been conducted in aquaria to understand the physiological effects of OA on organisms (Fig. 27). A single (most often) or a few species were maintained together under different controlled pH conditions. Results provided first insights to understand future OA effects and mechanisms. Laboratory experiments in aquaria are relatively easy to set up. They are replicable and can be finely controlled. However, results are hardly transposable to field conditions, due to the very artificial environment and to the absence of ecosystemic interactions.

The opposite strategy is to study the effects of pH in environments characterized by a naturally low pH as intertidal zones (e.g. Moulin et al., 2011; Egilsdottir et al., 2012), CO_2 seeps (or vents) (for example, Hall-Spencer et al., 2008; Cigliano et al., 2010; Fabricius et al., 2011; Calosi et al., 2013), upwelling zones (Feely et al., 2008) or the deep sea (Park, 1966; Roberts et al., 2006; Turley et al., 2007). However, several environmental parameters differ in these situations from the ongoing OA in most of the open ocean and benthic zones. For example, the pH decrease in rocky tidal pools occurs over short periods of time and varies in intensity over seasons. Around volcanic vents and in upwelling zones, many marine organisms could escape from pH stress. Indeed, the biomass of less CO_2 tolerant sessile species decreases there (Hall-Spencer et al., 2008). Conversely, recruitment of juveniles from outside could mask other potential effects. There is generally no possibility of replication. Measures can be performed on different organisms but as they originate all from the same location, they cannot be considered as independent replicates, leading to some degree of pseudo-replication and biased statistical analysis (Hurlbert, 1984). Finally, other physico-chemical factors (i.e. nutrients, trace elements, heavy metals and other pollutants, temperature, pressure,...) may create unwanted interactions with pH in the natural environment. For example, Vizzini et al. (2013) observed a trace element contamination around the CO_2 vents from Vulcano Island. The change in species distribution and the prospective physiological effects could therefore be falsely attributed to the pH decrease, due to the existence of other confounding factors.

Microcosm and mesocosm studies represent compromises between aquaria experiments in the laboratory and field surveys (Fig. 27). Historically, a "microcosm" was defined as an artificial, simplified ecosystem that was used to simulate and predict the behavior of natural ecosystem under controlled conditions (Odum, 1983). These are usually built in the laboratory for easy access. On the other hand, "mesocosm" was defined as partially enclosed outdoor experimental setup that closely simulates the natural environment (Odum, 1984). The advantages of these setups are numerous: possible replication, consideration of species interactions, tight (microcosms) or realistic (mesocosms) control of physico-chemical parameters and limitation of confounding factors.

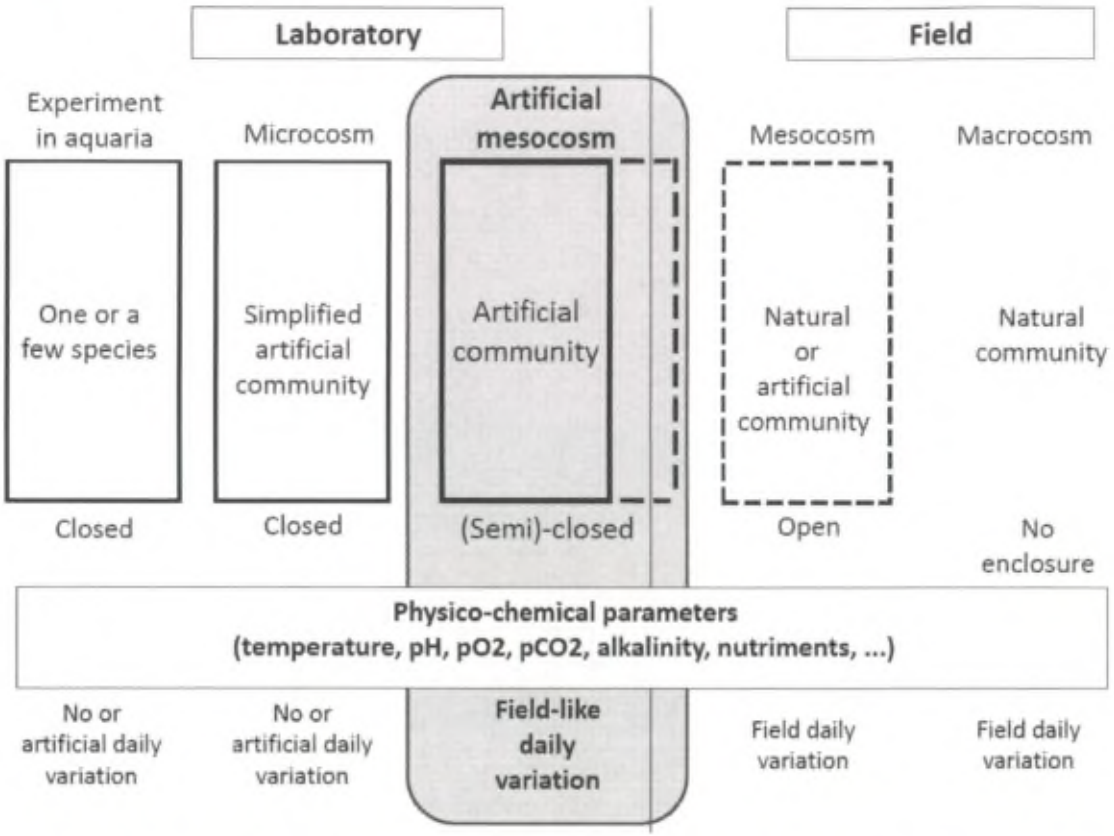

Figure 27 : Different methods which could be used in the studies of the effects of ocean acidification on marine organisms and ecosystems (as discussed in the text).

The growing concern about the effects of OA on marine ecosystems, including on tropical coral reefs, led scientists to favor a mesocosm approach since 1995 (Stewart et al., 2013). For instance, a continuous flow coral reef mesocosm (475 L) was used in studies investigating the impact of OA on a natural coral reef community at long-term (nine months) (Jokiel et al., 2008; Kuffner et al., 2008). Mesocosms like this one, used for long-term experiments allow also to take into account acclimation in a naturally fluctuating environment: seasonal and daily variations of physico-chemical parameters (i.e. light, temperature, carbonate system parameters, oxygen,...). The

mesocosm daily cycle followed the natural cycle thanks to a high flow rate of seawater input pumped from the adjacent reef. Natural recruitment was also possible through this seawater inflow. However, the high flow rate required that OA conditions were reached by HCl addition and total alkalinity (A_T) was therefore lower in the acidified mesocosms1 compared to control ones. Yet, OA does not imply such a change in A_T (at least, not when due to an increased pCO_2) which is known to affect biogenic calcification, particularly in corals (Jury et al., 2009). Recent studies using CO_2 manipulation to modify pH were also conducted in relatively small containers (150 L) which were called "open mesocosm" at short time scales (less than three months) (Comeau et al., 2013a, b; Leclercq et al., 2000, 2002). Moreover, field-like daily variations were not applied to the system. These studies were indeed not performed in mesocosms, according to Odum's definition (1983), but in microcosms.

A recent study by Dove et al. (2013) proposed a longer experiment (nine months) in open artificial mesocosms where small communities were submitted to different pCO_2 conditions. These different conditions were applied progressively during 2.5 months. In the nineties, a large closed reef "mesocosm" was developed (Biosphere 2, Atkinson et al., 1999; Langdon et al., 2000; Marubini et al., 2001). Being artificial, it does not strictly match Odum's definition of a mesocosm. The proposal is thus to refer to this type of "mesocosm" as an "artificial mesocosm" (Fig. 27) as it also differs from microcosms (still according to Odum's definition) by daily variations of physico-chemical parameters which closely mimic the water environment in the field. This was achieved through community activity (respiration and photosynthesis principally) and not through inflow of water from the corresponding natural ecosystem. However, Biosphere 2 was a large and costly project, leading to little possibilities of replication. Therefore, the method used was a "time series intervention analysis" with time intervals of stable conditions of no longer than 2 months and physico-chemical parameters, like A_T and pCO_2,being changed randomly for each time interval and applied to the same artificial ecosystem. Unfortunately, this does not allow for long-term acclimation of the studied organisms and communities.

Recent literature revealed new tools to investigate the impact of OA at the ecosystem level in situ. The "Free CO_2 Enrichment System" (FOCE) experimental devices (Kline et al., 2012; Gattuso et al., 2014) were recently developed. They are encapsulated open natural ecosystems with modern and sophisticated techniques to simulate a pH decrease similar to the one due to OA. Numerous advantages are highlighted: field recruitment, field physico-chemical daily variations, natural community, precise control of stress condition, replication, etc. Nevertheless some of these

promising tools encompass other disadvantages such as elevated costs and more limited accessibility than laboratory-based systems. Being run at relatively large scales and most of the time in the field, it is hard to combine pH decrease with another key factor in global change studies: temperature. Indeed, heating the large seawater masses that run through most FOCE systems would require too much energy (Gattuso et al., 2014). For this particular case, aquaria, microcosms and artificial mesocosms may be better suited. Among these alternatives, the artificial mesocosms best mimicks the natural environment variations and interspecific interactions in the ecosystem.

In the present paper, the design and evaluation of small-scale artificial reef mesocosms are described. The objective was to construct an experimental design which combines most of the advantages of both microcosms and (field) mesocosms at a relatively low cost, which does not necessarily require water input from the natural environment, and which is easily replicable.

2. Design

2.1. Artificial reef mesocosm

The main concern in the design of an artificial reef mesocosm is to build a closed-system in the laboratory (microcosms characteristics: relatively cheap, replicable and easy to access for measurements and observations) with more realistic variations of main physico-chemical characteristics of the water environment (closer to properties usually attributable to field mesocosms), including daily variations. The challenge can thus be summarized by the following question: given data from the monitoring of oxygen, pH, total alkalinity and major nutrients, like N and P in a given location in a natural tropical coral reef, is it possible to closely mimic these values in an artificial system in the laboratory? And if yes, how would the living community organize in such a system? Finally, how useful would it be for scientific investigations, like OA studies?

Figure 28 presents a simplified diagram of the system that was designed. Two identical artificial mesocosms were built in 2005 at UMONS (http://econum.umons.ac.be) and refined/tested until end of 2006. Each one consists of a closed system constituted of one main tank (500 L), 2 experimental aquaria (300 L) and common parts (sump, skimmer, etc). The main tank is holding a diverse community of coral reef microbes, plants and animals. It contains reef substrate handled with the same care as for fish or coral transportation to the laboratory, and its surface is almost completely covered with fast growing coral colonies (Seriatopora hystrix, Acropora muricata, *A. digitifera*, *A. tenuis*, *A. millepora*, *Pocillopora damicornis*, *Montipora patula*, *Stylophora*

pistillata,...). It also contains algivorous animals in such density that it avoids the overgrowth of algae and maintains coral cover (echinoderms, mollusks, crustaceans, reef fishes, ...). Detritivorous animals complete the community to recycle organic matter. The size of this tank is unfortunately not large enough to contain a few predators, hence the biomasses of the different ecological components are controlled manually (addition or elimination of items depending on the change of the community over several months). The installation of the reef community took two years before some ecological equilibrium was reached, characterized by a natural control of N and P macronutrients to concentrations close to those observed in the field (proxy used to assess that N and P cycles were established and stabilized, see results). The flow rate between the sump and the main tank is 14 ± 0.1 L min^{-1}. Experimental aquaria (300 L each) are connected to the common part, which makes the system a paired design. Each experimental aquarium is connected to the sump, but physico-chemical parameters such as pH, pCO_2 or temperature can be controlled independently for each of them. Of course, more experimental aquaria can be connected to the system if required. The flow rate between the sump and each experimental aquarium is 0.8 ± 0.5 L min^{-1}.

According to the present definition of an artificial mesocosm, the whole setup must be carefully controlled, either by biological ways, or by technical systems, to mimic changes in temperature, lighting, pH, pO_2, pCO_2, total alkalinity and macronutrients as observed in natura. The following part of the design section explains how this was possible. The reference location is a lagoon at Réunion Island, the back reef of La Saline fringing reef (21°70'S, 55°32'E). This lagoon offers a great diversity of reef organisms, and environmental data are available thanks to monitoring devices deployed on the site (Cuet, pers. comm., see also Chauvin et al., 2011).

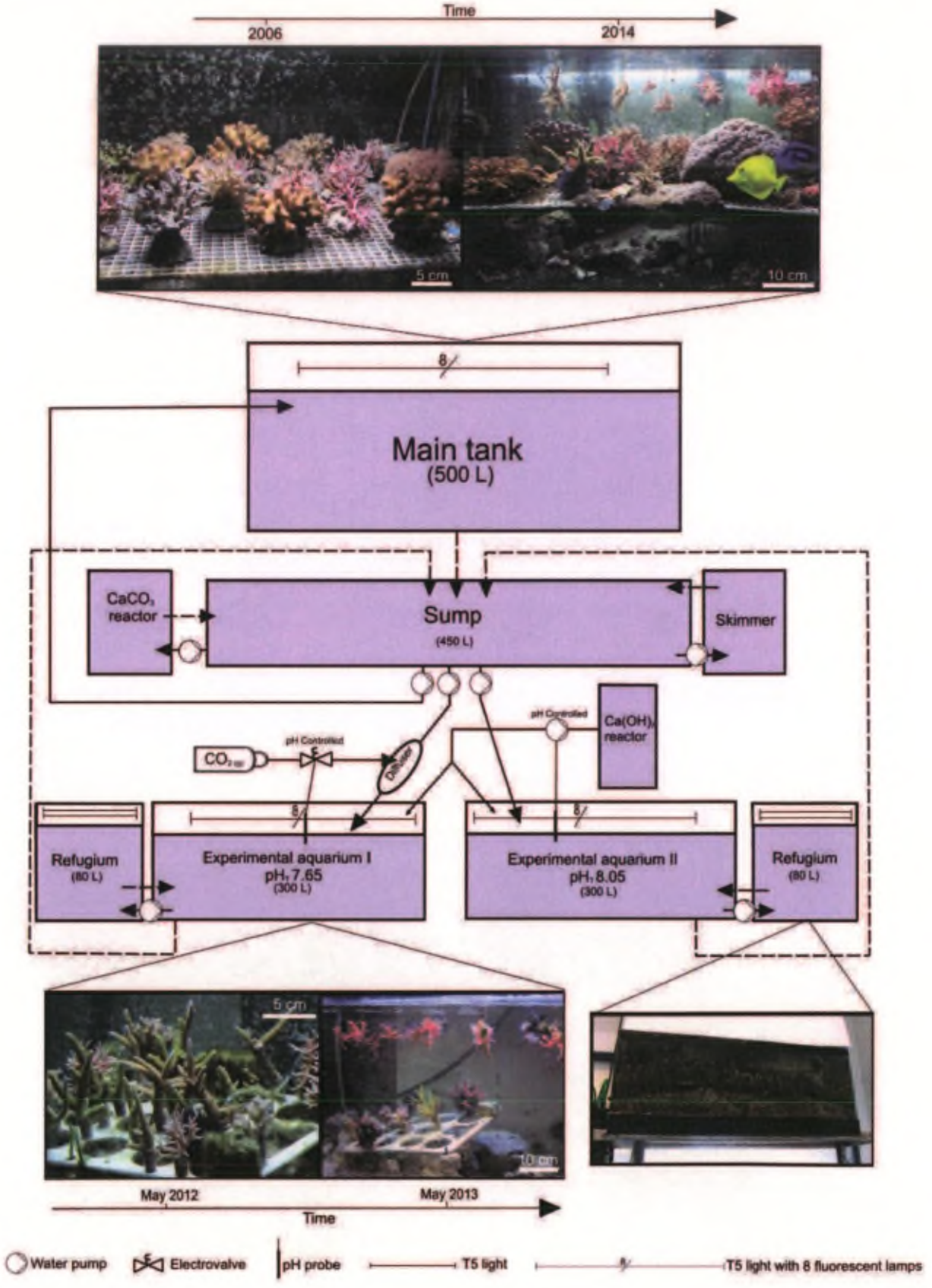

Figure 28 : Artificial reef mesocosm. Main tank is connected to the sump (waterchange= 14 L.min^{-1}). A_T is stabilized using a $CaCO_3$ reactor. A skimmer eliminates the excess of dissolved, colloidal and particulate organic molecules in the water column. pH in experimental aquaria is controlled using CO_2 bubbling and $Ca(OH)_2$ additions. Each of the two experimental aquaria is connected to the sump (waterchange= 0.8 L.min^{-1}). Refugiums connected to each experimental aquarium limit daily oxygen fluctuations. Experimental aquaria as well as main tank temperature are controlled with resistances and electric fans. Pictures illustrate the different parts and their evolution with time.

2.2. Real time monitoring

The artificial mesocosm is fully monitored and controlled using IKS Aquastar devices. These devices are connected to a computer and record the main physico-chemical parameters every 20 seconds (i.e. temperature and pH of each aquarium). In order to be able to check the stability of the system at any time, temperature and pH charts are created automatically and continuously using the R software (R Core Team, 2013). These charts are continuously displayed in the mesocosm room as well as a webpage through the intranet. They are thus available to every mesocosm user.

2.3. Light, temperature, water flow

Light is provided via T5 fluorescent lamps (25:75 actinic blue 420 nm:trichromatic 10000 K, Aqua Medic, Germany) for more flexibility. This allows to switch light on and off 150 progressively by managing groups of T5 lamps with different light duration (Fig. 29) to mimic natural intensity and spectral variation of light.

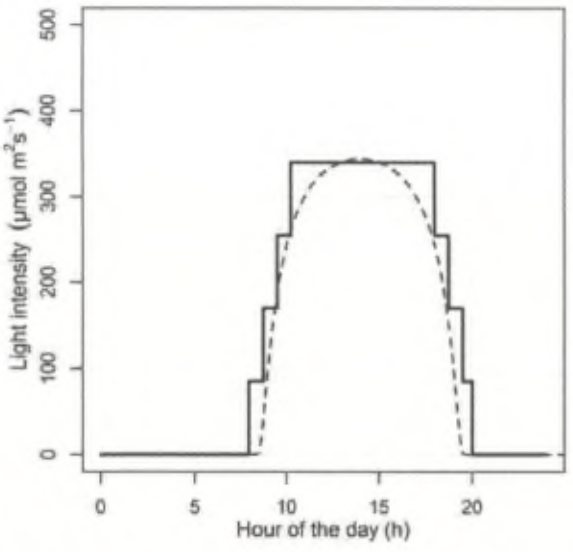

Figure 29 : Light intensity daily cycle. Black line represents the irradiance measured with a Apogee Quantum Meter inside main tank. Dotted line represents the Réunion solar irradiance ajusted with field measurements at 1 m depth(also performed with the same Quantum Meter) .Total day/night time is 12h/12h.

A closed-system allows an easy control of temperature in each experimental aquarium and in the main tank independently. Temperature probes (Aquastar, Germany) are connected to a computer that control both heaters (Eheim Jäger, Germany) and air fans (allowing for slight temperature decrease by water evaporation) or cooling units (for larger temperature decrease, not necessary in our temperate lab) in each experimental aquarium and the main tank. Temperature hysteresis is equal to 0.3 °C. Differential day and night temperatures are obtained by

changing target value as a function of the time of the day. Moreover, water motion is really important in tropical reefs. Many physiological parameters rely on water flow around coral colonies (Badgley, 2006; Carpenter et al., 2007; Finelli et al., 2006; Sebens et al., 1998, 2003; Schutter, 2010). Each aquarium was equipped with two variable speed Tunze - Turbelle stream 6100 driven by a Tunze wave maker to simulate action of waves (from 0 to 40 m^3/h of water flow). The reference site being located in a lagoon, hydrodynamism is relatively low in comparison to, say, the reef crest. It is thus more easily simulated in aquarium.

2.4. Seawater composition and salinity

As the experiment was run far away from tropical reefs, two alternatives were available to obtain seawater: natural seawater from a temperate coast, or artificial seawater. According to the constraint that the system should be easily replicable (including elsewhere), artificial seawater was preferred. It was prepared from ASTM type II water (Milli-G Direct, Millipore, Germany) and a mixture of mineral salts (Reef crystals, Instant Ocean, USA). Before adding it into the mesocosms, newly prepared artificial seawater was mixed and aerated overnight. Ten percent of the mesocosm water volume was changed every two weeks. The evaporation was compensated by addition of the same ASTM type II water using a Tunze 5017 osmolator. This device allows to keep water volume constant, and thus, also stabilizes salinity. Salinity was also checked every two days using a WTW salinometer.

2.5. Oxygen

Oxygen concentration in the field follows a daily cycle around saturation (Kline et al., 2012; see also Fig. 33). It is mainly driven by biological activities (net photosynthesis of photoautotrophs during the day, respiration of all organisms during the night). Daily fluctuations in pO_2 are also observed in closed systems (aquarium, microcosm). Nevertheless, the amplitude of oxygen variations in the laboratory can easily excess natural fluctuations, because of the water volume to biomass ratio that is much lower inside an aquarium than in the field. To avoid unnatural extremes in day versus night pO_2, each experimental aquarium is coupled with a 80 L-refugium. The refugia contain photoautotrophs (Caulerpa spp, Halimeda spp, etc.) and no grazers (hence the name, refugium) and are lighted with an inverted nycthemeral cycle compared to the mesocosm (T5 fluorescent lamps, 10000 K trichromatic, Aqua Medic, Germany). This setup limits the oxygen fluctuations between day and night in the experimental units. Its effects can be modulated through (1) the water flow between the aquarium and the refugium, (2) the biomass of photoautotroph and (3) the light intensity and duration over the refugium.

2.6. Macronutrients

Experience from aquariology and public aquaria demonstrates that it is possible to reproduce to some extend the natural cycles of macronutrients as N and P in closed systems (Adey & Loveland, 2011). A good balance between photoautotrophs, grazers and possibly, some predators, together with efficient recycling of the organic matter can lead to stabilized concentrations in ammonium/ammoniac, nitrites, nitrates and orthophosphates. However, stabilization of these inorganic species close to their natural levels is an additional challenge. Here, the goal is to obtain and maintain near micro-molar concentrations of NH3 + NO_2^- + NO_3^-, and submicromolar concentrations in orthophosphates. The main goal here is thus to set up progressively an equilibrated community of organisms in order to establish N and P cycles close to those observed in the ocean (of course, exchanges with other ecosystems like plankton arriving on a reef with the water currents, or exportation of sinking organic matter to the deep sea have to be simulated by artificial means -e.g., feeding and mechanical filters or settling tank, respectively). This adjustment takes time and is probably one of the hardest and longest stage in the establishment of an equilibrated artificial mesocosm. In our test system, it took two years to reach stability together with correct inorganic N and P concentrations.

Macronutrients cycles are established thanks to four items:

- Feeding the main tank with plankton (frozen Artemia and mysids) to simulate plankton importation from the open ocean. The amount of food provided is dictated by inorganic nitrogen and phosphorus concentrations (more food increases them, whereas less food has the opposite effect over a few weeks period).
- Simulating exportation of a fraction of the particulate matter produced out of the reef by a simple mechanical filter (perlon filter inside the sump weekly changed).
- Simulating the dilution of the organic matter produced in the water column by the living organisms in a similar way as on the reef is impossible, because the ratio water volume to biomass is much lower in the artificial mesocosms. However, a skimmer is a filter able to eliminate a portion of this organic matter, especially amphiphilic molecules. To date, it is the best system available to lower the loading in organic matter in a seawater aquarium (Delbeek & Sprung, 2007), and it is considered as an effective system to keep scleractinian corals healthy in aquarium conditions. One Deltec AP850 skimmer was thus installed in each artificial reef mesocosm.

- Finally, the most important part is the community of photoautotrophs, heterotrophs and bacteria, as well as their respectiver biomasses.With a correct adjustment of the community, by trial and error, we were finally able to stabilize inorganic N and P concentrations to target values and to maintain them over several years.

2.7. Carbonate system

Recent studies highlighted the importance of daily pCO_2 fluctuations in the field (Comeau et al., 2014; Shaw et al., 2013). These daily variations are mainly driven by biological activities, as for oxygen. The aim here was once more to simulate these natural fluctuations, also mainly by biological activities. Furthermore, a pH difference had to be installed between the two experimental aquaria for the purpose of OA experimentations. Therefore, CO_2 bubbling in the inflow water of the high pCO_2 aquarium was used. This bubbling is computer-controlled by a pH probe (Aquastar, Germany) by means of a solenoid valve. In the control aquaria, the pH had to be slightly increased. Calcium hydroxide saturated ASTM type II water was added, also computer-controlled by a pH probe in the control aquarium. Nevertheless, calcium hydroxide has a side-effect as it also increases A_T following:

$$Ca(OH)_2 \Leftrightarrow Ca^{2+} + 2\ OH^-$$

$$(Ca^+ + 2\ OH^-) + 2\ CO_2 \Leftrightarrow Ca^{2+} + 2\ HCO^{3-}$$

In order to keep the same A_T in each experimental aquarium, the problem was solved by adding the same amount of $Ca(OH)_2$ in the high pCO_2 aquarium as well.

In these artificial mesocosms, alkalinity tends to decrease regularly, due to bioaccretion by corals, urchins, mollusks, crustose coralline algae, and other calcifying organisms, despite the addition of $Ca(OH)_2$ that limits this effect. Alkalinity was stabilized globally in each artificial mesocosm by the use of a calcium reactor. The later is a container with solid calcium carbonate material maintained at low pH (around 6, or even less) by CO_2 bubbling controlled by a pH probe. In these conditions, the calcareous material progressively dissolves. A very slow water flow between the reactor and the mesocosm allows an increase of alkalinity in the water. The compensation of alkalinity is controlled by two parameters: the water flow between the reactor and the artificial mesocosm, and the pH maintained inside the reactor. These are adjusted by trial and error following alkalinity measurements in the mesocosms.

2.8. Test case in the experimental aquaria

For the test case OA experiment, a simplified reef community equal in biomass, was introduced progressively in each experimental aquarium. Sea urchins Echinometra mathaei (*E. mathaei violacea* (Mortensen, 1943), violet *Echinometra* (see Arakaki et al. (1998)) were collected at Réunion Island in the Indian Ocean, in the back-reef of Saint Pierre fringing reef (21°33'S, 55°47'E). Corals *Seriatopora hystrix*, *Acropora tenuis* and a half of the coral reef substrate (rocks) came from the aquarium market (Dejong Marinelife, Holland). Other coral species (*Acropora muricata*, *Acropora digitifera*, *Pocillopora damicornis*) and the other half of substrate were collected at Réunion Island in the back-reef of La Saline fringing reef (21°70'S, 55°32'E). Permits were obtained before field collections from "Réserve Naturelle Marine de La Réunion" (RNN164) and "Direction de l'Environnement, de l'Aménagement et du Logement" (DEAL). Organisms collected at Réunion Island were transported to the mesocosm facilities in Belgium (transport duration: 24h) in seawater using Styrofoam boxes. They were acclimated in control conditions during seven months before the beginning of the experiment. Sixteen sea urchins, 0.8 kg of hermatypic scleractinians and 20 kg of reef calcareous substrate were installed in each experimental aquarium. The main unit of each artificial mesocosm contained the same organisms as the experimental aquaria but sea urchins were green *Echinometra* sp B-like (Arakaki et al. (1998)). The main tank was fed five times a week with frozen Artemia and mysis aquarium food (5 g; Ocean Nutrition) and dehydrated red algae (1 g; Nori). Sea urchins fed on macro algae and coralline algae attached to the reef substrate.

The OA experiment consisted of six months of progressive pH decrease in the acidified aquaria followed by seven months of stabilized pH. Major parameters such as temperature, pH, A_T, oxygen, nutrients, calcium and magnesium were monitored/controlled during the whole duration of the experiment.

2.9. Physico-chemical measurements

2.9.1. *Seawater physico-chemical parameters*

The electromotive force (e.m.f) was measured once a day using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor). The e.m.f was then converted to total scale pH value (pH_T) using calibration curves of standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS) (DOE, 1994; DelValls et al., 1998; Dickson et al., 2007). The salinity and temperature were measured once a day using a salinometer pH/Cond 340i WTW (USA). These measurements of pH/T°/salinity were used as one-

point recalibration data for the continuous pH and temperature controllers. Seawater samples (50 mL) were collected daily and immediately filtered (0.22 µm GSWP, Millipore). Total alkalinity was measured by a potentiometric titration using 0.01 M HCl with 0.7 M NaCl following Dickson et al. (2007) but 280 adapted for a smaller volume (25 mL). Each titration was automatically performed by computer using a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyzer to record pH (Consort, Belgium) and a TW Alpha Plus autosampler (SI Analytics, Germany). Calibration was performed using certified reference seawater provided by A. G. Dickson (Scripps Institute of Oceanography, Dixon, batch 94). The pCO_2 was calculated from A_T, pH_T, temperature and salinity data using the R software (R Core Team, 2013) and the package seacarb (Lavigne & Gattuso, 2012; Lueker et al., 2000 's constants for K1 and K2; Perez & Fraga, 1987 's constant for Kf; Dickson, 1990 's constant for Ks). Every two weeks, seawater was sampled, filtered through a 0.22 µm filter (MilliPore), stored in polyethylene bottles and frozen at -20 °C until analysis. NH_4^+, NO_3^- +NO_2^- and PO_4^{3-} were analyzed through an automated colorimetric analysis using a QuAAtro nutrient analyzer coupled to a XY-2 auto sampler (Seal Analytical, Mecquon, Wisconsin, USA). Calibrations were done using standard solutions.

Calcium and total alkaline earth metals (magnesium + calcium + strontium) concentrations were determined monthly by a potentiometric titration method adapted from Kanamori and Ikegami (1980). The titration was automatically performed by computer using a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyzer to record e.m.f (Consort, Belgium) and a TW Alpha Plus auto sampler (SI Analytics, Germany). Calcium concentration was measured by an EGTA (molecular biological grade, VWR) titration using a calcium-selective electrode (Orion, Thermo Fisher Scientific, USA) and a calomel reference electrode (Schott B3510 Ch0, Germany). The total alkaline earth metals were determined by an EDTA (Merck) titration using a divalent cation electrode (Consort, Belgium) and a reference electrode (Schott B3510 Ch0, Germany). Calibrations were performed using certified reference seawater (High-purity standards, USA).

2.9.2. *Modelling of oxygen fluctuations*

The oxygen daily cycle was checked over 5 days at the beginning and monitored in each experimental aquarium at the end of the experiment. Oxygen was recorded using Clark oxygen electrodes connected to the IKS control system. Each probe was calibrated before the monitoring in the air (100 % saturation) and the zero was checked using saturated sodium sulfite seawater solution. Moreover, each probe signal was one-point corrected every day using a WTW oxymeter

and an WTW Oxycal probe, calibrated using 100% O_2 and 0% O_2 ($NaSO_2$ solution in seawater kept away from air contact). Oxygen net fluxes (i.e. net photosynthesis and dark respiration) at the ecosystem level were estimated by calculation using the R statistical software and the simecol package (Petzoldt & Kline, 2007). Net photosynthesis was defined from the following equation:

$$P = P_{max} \cdot (1 - e^{(E/EK)}) + R_{dark} \quad (1)$$

Where P is the net photosynthesis in mmol O_2 . min^{-1}, P_{max} is the maximum net photosynthesis in mmol O_2 . min^{-1}, E is the irradiance in PAR (µmol photons m^2s^{-1}), EK is a constant that define the efficiency of the photosynthesis as a function of irradiance and R_{dark} is the dark respiration in mmol O_2 . min^{-1}.

Considering an aquarium, the oxygen carried in or out of the aquarium by seawater change in the tank is define as

$$\frac{dO2_{exp}}{dt} = \frac{waterchange}{Vol} * (O2_w - O2_{in}) \quad (2)$$

Where waterchange is the volume of water exchanged between the aquarium and the main unit (in $L.min^{-1}$), Vol is the net volume of water in the aquarium (in L), Ox_w is the oxygen concentration in the aquarium and Ox_{in} is the oxygen concentration of the water entering in the aquarium. Since that water is pumped off the skimmer, O_2 is very close to saturation at any time (checked using the WTW oxymeter) and its [O_2] is computed from salinity and temperature of the tank using the R package marelac (Soetaert et al., 2012).

The oxygen exchanged with the air at the surface of the aquarium is calculated as:

$$\frac{dO2_{air}}{dt} = \frac{O2_{in} - O2_w}{\tau} \quad (3)$$

Where the variation of oxygen inside the aquarium is then defined as:

$$\frac{dO2_w}{dt} = \frac{P}{Vol} - \frac{dO2_{exp}}{dt} - \frac{dO2_{air}}{dt} \quad (4)$$

The mathematical model was fitted to real oxygen concentration data and an optimizer was used to find best estimates for P_{max} end P_{dark} (package R simecol, Petzolt & Kline, 2007).

2.10. Data analysis

Statistical analyses were performed using the statistical software R and was fixed to 0.05 for all tests. A_T, pH, salinity, alkaline earth metals and temperature were each analyzed using linear models. Each parameter was tested as a dependent variable of the model and time was the independent variable. Slopes were then tested using t-tests to check if changes with time were significant. Residuals analyses were graphically performed for each model to check normality, homoscedasticity and linearity of the residuals. Comparisons of pH during day and night were performed using paired t-test with the Welch approximation to the degrees of freedom. Comparison of A_T, alkaline earth metals, nitrates, nitrites, ammonium and orthophosphates concentrations between control and treatment aquaria were performed using paired t-tests with the Welch approximation to the degrees of freedom. When possible (enough replicates), normality and homoscedasticity of the residuals were verified using, respectively, a quantile-quantile plot and a Bartlett's test.

3. Results

3.1. Carbonates system

5.2.6. Diurnal variation of pH

The principal physico-chemical parameters are presented in Table 7 by averaged periods: during the acclimation period before the pH decrease (3 months), during the six months decrease and after the decrease, during the seven months stabilized period. It was possible to reasonably simulate natural diurnal variation of pH in each experimental aquarium during the OA experiment (Fig. 30), although it is hard to obtain realistic daily pH changes in acidified conditions. Both mesocosms presented a significantly different pH during the day and the night for each aquarium (paired t-tests, all p-values < 0.001), obtained mainly by biological activities (net photosynthesis during the day, dark respiration during the night, calcification), and just slightly facilitated by increased CO_2 and $Ca(OH)_2$ additions when needed (computer monitored and controlled). The amplitude between night and day in control aquaria was equal to 0.2 pH unit while it was equal to 0.1 pH unit in high pCO_2 aquaria. The amplitude of the pH variations observed in the control aquaria was just slightly larger than that recorded in the reference lagoon as it was adjusted to get an average daily pH_T around 8.1 (Fig. 30). Community-driven pH changes appear lower in acidified aquaria than in control (most of the changes account for a day/night switch in the trigger level for CO_2 and $Ca(OH)_2$ additions).

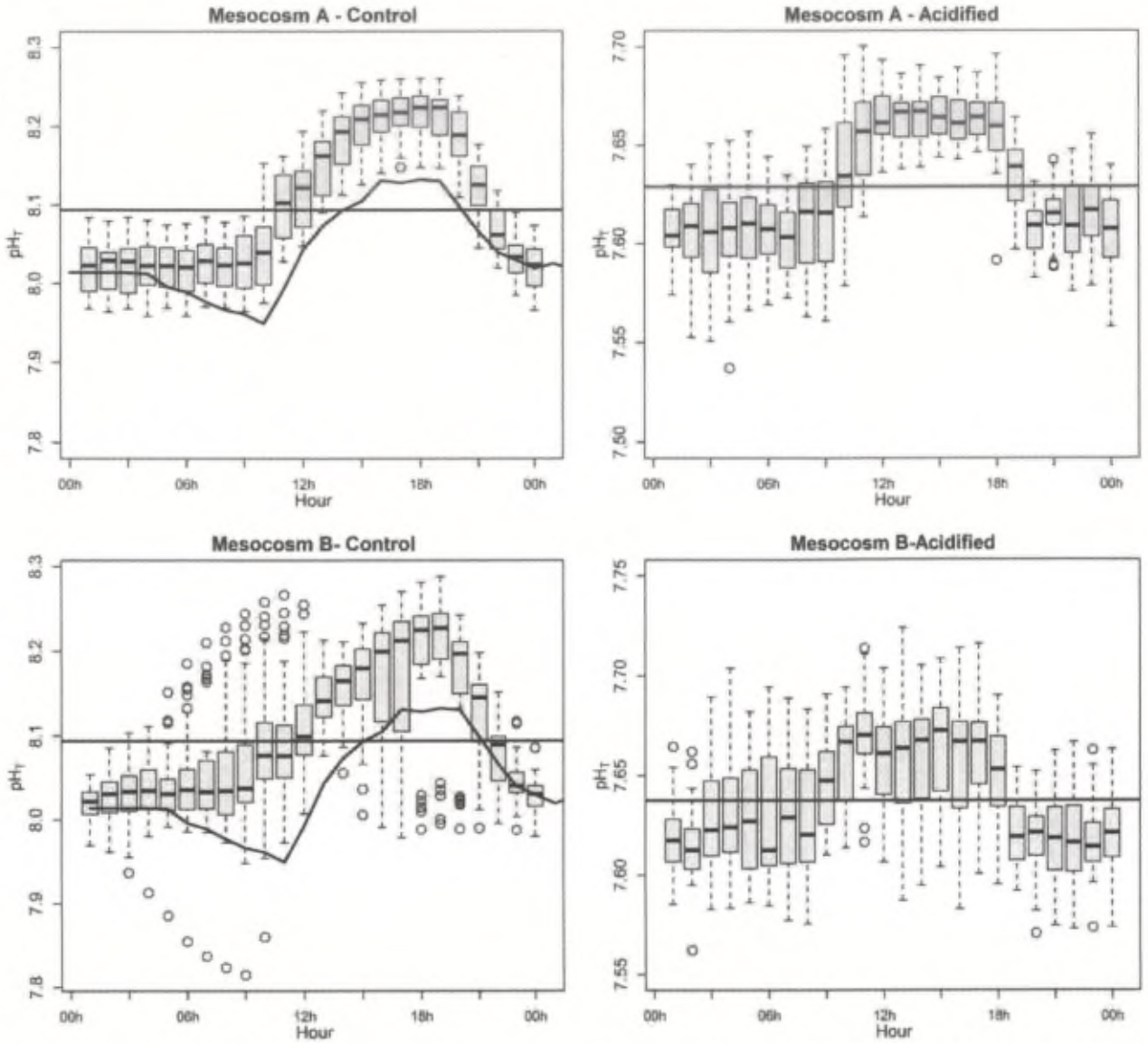

Figure 30 : pH_T diurnal variation inside each experimental aquarium. Boxplots represent median (blackline), interquartile range (box), 1.5 times the interquartile range from the box edges (whiskers) and outliers (individual points). Each boxplot correspond to data recorded each hour of the day during 3 months establishment of contrasted pCO_2 conditions. Median were calculated from measurements recorded every 20 seconds.Black horizontal lines represent the global pH_T mean. Black curves in control aquaria graphs represent the median field variation per hour (La Saline Lagoon, Réunion Island)(Cuet, pers. comm., see also Chauvin et al., 2011). Light is provided from 8h until 20h.

Table 7 : Mean physico-chemical parameters recorded in each aquarium before pH decrease (1 month monitoring), during pH decrease (6 months monitoring) and after pH decrease (7 months monitoring). Values represent means ± standard deviations. Mean temperature and pH were calculated on measurements recorded every 20 seconds. Mean salinity, A_T and pCO_2 were calculated on values measured every day. Calcium and total alkaline earth metals (Ca+Mg+Sr) were calculated on value mesured monthly.

Parameter	Mesocosm A - Control			Mesocosm A - Acidified			Mesocosm B - Control			Mesocosm B - Acidified		
	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease
pH_T	8.04±0.02	8.06±0.02	8.08±0.03	8.07±0.01	7.82±0.11	7.63±0.02	7.99±0.02	8.09±0.03	8.09±0.04	8.05±0.03	7.83±0.09	7.62±0.02
*p*CO2 (ppm)	420±21	388±61	381±40	378±18	784±224	1294±125	402±23	388±61	356±47	484±32	806±249	1263±92
Total alkalinity ($mmol.kg^{-1}$)	2.363±0.110	2.400±0.118	2.406±0.147	2.353±0.107	2.372±0.141	2.447±0.200	2.373±0.123	2.511±0.331	2.350±0.109	2.373±124	2.495±0.204	2.379±0.131
HCO_3 ($mmol.kg^{-1}$)	1.833±0.084	1.836±0.102	1.826±0.128	1.789±0.081	2.009±0.141	2.225±0.177	1.824±0.095	1.899±0.259	1.766±0.105	1.886±0.089	2.115±0.228	2.140±0.121
CO_3 ($mmol.kg^{-1}$)	0.215±0.014	0.229±0.013	0.236±0.021	0.229±0.014	0.148±0.034	0.101±0.009	0.223±0.016	0.251±0.039	0.237±0.015	0.199±0.019	0.156±0.022	0.098±0.006
Ωaragonite	3.843±0.228	4.164±0.246	4.269±0.381	4.406±0.217	2.664±0.561	1.819±0.166	3.936±0.266	4.629±0.786	4.289±0.298	3.487±0.322	2.864±0.374	1.760±0.105
Ωcalcite	5.841±0.344	6.323±0.373	6.480±0.578	6.174±0.327	4.047±0.856	2.274±0.254	5.979±0.402	7.018±1.190	6.511±0.451	5.299±0.488	4.344±0.567	2.672±0.159
Temperature (°C)	25.07±0.19	25.24±0.32	25.13±0.19	24.98±0.07	25.22±0.32	25.06±0.11	25.13±0.12	25.36±0.35	25.10±0.22	25.03±0.11	25.31±0.33	25.04±0.10
Salinity	34.47±0.44	34.14±0.79	34.47±0.44	34.37±0.95	34.13±0.78	34.37±0.95	34.50±0.43	34.70±0.46	34.50±0.43	34.50±0.42	34.70±0.45	34.50±0.42
Ca ($mmol.kg^{-1}$)	11.35±0.02	11.49±0.32	11.55±0.15	11.37±0.01	11.49±0.35	11.56±0.14	11.14±0.09	11.70±0.28	11.54±0.20	11.13±0.09	11.70±0.29	11.56±0.20
Ca + Mg + Sr ($mmol.kg^{-1}$)	64.22±0.52	65.29±2.47	66.08±1.41	64.54±0.85	65.38±2.13	65.79±1.10	63.74±0.44	67.16±2.07	66.02±1.22	63.78±1.16	66.93±2.05	66.39±1.69

5.2.7. *Control of pH and total alkalinity*

The pH was recorded during the thirteen months of the experiment. pH in each control aquarium showed a very small, but significant, increase throughout the experiment (Fig. 31, linear model, slope p-values <0.001). Nevertheless, this increase is lower than 0.02 units.year^{-1}. The acidified aquaria showed a stable pH during the seven last months (stable conditions, linear model, slope p-values ≥ 0.07). During the pH decrease in the acidified mesocosms, the pH slope was fixed to -0.03 unit every two weeks, which is much lower than the diurnal variation. Despite the increasing delta pH between control and treatment aquaria, total alkalinity (Fig. 32) showed no significant difference (paired t-test, all p-values ≥ 0.11) and remained stable during the thirteen month experiment (linear models, all slope p-values ≥ 0.15). Moreover, total alkalinity in the two mesocosms remained very close (within 5 % of variation).

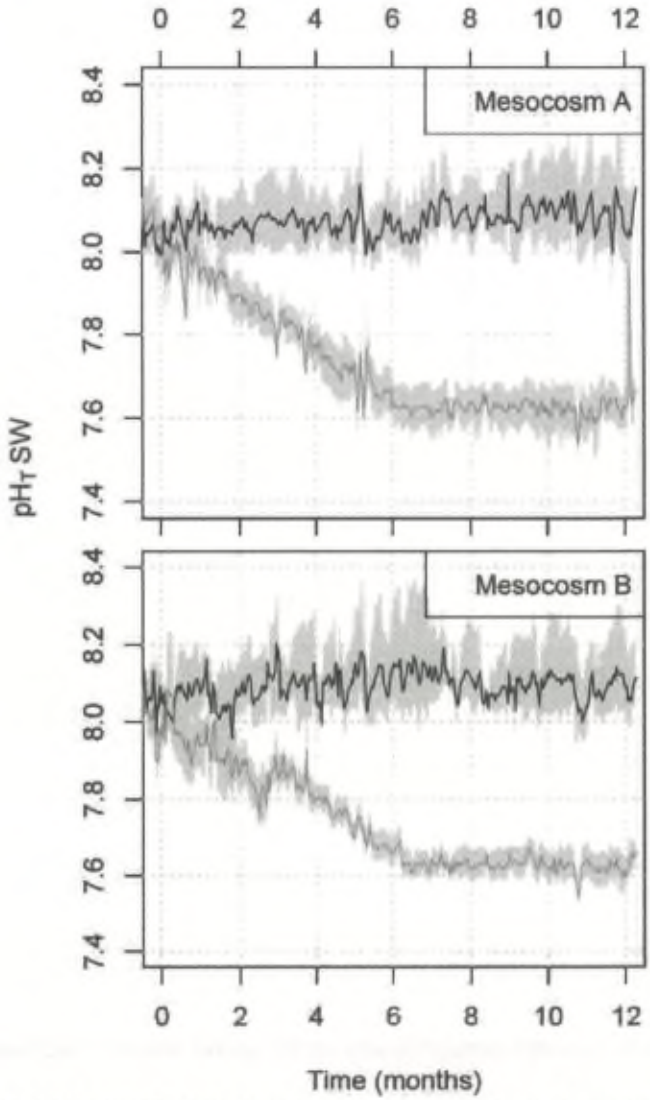

Figure 31 : pH$_T$ evolution inside in each experimental aquaria of both mesocosms during the experiment. Values represent pH$_T$ recorded every 20 seconds and then averaged by days (black lines for controls and grey lines for treatment aquaria). Envelopes correspond to minimum and maximum values per day.

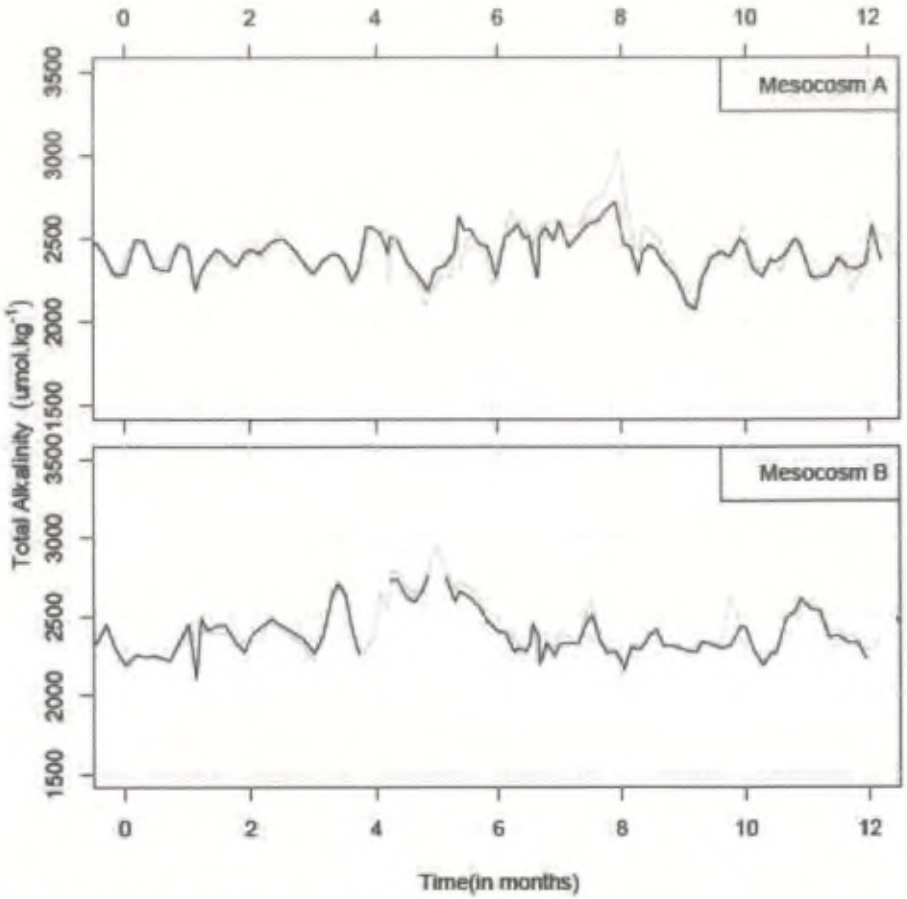

Figure 32: Total alkalinity (A_T) evolution in each experimental aquaria of both mesocosms. Black lines represent the control aquaria, grey lines represent high-CO_2 aquaria. Total alkalinity was measured every 2 days.

3.2. Salinity and temperature

Temperature remained within 1 degree of variation over the whole experiment in all experimental aquaria (Table 7). All experimental aquaria showed a slight temperature decrease during the experiment (slope p-values < 0.001). Nevertheless this decrease was lower than -0.1 °C $year^{-1}$. No difference was observed between experimental aquaria (paired t-tests, p-values ⍰ 0.06). A slight diurnal variation was observed, but only during the period when the highest temperatures were recorded. In this OA experiment test case, slight seasonal changes in temperature (and light) were not taken into account to avoid interference with the decreasing versus stabilized pH phases. However, adjustments for seasonal changes would not be a technical problem. Salinity was also monitored throughout the experiment (Table 7). One mesocosm showed no significant variation through time (slope p-values ≥ 0.21). The second mesocosm showed a slight and significant increase in salinity (slope p-values ≤ 0.001), but under 1 PSU $year^{-1}$. No difference was observed between experimental aquaria (paired t-tests, p-values ≥ 0.43).

3.3. Nutrients

The inorganic nitrogen concentration was studied throughout the experiment. Nitrates, nitrites as well as ammonium concentration remained at concentration levels comparable to that observed in the field (Table 8) and did not vary significantly during the 13 month experiments (slope p-values ≥0.07) except for ammonium, which was slightly higher at the beginning of the experiment. The inorganic phosphorus, i.e. orthophosphates, concentration also remained comparable to target concentration levels and did not vary during the experiment (slope p-value ≥0.07). No difference was observed between aquaria (paired t-tests, p-values ≥ 0.30)

3.4. Calcium and total alkaline earth metals

The calcium concentration (Table 7) did not vary significantly according to time throughout the experiment in all aquaria, nor the total alkaline earth metals concentration (slope p-values ≥ 0.07). Similarly, the ratio Ca/total alkaline earth metals was constant throughout the experiment in all aquaria (slope p-values ≥ 0.19). The mean value recorded in mesocosms (5.71±0.09) was lawer than that in field (6.38). All these parameters did not vary significantly between contrasted pH conditions in both mesocosms (paired t-tests, p-values > 0.11).

Table 8 : Mean nutrients concentrations (in µmol kg^{-1}) in in each aquarium before pH decrease (3 month monitoring), during pH decrease (6 months monitoring) and after pH decrease (7 months monitoring). Values represent means ± standard deviations. Nutrients were quantified every 2 weeks. Maximum field measurements are from Chazotte et al., (2002)

	Mesocosm A - Control			Mesocosm A - Acidified			Mesocosm B - Control			Mesocosm B - Acidified			
Nutrient	**Before decrease**	**During decrease**	**After decrease**	**Before decrease**	**During decrease**	**After decrease**	**Before decrease**	**During decrease**	**After decrease**	**Before decrease**	**During decrease**	**After decrease**	**Max field measurement**
NO_3	0.52±0.78	0.96±0.78	0.87±0.89	0.74±0.49	0.43±0.63	0.47±0.69	1.30±1.25	0.55±0.67	0.84±0.77	0.67±1.11	1.31±0.95	0.80±0.74	2.26 (NO_3 + NO_2)
NO_2	0.11±0.08	0.18±0.12	0.17±0.12	0.11±0.05	0.15±0.06	0.16±0.09	0.14±0.15	0.13±0.06	0.15±0.09	0.15±0.11	0.17±0.11	0.18±0.22	
NH_4	0.80±0.88	0.44±0.32	0.52±0.69	0.90±1.24	0.73±1.04	0.44±0.56	1.20±1.20	0.50±0.49	0.32±0.45	0.91±0.81	0.73±0.66	0.56±0.64	1.08
PO_4	0.42±0.18	0.17±0.24	0.30±0.44	0.30±0.35	0.18±0.17	0.31±0.44	0.13±0.32	0 .49 ± 0.60	0.25±0.34	0.76±0.90	0.18±0.18	0.34±0.44	0.33

3.5. Oxygen

The oxygen concentration in each aquarium was monitored over 5 days at the end of the experiment (Fig. 33). It followed a daily cycle principally driven by biological activities in each aquarium. Oxygen saturation state oscillated between 85 % and 130 %. The oscillations were more important in the control aquaria for both mesocosms. The overall balance of net oxygen fluxes were modeled for each aquarium (Table 9). Biological systems in each aquarium were global sources of O_2, except for the control aquarium of the mesocosm B. No difference was observed between control and treatment aquaria (Table 9) for dark respiration as well as for net photosynthesis (paired t-tests, p-values > 0.05).

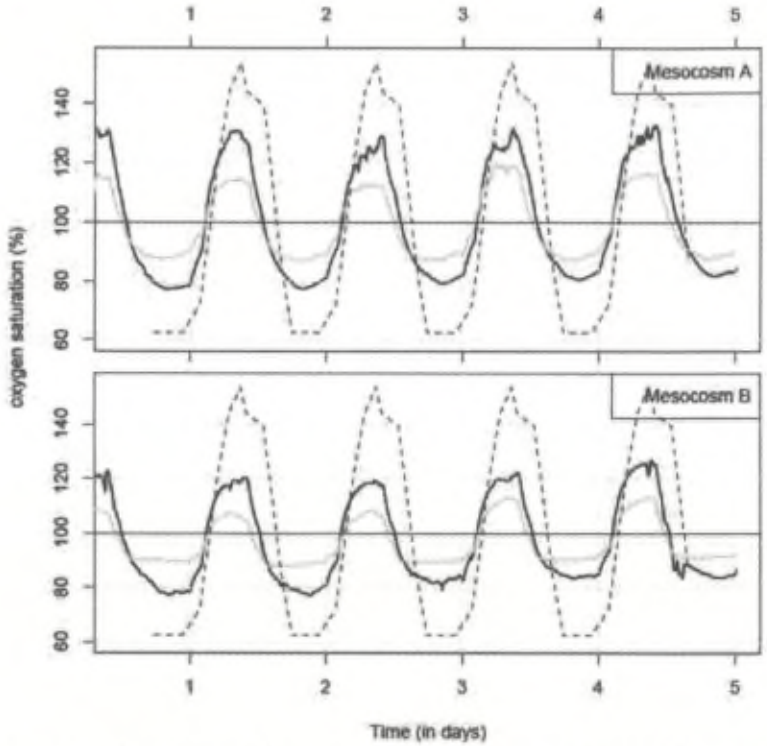

Figure 33: Oxygen saturation in each experimental aquarium of both mesocosms during a 5 days monitoring at the end of the experiment. Black lines represent the control aquaria, grey lines represent acidified aquaria. Dotted lines represent field measurements from Clavier et al. (2008) at La Saline Lagoon, La Réunion. Oxygen concentration was recorded every 20 seconds.

Table 9 : Result from the oxygen net fluxes modeling. Values are best estimates for parameters used in model described at eq. 1

	Mesocosm A - Control	Mesocosm A - Acidified	Mesocosm B - Control	Mesocosm B - Acidified
Net photosynthesis (P; $mmol.h^{-1}$)	11.2	7.7	10.3	7.7
Dark respiration (R_{dark}; $mmol.h^{-1}$)	-7.2	-5.8	-4.7	-7.9
Daily balance ($mmol.h^{-1}$)	4.0	1.9	5.65	-0.2

4. Discussion

Here "artificial mesocosms" are defined as intermediary systems between laboratory-based microcosms and *in situ* mesocosms. Artificial mesocosms can be laboratory-based closed systems (or semi-closed systems on site), but unlike microcosms, they must mimic the physico-chemical environment as closely as possible, including daily changes. This requires first to define a target site in natura and to get it instrumented to obtain records of temperature, oxygen, pH, nutrients, etc. The test case here used a station in the fringing reef of Réunion Island at La Saline as a reference site (Cuet, pers. comm.).

A second requirement is to obtain such variations in the most natural way possible. Consequently, the community of living organisms that establish and thrive in the artificial mesocosms must be directly responsible of much of the daily fluctuations of oxygen, pCO_2 and thus, pH. This is only achieved by a correct balance between photosynthesis and respiration, meaning that photoautotrophs versus heterotrophs biomasses must be carefully adjusted. Macronutrients (N and P in the test case, but also Si if diatoms play a major role) must also be community-controlled as much as possible. N and P cycles must establish as completely as possible and the resulting concentrations in ammonium, nitrites, nitrates and orthophosphates must adjust at levels close to those found in the field. This is a challenge for very oligotrophic ecosystems, like tropical coral reefs, but present results highlighted its feasability.

Obtaining realistic values of oxygen, carbon dioxide and macronutrients was not possible without a small technical input: (1) a refugium with inverted photoperiod was used to limit oxygen (and carbon dioxide) amplitude between day and night due to a lower water volume per biomass unit in the mesocosm than in the field, (2) artificial systems and manipulations were also required to mimic specific parts of N and P natural cycles: feeding with plankton to simulate imports, and filtering water mechanically (perlon) and chemically (skimmer) to simulate exports and dilution of the cocktail of organic matter produced in the water column. However, these are minimal interventions in comparison to heavy biological filters that lead to the accumulation of nitrates, or artificial denitrification filters or chemical phosphate filters that are proposed in the aquarium and aquaculture markets.

A mainly community-driven regulation of the environment is thus possible and can mimic physico-chemical conditions in the field, even in small (1700 L in total) 435 artificial mesocosms that are relatively cheap and easily replicable. Moreover, the twin mesocosms have been in a

steady state now for over five years, indicating that such an equilibrium is sustainable on the long-term. The community had to be adjusted manually from time to time, by eliminating a part of the organisms that grew too much, such as a few coral colonies, or *Caulerpa* algae out of the refugia; or by adding missing components, as replacing dead fish, mollusks or echinoderms. This simulates predation and recruitment that lack for obvious reasons in the present artificial mesocosms. It should be noted that the equilibration of an artificial mesocosm takes time. Two years were required to achieve the described experimental device. However, with experience gathered here, it may be possible to obtain it faster in the future (but probably in no less than one year).

Due to the sometimes excessive use of the term "mesocosm" in the literature to qualify poorly equilibrated communities in more or less artificial environments, the need to preserve Odum's original definitions of microcosms and mesocosms is here emphasized as well as the lack of a term for items in between those two situations."Artificial mesocosm" is proposed. To qualify for the artificial mesocosm "label", a system must contain healthy and fully acclimated living organisms, be community-driven as much as possible (artificial filtration techniques limited to a strict minimum and justified), equilibrated on the long-term (several months, if not years), and should match physico-chemical changes of a reference site in the field (at least for major chemical parameters like oxygen, carbon dioxide, pH, alkalinity, and macronutrients). Clearly, this rules out many laboratory-based systems that must rather be called microcosms.

The next key question, once one got a working artificial mesocosm, is what to do with it. Is it possible to run OA experiments with it? What happens if pH is lowered in the whole artificial mesocosm, or in a part of it like it was done here? Would this break completely the equilibrium, or would the artificial ecosystem be resilient enough to withstand such a change with organisms that remain observable in good conditions? To answer these questions, an original paired design was used with acidified and control conditions installed inside the same artificial mesocosms, using experimental aquaria connected to the main unit. It was shown that a paired design is technically possible, and a way to achieve it was proposed by addition of CO_2 or $Ca(OH)_2$. The latter also brings alkalinity to the system, which was something which would have had to be done anyways to counterbalance net bioaccretion in the coral reef community. However, stripping CO_2 is probably a viable alternative that does not impact alkalinity in systems with no (positive) bioaccretion. The main physico-chemical parameters did not vary between experimental aquaria of the same type, nor between mesocosms during the experiment. Moreover, at the beginning of the experiment, the same simplified biological community was introduced in these aquaria (same species

assemblages and biomasses). The test case OA experiment was a relatively long-term one (over one year), and with a gradual decrease of pH in the acidified aquaria. The purpose of this study was to avoid a brutal change (stress) and to take into account acclimation over several months, both for individual species and for the whole community. Such an experiment is clearly achievable in artificial mesocosms and the living community in the main tank remained stable despite these changes. It should be noted that pathogens are part of the community and episodes of white band disease were observable from time to time. Metagenomic analyses of the coral mucus revealed a large quantity of herpes-like viruses during one such episode (Laghdass and Gillan, pers. comm.). Herpes-like viruses 475 have already been shown to be related to some forms of the white band disease in the field (Soffer et al., 2014). During this OA experiment, the ecophysiological response of the scleractinians and the sea urchins were also studied. Some of these results were published study (Moulin et al., 2014) and more will be published soon. This demonstrated that such community and long-term OA experiments can be run in artificial mesocosms. However, the word "artificial" must be kept in mind. Indeed, whatever the degree of realism, observations are only cautiously transposable to the field and must be verified thanks to in situ observations or experiments.

One particular point concerns the macronutrients. Tropical coral reefs require very low nutrient concentrations (i.e. oligotrophic waters; Cooper et al., 2009). Generally, nutrient concentrations can be very difficult to maintain in closed systems and can rapidly rise to unrealistic concentrations, due to very limited volumes and water changes. An increase in the concentration of macronutrients in the water threatens coral reefs (Szman, 2002), by decreasing calcification rates of hermatypic scleractinians (Marubini & Davies, 1996; FerrierPages et al., 2000), or by increasing the severity of coral diseases (Bruno & Petes, 2003). The present artificial mesocosms did not suffer from these effects because low concentrations of macronutrients were obtained and maintained over several years. On the contrary, limitation of macronutrients is another possibility in such a closed system. The only N and P input here was through feeding (mainly with frozen plankton). Determination of correct feeding levels was done by indirect observations of macronutrient concentrations in order to come close to target levels. This required adjustments over a week, or even multi-weeks time scale. It should also be possible to fix a feeding level per time unit to a given N and P influx (for instance, to match values measured or estimated in the field), and then, to adjust the biomass of the various trophic components of the living community

to reach target macronutrient concentrations in the water. This kind of approach was not tested in the present study.

The concentrations of Ca and Mg ions in seawater partly affect the calcification rate of scleractinian corals and the nature of the precipitated mineral of sea urchins. Indeed, several studies showed that the calcium concentration influenced the calcification rate of corals (Langdon et al., 2000; Marshall & Clode, 2002). Furthermore, their concentration could even increase biological/metabolic effects (Mitsuguchi et al., 2003). Therefore, it is crucial to maintain constant concentration of calcium independently of pH conditions in order to avoid the effect of confounding factors on the calcification rate of corals. The mesocosm approach used here allowed to prevent this problem as concentrations of Ca ions remained constant throughout the experiment.

Daily changes in pH is undetermined for future OA conditions. A recent study showed that this fluctuation could be amplified by ocean acidification (Shaw et al., 2013). Jokiel et al. (2008) worked with an open system and observed daily pH fluctuations. (Wisshak et al., 2012) also took into account these fluctuations mediated through biological activities. More importantly, recent studies have also shown that these fluctuations could modulate the response of a scleractinians coral to OA (Comeau et al. (2014)). In the present artificial mesocosms, the same refugia were used to buffer oxygen and carbon dioxide day/night fluctuations in both control and acidified conditions. The acidified conditions exhibited lower amplitude, but that may be linked with a slightly lower photosynthesis rate at the community level (thus considering both zooxanthellate corals and algae on and inside the substrate). In any way, these results should be considered carefully because the refugia contain algae that are also impacted by OA. Higher photosynthesis activity in the acidified refugia may better buffer global O_2 decrease and CO_2 increase in the experimental aquaria. This constitutes an unwanted side-effect that may be eliminated by deciding amplitudes to use and tuning the refugia (algae biomasses, intensity and duration of light, and water flow to the aquaria) separately in the different subsystems. The same warning probably applies for different temperature conditions.

Species interaction can be very important when dealing with OA. Andersson et al. (2009) working on hermatypic coral calcification showed that the net balance of $CaCO_3$ accretion was declining in higher pCO_2 conditions. This shift demonstrated how the balance between calcifiers and eroders is important, highlighting the importance to conduct experiments at the ecosystem level to take into account interspecific interactions (Kroeker et al. (2012)). Balance of calcifiers and

bioeroders in 525 the community must be considered, especially when $Ca(OH)_2$ is used to increase pH, because consumption of alkalinity by the community must be higher than the alkalinity introduced to the system by $Ca(OH)_2$. The resulting unbalance is better compensated by an additional calcium reactor connected to the main unit.

In the present study, algae, sea urchins, scleractinians corals and all the other organisms had the opportunity to acclimatize to new physicochemical conditions before the OA experiment started. Sea urchins, for instance, were placed in each mesocosm 7 months before the start of the experiment. Scleractinians corals were also introduced 6 months before the start of the experiment. Moreover only neo-formed coral branches (i.e. calcification occuring within the artificial mesocosm) were used for ecophysiological measurements. It allowed to ensure as much as possible that observed effects are due to the treatment. In conclusion, artificial reef mesocosms can be designed, maintained and used for OA experiments. It is certainly possible to connect more experimental aquaria to test a suite of pH values (say 8.2, 8.0, 7.6 and 7.3, for instance). This would allow a much more powerful statistical approach of the analysis than with ANOVAs by means of linear or nonlinear modeling of biological responses in function of pH. On the other hand, four experimental aquaria would also allow to combine pH and temperature changes in a cross-factorial design with mesocosm as a repeated factor (being a paired design). Thanks to its flexibility, replicability, easy access and independence from extreme meteorological factors in the field, artificial mesocosms are a complementary tool to observations and experiments undertaken directly in the field (for instance future studies with FOCE systems; Gattuso et al. (2014).

The present study highlighted that artificial reef mesocosms were a complementary tool of field experiments, allowing an easy manipulation of seawater physico-chemistry and the study of ecophysiological effects on simplified reef ecosystems.

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Impact of elevated pCO_2 on acid-base regulation of the sea urchin *Echinometra mathaei* and its relation to resistance to ocean acidification: a study in mesocosms.

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Abstract

Due to their low metabolism and apparent poor ion regulation ability, sea urchins could be particularly sensitive to ocean acidification resulting from increased dissolution of atmospheric carbon dioxide. Therefore, we evaluated the acid-base regulation ability of the coral reef sea urchin *Echinometra mathaei* and the impact of decreased pH on its growth and respiration activity. The study was conducted in two identical artificial reef mesocosms during seven weeks. Experimental tanks were maintained respectively at mean pH_T 7.7 and 8.05 (with field-like night and day variations). The major physico-chemical parameters were identical, only pCO_2 and pH_T differed. Results indicate that *E. mathaei* can regulate the pH of its coelomic fluid in the considered range of pH, allowing a sustainable growth and ensuring an unaffected respiratory metabolism, at least at short term.

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1. Introduction

From the beginning of the industrial era, human activities have caused an increase of atmospheric carbon dioxide (CO_2), whose concentration has now reached ca. 400 ppm, an unprecedented level in the last 420,000 years (Petit *et al.*, 1999). This increase of atmospheric CO_2 has several consequences, including global warming and ocean acidification (OA) (IPCC, 2013). Indeed, atmospheric CO_2 dissolves in seawater leading to a decrease in pH and carbonate ion concentration, a phenomenon known as OA (IPCC, 2007; Sabine *et al.*, 2004). As a consequence, surface ocean pH is expected to decrease by 0.3-0.4 units by the end of the century according to the "business-as-usual" IS92a scenario (Caldeira & Wickett, 2003; 2005; IPCC, 2007).

OA will impact numerous marine organisms (see e.g. Andersson *et al.*, 2011; Barry *et al.*, 2011). Several physiological processes will be directly or indirectly impacted by the induced acidosis and hypercapnia (Pörtner, 2008). Studies showed that the acid-base balance of extracellular fluids is the first physiological parameter directly affected when the chemistry of seawater is modified (see Pörtner, 2008 for a review). The pH of these fluids appears to be the key parameter at the basis of metabolic effects of OA. Extracellular acidosis can lead, e.g., to reduction of metabolism, ion exchange and protein synthesis (Fabry *et al.*, 2008; Melzner *et al.*, 2009; Pörtner *et al.*, 2004; Pörtner, 2008). In turn, it can affect calcification processes either through metabolic depression or due to the energy cost of H+ elimination (Pörtner, 2008). Thus, the ability of an organism to resist OA depends on its ability to regulate its extracellular pH. Species with a low metabolism and inefficient ion regulatory abilities were suggested to be more sensitive to a high seawater pCO_2 (Melzner *et al.*, 2009).

Sea urchins possess a low metabolism and low ion regulation ability (Shick, 1983). Indeed, the composition of their main extracellular fluid, the coelomic fluid (CF), mainly varies with that of the surrounding seawater (Farmanfarmaian, 1966). Furthermore, gas exchange, e.g. elimination of CO_2, is limited due to the lack of respiratory pigment and active respiratory mechanism, relying only on a favourable diffusion gradient for gas exchange (Farmanfarmaian, 1966). These features could make sea urchins particularly susceptible to OA. Several authors have studied the acid-base balance of the CF in adult sea urchins. Depending on the species, compensation of the pH of the CF (pH_{CF}) was absent, partial or complete. In *Psammechinus miliaris*, the pH_{CF} was reduced after 8 days exposure at seawater pH (pH_{SW}) 7.44 despite some bicarbonate buffering (Miles *et al.* 2007). Spicer *et al.* (2011) also observed a reduction in pH_{CF} which was not fully compensated by an increase in bicarbonate when exposed to pH_{SW} 7.6 during 5 days in *Strongylocentrotus*

droebachiensis. In *Hemicentrotus pulcherrimus*, after a nine-months experiment, the pH_{CF} was reduced from 7.6 to 7.0 when the the pH_{SW} was decreased from 8.1 to 7.8 (Kurihara *et al.*, 2013). On the other hand, Collard *et al.* (2013) showed that in *Paracentrotus lividus*, the pH_{CF} was fully compensated at pH_{SW} 7.7 and partially compensated at pH_{SW} 7.4 after 6 days of exposure. Calosi *et al.* (2013) also reported a complete compensation of the pH_{CF} by accumulation of bicarbonates in *P. lividus* and *Arbacia lixula* after 4 days at pH_{SW} 7.73. In *S. droebachiensis*, the pH_{CF} was totally compensated within 5 days when exposed at pH_{SW} 7.7 (Dupont & Thorndyke 2012). Similarly, Stumpp *et al.* (2012) observed in this species a complete compensation of the pH_{CF} by accumulation of bicarbonates after a ten-days exposure to pH_{SW} 7.51 and 45 days at pH_{SW} 7.7.

All these studies focused on boreal, temperate or Mediterranean-like species. Although several studies addressed the effects of OA on tropical sea urchins, none studied the response of the CF despite the ecological importance of some of those species in tropical ecosystems, including coral reefs (Albright *et al.*, 2012; Courtney *et al.*, 2013; Shirayama & Thornton, 2005; Uthicke *et al.*, 2012). Moreover, seawater pCO_2 in shallow coral reef fluctuates daily and naturally through community respiration and photosynthesis. Recent study showed that this cycle could be amplified by OA (Shaw *et al.*, 2013), highlighting the importance to conduct experiment in more natural fluctuating conditions. It is also noteworthy that all the previous studies were conducted on sea urchins fed ad libitum, a condition not always encountered in natural conditions. Now, food limitation is known to reduce respiration rate (McClanahan & Kurtis, 1991) and the buffer capacity of the CF (Collard *et al.*, 2013), both factors being involved in the acid-base balance of the CF.

In the present study, we evaluated the effect of moderate OA (pH 7.7), as expected in 2100, on the sea urchin *Echinometra mathaei*, a key species of coral reef ecosystems. The study was conducted in artificial reef mesocosms. Sea urchins fed only on macroalgae and coralline algae attached to the reef substrate to obtain more natural food conditions. We studied the response of growth, metabolism and acid-base parameters of the CF (pH, pCO_2 and total alkalinity) after a two-weeks gradual decrease of pH followed by a five-weeks maintenance of pH contrasted condition (in order to allow acclimatization).

2. Materials and methods

2.1. Experimental design and specimen origin

The experiment was conducted in two separated, independent but technically identical, artificial reef mesocosms. Each mesocosm included one main unit (500 L) and two experimental

aquaria (each 300 L): one control at target pH expressed in total scale (pH_T) 8.05 and one acidified at target pH_T 7.65. Both experimental aquaria were connected to the same main unit and the water flow was the same for both aquaria (800 ± 50 mL min-1). Thanks to such a paired design, they share very close physico-chemical parameters except for pH and pCO_2. The physico-chemical parameters were (mean (± SD)): salinity 35 (±1), temperature 25 (±1) °C, photosynthetically active radiation (PAR) 200 (±50) µmol photons.m-2.s-1, total alkalinity (AT) 2600 (±200) µmol.kg-1, oxygen near saturation, total inorganic nitrogen (NH3/NH4+ + NO2- + NO3-) <2 µmol kg-1 and orthophosphates <1 µmol kg-1.

A pH electrode (Aquastar, Germany) and a temperature sensor (Aquastar, Germany) were immersed in each experimental aquarium and connected to a control system (IKS, Aquastar, Germany). The pH and temperature were recorded every 20 seconds. The pHNIST (National Institute of Standards and Technology) and the electromotive force (e.m.f) were measured once a day using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor) calibrated with pH_{NIST} buffers 4.01 and 6.87 (SI Analytics GmbH, Germany). The values of e.m.f. and sequential measurements of the e.m.f. of the cell using standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS), were applied on the calculation of the pH_T (DOE, 1994; Del Valls & Dickson, 1998; Dickson *et al.*, 2007). The target pH value set up in the control system was calculated according to the difference observed between the pH measured by the pH electrode connected to the IKS and the pH_T. The A_T and pH of the control aquaria were maintained by addition of calcium hydroxide (Merck EMSURE®, Germany) saturated in distilled water using a "kalkwasser" stirrer (Aqua Medic, Germany) and a peristaltic pump (MS-CA 2/820, Ismatec, Germany) controlled by the control system. The acidified condition was obtained by addition of CO_2 (Air Liquide) using a gas/water exchanger (reactor 1000, Aqua Medic, Germany) and a solenoid valve (Aqua Medic, Germany) controlled by the IKS system. The contrasted conditions of pH were established gradually during 2 weeks (-0.03 units of pH_T every day) and then maintained during 5 weeks. The daily variation of pH_T was maintained at a level similar to field conditions as measured in La Saline fringing reef (Réunion Island, 21°70'S, 55°32'E) (mean ± SD = 8.04 ± 0.06, N=1344, from 2011/05/10 to 2011/06/13, pH measured every 15 minutes, Cuet, pers comm, see also Chauvin *et al.*, 2011). In the field, pH increased between sunrise and zenith time on average by 0.18 units (± 0.05). From this moment and sunrise, the pH decreased approximately by the same value. On our mesocosm, pH variation was principally due to community respiration/photosynthesis and for a minor part to the precision of the control

system. Indeed, addition of calcium hydroxide and CO_2 was triggered when seawater pH respectively decreased and increased by 0.05 units from target pH value. Moreover, in acidified aquaria, the target pH value differed between night and day from 0.05 units to obtain the same diurnal pH fluctuations than in control aquaria.

The dissolved oxygen was always maintained near saturation. Light was supplied by T5 fluorescent lamps (25:75 actinic blue 420 nm:trichromatic 10000 K, Aqua Medic, Germany) at a 12h dark:12h light cycle and lamps were switched on/off progressively to reproduce sunrise and sunset. An inversed nycthemeral cycle compared to the mesocosm lighting (T5 fluorescent lamps, 10000 K trichromatic, Aqua Medic, Germany) was established in a refugium (80 L) containing Caulerpa sp., connected to each experimental aquarium, allowing to keep oxygen near saturation during night.

The salinity and temperature were measured once a day using a conductivity meter pH/Cond 340i WTW (USA). The target temperature value set up in the control system was calculated according to the difference observed between the temperature measured by the IKS sensor and that measured by the WTW device. Seawater was filtered mechanically (perlon® filter mat) and using a skimmer (Deltec AP850). Moreover, a tank (240 L) filled with reef substrate received seawater from the main unit and experimental aquaria and acted as a biological filter (Delbeek & Sprung, 1997). Waves were simulated by an aquarium wave maker (two Tunze - Turbelle® stream 6100 driven by a Tunze wave maker). Artificial seawater was prepared from distilled water and a mixture of mineral salts (Reef crystals, France). Before inflow in mesocosms, newly prepared seawater was mixed and aerated overnight. Ten percent of seawater was changed every 2 weeks and the evaporation was compensated by distilled water.

Seawater samples were collected every day and immediately filtered (0.22 µm GSWP, Millipore) in order to determine AT. This was carried out by a potentiometric titration with HCl following Dickson *et al.* (2007) adapted for a smaller volume (25 mL). Calibration was performed using certified reference seawater provided by A. G. Dickson (Scripps Institute of Oceanography, Dixon, batch 94). The pCO_2 was determined from A_T, pH_T, temperature and salinity data using the program R (R Core Team 2013) and the package seacarb (Lavigne & Gattuso, 2012) (Lueker *et al.* 2000 's constants for K1 and K2; Perez & Fraga 1987 's constant for Kf; Dickson 1990 's constant for Ks).

A simplified reef community, equal in biomass, was introduced in each experimental aquarium. It consists of three species of hermatypic scleractinians (*Seriatopora hystrix, Acropora tumida* and *Montipora patula*), sea urchins (*Echinometra mathaei violacea* (Mortensen, 1943), violet Echinometra, see Arakaki *et al.* 1998) and reef calcareous substrate with its diverse communities of algae, bacteria, archae, fungi and meiofauna (sponges, crustaceans, molluscs and polychetes). Corals and substrate came from aquarium market (Dejong, Holland). *E. mathaei* (test diameter: 20-40 mm) were collected at Réunion Island in the Indian Ocean, in the back-reef of Saint Pierre fringing reef (21°33'S, 55°47'E). This zone, in major part covered with detritic sediment, is characterized by a low algae cover (coralline algae and encrusting pheophyta), a low coral cover (10-15 %) and a high sea urchins density (ca. 30 individuals.m-2) resulting in low food availability and competition between sea urchins. The experimental community composition was similar to field conditions. Immediately after collection, sea urchins were transported in seawater in styrofoam boxes to mesocosm facilities in Belgium (transport duration: 24h). They were acclimated in control condition during 1 month before the beginning of the experiment. Twelve sea urchins were distributed randomly in each experimental aquarium to obtain high sea urchins density like in natural collection site. Sea urchins fed on macroalgae and coralline algae attached to the reef substrate (no additional food).

2.2. Growth and respiration rate measurement

The growth (%) of the skeleton of sea urchins was assessed by the buoyant weighing method adapted from Jokiel *et al.* (1978). The dry skeletal weight of sea urchins in air (Wa in g) was computed by applying Archimedes' principle:

$$Wa = \frac{\mathrm{Wsw}}{1 - (\frac{\rho sw}{\rho s})}$$

Sea urchins were weighed (± 0.003 g) in seawater (Wsw in g) at the beginning and at the end of the experiment. The density of seawater (ρsw in g.dm-3) was calculated using its temperature and salinity (measured as previously described in section 2.1.). Twenty sea urchins of the same batch as those used in the experiment were sacrificed. Their skeleton was cleaned using NaOH 2M and then dried for 24 h at 70 °C. The density of the skeleton (spine, test and Aristotle's lantern) (ρs in g.dm-3) was determined by buoyant weighing method. The growth was calculated as the change in dry skeletal weight divided by the initial dry skeletal weight. Sea urchins were not starved prior to buoyant weight measurements in order to avoid an effect on AT of CF (see Collard *et al.* 2013). Because the final measure is relative, the impact of ingested calcareous material is low.

The respiration rate was determined using homemade intermittent respirometers once a week after 3 weeks of establishment of the contrasted conditions. The wet weight of sea urchins (6 individuals per experimental aquaria) was measured (± 0.003 g) and three individuals were placed in each respirometer (1.2 L). There were 2 respirometers per experimental aquaria. Each respirometer was placed inside a thermostated water bath at a controlled temperature of 25 °C on a magnetic stirring unit. Oxygen electrode (Aquastar, Germany) was immersed inside the respirometer chamber through a sealed operculum and oxygen concentration was recorded by means of a control system (IKS Aquastar, Germany). Oxygen electrode was calibrated by water-saturated air as 100 % O2. Water inflow came from the experimental aquarium corresponding to the origin of sea urchins. It was open for 1h (acclimation phase) and then stopped for 1h (respiration measure phase). Oxygen concentration (mg L-1) was recorded every 8 seconds and never fell under 80 % saturation. The sequence open/closed respirometer was carried on 3 times. Linear regression (R2 > 0.9) of seawater oxygen concentration over time was computed for the 3 successive respiration measures in closed phase and the median was calculated as representative respiration rate. This value was converted into µmol.L-1.h-1 O2, multiplied by water volume of respirometer and divided by the sea urchin wet weight. As the rate of oxygen consumption by the electrode itself was very low comparatively to rates of oxygen consumption by sea urchins (< 0.01 %), no correction was applied. Background respiration was not cheeked during respiration rate measure as seawater circulating was the same regardless the pH of seawater (due to mesocosm design, see 2.1).

2.3. Carbonate chemistry of the coelomic fluid

After 3 weeks in final established conditions, the pH_T, the pCO_2 and the A_T of the CF were measured once a week during 3 weeks. About 2.5 mL of CF (6 individuals per experimental aquaria, different from those using for respiration rate measurements) were collected in each individual through the peristomial membrane using a syringe (21G, Terumo, Japan). The CF pH_{NIST} and e.m.f. were immediately measured after collection using a 827 pH Lab Metrohm meter (Switzerland) with a microelectrode (6.0224.100, Metrohm). Calibration and e.m.f. values conversion to pH_T were done as previously described (section 2.1). Salinity was assumed to be the same as seawater of experimental aquaria from which sea urchins came as the composition of CF of sea urchins is known to be close to that of seawater (Farmanfarmaian 1966).

The pCO_2 of the CF was measured by equilibration of about 1g of samples (± 0.2 mg) with a known volume of air (49 mL) at a known pCO_2 in a closed system (syringe 50 mL, Terumo, Japan)

maintained at a constant, known temperature and pressure. Air came from a scuba tank. The system was agitated during 1h using a shaking platform (POS-300, Grant Bio) at 300 rpm in order to achieve equilibration. Time agitation was first tested to ensure that a full equilibration with different pCO_2 samples was obtained. The pCO_2 of the air before inclusion in the closed system and after equilibration was measured using a CO_2 infrared gas analyser (Li-840, LI-COR, USA). Air contained in the 50 mL syringe was introduced in gas analyser through constant hand pressure during 1 minute until a constant pCO_2. The pH_T of the sample after equilibration was measured as described previously. After equilibration, the pCO_2 of the air was assumed to be equal to the pCO_2 of the sample. The total carbon (C_T) of the sample after equilibration was then determined from pH_T, pCO_2, temperature and salinity after equilibration data using seacarb. C_T after equilibration was converted into amount of carbon by multiplying by the weight of the sample. The difference between the pCO_2 of air before and after equilibration was converted into amount of carbon through the equation of ideal gas law (P.V = n.R.T). This corresponds to the amount of carbon flowing from the sample to air and was added to the amount of carbon after equilibration. This sum corresponds to the amount of carbon present in the sample before equilibration. C_T before equilibration was calculated by dividing the amount of carbon before equilibration by the weight of the sample. The pCO_2 of the sample before equilibration was finally determined from pH_T (measured just after collection of CF), C_T, temperature and salinity before equilibration data using seacarb. This method was tested on seawater with a known A_T (certified reference seawater by Andrew G. Dickson, Scripps Institute of Oceanography, Dixon, batch 94). The pCO_2 measured with our method had a random deviation of 4.7 % from the pCO_2 determined from pH_T (measured just before introduction of sample in the closed system), A_T, temperature and salinity data as previously described.

In order to determine the A_T of the CF, a manual titration was performed on 0.5 mL samples using HCl 0.02 N in NaCl 0.7 mol kg-1 and calculations were done using the Gran function (Gran, 1952) (see Collard *et al.* 2013). Certified reference seawater (by Andrew G. Dickson, Scripps Institute of Oceanography, Dixon, batch 94) were titrated using the same method. Certified reference seawater measurements had a random deviation of 2 % of the original batch value. The theoretical A_T due to seawater compounds was also calculated from pH_T, pCO_2, temperature and salinity data using seacarb. A_T of the CF was also determined from sea urchins immediately after collection on the field.

2.4. Data analysis

Normality and homogeneity of variances were checked before all analysis. In order to determine if the variation of salinity, temperature and alkalinity during the experiment was significantly different between mesocosms, a two-factor ANOVA with repeated measures on one factor (cross factors mesocosm and pH condition, repeated fixed factor time) was performed. The pH_T, A_T and pCO_2 of the CF were analyzed using a three-factor ANOVA with repeated measures on one factor (random factor individual nested in cross factors mesocosm (random) and pH condition (fixed), repeated fixed factor time). In order to determine if growth of sea urchins was significant between the beginning and the end of the experiment, a paired Student's t-test with Welch correction was performed. The effect of seawater pH on growth of sea urchins was tested using a two-factor ANOVA (cross factors mesocosm (random) and pH condition (fixed)). The analysis of respiration rate was performed by means of a three-factor ANOVA with repeated measures on one factor (random factor respirometer nested in cross factors mesocosm (random) and pH condition (fixed), repeated fixed factor time). All mean multiple comparisons were performed using Tukey tests. All tests were conducted according to Doncaster & Davey (2007) and using the program R. The level of significance α was set at 0.05.

3. Results

3.1. Physico-chemical parameters in mesocosms

The pH_T of experimental aquaria was maintained closed to target values throughout the experiment, as well in control conditions than in acidified conditions (Fig. 34, Table 10). The daily variation of pH_T was maintained to at a level similar to field conditions (see Methods section). A_T, salinity and temperature were closed to desired values (Table 10) and did not differ significantly according to pH treatment (due to experimental aquaria conception). A_T and salinity did not vary significantly according to mesocosm (ANOVA, $F(1,167) \leq 1.244$, $P \geq 0.268$). However, alkalinity increased throughout the experiment, in all aquaria, due to experimental setup difficulties to maintain this parameter. Temperature varies significantly with mesocosm factor (ANOVA, $F(1,167) = 14.404$, $P < 10\text{-}3$) but the mean of the difference was small and in the range of precision of the control system (< 0.3 °C).

Table 10 : Mean seawater conditions during the 42 days of the experiment. Values between brackets are standard deviations. Mean temperature and pH was calculated on measurements recorded every 20 seconds. Mean salinity, A_T and pCO_2 were calculated on values measured every day

Time (days)	pH condition	Mesocosm	Salinity	Temperature (°C)	pH_T	A_T (µmol.kg-1)	pCO_2 (ppm)
0-3 (before pH decrease)	control	A	33.5 (1.9)	25.2 (0.6)	8.05 (0.05)	2226 (66)	384 (61)
		B	35.0 (0.3)	25.1 (0.6)	8.07 (0.04)	2293 (76)	367 (47)
	acidified	A	33.5 (1.8)	25.6 (0.8)	8.04 (0.06)	2229 (67)	401 (96)
		B	35.0 (0.3)	25.3 (0.7)	8.05 (0.07)	2292 (70)	399 (82)
4-10	control	A	34.5 (0.2)	25.1 (0.4)	8.03 (0.06)	2630 (93)	478 (78)
		B	34.4 (0.1)	24.7 (0.3)	8.05 (0.05)	2506 (69)	430 (60)
	acidified	A	34.5 (0.1)	25.4 (0.7)	7.90 (0.14)	2638 (93)	716 (63)
		B	34.4 (0.1)	24.9 (0.6)	7.93 (0.07)	2490 (64)	601 (119)
11-17	control	A	34.3 (0.1)	25.3 (0.5)	8.07 (0.05)	2683 (41)	437 (63)
		B	34.3 (0.1)	24.9 (0.4)	8.07 (0.06)	2715 (31)	439 (71)
	acidified	A	34.3 (0.1)	25.7 (0.8)	7.76 (0.09)	2711 (61)	1061 (286)
		B	34.2 (0.1)	25.3 (0.7)	7.77 (0.15)	2611 (163)	1003 (292)
18-42 (contrasted pH conditions established)	control	A	34.7 (0.4)	25.1 (0.5)	8.02 (0.06)	2635 (268)	498 (85)
		B	35.0 (0.3)	24.8 (0.4)	8.02 (0.05)	2685 (72)	501 (67)
	acidified	A	34.7 (0.4)	25.5 (0.8)	7.61 (0.06)	2699 (311)	1502 (299)
		B	34.9 (0.3)	25.0 (0.6)	7.62 (0.05)	2685 (112)	1445 (193)

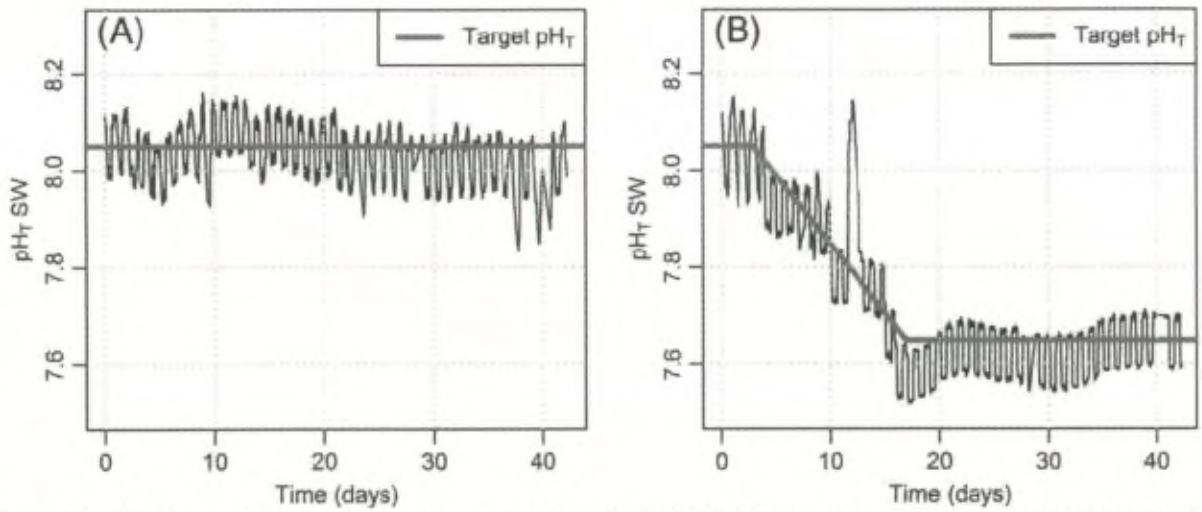

Figure 34 : pHT of seawater in a representative control (A) and acidified (B) aquarium during the experiment (days since the beginning of the experiment). The grey line represents the target pHT (8.05 in (A), 7.65 after progressive establishment in (B)). The daily variation of pHT was purposely maintained to match daily changes in the field.

3.2. Physiological parameters

No mortality was observed during the experiment. Growth of sea urchins at the end of the experiment was significant (mean ± SD = 7.00 ± 9.09 % of initial dry skeletal weight, Paired t test, t42 = 5.19, P< 10-3) (Fig. 35). However, some individuals exhibited a negative growth, explained by loss of spines due to manipulation, although sea urchins appeared to be in good health (active movement of spines and tube feet). These outliers (corresponding to sea urchins that lost spines identified during the experiment, n= 2, growth < -5 % of initial dry skeletal weight, represented in the Fig. 35) were removed from growth data to respect normality and homogeneity of variances.

Neither the pH of seawater (ANOVA, F(1,42) = 0.27, P= 0.61) nor the mesocosm (ANOVA, F(1,42) = 2, P= 0.12), nor the interaction of both factors (ANOVA, F(1,42) = 2.55, P= 0.12) had a significant effect on growth of sea urchins.

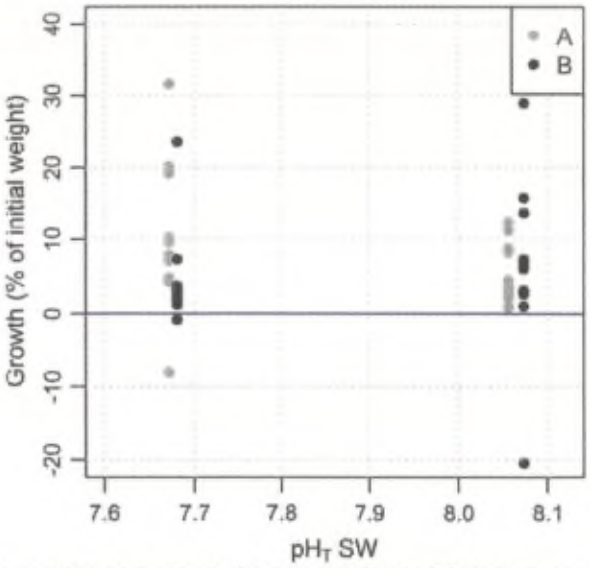

Figure 35 : Skeletal growth (as percentage of the initial skeleton weight) at different pH conditions in mesocosm A (grey) and mesocosm B (black) after the 7 weeks of experiment.

Time had not effect on the respiration rate of sea urchins once final conditions were established (ANOVA, interaction of mesocosm, pH and time, F(1,23) = 0.26, P= 0.78). So, results are presented as means of measurements made during the 3 last weeks of the experiment (Fig. 36). Respiration rate did not differ according to pH (ANOVA, F(1,23) = 14.7, P= 0.16) or mesocosm (ANOVA, F(1,23) = 3.25, P= 0.15) or the interaction of both factors (ANOVA, F(1,23) = 0.019, P= 0.90).

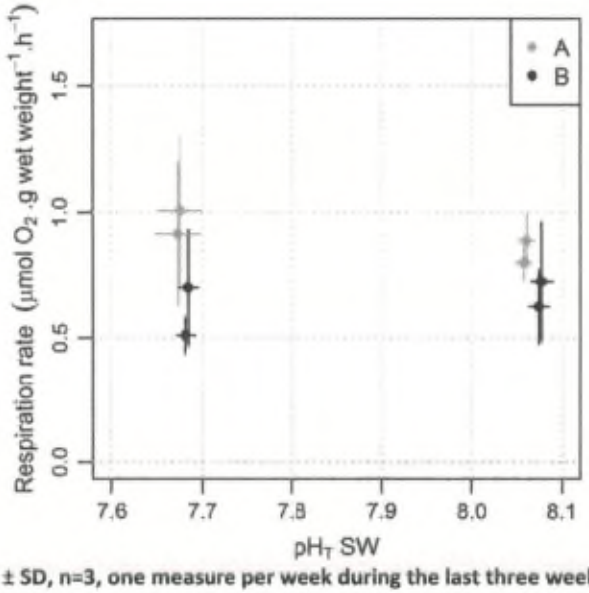

Figure 36 : Respiration rate (mean ± SD, n=3, one measure per week during the last three weeks of experiment) (2 respirometers per pH and mesocosm, 3 individuals per respirometer) at different pH conditions (mean ± SD) in mesocosm A (grey) and mesocosm B (black). The mean of seawater pHT was calculated on values recorded during the respiration rate measure. The mean seawater pHT of respirometer from the same aquarium was shifted by 0.003 unit to make the interpretation easier

3.3. Acid-base regulation of the coelomic fluid

The difference between the A_T of the CF and the A_T of the seawater (at the moment of the measurement in CF) was calculated as ΔA_T, in order to take into account variation of A_T in the mesocosms during the experiment. For all aquaria, time had no effect on pH_T, ΔA_T and pCO_2 of the CF once final conditions were established (interaction of mesocosm, pH and time, ANOVA, $F(1,23) \leq 1.479$, $P \geq 0.241$). Consequently, results are presented as means of measurements made during the 3 last weeks of the experiment. The pH_T of the CF did not vary significantly with the pH of seawater (ANOVA, $F(1,23) = 2.54$, $P = 0.36$), nor with the mesocosm (ANOVA, $F(1,23) = 0.95$, $P = 0.34$) (Fig. 37B). On the other hand, the pCO_2 of the CF was significantly higher at low pH (ANOVA, $F(1,23) = 526$, $P=0.028$) whatever the mesocosm (ANOVA, $F(1,23) = 0.017$, $P = 0.90$) (Fig. 37C). The ΔA_T was also significantly higher at low pH (ANOVA, $F(1,23) = 5073$, $P=0.009$) with no difference between mesocosms (ANOVA, $F(1,23) =0.994$, $P = 0.33$) (Fig. 37D). ΔA_T of sea urchins determined at collection site in the field was 2234 ± 406 µmol.kg-1 (mean ± SD). The difference between the measured A_T and the calculated A_T of the CF (from pH_T and pCO_2 using seacarb) was not significantly affected by the pH, the mesocosm nor by the interaction of both factors (ANOVA, $F(1,23) \leq 3.48$, $P \geq 0.33$). Moreover, for all aquaria, time had no effect on this difference (ANOVA, $F(1,23) = 1.48$, $P \geq 0.24$). Results are therefore presented as means calculated on measurements made during the 3 last weeks of the experiment (Fig. 38).

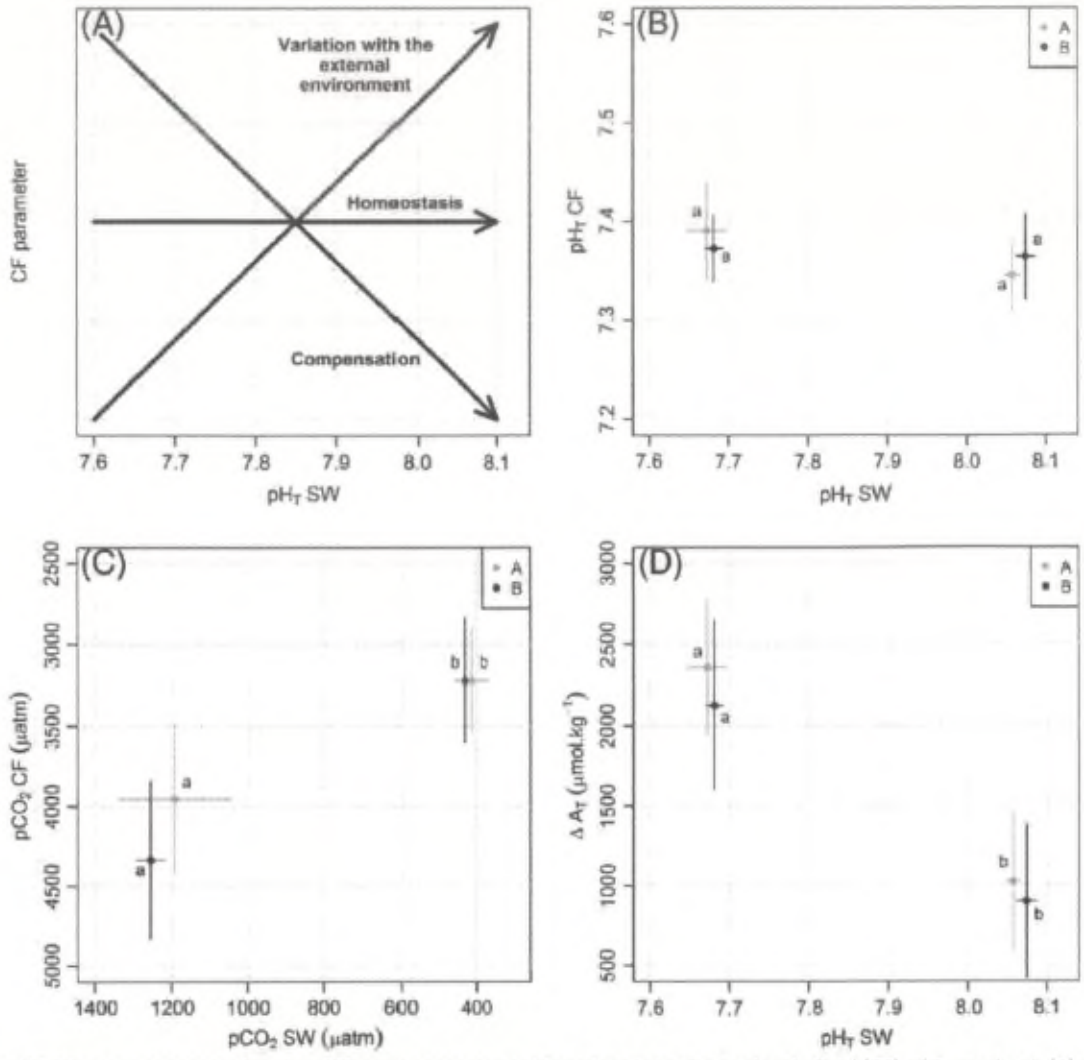

Figure 37 : (A) Diagram representing the different processes taking place into the coelomic fluid (CF) of E. mathaei, (B) pHT of the CF, (C) pCO2 of the CF and (D) difference between the total alkalinity (AT) of seawater and the AT of the CF (ΔAT) (mean ± SD, n=18) at different pH conditions (mean ± SD) in mesocosm A (grey) and mesocosm B (black) during the last three weeks of experiment. The mean of seawater pHT was calculated on values recorded during the measure of the different parameters. Means that share the same letter are not significantly different (ANOVA, α = 0.05). Note that scales are inverted in (C) to make comparisons with (B) and (D) easier.

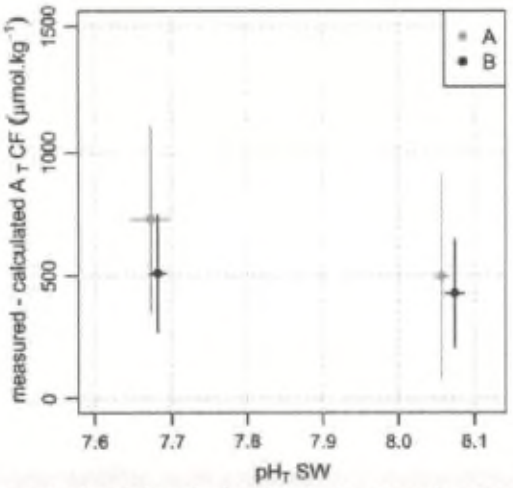

Figure 38 : Difference between the measured and the calculated A_T (using seacarb, see text) of the coelomic fluid (CF) (mean ± SD, n=18) at different pH conditions (mean ± SD) in mesocosm A (grey) and mesocosm B (black) during the last three weeks of experiment. The mean of seawater pH_T was calculated on values recorded during the measure of A_T.

4. Discussion

Figure 37A represents the various processes taking place into the CF of the sea urchin *Echinometra mathaei*. In this experiment, the pH_{CF} did not vary significantly according to the pH of seawater after two weeks at final pH. So, this indicates that, at pH predicted for 2100 (pH 7, 7), *E. mathaei* is able to maintain its internal pH at current levels despite the decrease in external pH, at least at short term. Conversely, there was an increase in pCO_2 of the CF of the same order of magnitude as the increase in pCO_2 in seawater. This is not surprising because sea urchins rely only on favorable diffusion gradient for CO_2 exchange (Farmanfarmaian, 1966). A constant pCO_2 difference between CF and seawater has to be maintained in order to maintain constant rates of diffusive CO_2 elimination. As respiration rate did not differ according to pH_T of seawater, this indicates that the increase in pCO_2 in the CF is probably due to the entry of seawater CO_2. Indeed, as background respiration rate was not checked, impacts of pH treatment on background respiration could not be ruled out. This increased pCO_2 of the CF is compensated by the significant increase in A_T of the same fluid at low pH. This is for a large part due to the bicarbonate buffer (Collard *et al.*, 2013; Holtmann *et al.*, 2013; Stumpp *et al.*, 2012). However, other compounds contribute to the buffer capacity of the CF as evidenced by the difference between the measured A_T and the calculated A_T using CF pH and pCO_2 in seacarb. Indeed, seacarb is compiled for seawater and therefore does not take into account biological buffering compounds. Although the exact nature of the buffer capacity of the CF of sea urchins has yet to be determined, coelomocytes, proteins, inorganic and organic phosphate, succinate, lactate, ammonia or other bases and acids produced metabolically or exchanged against ions could contribute (Catarino *et al.*, 2012; Collard *et al.*, 2013).

However, it seems that the carbonate buffer system is mainly responsible for increasing A_T of CF in acidified conditions. Indeed, the difference between the calculated A_T and the measured A_T did not vary significantly depending on pH conditions. The increase of bicarbonates concentration to compensate the pH_{CF} was recorded or suspected in other sea urchin species (Calosi *et al.*, 2013; Collard *et al.*, 2013; Dupont & Thorndyke 2012; Holtmann *et al.*, 2013; Spicer *et al.*, 1988; Stumpp *et al.*, 2012). A passive dissolution of the test was hypothesized by Spicer *et al.* (1988) as a source of carbonate buffer to compensate the emersion-related respiratory acidosis and further supported by Holtmann *et al.* (2013). However, Miles et al (2007) suggested that the test contributes little to the pH compensation in sea urchins immersed in seawater as they have access to the available ion pool containing bicarbonate ions that could be exchanged and used in acid–

base compensation. Another hypothesis is an increase of carbonic anhydrase (CA) activity or expression. Carbonic anhydrase plays an important role in calcification but it also facilitates CO_2 transport and participates to pH regulation processes (Chen & Lawrence, 1986; Donachy *et al.*, 1990; Heatfield, 1970; Livingston *et al.*, 2006; Mitsunaga *et al.*, 1986). Todgham & Hofmann (2009) observed an increased expression of one gene coding for CA in *S. purpuratus* larvae exposed to high CO_2. However, this was not observed in another study on the same species larvae for the same gene (Stumpp *et al.*, 2011). Finally, enhanced buffer capacity could be explained by the activation of a transport system that brings bicarbonates from the surrounding seawater. Indeed, reliance on bicarbonate ion transporter as a primary mechanism for compensating acid-base disturbance was observed in many marine animals (Pörtner *et al.*, 2004). The upregulation of one gene involved in acid-base regulation coding for a bicarbonate transporter was also shown in sea urchin larvae raised under high pCO_2 (O'Donnell *et al.*, 2010). Recently, Holtmann *et al.* (2013) suggested that test dissolution contribute only partially to pH compensation in the CF during initial acute stress phases. The long-term pH compensation by bicarbonate would be the result of active processes, which might partially be mediated by intestinal epithelia.

The present results indicate that, at the considered time scale, the tropical *E. mathaei* is able to compensate a pH_{SW} decrease expected in 2100, similarly to several boreal and temperate species. Actually, all sea urchin species tested so far appeared to be able to compensate their pH_{CF} when the $pHS_{W\text{-}NBS}$ (pH in NBS scale) was decreased from control to 7.7 (see introduction part for references). Below this value, most species were only able to partially compensate. The only exception is *H. pulcherrimus* which did not compensate its pH_{CF} after a 9 month exposure to $pHS_{W\text{-}NBS}$ 7.8 (Kurihara *et al.*, 2013). Interestingly, this is the longest experiment reporting pHC_F. It would be definitely worth to have such long exposure time for other species.

It is noteworthy that the present study is apparently the first to assess the acid-base regulation of sea urchins in condition where food was not provided ad libitum. Indeed, sea urchin density was high (12 individuals per m^2) and no additional food was supplied (other than algae produced in the mesocosms). Consistent by, ΔA_T of the coelomic fluid of *E. mathaei* maintained at control conditions (mean ± SD = 962 ± 458 µmol.kg-1) was lower from ca. 47 % than that measured for field-collected sea urchins from the same population (mean ± SD = 2234 ± 406 µmol.kg-1). This difference could be representative of starved sea urchins. Indeed, Collard *et al.* (2013) observed a decrease of ca. 40 % of ΔA_T when P. lividus is unfed during 5 weeks. However when exposed to a decreased pH, *E. mathaei* was able to increase its $A_{T\text{-}CF}$. So, even in probably food limiting

conditions, *E. mathaei* is apparently able to regulate its acid-base balance when facing pH 7.7. This is relevant to natural conditions as high densities of *E. mathaei* is encountered in nature, in particular in Reunion Island (as high as 73.6 individuals per m^2 observed by Conand *et al.*, 1997; field personal observations) but also in other reefs (McClanahan & Muthiga, 1988; McClanahan, 1998). This should have an energetic cost. However, growth was significant and of the same order of magnitude than that measured in similar *E. mathaei* size range and duration experiment using sea urchins fed ad libitum (Uthicke *et al.*, 2012). Higher growth was observed in the same species by several authors (Courtney *et al.*, 2013; Shirayama & Thornton, 2005) but juveniles were used in these studies. This difference is not surprising as juveniles exhibit a higher growth than adults (Ebert, 1982). Concerning effect of OA, decrease of growth was only observed in juveniles of *E. mathaei* and after longer period than in the present study. For example, Shirayama & Thornton (2005) observed a significant decrease of growth only after a 12 weeks exposure to OA. Moreover, in our study, sea urchins fed on macroalgae and coralline algae attached on calcareous substrate while in most other studies, organisms were fed artificially with fleshy algae. Now, Asnaghi *et al.* (2013) showed that carbonate in the diet of *P. lividus* could modulate sea urchin response to ocean acidification. These authors showed that in presence of the coralline algae Corallina elongata, even at the higher pCO_2, the test of the juveniles was much stronger and their jaws larger compared to individuals fed with non-calcifying algae. Furthermore, Shirayama & Thornton (2005) fed their sea urchins with "seaweed and chopped krill". The latter is known to be rich in fluoride which can significantly reduce calcification (Young, 1958). If food was limiting but neither acid-base regulation nor growth were affected, this does not rule out an energetic cost of OA on gonad development. Evidencing such effect requests a longer term experiment encompassing the whole gametogenetic cycle. In one of the few long term OA experiments carried out on sea urchins, Kurihara *et al.* (2013) showed that maturation and spawing of *H. pulcherrimus* fed ad libitum was delayed by 1 month but that the number of ova was not affected. Long term experiments with realistic feeding regimes are needed to assess the possible impact of OA on gonad development in sea urchins.

The present study highlights that, under a moderate acidosis and at short term, *E. mathaei* is able to regulate the pH of its CF and growth was not significantly affected even if sea urchins were not fed ad libitum. However, this pH regulation could have an energetic cost that could be observable only at longer term. This is of particular importance because the long term effect of OA on the ecological role of *E. mathaei* as bioeroder and grazer and on the balance bioaccretion-

bioerosion could also determine the future of coral reef ecosystems, particularly reefs where *E. mathaei* is one of the major bioeroders.

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Long term mesocosms study of the effects of ocean acidification on growth and physiology of the sea urchin *Echinometra mathaei*

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Abstract

Recent researches on the impact of ocean acidification (OA) on echinoderms highlighted the importance to conduct long-term experiments taking into account ecosystemic interaction in order to better predict possible effects of elevated pCO_2. The goal of this study was to evaluate the physiological effects of OA on the sea urchin *Echinometra mathaei* taking into account these considerations. A long-term experiment was conducted in artificial reef mesocosms in which urchins fed principally on algae attached to reef calcareous substrate. Contrasted pH conditions (pH 7.7 vs control) were established gradually during six months and then maintained during seven more months. Acid-base parameters of the coelomic fluid, growth and respiration rate were monitored throughout the experiment. Results indicate that *E. mathaei* should be able to regulate its extracellular pH at long term, through bicarbonate compensation. We suggest that this ability depends on phylogenetic aspect and on the quantity and quality of available food. Growth, respiration rate and mechanical properties of the test were not affected. This ability to resist OA expected in 2100 at long term could determine the future of coral reefs, particularly reefs where *E. mathaei* is the major bioeroder.

1. Introduction

For the last century, human activities have produced large amounts of CO_2 through fossil fuels burning, intensive agriculture and deforestation. Changes in ocean surface water chemistry (pH and carbonate saturation decrease) have been evidenced and unequivocally linked to the rise of atmospheric CO_2 concentration (IPCC, 2013). This raised concerns about the possible impact of these changes on marine organisms.

Responses of organisms to ocean acidification (OA) greatly differ according to taxa (e.g. Wittmann & Pörtner, 2013). Due to their low metabolism and poor osmoregulation abilities, sea urchins have been considered as potential victims of OA (Dupont *et al.*, 2010; Dupont & Thorndyke, 2013; Melzner *et al.*, 2009; Pörtner *et al.*, 2004). The studies carried out so far produced contrasted results. Some adult sea urchins appear to be able to maintain the pH of their coelomic fluid (the main extracellular fluid compartment in echinoderms) when facing a decrease of the seawater pH (Dupont & Thorndyke, 2012; Calosi *et al.*, 2013; Collard *et al.*, 2013, 2014; Moulin *et al.*, 2014; Stumpp *et al.*, 2012). On the contrary, other studies reported an incomplete or ineffective extracellular pH regulation (Catarino *et al.*, 2012; Kurihara *et al.*, 2013; Miles *et al.*, 2007; Spicer *et al.*, 2011). Organisms which cannot avoid extracellular acidosis could be directly affected by OA (Fabry *et al.*, 2008; Melzner *et al.*, 2009; Pörtner *et al.*, 2004; Pörtner, 2008). For those which regulate their extracellular pH, the energy cost of proton elimination or buffering could modify resource allocation leading to reduced calcification and somatic or gonad growth. Indeed, several authors observed a decreased growth when sea urchins were submitted to elevated pCO_2 (Albright *et al.*, 2012; Courtney *et al.*, 2013; Holtmann *et al.*, 2013; Shirayama & Thornton, 2005; Stumpp *et al.*, 2012; Wolfe *et al.*, 2013) while in other studies, no effect was reported (Kurihara *et al.*, 2013; Moulin *et al.*, 2014; Uthicke *et al.*, 2013). Concerning reproduction allocation, it seems that OA leads to a gonad growth decrease and/or a gametogenesis delay (Kurihara *et al.*, 2013; Siikavuopio *et al.*, 2007; Stumpp *et al.*, 2012; Uthicke *et al.*, 2013). Crucial aspects when dealing with energy related effects are the duration of the experiment and the provided food. Until now, only three studies considered acclimatization by conducting long term experiments (over 6 months, Dupont *et al.*, 2013; Kurihara *et al.*, 2013; Shirayama & Thornton, 2005). All three reported significant effects either on growth or on reproductive output although the latter was modulated according to acclimatization duration (Dupont *et al.*, 2013). The need for more long-term studies has been recently emphasized (Dupont & Thorndyke, 2013). Furthermore, in all studies carried out so far on postmetamorphic sea urchins, food was supplied ad libitum and

used algae were not submitted to OA before being provided to sea urchins. Now, the biochemical composition and palatability of algae may be affected by OA (e.g. Borell *et al.*, 2013). Asnaghi *et al.* (2013; 2014) reported more severe effects of OA on *Paracentrotus lividus* when fed non-calcified algae instead of calcified algae *Corallina* and OA is known to significantly reduce growth of coralline algae (Kuffner *et al.*, 2008) .

Therefore, the goal of the present study was to assess the impact of OA at long-term on a suite of physiological parameters of a sea urchin in realistic food conditions. The sea urchin *Echinometra mathaei* was submitted during six months to a gradual pH decrease until reaching a moderate OA level (pH 7.7). Sea urchins were then maintained at target pH during seven more months. Sea urchins were placed in two replicated artificial reef mesocosm consisting of hermatypic scleractinians and reef calcareous substrate with its diverse communities of algae as principal food. The growth, the carbonate chemistry of the coelomic fluid (pH, dissolved inorganic carbon and total alkalinity) and the respiration rate of sea urchins were monitored throughout the experiment. Biomechanical resistance of the skeleton was also tested at the end of the experiment to assess test robustness to fish predation.

2. Materials and methods

2.1. Mesocosms design and seawater physico-chemistry

The technical setup of artificial reef mesocosms was previously described in Moulin *et al.* (2014). Briefly, the study was conducted in two separated mesocosms (true replicates). Each mesocosm is composed of one main unit and two experimental aquaria. Both experimental aquaria were connected to the main unit and the water flow was the same for both aquaria (800 ± 50 mL min^{-1}). This design allowed to maintain the same physico-chemical parameters in both aquaria. Only pCO_2 and pH differed: one was maintained at control conditions (mean target pH in total scale, pH_T, 8.10) and another was acidified (mean target pH_T 7.65). The contrasted conditions of pH were established gradually during six months (approximately -0.03 units of pH_T every two weeks) and then maintained during seven more months. In our reference site in the field (La Saline fringing reef, Réunion Island, 21°70'S, 55°32'E), pH increased between sunrise and zenith time on average by 0.18 units (± 0.05, N=1344, from 2011/05/10 to 2011/06/13, pH measured every 15 minutes, Cuet P., pers comm, see also Chauvin *et al.*, 2011). From zenith to sunrise, the pH decreased approximately by the same value. In order to get more realistic conditions in our mesocosms, daily variation of pH_T was maintained at a level similar to field values through

community respiration/photosynthesis and change in the day/night pH setpoint of the control system.

In each experimental aquarium, a temperature sensor (Aquastar, Germany) and a pH electrode (Aquastar, Germany) were connected to an IKS control system (IKS, Aquastar, Germany) which record values every 20 seconds. Seawater samples were collected every other day and immediately filtered (0.22 µm GSWP, Millipore). Total alkalinity (A_T) was measured by a potentiometric titration with 0.01M HCl in NaCl 0.7M following Dickson *et al.* (2007) adapted for a smaller volume (25 mL). Each titration was automatically performed by computer using a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyzer to record pH (Consort, Belgium) and a TW Alpha Plus auto sampler (SI Analytics, Germany). Calibration was performed using certified reference seawater provided by A. G. Dickson (Scripps Institute of Oceanography, Dixon, batch 94). Once a day, the electromotive force (e.m.f) was measured using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor). The e.m.f was then converted to pH_T using calibration curve of standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS) (Del Valls & Dickson, 1998; Dickson *et al.*, 2007; DOE, 1994). The salinity and temperature were measured once a day using a conductivity meter pH/Cond 340i WTW (USA). The intercalibration between these measures (pH_T and temperature) and those recorded by the IKS control system allow to recalculate the real 24h cycle of pH and temperature and to adjust setpoints of the controllers. The pCO_2 was determined from A_T, pH_T, temperature and salinity data using the program R (R Core Team, 2013) and the package seacarb (Lavigne & Gattuso, 2012) (Lueker *et al.* (2000) constants for K1 and K2; Perez & Fraga (1987) constant for Kf; Dickson (1990) constant for Ks).

2.2. Mesocosms community

Sea urchins (*Echinometra mathaei violacea* (Mortensen 1943), violet *Echinometra*, see Arakaki *et al.*, 1998) were collected at Réunion Island in the Indian Ocean, from the back-reef of Saint Pierre fringing reef (21°33'S, 55°47'E). Hermatypic scleractinians and reef calcareous substrate with its diverse communities of algae, bacteria, archae, fungi and meiofauna were also introduced in the mesocosms. Corals and substrate came from Réunion Island in the back reef of La Saline fringing reef (21°70'S, 55°32'E) (*Acropora muricata*, *Acropora digitifera*, *Pocillopora damicornis*) and from aquarium market (Dejong, Holland) (*Seriatopora hystrix*, *Acropora tenuis*). Before field collection, permits were obtained from the Marine Nature Reserve of Réunion Island (RNN164) and the "Direction de l'Environnement, de l'Aménagement et du Logement" (DEAL) and CITES

permits were checked for aquarium market corals. Organisms were acclimated to the mesocosms in Belgium in control condition for seven months before the beginning of the experiment. Sixteen sea urchins (test diameter: 28-40 mm), 0.4 ± 0.04 kg of corals and 22 ± 1 kg of reef substrate were distributed randomly in each experimental aquarium. When a sea urchin died, it was replaced by a green *Echinometra sp.* B-like (Arakaki *et al.*, 1998) in order to maintain sea urchins density and to identify individuals that were present from the beginning of the experiment at any time. Sea urchins fed principally on macroalgae and coralline algae that grow on the reef substrate. As mortality occurred, excessive starvation was suspected and additional food (dehydrated *Porphyra sp.*, Japanese edible seaweed "Nori") was supplied from the fifth month of the experiment (ca. 0.35 ± 0.05 g every other day).

2.3. Growth and respiration rate measurements

Growth and respiration rates were only measured in violet *Echinometra*. The respiration rate was determined every 3 months using homemade intermittent respirometers. Respirometers (1.2L) consisted of a Plexiglas cylinder with a waterproof gas tight cap provided with a water inlet and outlet. A Clarck oxygen electrode (Aquastar, Germany) was immersed inside the respirometer chamber through a sealed operculum. To ensure water mixing and homogeneous distribution of the oxygen, a magnetic stir bar was introduced in each respirometer. Water inflow came from the experimental aquarium from which the sea urchins studied originated. Respirometers were placed inside a thermostated water bath (25 ± 0.5 °C) on a magnetic stirring unit. Water inflow was open for 1h and then stopped for 1h during the respiration measurement phase. Oxygen concentration was recorded by means of a control system (IKS Aquastar, Germany) every 8 seconds. The sequence open/closed water inflow was carried on during 24h before the introduction of sea urchins. Then, two sea urchins were introduced into each respirometer for 48h. After the sea urchins were removed, the system wasmaintained for an additional 24h. Oxygen electrodes were calibrated with water-saturated air as 100 % O_2. Moreover, each probe signal was corrected using a WTW oxymeter and an WTW Oxycal probe, calibrated using 0 % O_2 $NaSO_2$ solution and 100 % O_2 water-saturated air. Linear regression ($R^2 > 0.9$) of seawater oxygen concentration over time was computed during the closed phases. This value was converted into µmol O_2 $L^{-1}.h^{-1}$ and multiplied by the water volume of the respirometer. Background respiration and oxygen consumption by the electrode itself were assessed by linear regression between the first 24h (before the sea urchin respiration measurements) and last 24h measurement (after the sea urchin respiration measurements). The correction was then applied to sea urchin respiration rate measures. Wet

weight of sea urchins was measured after the respiration rate measurements (± 0.1 g) to prevent the influence of gut content. Indeed, twenty sea urchins were dissected after starvation during 48h and gut was always observed empty. Respiration rate was normalized to sea urchin wet weight.

Skeletal weight was assessed by the buoyant weighing method (Jokiel *et al.*, 1978) (± 0.005 g) after the respiration rate measure (no gut content influence). The density of the skeleton was determined from the whole skeleton (spine, test and Aristotle's lantern) of twenty sea urchins coming from the same population by buoyant weighing method. Growth rate was calculated following an exponential growth curve using the equation:

$$\log Y = log\ Y_0 + k.t$$

with Y being the skeletal weight at time t , Y0 the initial skeletal weight at time t = 0, k the growth rate and t the time (in month).

2.4. Carbonate chemistry of the coelomic fluid

The pH_T, the dissolved inorganic carbon concentration (DIC) and the A_T of the CF (respectively pH_{CF}, DIC_{CF} and $A_{T\text{-}CF}$) were measured every 3 months in violet *Echinometra*. Samples of CF were collected by puncture through the peristomial membrane (syringe 21G, Terumo, Japan). The e.m.f. of the CF was immediately measured by immersing a microelectrode in 1 mL aliquot (microelectrode 6.0224.100 coupled to a 827 pH Lab meter, Metrohm, Switzerland). Calibration and e.m.f. values conversion to pH_T were done as previously described (section 2.1). Salinity was assumed to be the same as that of seawater in the experimental aquaria from which sea urchins came as the composition sea urchins CF is known to be close to that of seawater (Farmanfarmaian, 1966; Russell, 2013). Remaining samples were transferred to a microcentrifuge tube filled to the top and closed to avoid sample-air exchange. Samples were always kept on ice to prevent clotting. Sample were then centrifuged for ten minutes (4000 G) using a microcentrifuge (Eppendorf, Germany) at 4 °C. The supernatant was used to determine DIC_{CF} and $A_{T\text{-}CF}$.

A homemade method was developed to determine DIC_{CF}. A known amount of sample (ca. 0.5 mL, weighted on an analytical balance, ± 2 mg) was isolated in a homemade closed system containing air with an initial known amount of CO_2 and maintained at a constant temperature and pressure. Concentrated 32.5 % nitric acid was added in order to acidify the sample and thus to transform all DIC species into CO_2. Once the sample and air were in equilibrium, air was analyzed for carbon dioxide content using a CO_2/H_2O LI-840 analyzer (LI-COR, USA). The method was

calibrated using standard solutions ($NaHCO_3$, normapur®, VWR) and then corrected by an offset estimated from a standard certified material provided by Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control Laboratory. Certified reference seawater measurements had a random deviation of 1.5 % of the original batch value.

$A_{T\text{-}CF}$ was determined by a manual potentiometric titration with HCl 0.02 N in NaCl 0.7 mol kg^{-1} on 0.5 mL CF sample following Collard *et al.* (2013). The quality of the method was assessed using certified reference seawater (by Andrew G. Dickson, Scripps Institute of Oceanography, Dixon, batch 9). Certified reference seawater measurements had a random deviation of 1.5 % of the original batch value. Results were reported as the difference between the $A_{T\text{-}CF}$ and A_T of seawater (ΔA_T). Theoretical $A_{T\text{-}CF}$ was also calculated from pH_T, DIC, temperature and salinity data using the seacarb package.

2.5. Biomechanical properties

At the end of the experiment, sea urchins were dissected. The test skeleton was cleaned from its associated soft tissues in gently shaken solutions of 2.5 % and 5.25 % NaOCl (Loda, professional quality) for, respectively, 1h30 and 2h30, rinsed in MilliQ water (Millipore) and air-dried. Three ambital and three apical plates coming from three interambulacral zones from each sea urchin were selected for mechanical tests. The ambital and apical plates chosen were respectively the largest and the smallest ones. The plate effective length (between external supporting points) was measured on enlarged photos by image analysis (ImageJ software, National Institutes of Health, USA). Three-point bending tests were performed on ambital plates following Moureaux *et al.* (2011). Briefly, each ambital plate was placed on a stainless steel stand. A noncutting blade fixed on the load frame (Instron 5543) was lowered on the primary tubercle at a speed of 0.01 mm/min to bend the plate. Displacement and force were recorded at a frequency of 10 Hz. The Young's Modulus was determined using the equation:

$$Young's\ modulus\ E\ (Pa) = \frac{F_{max}\ L_e^3}{48\ \Delta L\ I_2}$$

where F_{max}: force at rupture, L_e: effective length, ΔL: displacement, I_2: second moment of area.

The macro MomentMacro (Ruff C., Johns Hopkins University School of Medicine, MD, USA) for ImageJ software was used to calculate the second moment of area I_2 of the skeleton cross sections. Apical plates underwent simple compression test. Each apical plate was placed on a stainless steel block. A second similar block was fixed on the load frame of the bending machine

(Instron 5543) and was lowered on the main tubercle of the plate at a speed of 0.3 mm/min until rupture. Displacement and force were recorded at a frequency of 10 Hz. The Young's Modulus was determined using the equation:

$$Young's\ modulus\ (Pa) = \frac{\text{stress}}{\text{strain}} = \frac{\frac{F}{A}}{\frac{\Delta L}{L_e}}$$

where F: force at rupture, A: area of the tubercle, , ΔL: displacement, L_e: effective length.

2.6. Data analysis

Normality and homogeneity of variances were checked before analysis, using respectively Shapiro-Wilk's and Levene's tests. Temperature, A_T and salinity of seawater were analyzed using a paired Student's t-test in order to examine possible differences between aquaria within each mesocosm. In order to determine if growth rate of sea urchins was significantly different from zero, a paired Student's t-test was performed. Mortality, growth and biomechanical properties were analyzed using a one way ANOVA with repeated measures on one factor (factor nominal pH nested in repeated factor mesocosm). The respiration rate and CF acid-base parameters (pH_{CF}, DIC_{CF} and ΔA_T) were treated as the difference between means from control and acidified aquaria in each mesocosm to take into account the paired design of the experiment. During the gradual pH decrease, this difference was fitted using a linear regression with ΔpH (difference of mean seawater pH between control and acidified aquaria at the moment of the measure) and mesocosm as explanatory variables. The y intercept and the slope correspond, respectively, to the difference between control and acidified aquaria at the beginning of the experiment and during the gradual pH decrease. After establishment of contrasted conditions, this difference was fitted using a linear regression with time and mesocosm as explanatory variables. The slope provided information about the possible change of this difference when contrasted conditions were clearly established. All tests were conducted using the program R. The level of significance α was set at 0.05.

3. Results

3.1. Physico-chemical parameters in mesocosms

The pH_T of experimental aquaria was maintained close to target values, in control conditions as well as in acidified conditions throughout the experiment (Fig. 39, Table 11). In control aquaria, mean pH were respectively 8.08 ± 0.08 and 8.09 ± 0.09 in mesocosm A and B. In acidified aquaria, after the progressive decrease of seawater pH, mean pH were respectively 7.63 ± 0.03 and 7.62 ±

0.04 in mesocosm A and B. Mean value of A_T, salinity and temperature during the whole experiment are presented in Table 11. Temperature varies significantly between contrasted conditions in mesocosm A (paired t test, $t_{(9638)} = 11.34$, $p < 10^{-3}$) but not in mesocosm B (paired t test, $t_{(9832)} = -1.51$, $p = 0.13$). However, means of these differences are small and in the range of precision of the control system (< 0.1 °C). A_T and salinity did not differ significantly between contrasted conditions in both mesocosms (paired t test, $t_{(210)} \leq 1.88$, $p \geq 0.06$).

Table 11 : Mean seawater conditions during the experiment. Values between brackets are standard deviations. Mean temperature and pH_T were calculated on measurements recorded every 20 seconds. Mean salinity, A_T and pCO_2, DIC, CO_3^{2-}, HCO_3^- and Ω (calcite and aragonite) were calculated on values measured every day short after mesocosms sunrise. Mean salinity, temperature and A_T correspond to the data during the whole experiment. Other parameters correspond to the data after establishment of contrasted conditions (last 7 months).

Treatment	Mesocosm	Salinity	Temperature (°C)	A_T ($\mu mol.kg^{-1}$)	pH_T	pCO_2 (ppm)	DIC ($\mu mol.kg^{-1}$)	CO_3^{2-} ($\mu mol.kg^{-1}$)	HCO_3^- ($\mu mol.kg^{-1}$)	Ω_{Cal}	Ω_{Ar}
control	A	34.3 (0.6)	25.2 (0.3)	2400 (133)	8.08 (0.08)	382 (40)	2074 (130)	234 (21)	1829 (117)	6.48 (0.58)	4.27 (0.38)
	B	34.6 (0.5)	25.2 (0.4)	2421 (237)	8.09 (0.09)	359 (47)	2024 (110)	236 (16)	1778 (107)	6.51 (0.45)	4.29 (0.30)
acidified	A	34.3 (0.9)	25.2 (0.3)	2409 (177)	7.63 (0.05)	1288 (124)	2335 (194)	100 (9)	2198 (183)	2.27 (0.25)	1.82 (0.17)
	B	34.6 (0.5)	25.2 (0.3)	2432 (175)	7.62 (0.04)	1269 (93)	2286 (134)	97 (6)	2153 (127)	2.67 (0.16)	1.76 (0.11)

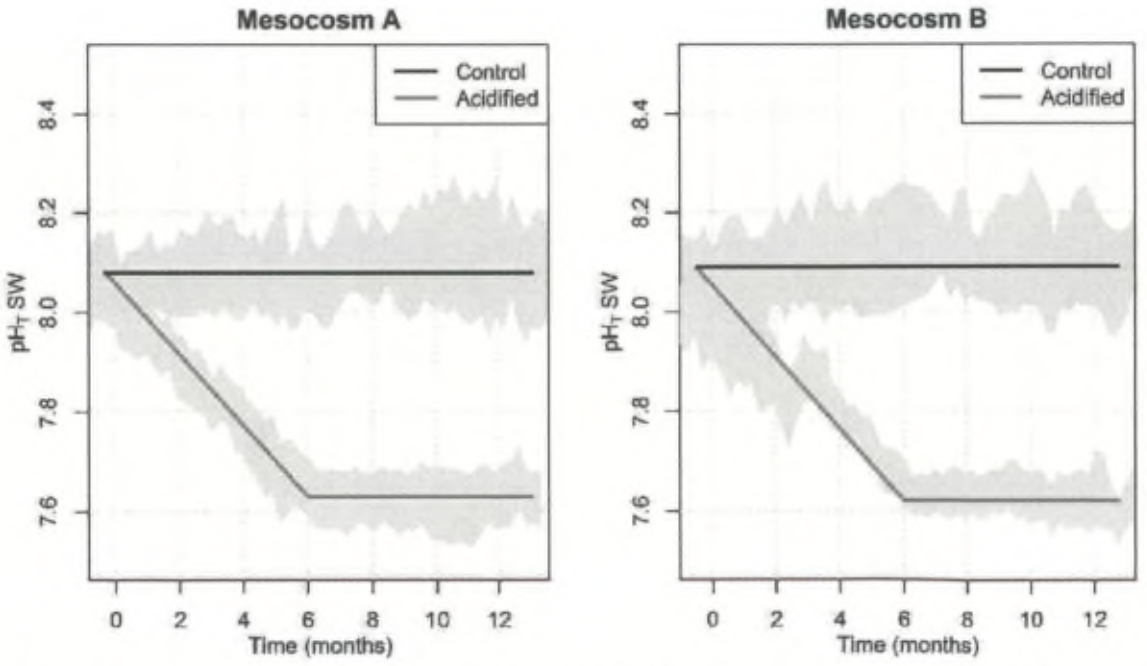

Figure 39 : pH_T of seawater in control (black) and acidified (grey) aquaria during the experiment (months since the beginning of the experiment). The lines represent target pH. The grey zones represent minimal and maximal value (respectively during night and day) measured once a week throughout the experiment

3.2. Mortality and growth

Some mortality occurred during the experiment. At the end of the experiment, 7 and 10 sea urchins remained in acidified aquaria in mesocosm A and B respectively. In control aquaria, 5 and 4 sea urchins were alive in mesocosm A and B respectively. Mortality did not differ significantly according to the pH condition (ANOVA, $F_{(1,3)} = 4$, $p = 0.30$). From the fifth month of the experiment, additional food was supplied but mortality continued to take place independently. Sea urchins were not tagged individually and mortality occurred during the whole experiment except the last three months. Consequently, growth rate was only assessed at the end of the experiment by calculation using the individual skeleton weight after 10 and 12 months of experiment, i.e. during a period with established contrasted conditions. Growth rate of sea urchins at the end of the experiment was significantly different from 0 (mean ± SD = 1.1 ± 1.3 % per month, t test, t(24) = 4.18, $p < 10^{-3}$) (Fig. 40). The pH of seawater did not have a significant effect on growth rate of sea urchins (ANOVA, $F_{(1,24)} = 0.26$, $p = 0.70$).

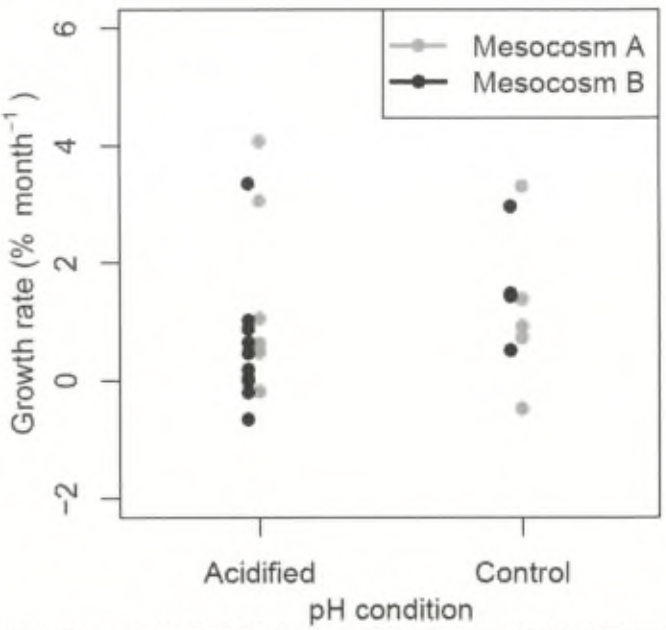

Figure 40 : Skeletal growth (as percentage of the initial skeleton weight) of *E. mathaei* at different pH conditions in mesocosm A (grey) and mesocosm B (black)

3.3. Metabolism

The respiration rate of sea urchins was measured every 3 months throughout the experiment (Fig. 41). As sea urchins exhibit greater activity during the night, respiration rate data were separated between night and day data. At the beginning of the experiment, the difference of respiration rate between control and future acidified aquaria was not significant in both mesocosms (Table 12, y-intercepts). During the gradual pH decrease, the pH of the seawater did

not affect significantly the respiration rate in both mesocosms and the mesocosm factor was not significant (Table 12, slopes). After the establishment of contrasted conditions, the respiration rate of sea urchins did not vary significantly with time (Table 12, slopes).

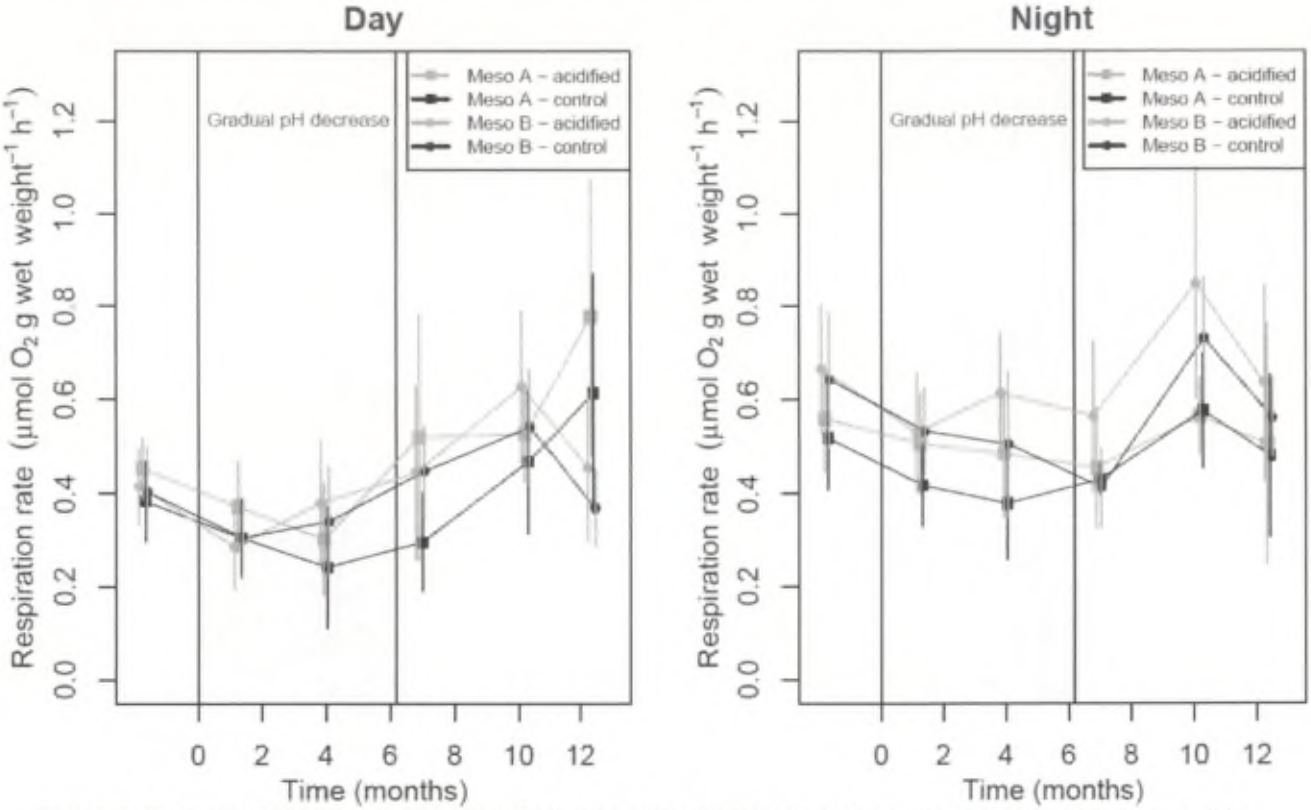

Figure 41 : Respiration rate (mean ± SD, n>4) of E. mathaei in the different pH conditions (grey: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) during the experiment (months since the beginning of the experiment) during day and night.

Table 12 : Linear models of the difference of respiration rate between acidified and control conditions. The difference of pH between contrasted conditions was used as predictive variable during the gradual pH decrease. The time was used as predictive variable after the establishment of contrasted conditions. Models were computed for day and night data separately. Parameters of the linear regression are showed for the mesocosm A (A) and for the difference between mesocosm A and B (B-A).

		Day			Night		
		Estimate	t value	*p*	Estimate	t value	*p*
Gradual pH decrease							
A	y-intercept	-0.04	-1.07	0.35	-0.07	-2.27	0.09
	slope	0.33	2.02	0.11	-0.03	-0.23	0.83
B - A	y-intercept	0.04	0.71	0.52	0.07	0.17	0.17
	slope	-0.30	-1.32	0.26	0.41	0.08	0.08
model R^2			0.74			0.70	
Contrasted conditions established							
A	y-intercept	-0.29	-1.46	0.28	-0.03	-0.43	0.71
	slope	0.01	0.73	0.54	0.001	0.22	0.85
B - A	y-intercept	0.40	1.44	0.29	-0.23	2.78	0.11
	slope	-0.03	-1.14	0.37	0.01	1.59	0.25
model R^2			0.64			0.95	

3.4. Acid-base parameters of the coelomic fluid

pH_{CF}, DIC_{CF} and ΔA_T were measured every three months throughout the experiment. At the beginning of the experiment, the pH_{CF} did not differ significantly between experimental aquaria in both mesocosms (Fig. 42, Table 3, y-intercept). During the gradual pH decrease, the pH of the seawater did not affect significantly the pH_{CF} in both mesocosms and the mesocosm factor was not significant (Fig. 42, Table 3, slope). After the establishment of contrasted conditions, pH_{CF} did not vary significantly with time (Fig. 42, Table 13, slope). The DIC_{CF} (Fig. 43) and the ΔA_T (Fig. 44) did not differ significantly between experimental aquaria in both mesocosms before gradual pH decrease (Table 13, y-intercepts). The DIC_{CF} and ΔA_T were significantly higher in acidified condition during establishment of contrasting conditions independently of the considered mesocosm (Table 13, slopes). The adjusted linear model showed that pH of seawater predicted well the DIC_{CF} and ΔA_T ($R^2 > 0.94$). Once the contrasted conditions were established, the difference in DIC_{CF} and ΔA_T between contrasted conditions remained constant throughout the experiment (Table 13, slopes). The difference between the measured and the calculated A_T (Fig. 45) did not vary significantly between between experimental aquaria in both mesocosms at the beginning of the experiment (Table 3, y-intercept), nor during the gradual pH decrease and after, when contrasted pH conditions were maintained (Table 3, slopes).

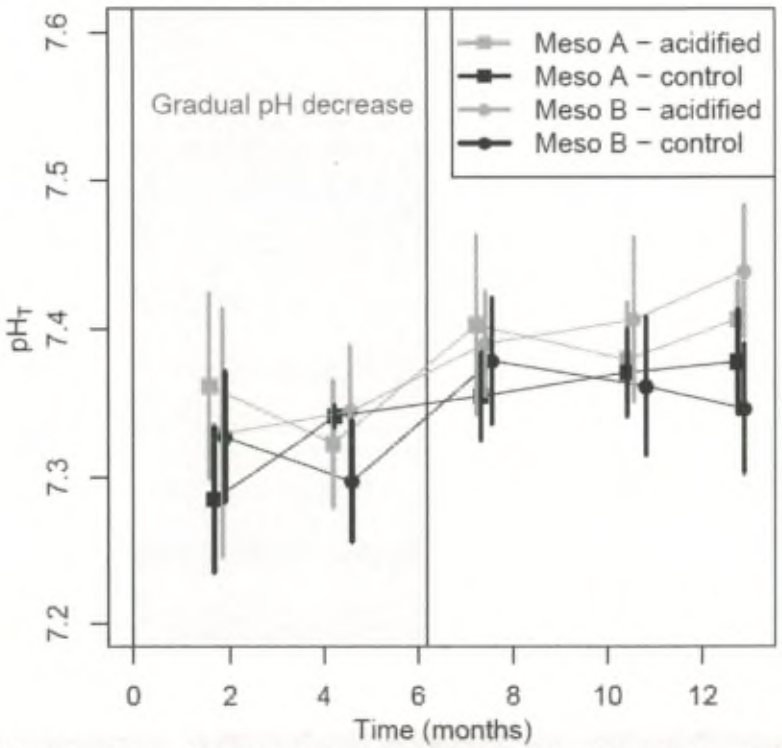

Figure 42 : pH_T of the CF (mean ± SD, n ≥ 4) of *E. mathaei* in the different pH conditions (grey: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) during the experiment (months since the beginning of the experiment)

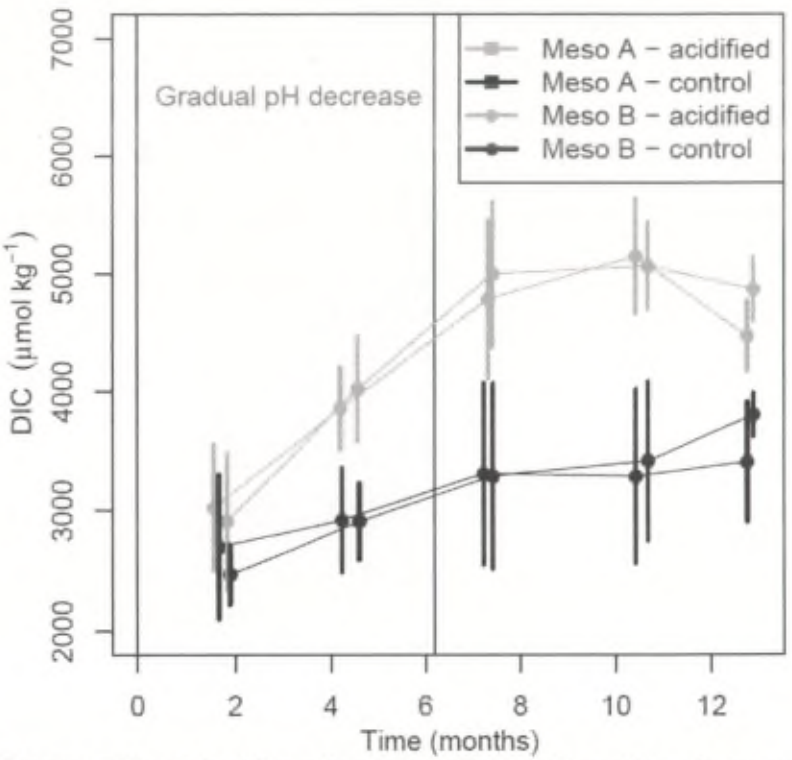

Figure 43 : DIC of the CF (mean ± SD, n ≥ 4) of *E. mathaei* in the different pH conditions (grey: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) during the experiment (months since the beginning of the experiment)

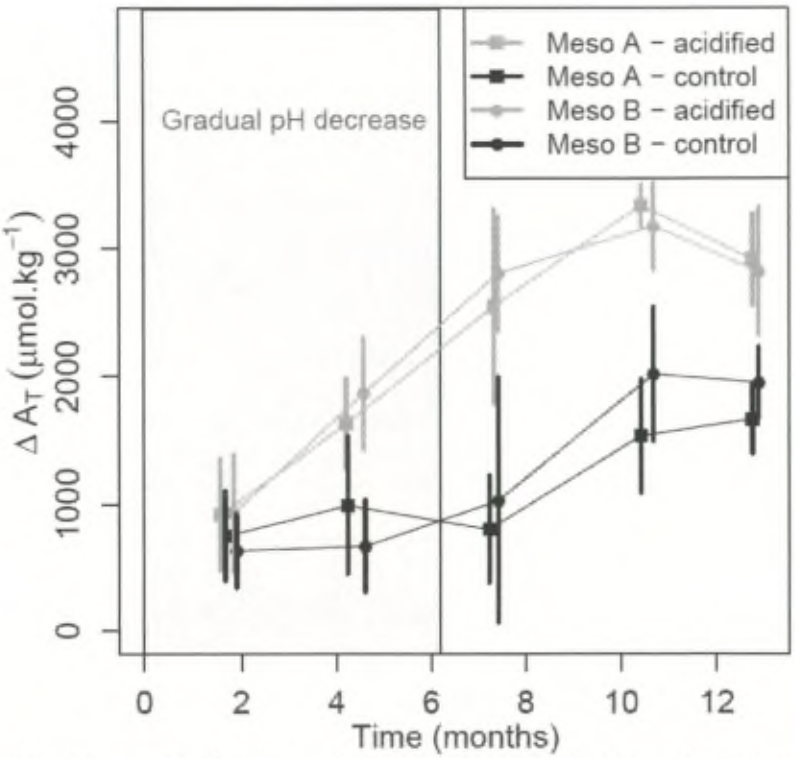

Figure 44 : ΔA_T (difference between the A_T of seawater and the A_T of the CF of *E. mathaei*) (mean ± SD, n ≥ 4) in the different pH conditions (grey: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) during the experiment (months since the beginning of the experiment)

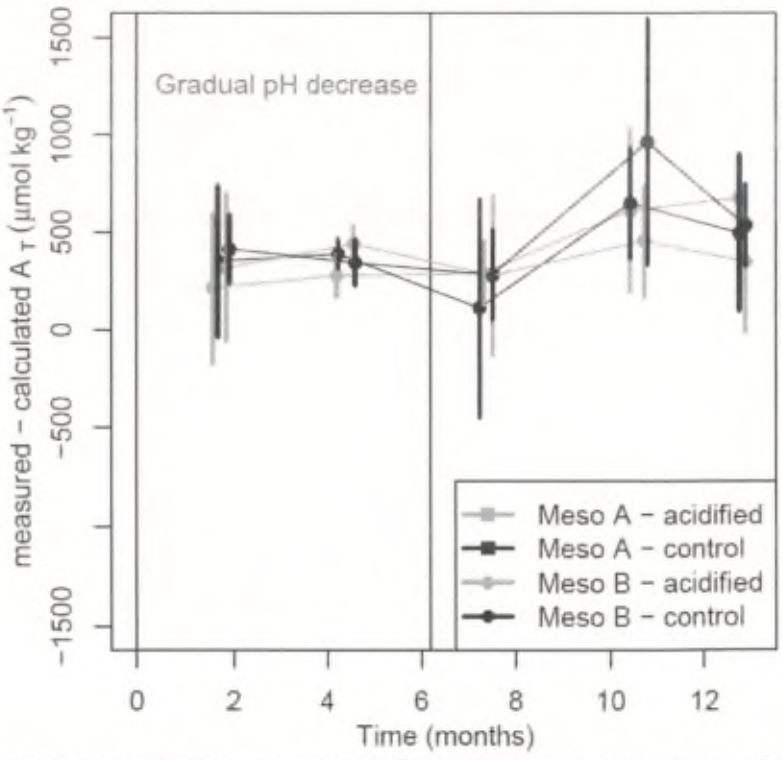

Figure 45 : Difference between the measured and the calculated A_T (using seacarb, see text) of the CF (mean ± SD, , n ≥ 4) of *E. mathaei* in the different pH condition (darkgrey: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) during the experiment (months since the beginning of the experiment)

Table 13 : Linear models of the difference of CF parameters (pH, DIC, A_T and difference between measured and calculated A_T) between acidified and control conditions. The difference of pH between contrasted conditions was used as predictive variable during the gradual pH decrease. The time was used as predictive variable after the establishment of contrasted conditions. Parameters of the linear regression are showed for the mesocosm A (A) and for the difference between mesocosm A and B (B-A).

		pH			DIC			A_T			mesaured - caclulated A_T		
		Estimate	t value	*p*	Estimate	t value	*p*	Estimate	t value	*p*	Estimate	t value	*p*
Gradual pH decrease													
A	y-intercept	0.07	0.79	0.51	-17	-0.26	0.82	-1923	-1.08	0.34	245	1.88	0.20
	slope	0.04	0.12	0.92	-3665	-16	**0.004**	-4290	-5.90	**0.004**	-125	-0.27	0.81
B - A	y-intercept	-0.05	-0.42	0.72	82	0.90	0.46	250	0.99	0.38	114	0.62	0.60
	slope	-0.09	-0.19	0.87	-372	-1.16	0.37	64	0.06	0.95	-2	-0.003	0.998
	model R^2		0.15			0.997			0.95			0.51	
Contrasted conditions established													
A	y-intercept	0.19	2.29	0.15	2122	1.96	0.19	2469	4.48	0.046	-213	-0.89	0.47
	slope	-0.01	-1.79	0.22	-65	-0.62	0.60	-85	-1.61	0.25	71	3.11	0.09
B - A	y-intercept	-0.26	-2.16	0.16	503	0.33	0.77	521	0.67	0.57	651	1.93	0.19
	slope	0.03	2.29	0.15	-49	-0.33	0.77	-84	-1.12	0.38	-79	-2.43	0.14
	model R^2		0.73			0.44			0.89			0.89	

3.5. Mechanical tests

Mechanical tests were carried out on ambital and apical plates (Figs. 46 and 47 respectively). The pH of seawater did not have a significant effect on the force at rupture F_{max} and on the Young's modulus E in both types of plates (ANOVA, $F_{(1,25)} \leq 38.2$, $p \geq 0.10$) nor on the flexural stiffness EI_2 of ambital plates (ANOVA, $F_{(1,25)} = 1.09$, $p = 0.49$).

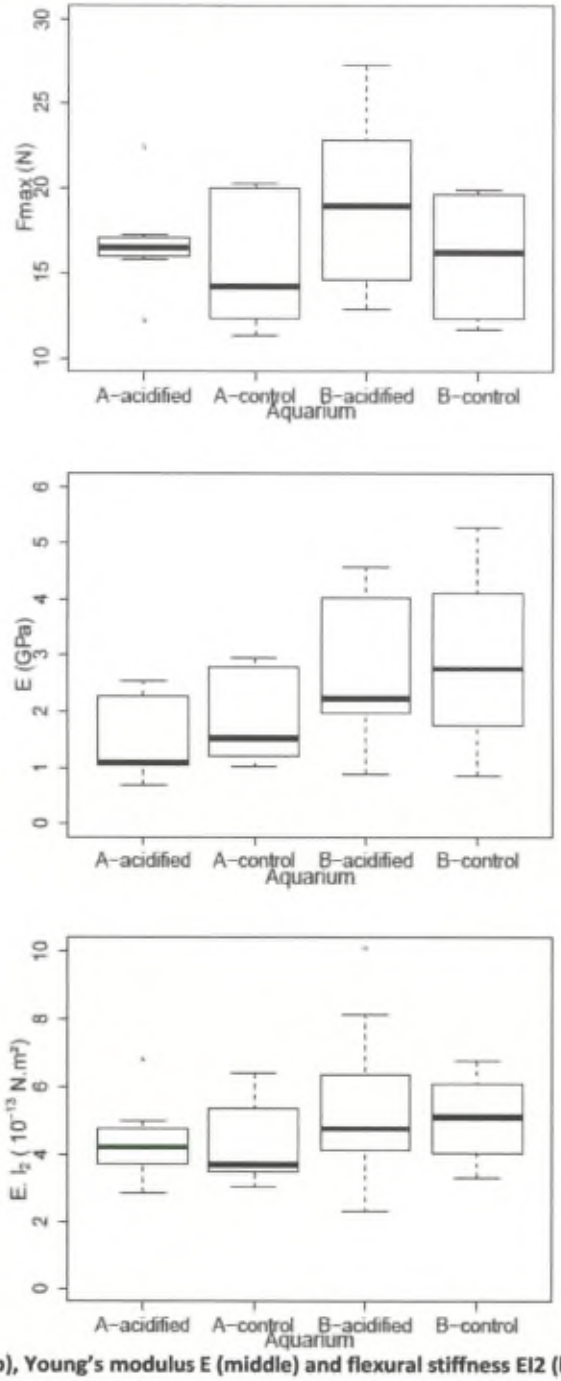

Figure 46 : Force at rupture Fmax (top), Young's modulus E (middle) and flexural stiffness EI2 (bottom) of skeletal ambital plates of E. mathaei coming from the different aquaria (A for mesocosm A and B for mesocosm B) at different pH conditions at the end of the experiment. Boxplots represent median (blackline), interquartile range (box), extend up to 1.5 times the interquartile range from the box edges (whiskers) and outliers (individual points)

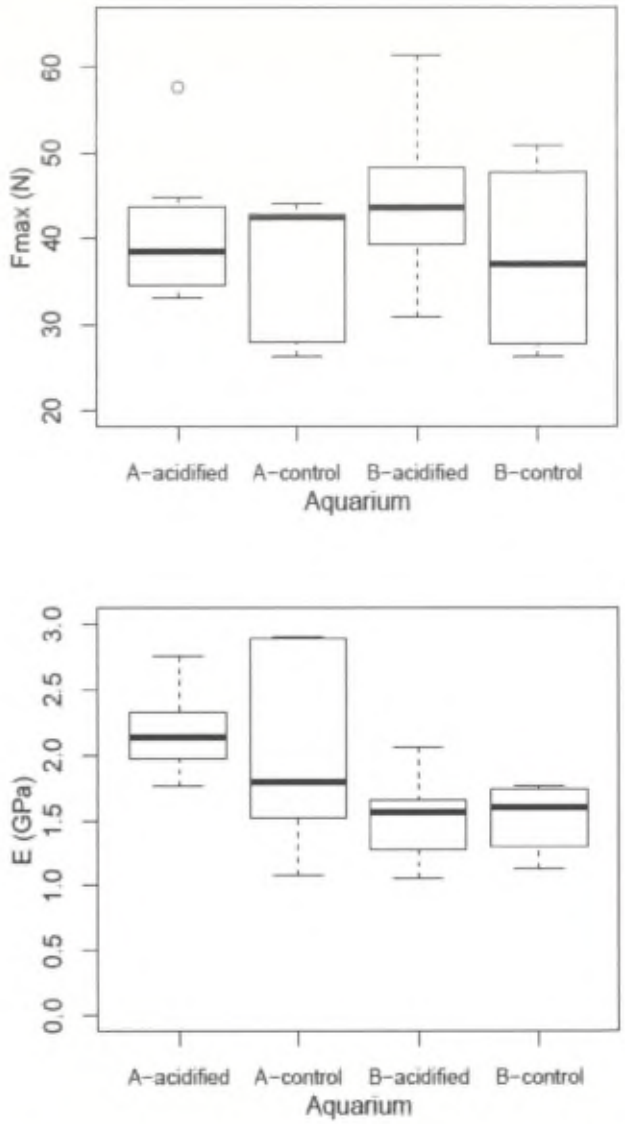

Figure 47 : Force at rupture F_{max} (top) and Young's modulus E (bottom) of skeletal apical plates of *E. mathaei* coming from the different aquaria (A for mesocosm A and B for mesocosm B) at different pH conditions at the end of the experiment. Boxplots represent median (blackline), interquartile range (box), extent up to 1.5 times the interquartile range from the box edges (whiskers) and outlier (individual points).

4. Discussion

During a 6 months gradual pH decrease and after 7 months at mean ΔpH -0.46, the pH_{CF} in *Echinometra mathaei* was maintained similar in control and treatment sea urchins. The increase of the ΔA_T is well predicted by the ΔpH of seawater, indicating that the pH regulation was conducted by an increase of the A_T. The measures of pH_{CF} and DIC_{CF} allowed to calculate the theoretical value of A_T using carbonate system equilibrium equations in seawater, without taking into account biological compounds of the CF. As the difference between the calculated $A_{T\text{-}CF}$ and the measured $A_{T\text{-}CF}$ did not vary significantly with pH of seawater, the buffering increase is principally due to an increase of bicarbonates as evidenced by the increase in DIC_{CF}. The compensation by bicarbonates was already observed, at short term, in *E. mathaei* (Moulin *et al.*, 2014) and in other species (*Psammechinus miliaris, Paracentrotus lividus, Strongylocentrotus droebachiensis, Tripneustes ventricosus*, Table 14 and *Echinus esculentus* in Spicer *et al.*, 1988). In most of studies, this compensation by bicarbonates allowed to restore pH_{CF} at control value despite a transient decrease during the first days of exposure at $pH_{T\text{-}SW} \geq 7.6$ (Table 14). For example, in *P. lividus*, pH_{CF} was decreased after 24h exposition at $pH_{T\text{-}SW}$ 7.6 (Calosi *et al.*, 2013) but not after more than 7 days (Catarino *et al.*, 2012; Collard *et al.*, 2013; 2014). Similarly, pH_{CF} was fully compensated after more than 7 days in almost all regular euechinoid species tested until now when facing OA predicted for 2100 (Table 14). On the contrary, the cidaroid *Eucidaris tribuloides* has a naturally low pH_{CF} which does not change when the sea urchin faces acidified conditions (Collard *et al.*, 2014). The basal euechinoid *Arbacia lixula* also has a low pH_{CF} which did not change after 4 days of exposure to $pH_{T\text{-}SW}$ 7.6 (Calosi *et al.*, 2013). However, longer exposure duration should be tested before a conclusion could be drawn for this species. The present results show that *E. mathaei* is able to maintain this compensation at long term when facing a $pH_{T\text{-}SW}$ of 7.6. In contrast, a decrease of the pH_{CF} occurred in *Hemicentrotus pulcherrimus* after a 9 month exposure to $pH_{T\text{-}SW}$ 7.7 (Kurihara *et al.*, 2013). Interestingly almost all these contrasted responses match the phylogeny of sea urchins (Fig. 48). *E. tribuloides* and *A. lixula* do not need regulation due to their naturally low pH_{CF} making ocean acidification negligible. *P. lividus* and *P. miliaris* are able to regulate their pH_{CF} at $pH_{T\text{-}SW} \geq 7.6$ but not at lower pH (Catarino *et al.*, 2012, Collard *et al.*, 2014; Miles *et al.*, 2007).

Table 14 : Impact of ocean acidification on the pH and the concentration of bicarbonates in the coelomic fluid (CF) of regular echinoid species. pH of seawater is expressed in total scale (pHT-SW).

Species	Exposure time	Tested pH_{T-SW}	pH_{CF} change	Bicarbonate compensation	Reference
Eucidaris tribuloides	1 month	7.65 – 7.5	no - no	yes - yes	Collard *et al.*, 2014
Arbacia lixula	2-4 days**	7.6*	no	no	Calosi *et al.*, 2013
	24hours	7.6*	no	no	
Paracentrotus lividus	19 days	7.7 – 7.4	no - yes	-	Catarino *et al.*, 2012
	2-4 days**	7.6*	no	yes	Calosi *et al.*, 2013
	24hours	7.6*	yes	yes	
	7 days	7.7 – 7.4	no - yes	yes - yes	Collard *et al.*, 2013
	1 month	7.65 – 7.5	no – yes(a)	yes - yes	Collard *et al.*, 2014
Psammechinus miliaris	8 days	7.45*	yes	yes	Miles *et al.*, 2007
Echinometra mathaei	14 + 35 days***	7.65	no	yes	Moulin *et al.*, 2014
	6 + 7 months***	7.65	no	yes	Present study
Hemicentrotus pulcherrimus	9 months	7.7*	yes	-	Kurihara *et al.*, 2013
Strongylocentrotus droebachiensis	5 days	7.35*	yes	yes	Spicer *et al.* 2011
	3 - 5 days	7.6*	yes - no	-	Dupont & Thorndyke 2012
	10 days	7.5 – 7.1	no - yes	yes - yes	Stumpp *et al.*, 2012
	45 days	7.7 – 7.25	no – no	yes – yes	
		7.25 (b)	yes	no	
Strongylocentrotus fragilis	7 - 31 days	7.5	yes - no	no - no	Taylor *et al.*, 2014
Tripneustes ventricosus	1 month	7.7 – 7.4	no - no	yes - yes	Collard *et al.*, 2014

(a) pH_{CF} was reduced from 7.4 to 6.8 at pH_{T-SW} 7.5 but this decrease was not significant probably due to small sample size (n=3)
(b) Fasted individuals
* pH_T was calculated from carbonate system parameters presented in the study using seacarb
** In situ transplantation to volcanic vent
*** Respectively gradual pH decrease + maintenance of contrasted conditions

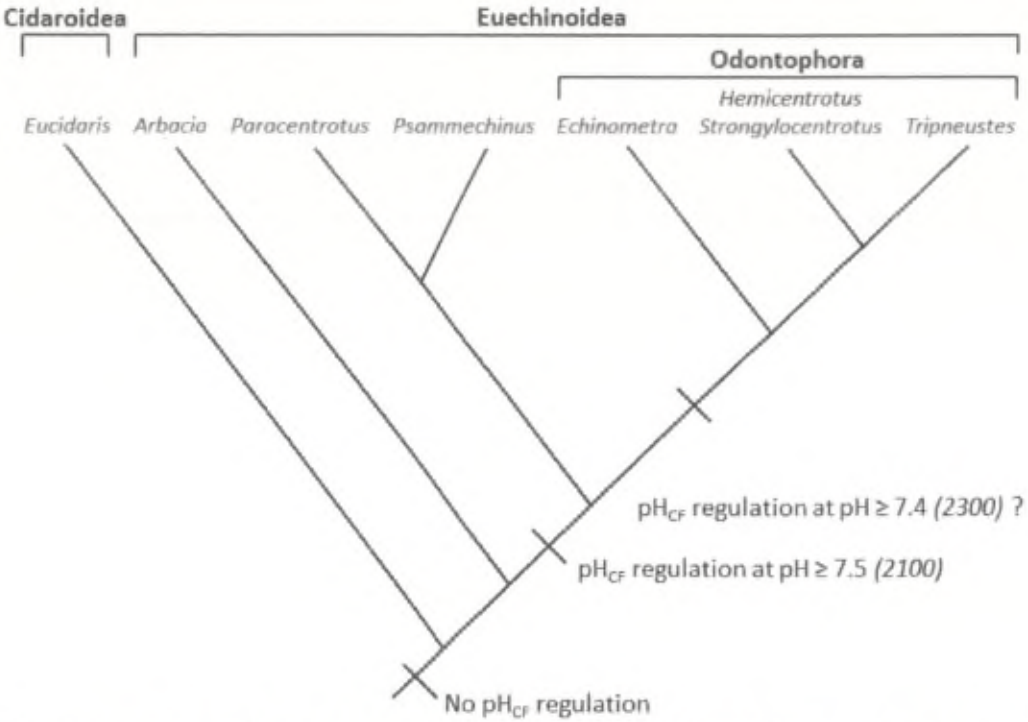

Figure 48 : The phylogeny of sea urchin genera (according to Kroh & Smith, 2010) in relation to the ability to regulate the pH of the coelomic fluid (pHCF) under ocean acidification. Genera represented in the tree are those for which CF compensation capacity has been studied (Table 4 and as discussed in the text).

Odontophora species tested at $pH_T \leq 7.4$ (*S. droebachiensis*, *T. ventricosus)* were shown to regulate their extracellular fluid acid-base status after a minimum acclimation period of 7 days (Collard *et al.*, 2014; Stumpp *et al.*, 2012). This hypothesis has to be verified for *E. mathaei* and *S. fragilis*. Based on this phylogenetic hypothesis, *H. pulcherrimus* should be able to regulate its pH_{CF} at long term but this was not observed by Kurihara *et al.* (2013). We suggest that the nutrition status is another parameter that should be taken into account. Stumpp *et al.* (2012) observed that *S. droebachiensis* was not able to compensate its pH_{CF} by increasing bicarbonate ion concentration when fasted. Most other studies involved sea urchins fed *ad libitum* and at rather low density. In the study of Kurihara *et al.* (2013), *H. pulcherrimus* density was very high (100 urchins in a 45L aquarium) and competition for food could have affected their pH regulation ability. Indeed, intraspecific competition was demonstrated in sea urchins living in large aggregation even when supposedly fed *ad libitum* (see Grosjean *et al.*, 1996 and references therein). Furthermore, food limited sea urchins have a lower buffer capacity in the coelomic fluid (Collard *et al.*, 2013). In the present study, sea urchin density (16 individuals per m^2 of bottom substrate) was voluntarily high, probably inducing also competition for food. A high density of *E. mathaei* is encountered in nature, in particular in Réunion Island (up to 74 individuals per m^2 observed by Conand *et al.*, 1997; field personal observations). This species is relatively tolerant to starvation and resource competition due to its low resource consumption rates (McClanahan & Kurtis, 1991). McClanahan & Kurtis

(1991) showed that *E. mathaei* population regulation occurs at the level of the individual organism, allowing high population densities through decreased energy availability to all individuals (respiration rate and growth reduction). Furthermore, sea urchins were not fed *ad libitum*. Indeed, ΔA_T of the coelomic fluid of *E. mathaei* maintained at control conditions was lower than in the field (mean ± SD = 2234 ± 406 $\mu mol.kg^{-1}$, Moulin *et al.*, 2014). This difference is representative of starved sea urchins as observed by Collard *et al.* (2013). Reduced food availability could explain the low respiration rate and low growth observed during this experiment as compared with other studies where *E. mathaei* was fed *ad libitum*. A low quantity of artificial food was provided from the fifth month of the experiment but this did not modify the pattern of acid-base regulation. Despite the high density/low food availability condition, *E. mathaei* was able to maintain their pH_{CF}. We suggest that this is because they were able to graze on calcareous substrate and ingest calcium carbonate which could provide buffering bicarbonate ions. Holtmann *et al.* (2013) showed that the intestine wall prevents the loss of bicarbonate ions from the CF to the digestive lumen but hypothesized a bicarbonate transport from the intestine to the CF. Results of Asnaghi *et al.* (2013) support a role of calcareous food in mitigating the impact of ocean acidification. On the contrary, it is noteworthy that *H. pulcherrimus* studied by Kurihara *et al.* (2013) were fed non calcareous *Undaria pinnatifida*. Thus, we suggest that the ability to maintain the pH_{CF} in front of ocean acidification is linked to phylogenetic aspects, feeding status and nature of available food and/or substrate to graze (calcareous or not). This emphasizes the importance of conducting experiments in realistic environmental conditions, especially feeding conditions.

Even food limited, the respiration and growth rate of *E. mathaei* was not affected by the seawater pH. This indicates that individuals that survived until the end of the experiment are resilient in front of ocean acidification. Mortality was indeed important during the experiment. This could be explained by natural population senescence. Indeed, *E. mathaei* longevity is relatively low (ca. 7 years) and the test diameter range studied in this experiment was close to the maximum length (40 mm) observed for this species close to the Equator (Ebert *et al.*, 2008). Additional food (nori) was supplied from the fifth month of the experiment but mortality was not affected and continued to occur. Indeed, this supplementary food was added in low quantity (0.02 g dry algae $individual^{-1}$ day^{-1}) in comparison with the grazing rate of *E. mathaei* (0.04- 0.24 g algae $individual^{-1}$ day^{-1}; Bronstein & Loya, 2014). Moreover, the buffer capacity in the coelomic fluid in control conditions did not increase in parallel to food addition. This indicates that sea urchins were always starved despite food addition which may explain the high mortality observed during the

experiment resulting in the selection of more "starvation resistant" individuals. Such selection could also account for the absence of pH effect on respiration and growth rates. However, growth/calcification of adult regular echinoids are in general not affected by pH ≥ 7.7 (Kurihara *et al.*, 2013; Moulin *et al.*, 2014; Ries *et al.* 2009; Stumpp *et al.*, 2012; Taylor *et al.*, 2013; Uthicke *et al.*, 2013; Wang *et al.*, 2013). Only two studies conducted on adults showed that growth was affected by OA (Courtney *et al.*, 2013; Holtmann *et al.*, 2013). Similarly, respiration rate did not vary significantly in adults at pH ≥ 7.7 (Catarino *et al.* 2012; Kurihara *et al.*, 2013; Moulin *et al.*, 2014; Stumpp *et al.*, 2012; Wang *et al.*, 2014). Only Uthicke *et al.* (2013) observed a slightly decreased respiration rate at pH 7.7 but not at pH 7.5. So growth and respiration rates appear as not or weakly affected by acidification up to pH 7.7 in adult sea urchins including at long-term.

Mechanical properties of the test did not differ significantly between control and treatment sea urchins at long-term. Similarly, no difference in mechanical properties of the test of *P. lividus* was observed after one year at pH 7.8 (Collard *et al.*, pers comm). This agrees with short-term results obtained on *S. droebachiensis* in which test plate strength was not affected after 45 days at pH 7.7 (Holtmann *et al.*, 2013). Asnaghi *et al.* (2013) reported that the whole test (crushing) of *P. lividus* was less robust at pH 7.7. However, these tests were conducted on dried test and mainly reflect the strength of dried ligaments maintaining the plates together (see Dubois, 2014, Ellers *et al.*, 1998). So results obtained so far indicate that mechanical properties of the test are not affected by acidification around pH 7.7.

The last principal biological function which could be affected is reproduction. Kurihara *et al.* (2013) observed a delay in gonad maturation and spawning following a 9 months pH decrease but no effect on the maximum number of ova by *H. pulcherrimus*. *S. droebachiensis* fecundity was not affected after 16 month exposure to elevated pCO_2 (Dupont *et al.*, 2013). In our study, gonad development (observed during dissections for sampling of test plates) was zero or low. It is unclear if this was due to feeding conditions, to the cost of bicarbonate buffering or simply because sea urchins were sacrificed out the spawning season.

In conclusion, *E. mathaei* appears resilient in front of ocean acidification expected to occur around 2100 for what concerns acid-base regulation, growth, respiration rate and test mechanical properties. Effects on reproduction are unclear and respective impact of food and cost of the regulation cannot be distinguished. Sea urchins, of which *E. mathaei*, actively participate to the resilience of coral reef by its grazing activity, preventing the overgrowth of coral by algae (Valentine & Edgar, 2010). On the other hand, sea urchins, in some location, are the major

bioeroder of coral reef substrate (Bak, 1994; Carreiro-Silva & McClanahan, 2001; Mokady *et al.*, 1996), weakening the reef structure. The ability of *E. mathaei* to resist long term moderate OA could therefore in turn affect its ecosystemic interaction with algae and coral, making difficult the prediction of the future of coral reefs.

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Bioerosion of tropical coral reefs by the sea urchins *Echinometra mathaei* increases under ocean acidification

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Abstract

Recent studies demonstrated that sea urchins could be more resistant to ocean acidification (OA) than previously postulated. In coral reef, they could be key species through their grazing and bioerosive activities. A change in these processes could strongly influence the competition between coral and macroalgae and the net calcification rate of this ecosystem, particularly under OA. Therefore, in this study, we estimated the effect of a long term (more than 1 year) ocean acidification (pH control 8.1 vs. pH treatment 7.65) on the bioerosion rate of the sea urchin *Echinometra mathaei* using artificial reef mesocosms. This experimental design allowed taking into account ecosystemic interactions and acclimation processes. Results indicate that bioerosion rate of *E. mathaei* is increased by more than 170 % under OA expected in 2100. We suggest that this increase was mediated through direct effect (enhanced grazing rate) but also by indirect effects of elevated biogenic dissolution of the coral substrate that facilitate bioerosion. This could have an impact on the dynamic balance between bioerosion and bioaccretion of corals and could determine the future of coral reef ecosystem, particularly reefs where *E. mathaei* is the major bioeroder.

1. Introduction

Ocean acidification (OA), a consequence of anthropogenic atmospheric carbon dioxide rise, could impact marine organisms (see e.g. Andersson et al., 2011; Barry et al., 2011; Fabry et al., 2008). OA effects on echinoderms were well studied in the last decade (see reviews by Byrne, 2011; Byrne & Przeslawski, 2013; Dubois, 2014; Dupont et al., 2010; Dupont & Thorndyke, 2013). Several physiological parameters (somatic and skeleton growth, metabolism, acid-base regulation, reproduction, energy allocation,...) were studied in adult, post-metamorphic stages and larvae. Initially, this phylum was thought to be particularly sensitive to OA due to their calcium carbonate skeleton, and their apparent poor ion and acid-base regulation ability (Dupont *et al.*, 2010; Dupont & Thorndyke, 2013; Melzner *et al.*, 2009; Pörtner *et al.*, 2004). However, recent studies demonstrated that post-metamorphic sea urchins could be more resistant to OA than previously postulated. Indeed, several species are able to maintain the pH of their coelomic fluid (pH_{CF}) by increasing the buffer capacity of this fluid, at least at seawater pH (expressed in total scale -pH_{SW-T}) ≥ 7.6, i.e. values that are predicted to occur in 2100 (*Paracentrotus lividus* - Calosi et al., 2013; Collard et al., 2013; 2014, *Tripneustes ventricosus* - Collard et al., 2014, *Echinometra mathaei* - Moulin et al., 2014, *Strongylocentrotus droebachiensis* - Stumpp et al., 2012). The pH_{CF} of *Stongylocentrotus fragilis* was even regulated at pH_{SW-T} 7.5 after a sufficient acclimatization period (Taylor et al. 2014). Another study observed a decrease of the pH_{CF} but at lower pH_{SW-T} (7.45, *Psammechinus miliaris* in Miles et al., 2007). Finally, a reduction in pH_{CF} at pH_{SW-T} ≥ 7.6 was only evidenced in *Hemicentrotus pulcherrimus* (Kurihara et al., 2013). The buffer capacity of the coelomic fluid appears to be linked to an increase of the bicarbonate ion concentration possibly mediated by carbonic anhydrase or ion transporters (see references above). Such capacity to regulate the pH of the coelomic fluid has necessarily an energetic cost. Growth of adult appears to be affected in some studies (Courtney et al., 2013; Holtmann et al., 2013; Stumpp et al., 2012) but not in others (Kurihara et al., 2013; Moulin et al., 2014; Uthicke et al., 2012; 2014). Also, energy allocation to reproduction decreased in some cases (Kurihara et al., 2013; Siikavuopio et al., 2007; Stumpp et al., 2012). OA could also impact behaviour and activity of sea urchins on the long term. Borell et al. (2013) observed that exposure to elevated pCO_2 did not change the overall algal consumption by *Tripneustes gratilla*. However, a reduced preference for algae cultured at 4000 µatm pCO_2 was recorded. In contrast, Burnell et al. (2013) showed an increase of grazing activity under OA.

Hermatypic corals are potential victims of OA (Chan & Connolly, 2013; Hoegh-Guldberg et al., 2007; Kleypas & Langdon, 2006). Tropical coral reefs are highly dynamic communities whose existence depends mainly on the calcification rate of hermatypic corals that must remain greater than (bio)erosion (Andersson & Gledhill, 2013; Glynn, 1997; Hutchings, 1986; Tribollet & Golubic, 2011). The balance between construction and destruction of coral reefs is fragile. Even little changes in calcification rate could switch the system from net accretion to net erosion, leading to the rapid destruction of the reef substrate (Andersson et al., 2005). Moreover, coral reefs with a low accretion rate tend to shift more easily to an ecosystem dominated by fleshy algae. Healthy coral reefs are quite resistant to this shift, but much more sensitive under changing environment, like OA (Anthony et al., 2011; Hoegh-Guldberg et al., 2007). Indeed, corals and algae continually compete for light and space (for a review, see Cook et al. 2011). Grazers (principally fishes and sea urchins) are key species in these ecosystems as they prevent the overgrowth of corals by algae (see eg. Carpenter, 1990; Hatcher & Larkum, 1983; Johansson et al., 2010; Lewis, 1986; Lirman, 2001; Sotka & Hay, 2009; Valentine & Edgar, 2010; Williams & Polunin, 2001). Through this activity, grazers play a significant role in the resistance or resilience of coral reefs. For example, mass mortalities of the sea urchin *Diadema antillarum* were correlated to the very slow recovery of coral reefs after hurricanes, algae showing a faster growth than corals in the absence of their main grazer (Birkeland, 1989; Hughes et al., 1987). On the other hand, grazers, in particular sea urchins, are significant bioeroders of the coral reef substrate (Bak 1994; Carreiro-Silva & McClanahan, 2001; Mokady et al., 1996). For instance, at Réunion Island, *Echinometra mathaei* caused a bioerosion of 8.3 kg $CaCO_3$ m^{-2} $year^{-1}$ associated with high population density (73.6 ind. m^{-2}, Conand et al., 1997). A change in the rate of bioerosion, e.g. an increase due to OA, could take part in the weakening of the reef structure. In turn, this would increase the impact of storms and other physical phenomena. This is documented for the sponge *Cliona orientalis* whose bioerosion capacity raises with increasing pCO_2 (Fang et al., 2013; Wisshak et al., 2012; 2013; 2014). For sea urchins, no information on their bioerosive ability under OA is currently available.

Understanding on the one hand the subtle equilibria between coral calcification and bioerosion and on the other hand the participation of grazers, like sea urchins, to the resistance or resilience of tropical coral reefs is required to predict the indirect impact of OA on these ecosystems. Clearly, investigations of the complex ecosystemic interactions at long term are needed. Indeed, such prediction is even more complex if one takes into account the possible acclimatization of the different actors. The goal of the present study was to estimate the effect of a long term (thirteen

months) OA in the range of that predicted in 2100 on the bioerosion rate by the sea urchin *E. mathaei* in an artificial reef community assemblage.

2. Materials and methods

2.1. Experimental design and biological community of mesocosms

The technical setup of artificial reef mesocosms was previously described in Moulin et al. (2014). Briefly, the study was conducted in two independent mesocosms (true replicates). Each mesocosm is composed of one main 500L unit and two experimental aquaria of 300L each. Both experimental aquaria were connected to the main unit and the water flow was the same for both aquaria (800 ± 50 mL min^{-1}). It allowed to maintain the same physico-chemical parameters in both aquaria except for *p*CO2 and pH. One aquarium was maintained at control conditions (mean target pH in total scale, pH_T 8.10) and the other one was acidified using CO_2 injection (mean target pH_T 7.65). In treatment aquaria, pH was decreased gradually during six months (approximately -0.03 units of pH_T every two weeks) and then maintained during seven more months. Control conditions were maintained throughout the experiment. On a daily basis, pH_T was allowed to fluctuate around the target value with an amplitude equivalent to daily changes observed in the field. This was obtained by different day/night setpoint for the pH controllers (pH electrode connected to a control system, Aquastar, Germany) and through community respiration/photosynthesis. In the field (La Saline fringing reef, Réunion Island, 21°70'S, 55°32'E), pH increased between sunrise and zenith time on average by 0.18 units (± 0.05, N=1344, from 2011/05/10 to 2011/06/13, pH measured every 15 minutes, Cuet, pers comm, see also Chauvin et al. 2011) and inversely from zenith to sunrise. A similar pattern was established in experimental aquaria.

Seawater samples were collected every other day and immediately filtered (0.22 µm GSWP, Millipore). Total alkalinity (A_T) was measured by a potentiometric titration with 0.01M HCl in NaCl 0.7M following Dickson et al. (2007) adapted for a smaller volume (25 mL). Each titration was automatically performed by computer using a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyzer to record pH (Consort, Belgium) and a TW Alpha Plus autosampler (SI Analytics, Germany). Calibration was performed using certified reference seawater provided by A. G. Dickson (Scripps Institute of Oceanography, Dixon, batch 94). Once a day, the electromotive force (e.m.f) was measured using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor). The e.m.f was then converted in pH_T using calibration curve of standard buffers of known pH, 2-

aminopyridine/HCl (AMP) and tris/HCl (TRIS) (DOE 1994; Del Valls and Dickson 1998; Dickson et al. 2007). Salinity and temperature were measured once a day using a conductivimeter pH/Cond 340i WTW (USA). In each experimental aquarium, a temperature sensor (Aquastar, Germany) and a pH electrode (Aquastar, Germany) were connected to an IKS control system (IKS, Aquastar, Germany) which record values every 20 seconds. The hand measures were used to make a one-point recalibration of the IKS control system, and possible shift was linearly interpolated between two calibration dates. pCO_2 was claculated from A_T, pH_T, temperature and salinity using the program R (R Core Team 2013) and the package seacarb (Lavigne & Gattuso 2012) (Lueker et al. 2000 's constants for K1 and K2; Perez and Fraga 1987 's constant for Kf; Dickson 1990 's constant for Ks). Calcium and total alkaline earth metals (magnesium + calcium + strontium) concentrations were determined once every two weeks by potentiometric titration adapted from Kanamori & Ikegami (1980). The method was automatically performed by a custom-made computer program piloting a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyzer to record e.m.f (Consort, Belgium) and a TW Alpha Plus autosampler (SI Analytics, Germany). Calcium concentration was measured by an EGTA (molecular biological grade, VWR) titration using of a calcium-selective electrode (Orion, Thermo Fisher Scientific, USA) and a calomel reference electrode (Schott B3510 Ch0, Germany). The total alkaline earth metals were determined by an EDTA (Merck) titration using a hardness ion selective electrode (Consort, Belgium) and a calomel reference electrode (Schott B3510 Ch0, Germany). Calibrations were performed using certified reference seawater (High-purity standards, USA).

Sea urchins *Echinometra mathaei violacea* (Mortensen 1943), violet *Echinometra* (see Arakaki et al. 1998) were collected at Réunion Island in the Indian Ocean, in the back-reef of Saint Pierre fringing reef (21°33'S, 55°47'E). Hermatypic scleractinians and reef calcareous substrate with its diverse communities of algae, bacteria, archae, fungi and meiofauna were also introduced in the mesocosms. Corals and substrate came from aquarium market (Dejong Marinelife, Holland) (*Seriatopora hystrix*, *Acropora tenuis*) and from Réunion Island in the back reef of La Saline fringing reef (21°70'S, 55°32'E) (*Acropora muricata*, *Acropora digitifera*, *Pocillopora damicornis*). Permit was first obtained from "Réserve Naturelle Marine de La Réunion" (RNN164) and "Direction de l'Environnement, de l'Aménagement et du Logement" (DEAL) before field collection. Organisms were acclimated in mesocosm in control condition during seven months before the beginning of the experiment. Sixteen sea urchins (diameter range 28-40 mm), 0.4 ± 0.01 kg corals and 22 ± 1 kg reef substrate were distributed randomly in each experimental aquarium. When a sea urchin died,

it was replaced by one green *Echinometra sp.* B-like of approximately the same size (Arakaki et al. 1998) in order to maintain same grazing/bioerosive pressure. Color of sea urchins allowed us to identify urchins which are present or not from the beginning of the experiment. Mortality of sea urchins in each aquarium was recorded throughout the experiment. Sea urchins fed mainly on macroalgae and coralline algae growing on the reef substrate. As mortality occurred, excessive starvation was suspected and additional food (dehydrated *Porphyra sp.*, Japanese edible seaweed "Nori") was supplied from the fifth month of the experiment in low quantity (ca. 0.3 ± 0.02 g every other day).

Photos were taken throughout the experience in order to assess, qualitatively, the algae community assemblage.

2.2. Bioerosion

One large piece of dead coral colony was cut into regular blocks of ca. 4 x 4 x 3 cm. Blocks were cleaned into gently shaken solution of 2M NaOH (Merck) during 24h, rinsed in MilliQ water (Millipore) and oven-dried at 70 °C for 48h. Blocks were then weighted (± 0.003 g), tagged and fixed inside the experimental aquaria. In each experimental aquarium, six blocks were exposed free and six other blocks were fixed inside a cage (1cm mesh size) to avoid grazing by the sea urchins. Blocks were collected after the gradual pH decrease (7 months exposure), were submitted to the same cleaning/drying protocol and were re-weighted. These same blocks were then re-introduced into experimental aquaria until the end of the experiment. They were then cleaned, dried and weighted as previously described. The length, width and height of each block were measured using a digital caliper in order to estimate its surface. The $CaCO_3$ loss for each block was divided by its surface and reported to one year resulting in bioerosion rate expressed in kg m^{-2} $year^{-1}$. The bioerosion rate of sea urchins was calculated by subtracting the mean bioerosion rate measured in "caged" blocks from the mean bioerosion rate measured in freely exposed blocks.

2.3. Sea urchins tooth and coral skeleton nanohardness

At the end of the experiment, sea urchins were dissected. Their chewing apparatus, the Aristotle's lantern, was cleaned from its soft tissues and disarticulated in agitated solution of 2.5 % and 5.25 % NaOCl during respectively 1h30 and 2h30, rinsed in MilliQ water (Millipore) and air-dried. Similarly, coral growing tips (corresponding to skeleton newly formed in experimental conditions) were sampled and underwent same cleaning protocol. Sea urchins tooth and coral skeleton were embedded in epoxy resin (Epofix, Struers) and then pre-polished using SiC paper

(grain size from 220 to 4000) until reaching growing tips of the skeleton. Samples were finally polished with 3 and 1 µm thick diamond paste to avoid surface deformation (DP-Dac and DP-Nap polishing cloths with DiaPro paste, Struers).

A nanoindenter Hysitron (TriboIndenter) was used for mechanical analyses. Three series of single indentations applying a load of 3000 µN using a Berkovich tip (a low charge was chosen in order to avoid any confounding effect of the resin; Presser et al., 2010) along a line pattern were performed on the middle of the stone part of the sea urchin tooth (Fig.1) and along the surface of coral skeleton. The stone part of sea urchin tooth is composed of a parallel array of fibers coated by a thin organic sheath which are separated by polycrystalline calcitic structures named discs (Märkel, 1970, Wang et al. 1997). For each indentation, the reduced Young's modulus (E) (characterizing the stiffness of the material) and the hardness (H) were automatically determined on both structures from the unloading curve of indentation test (Oliver & Pharr, 1992). The median values were considered for each sample.

2.4. Sea urchins tooth energy dispersive X-ray analysis (EDX)

Elemental energy dispersive X-ray microanalysis was carried out on the same sea urchins tooth blocks used for the nanoindentation tests (see the part 2.3). The elemental analyses were realized in an environmental SEM (FEI XL30 ESEM-FEG), operating at 30 kV and at a working distance of 10 mm with a normalized acquisition time of 100 seconds. They were performed on delimited surfaces in the middle of the stone part of the teeth, taking into account both fibers and discs (Fig. 49). The elemental quantitative analyses used automatic background subtraction and ZAF correction matrix to calculate the elemental composition in atomic percent. The Mg percentage (%) was calculated from the atomic percentage of Mg divided by the sum of atomic percentage of Mg and Ca. The contribution of fibers and disks in the analysis area were determined as percentage on SEM images using ImageJ (after transformation to 8-bit images).

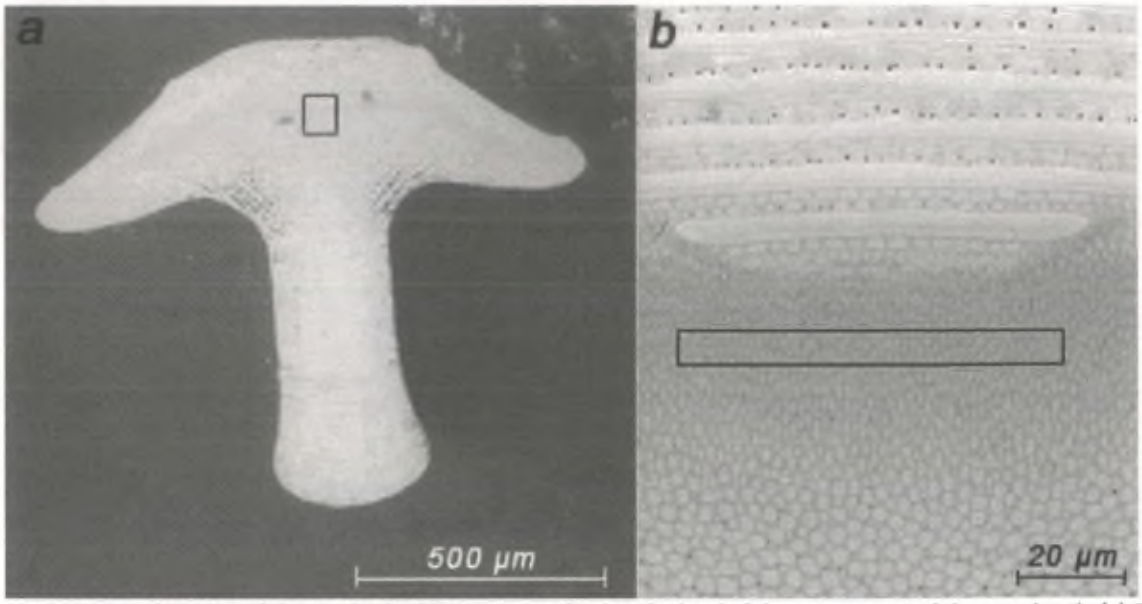

Figure 49 : Transversal section of the tooth of Echinometra mathaei at the level of the mature part of the growing tip (a) General view and (b) enlargement of the stone part (corresponding to the frame on the sub-figure (a)). The area for elemental analysis is delimited by the frame on the sub-figure (b).

2.5. Data analysis

Normality and homogeneity of variances were checked before analysis, using respectively Shapiro-Wilk's and Levene's tests. A_T and salinity of seawater were analyzed using a paired Student's t-test in order to examine possible differences between aquaria within each mesocosm. All parameters studied were analyzed in term of mean by experimental aquaria using a paired Student's t-test (paired factor: pH nominal in each mesocosm). All tests were conducted using the R software (http://www.r-project.org). The level of significance α was set at 0.05.

3. Results

3.1. Physico-chemical parameters in mesocosms and sea urchin mortality

The pH_T of experimental aquaria was maintained close to target values, as well in control conditions as in acidified conditions throughout the experiment (Table 15). In control aquaria, mean pH_T were respectively 8.08 ± 0.08 and 8.09 ± 0.09 in mesocosms A and B. In acidified aquaria, after the progressive decrease of seawater pH, mean pH were respectively 7.63 ± 0.03 and 7.62 ± 0.04 in mesocosms A and B. Mean value of A_T, salinity and temperature during the whole experiment are presented in Table 15. The difference of temperature between contrasted conditions in both mesocosms was not higher than the precision of the control system (< 0.1 °C). A_T and salinity did not differ significantly between contrasted conditions in both mesocosms (paired t test, $t_{(210)} \leq 1.88$, $p \geq 0.06$). The mean calcium and total alkaline earth metals concentrations were respectively 461 ± 13 mg kg^{-1} and 1298 ± 38 mg kg^{-1}. The ratio between total

alkaline earth metals and calcium was maintained between 4.48 and 5.29 throughout the experiment and did not vary significantly between contrasted conditions in both mesocosms (paired t test, $t_{(17)} \leq 0.36$, $p \geq 0.59$). The mortality of sea urchins was monitored throughout the experiment (Fig. 50). At the end of the experiment, mortality did not differ significantly according to the pH condition (paired t test, $t_{(1)} \leq 2$, $p = 0.30$).

Table 15 : Mean seawater conditions during the experiment. Values between brackets are standard deviations. Mean temperature and pH_T were calculated on measurements recorded every 20 seconds. Mean salinity, A_T and pCO_2, DIC, CO_3^{2-}, HCO_3^- and saturation levels of calcite and aragonite (Ω_{Cal} and Ω_{Ar} respectively) were calculated on values measured every day. Mean salinity, temperature and A_T correspond to the data during the whole experiment. Other parameters correspond to the data after establishment of contrasted conditions (last 7 months).

Treatment	Mesocosm	Salinity	Temperature (°C)	A_T ($\mu mol.kg^{-1}$)	pH_T	pCO_2 (ppm)	DIC ($\mu mol.kg^{-1}$)	CO_3^{2-} ($\mu mol.kg^{-1}$)	HCO_3^- ($\mu mol.kg^{-1}$)	Ω_{Cal}	Ω_{Ar}
control	A	34.3 (0.6)	25.2 (0.3)	2400 (133)	8.08 (0.08)	382 (40)	2074 (130)	234 (21)	1829 (117)	6.48 (0.58)	4.27 (0.38)
	B	34.6 (0.5)	25.2 (0.4)	2421 (237)	8.09 (0.09)	359 (47)	2024 (110)	236 (16)	1778 (107)	6.51 (0.45)	4.29 (0.30)
acidified	A	34.3 (0.9)	25.2 (0.3)	2409 (177)	7.63 (0.05)	1288 (124)	2335 (194)	100 (9)	2198 (183)	2.27 (0.25)	1.82 (0.17)
	B	34.6 (0.5)	25.2 (0.3)	2432 (175)	7.62 (0.04)	1269 (93)	2286 (134)	97 (6)	2153 (127)	2.67 (0.16)	1.76 (0.11)

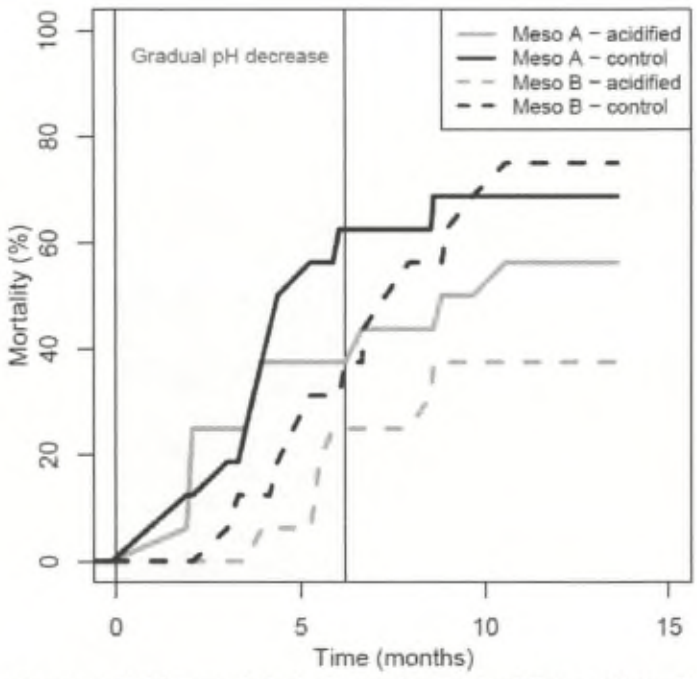

Figure 50 : Mortality (as percentage of initial population) of E. mathaei in the different pH conditions (grey: acidified, black: control) in mesocosm A (continuous line) and mesocosm B (dotted line) during the experiment (months since the beginning of the experiment).

3.2. Bioerosion rate

The bioerosion rate of sea urchins was measured twice during the experiment (Fig. 51). It ranged between 0.52 and 0.64 kg m^{-2} y^{-1} (acidified conditions) and 0.27 and 0.39 kg m^{-2} y^{-1} (control conditions) after the gradual pH decrease corresponding to seven months after the start of the experiment. The bioerosion rate was significantly higher, until more than by 90 % in acidified conditions (paired t test, $t_{(1)} = 77.7$, $p < 10^{-3}$). At the end of the experiment, the bioerosion rate ranged between 0.08 and 0.31 kg m^{-2} y^{-1} (acidified conditions) and -0.11 and 0.11 kg m^{-2} y^{-1} (control conditions). Again it was significantly higher in acidified conditions in both mesocosms (paired t test, $t_{(1)} = 236$, $p < 10^{-3}$), this time by more than 170 %. The bioerosion rates were significantly lower during the second part of the experiment (7- 14 months after start), in comparison to the first measure (paired t test, $t_{(3)} = 3.55$, $p = 0.038$).

The bioerosion rate of blocks not submitted to sea urchins was very low, corresponding to a mean of 0.07 and 0.04 kg m^{-2} $year^{-1}$ respectively after 7 months and 13 months exposition. This rate did not vary significantly between contrasted condition in both mesocosms throughout the experiment (paired t test, $t_{(1)} < 2.16$, $p > 0.28$).

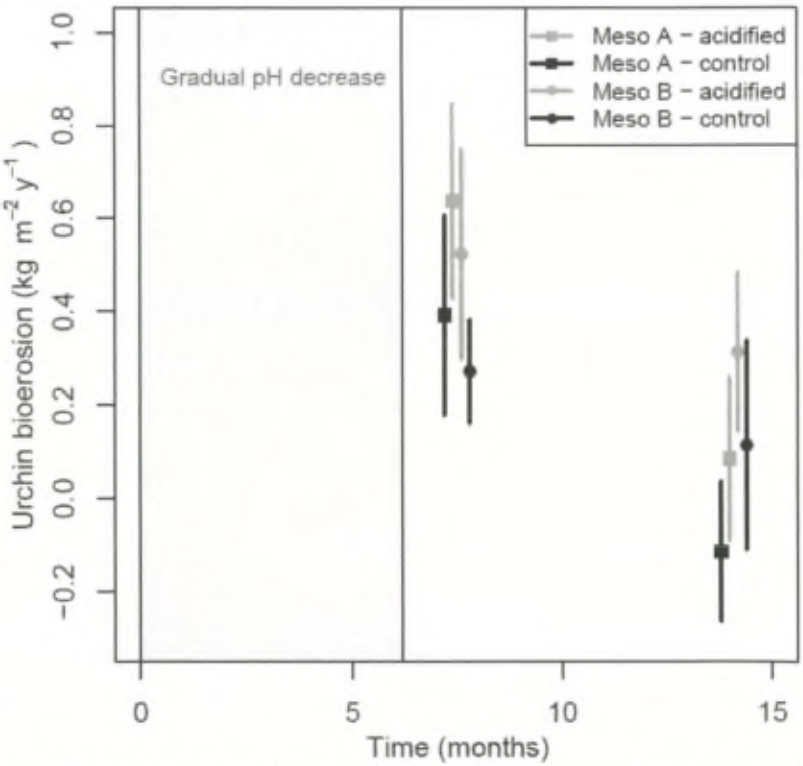

Figure 51 : Bioerosion rate (mean ± SD, n=6) in kg m^{-2} $year^{-1}$ of *E. mathaei* in the different pH conditions (grey: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) during the experiment (months since the beginning of the experiment).

3.3. Sea urchins tooth and coral skeleton mechanical properties

The hardness (H) and the reduced Young's modulus (E) of the sea urchin tooth and coral skeleton , were measured at the end of the experiment (Fig. 52 and Fig. 53 respectively). Mechanical properties did not significantly differ according to seawater pH neither sea urchins nor for corals (Table 16).

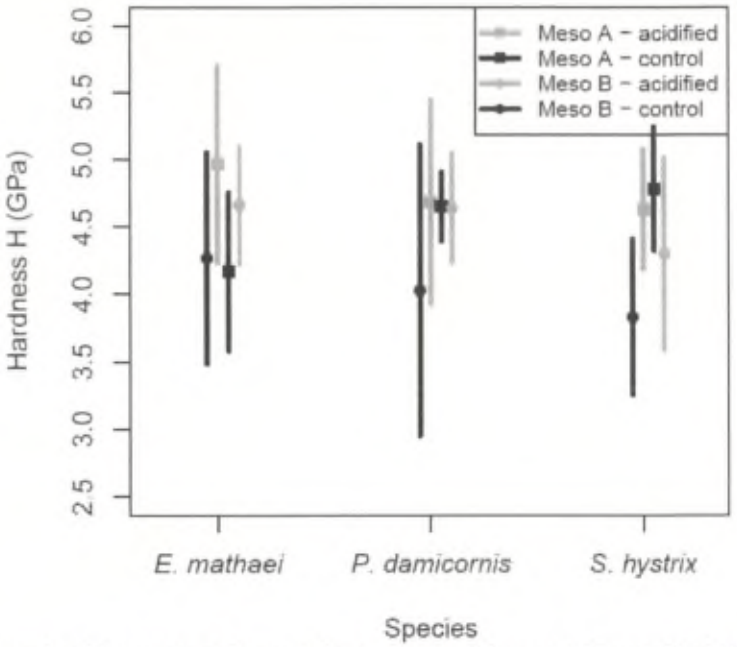

Figure 52 : Hardness (determined by means of nanoindentation test) (mean ± SD, n ≥ 3) of the stone part of the tooth of *E. mathaei* and of the skeleton of *P. damicornis* and *S. hystrix* in the different pH conditions (grey: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) at the end of the experiment.

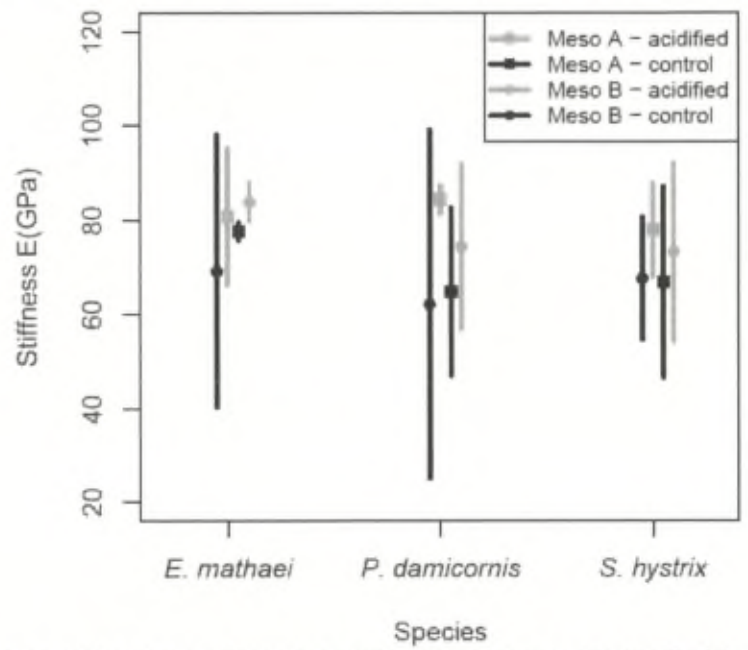

Figure 53 : Stiffness (determined by means of nanoindentation test) (mean ± SD, n ≥ 3) of the stone part of the tooth of *E. mathaei* and of the skeleton of *P. damicornis* and *S. hystrix* in the different pH conditions (grey: acidified, black: control) in mesocosm A (squares) and mesocosm B (circles) at the end of the experiment.

Table 16: Seawater pH effect using a paired Student's t-test for hardness (H) and Young's modulus (E) for the tooth (stone part) of the sea urchin *Echinometra mathaei* and for the skeleton of hermatypic coral *Pocillopora damicornis* and *Seriatopora hystrix*. d.f. : degrees of freedom.

	Hardness (H)			Young's modulus (E)		
Species	d.f.	t value	*p*	d.f.	t value	*p*
Echinometra mathaei	1	2.92	0.21	1	1.54	0.37
Pocillopora damicornis	1	1.12	0.46	1	4.38	0.14
Seriatopora hystrix	1	0.51	0.70	1	3.02	0.20

3.4. Mg content and structure of sea urchins tooth

The magnesium content and the percentage of fiber in the stone part were assessed from teeth of sea urchins sacrificed at the end of the experiment (Fig. 54). Both parameters did not vary significantly according to the pH of seawater (paired t test, $t_{(1)} = 1.77$, $p \geq 0.33$). Similarly, the percentage of fiber in the stone part of the tooth (Fig. 54) was not significantly different between contrasted pH condition (paired t test, $t_{(1)} = 2.65$, $p = 0.23$). However, even if not significant, we observe a trend in the distribution of sea urchins tooth parameters between control and acidified conditions: the fiber percentage was lower and the hardness was greater at low pH.

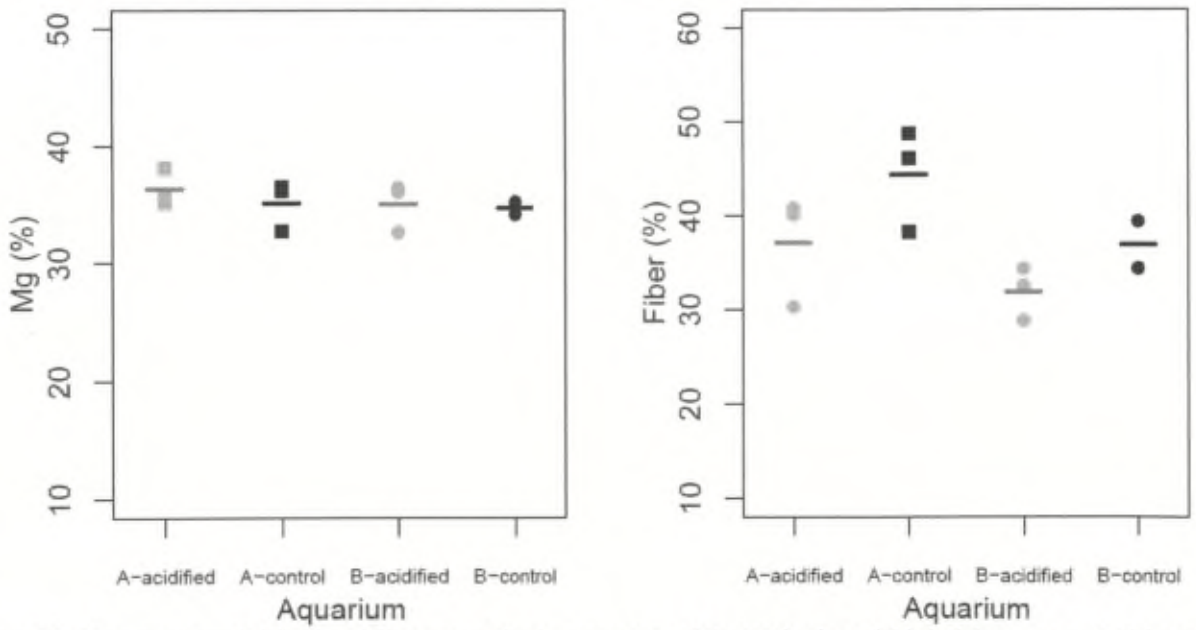

Figure 54 : Mg percentage (%) (left) and fiber proportion in percentage of the total volume (right) of the stone part of the tooth of *E. mathaei* from the different aquaria (A for mesocosm A and B for mesocosm B) at different pH conditions (acidified vs. control) at the end of the experiment. Horizontal black lines represent the mean.

4. Discussion

The mean bioerosion rates measured after seven months in our artificial reef mesocosms ranged between 0.3 and 0.6 kg $CaCO_3$ m^{-2} y^{-1} and are in agreement with previous field studies on *Echinometra mathaei* for individuals of comparable test diameter and density (Table 17). Nevertheless, at the end of the experiment, the bioerosion rates were comparatively low and even zero in control conditions. This could be explained by the introduction of additional food (nori) from the fifth month as sea urchins could prefer this food. Indeed, secondary metabolites like dimethylsulfide (DMS), which is produced when herbivore attacks algae, inhibits feeding by herbivores, including by the subtropical sea urchin *Echinometra lucunter* (Erickson et al., 2006; Lyons et al., 2007; Van Alstyne et al., 2001; Van Alstyne & Houser, 2003). Additional food may not contain it, or in lower concentration.

Moreover, several hypotheses could be formulated assuming that interactions between organisms in our mesocosms are similar to those observed in field conditions. As the grazing pressure was high in our mesocosms, less palatable algae could be selected over the experiment (Hay & Steinberg, 1992). This would result in a decrease of food available for sea urchins. Low bioerosion rate at the end of the experiment could also be the result of enhanced crustose coralline algae (CCA) growth due to sea urchins grazing activity. Indeed, encrusted algae are generally thought to dominate areas where sea urchin density is high. It has been suggested that encrusted algae can be overshadowed by macroalgae (Steneck, 1986) and therefore sea urchin grazing may facilitate access to light for CCA. Indeed, in tropical coral reefs, mass mortalities of the sea urchin *Diadema antillarum* throughout the Caribbean resulted in dramatic shifts from CCA benthic community to communities dominated by macroalgae (Carpenter, 1990; Hughes, 1994; Steneck, 1994). This agrees with qualitative observation of algae community during the experiment. Macroalgae were not visible during the experiment while several months after the removal of the sea urchins from experimental aquaria they became an obvious feature in all aquariums (pers. obs.).

Table 17: Estimation of the bioerosion rate of *Echinometra mathaei*.

Locality	Test diameter (mm)	Density (ind. m^{-2})	Bioerosion rate (g ind-1 day-1)	Bioerosion rate (kg m-2 year-1)	Reference
Zanzibar	34-38	1.26-12.47	0.20-1.57	0.12-0.66	Bronstein & Loya (2014)
Kenya	26-49	56.35	-	0.86	Careiro-Silva & McClanahan (2001)
Reunion	12-50	3.8 – 73.6	-	0.4-8.3	Conand et al. (1997)
Arabian Gulf	37.1	30	0.9-1.4	-	Downing & El-Zahr (1987)
Australia	-	12	-	0.4	Johansson et al. (2010)
Kenya	26-49	0.03-5.6	0.42	-	McClanahan & Kurtis (1991)
Kenya	-	-	0.5	-	McClanahan & Muthiga (1988)
French Polynesia	35	-	0.32	-	Mills et al. (2000)
Gulf of Aqaba	23.1 ± 4.5	3.7-10.5	0.12	-	Mokady et al. (1996)
French Polynesia	-	7.12 -10.10	-	0.6-7.5	Peyrot-Clausade et al. (2000)
Marshall Islands	-	-	0.1-0.2	-	Russo (1980)
Artificial reef Mesocosms (UMONS, Belgium	28-40	30	-	0.33 (pH 8.10, 7 months)	This study
			-	0.58 (pH 7.65, 7 months)	
			-	0 (pH 8.10, 14 months)	
			-	0.20 (pH 7.65, 14 months)	

It is noteworthy that a higher bioerosion rate by *E. mathaei* was observed in acidified conditions both after seven months and at the end of the experiment. Similarly, Burnell et al. (2013) observed an increase of the grazing rate by the sea urchin *Amblypneustes pallidus* at pH 7.93 (control pH: 8.11). This corresponds also to previous observations conducted on endolithic communities and sponges in which an increased bioerosion rate was recorded under elevated pCO_2 (Duckworth & Peterson, 2013; Fang et al., 2013; 2014; Reyes-Nivia et al., 2013; Tribollet et al., 2009; Wisshak et al., 2012; 2013; 2014).

Surprisingly, this increased bioerosion does not appear to be linked, in *E. mathaei*, with an increased metabolism, at the same pCO_2 but at short term (7 weeks, Moulin et al., 2014; 6 weeks, Uthicke et al., 2012; 10 weeks, 2014). In addtition to the present study, the effect of ocean acidification on the physiology of *E. mathaei* was also studied (Moulin et al, *in prep*). Results

showed that *E. mathaei* respiration rate remain unaffected in acidified conditions (pH_T 7.65), even at long term. However, the increased bioerosion rate could be associated with a subtle change in the respiration rate. It would be the case if that change remains relatively low compared to the total metabolism of the echinoid. In this case, it may be not revealed by the statistics, due to the high variability of respiration rate between individuals. Indeed, McClanahan & Kurtis (1991) observed a 20 % decrease in the rate of respiration in *E. mathaei* when it was starved for 2 days. McPherson (1968) found a similar 22 % decrease in respiration rate of the tropical urchin *Eucidaris tribuloides* unfed. So, the difference between the resting metabolic rate (when starved) and the active metabolic rate (when fed) is indeed, limited. The increase in metabolism in relation with a slightly higher grazing rate could therefore remain unnoticeable. Moreover, we suggest that compensation mechanisms may set up to compensate the energy cost of acid-base regulation of the coelomic fluid in *E. mathaei* (Moulin et al., 2014, Uthicke et al. 2014) by a different nutrition behaviour that readjusts the balance between energy spend to maintenance and grazing *versus* energy gathered from the food (optimal foraging theory). A diet richer in calcareous material may lead to an increase in bicarbonate concentration inside the digestive tract that may, in turn, ease the regulation of the pH in the coelomic fluid. Indeed, Holtmann et al. (2013) assumed that the compensation by bicarbonates ions for the acid-base balance of the coelomic fluid could be mediated by the intestinal epithelium, which is compatible with our hypothesis. Calcareous diet may also compensate protons increase in the digestive tract due their efflux from the coelomic fluid. Moreover, Asnaghi et al. (2013, 2014) showed that juveniles *Paracentrotus lividus* are not affected by pH when fed on coralline algae. Another explanation for higher bioerosion could be that sea urchins diet change to a greater contribution of endolith and encrusted organisms and less turf algae. Several authors have demonstrated that turf algae are less palatable for grazers under OA due to enhanced production of deterrent secondary metabolites (Borell et al., 2013; Kerrison et al., 2012). Such readjustments could lead globally to an apparently contradictory situation where acid-base regulation is maintained, together with higher bioerosion, but these energetically costly adaptations are not paired with significant change in respiration rate.

Another hypothesis could be the relative increase of sea urchin tooth hardness comparatively to that of substrate blocks. The stone part of sea urchin tooth is the main working part, directly implicated in grazing/erosive activity, and is also the hardest zone (Märkel & Gorny, 1973; Wang et al., 1997). Urchin tooth hardness has been shown to be correlated to its Mg content and to its structure (Wang et al., 1997). Indeed, the stone part is composed of fibers (about 13 mol% $MgCO_3$)

coated by a thin organic sheath and surrounded by polycrystalline calcitic discs containing as much as 35 mol% $MgCO_3$ (Wang et al., 1997). Moreover, the solubility of magnesian calcite increases with Mg content (Andersson et al., 2008; Morse & Mackenzie, 1990). Therefore, modifications of the Mg content and structure (proportion of fibers and discs) could affect the hardness, solubility and stiffness of the stone part. A trend towards decreased proportion of fibers in the stone part of the teeth which could lead to an enhanced hardness is observable under acidified conditions (Figs. 53 and 54). However, in the present study, the trend is not statistically significant. Hence, we cannot consider it, except we should keep in mind that a lack of power of the statistical test can also be suspected due to the low number of replicates. Comparable measures should be undertaken in the future on a greater number of replicates in order to invalidate or not this hypothesis.

Increased bioerosion rate under elevated pCO_2 could also be indirect. Biogenic dissolution of calcareous substrate by euendolith microborers (cyanobacteria, algae, and fungi) are increased under elevated pCO_2 (Tribollet et al., 2009; Reyes-Nivia et al., 2013). This is explained by a decreased cost of erosion as the gradient between ambient seawater and the site of dissolution decrease as well. Therefore, biogenic dissolution could facilitate and enhance the impact of sea urchins bioerosion, mainly at night when sea urchin feeding activity is the highest and seawater pH is the lowest.

The hardness and the stiffness of coral skeletons, that will form a major part of the reef substrate at the death of the coral, was assessed but did not vary according to seawater pH indicating that intrinsic mechanical properties should not be modified in the future acidified ocean, at least for species studied in this experiment, and at this time scale. Until now, no study has focused on the effect of OA on the mechanical properties of hermatypic coral skeleton. The only study which addressed this effect was conducted on the Mediterranean solitary coral *Balanophyllia europaea*. Goffredo et al (2014) observed that the stiffness, but not the hardness, of the skeleton of specimens growing near CO_2 vents was decreased. This was linked to a porosity increase of the skeleton. So, the increased porosity for some coral species could lead to an increased bioerosion effect under OA. Indeed, a study showed that, even if *Porites astreoides* coral colonies maintain their linear extension under the ocean acidification conditions expected by the year 2100, erosion and predation by boring organisms was significantly greater. This enhanced bioerosion was explained by a decreased density of the colonies (Crook et al., 2013).

The increased rate of bioerosion by *E. mathaei* could affect coral reef ecosystem sustainability. Indeed, it could lead to the weakening of the reef structure, increasing the impact of storms and other physical phenomena. Moreover, the bioerosion by sea urchins is an important factor in the reef carbonate budget in some locations. Echinoid erosion as percentage of reef gross production was measured at different locations and varied between 5 and 216 % (Bak, 1990; Bak et al., 1984; Carriero-Silva & McClanahan, 2001; Glynn & Wellington, 1983; Mokady et al., 1996; Scoffin et al., 1980). Therefore, in several locations where the balance between coral reef accretion and sea urchin bioerosion is already close to zero, ocean acidification could lead to a switch of this equilibrium towards net erosion (Andersson & Gledhill, 2013). Currently, several reefs whose mass budget has been studied have more than 50 % of their gross production bioeroded by sea urchins (Carriero-Silva & McClanahan, 2001; Herrera-Escalante et al., 2005). If OA induces the same increase in bioerosion as that recorded in the present study (at least a doubling), the net ecosystem calcification will become negative in these reefs, especially if all $CaCO_3$ loss processes (physical, chemical and biological erosion) increased likewise, leading ultimately to the fade-out of the coral reef (Andersson et al., 2005; Andersson & Gledhill, 2013). However, response of coral calcification to ocean acidification differs among species (Chan & Connolly 2012). Some species appear unaffected at pH around 7.7 (see eg. Comeau et al., 2013c; Fabricius et al., 2011; Venn et al., 2012). Moreover, in this study, coral calcification rate was unchanged for Acroporidae species (*Acropora muricata* and *Acropora digitifera*) and even doubled for *Seriatopora hystrix* and *Pocillopora damicornis* (Leblud J., pers. com.). Therefore, the increased bioerosion rate could be mitigated through coral calcification adaptation. Moreover, sea urchins and their impacts on macroalgae-coralline interactions could have cascading impacts on the settlement of corals since CCA facilitates coral recruitment (Heyward & Negri 1999; Doropoulos et al. 2012; Webster et al. 2013). However, the persistance of CCA thanks to sea urchin grazing could be ineffective under ocean acidification as coralline algae appear particularly sensitive to pH decrease (Kuffner et al. 2008; Johnson & Carpenter 2012). Moreover, some sea urchin species graze directly on live coral (e.g. *Eucidaris thouarsii*, Glynn et al., 1979) and increased sea urchin grazing could be therefore strongly negative for coral growth.

Conclusion

To our knowledge, this study is the first which estimated the effect of OA on a sea urchin bioerosion ability. *E. mathae*i bioerosion rate almost doubled after six month gradual decrease of

seawater pH until a mean value 7.65 and tripled after seven months maintenance at these conditions. If such increase occurs in the field, reefs where mass budget is already strongly affected by sea urchins bioerosion rate could turn to net erosion. We suggest that this increase was mediated by direct effect (enhanced grazing rate) but also by indirect effects of elevated biogenic dissolution of the coral substrate under OA that facilitates bioerosion. This result emphasizes the importance to conduct long experiment at ecosystem rather than at individual scale. Indeed, response to ocean acidification will vary with species interactions within the ecosystem and thus will vary also depending on direct and indirect effects driven by top-down and bottom-up processes taking place. In the future, it would be interesting to study OA effect on other major bioeroders, like sea urchins *Diadema sp.*, for which no information is currently available concerning OA resistance (including physiological effects and bioerosive ability).

Acknowledgments

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1. Impact du changement global sur les bioérodeurs de récifs coralliens

1.1. Les échinides

Jusqu'à présent, la plupart des études concernant les effets de l'acidification des océans (AO) sur les échinides se sont concentrées sur les espèces tempérées et boréales. En effet, seules huit des trente-deux espèces d'échinides étudiées dans le cadre de l'AO étaient tropicales et parmi celles-ci, une seule étude s'est intéressée aux effets à long terme (plus de six mois) sur des juvéniles. Parmi ces huit espèces tropicales, seules cinq sont reconnues comme des bioérodeurs des récifs coralliens (Table 18).

Les notions de court et long terme sont importantes dans la discussion suivante et nécessitent donc d'être plus clairement définies. Nous nous référons à des expériences à court terme comme celles qui sont réalisées à l'échelle individuelle et qui ne considèrent pas ou peu l'acclimatation de l'organisme aux conditions environnementales. Ainsi, une étude à court terme est généralement menée durant un court laps de temps et permet uniquement d'observer les mécanismes physiologiques. Le terme « long terme » est réservé aux expériences où une acclimatation sur une longue durée, de préférence supérieure au cycle reproductif de l'espèce, a été prise en compte. Dans celles-ci, les interactions symbiotiques et/ou écosystèmiques peuvent s'ajouter aux effets directs du paramètre étudié. Lorsque la durée de l'étude considère plusieurs générations, on parle d'études expérimentales à très long terme. Ces études concernant uniquement les espèces ayant une durée de vie relativement courte à l'échelle d'une vie humaine et sont donc non applicables dans le cas qui nous intéresse ici. Ces études à très long terme s'apparentent aux observations de terrain. Dans celles-ci, l'adaptation génétique des organismes est prise en compte.

Table 18 : Résumé des effets de l'acidification des océans (AO) chez les échinides bioérodeurs au pH prévu en 2100 ($pH_{EM-T} \geq 7,6$). O indique l'absence d'effet sur le paramètre étudié. ↑ et ↓ indiquent respectivement une augmentation et une diminution de celui-ci. Les références associées à chaque observation sont indiquées par des lettres et reprises en légende de la table. Le temps d'exposition au stress de pH y est indiqué entre parenthèses. Dans certaines études (f et j), l'AO a été étudiée en interaction avec un autre facteur (acclimatation préalable des parents à l'AO ou augmentation de la température).

Espèce	Stade	Effet	Paramètre étudié	Références
Euéchinoïdes réguliers				
Echinometra mathaei	Larve	O	Taux de fécondation	a, b, e
		O	Taille des larves	e **si acclimatation**
		O	pourcentage de larves anormales	a, b, e **si acclimatation**
		↓	Taille des larves (retard de développement)	a, b, e
		↑	pourcentage de larves anormales	a, b, e
	juvénile	O	Survie	c
		↓	Croissance	c
	adulte	O	Survie	e, j, k
		O	pH_{LC}	i, j, k
		O	Croissance	e, i, j, k
		O	Taux de respiration	i, j, k
		O	Propriétés mécaniques du squelette (test et dents)	j, k
		O	Excrétion	i
		O	Développement des gonades	i
		↓	Taux de respiration	e
		↓	Quantité de sperme	e
		↓	Développement des gonades	i **si + 3 °C**
		↑	Excrétion	i **si + 3 °C**
		↑	Taux de respiration	i **si + 3 °C**
		↑	Bioerosion	j, k
		↑	$[HCO3-]_{LC}$	j, k
Echinometra viridis	adulte	O	Survie	f
		O	Croissance	f **si + 10 °C**
		↓	Croissance	f
Cidaroïdes				
Eucidaris tribuloides	adulte	O	pH_{LC}	g
		O	$[HCO3-]_{LC}$	g
		O	Taux de nutrition	g
		O	Survie	g
		↓	Croissance	d
Phyllacanthus imperialis	adulte	O	Piquants matures	h
		↓	Jeunes piquants (dissolution)	h

[a]Kurihara & Shirayama, 2004 (3 jours), [b]Kurihara *et al.*, 2004 (3 jours), [c]Shirayama & Thornton, 2005 (6 et 9 mois), [d]Ries *et al.*, 2009 (2 mois), [e]Uthicke *et al.*, 2012 (parents exposés durant 6 semaines, larves 1 jour), [f]Courtney *et al.*, 2013 (2 mois), [g]Collard *et al.*, 2014 (1 mois), [h]Dery *et al.*, 2014 (3 semaines), [i]Uthicke *et al.*, 2014 (10 semaines), [j]Chapitre 2 (7 semaines dont 2 semaines de diminution progressive du pH), [k]Chapitre 3 (13 mois dont 6 mois de diminution progressive du pH).

Le présent travail a permis de mettre en évidence la capacité de résistance à l'AO à court terme (*chapitre 2*) mais également à long terme (*chapitre 3*) chez *Echinometra mathaei* au stade adulte lorsque celui-ci est soumis à un $pH_{EM\text{-}T} \geq 7{,}65$. Cette résistance est associée au maintien de la balance acide-base du fluide extracellulaire, un aspect clé chez les organismes marins faisant face à l'AO (Melzner *et al.*, 2009 ; Pörtner, 2008 ; Pörtner *et al.*, 2004). En effet, le pH du liquide cœlomique (pH_{LC}) est tamponné par accumulation de bicarbonates (HCO_3^-) dans ce même liquide. Plusieurs mécanismes pourraient expliquer leur origine : une augmentation de l'activité/expression de l'anhydrase carbonique et/ou l'activation de transporteurs ioniques. Les ions HCO_3^- seraient transportés du tube digestif vers le LC et/ou proviendraient de l'hydratation du CO_2 métabolique et de sa dissociation en HCO_3^- par l'anhydrase carbonique au niveau des cellules de l'épithélium du tube digestif. Les protons formés seraient alors transportés activement par ces mêmes cellules vers la lumière du tube digestif par le biais de pompes à protons. Cette hypothèse a été avancée pour un oursin tempéré, *Strongylocentrotus droebachiensis* (Holtmann *et al.*, 2013). Collard (2014) a observé chez un autre oursin tempéré, *Paracentrotus lividus,* que la signature isotopique de carbone $\delta^{13}C$ du LC suit de près celle de l'eau de mer, impliquant dès lors un échange important entre ces 2 compartiments. L'auteur fait l'hypothèse que des mécanismes de régulation comparable à ceux proposés par Holtmann *et al.* (2013) pourraient prendre place également au niveau de la paroi des podia. Cependant, cela doit encore être vérifié, à l'aide d'inhibiteurs de ces transporteurs ioniques par exemple.

Le processus de régulation acide-base du LC décrit précédemment entraîne inévitablement un accroissement des coûts énergétiques qui doit se répercuter sur d'autres processus physiologiques. Cependant, la croissance, la résistance du squelette et le métabolisme ne sont pas affectés de manière significative chez *E. mathaei* par la diminution du pH dans le présent travail, aussi bien à court terme qu'à long terme. Deux autres études menées sur la même espèce avaient également montré, à court terme, l'absence d'impact de l'AO sur la croissance (Uthicke *et al.* ,2012, 2014) malgré une diminution du taux de respiration dans l'une de ces études (Uthicke *et al.*, 2012). Néanmoins, dans cette dernière, la faible diminution du taux de respiration était significative à $pH_{EM\text{-}T}$ 7,8 et 7,6 (réduction moyenne de 7 % à ces deux pH) mais pas à 7,45. Ce résultat est en partie expliqué par l'usage d'un modèle polynomial, donc flexible mais purement empirique. Uthicke *et al.* (2014) a ensuite observé l'absence d'effet sur le taux de respiration et l'excrétion d'ammonium chez *E. mathaei* soumis à une diminution du pH_{EM} comparable durant 10 semaines. Par contre, lorsque la température était augmentée de 3 °C, ces deux paramètres

augmentaient bien qu'étant sans effet sur la croissance. On pourrait imaginer que les coûts énergétiques supplémentaires associés au maintien de la balance acide-base pourraient affecter la reproduction. En effet, une diminution de l'allocation des ressources aux gonades est souvent observée chez les échinides en milieu stressant (Byrne, 1990 ; Russel, 1998 ; Shpigel *et al.*, 2004 ; Siikavuopio *et al.*, 2004). Stumpp *et al.* (2012) ont émis cette hypothèse d'une réallocation des ressources au maintien de la balance acide-base au détriment de la reproduction chez l'espèce boréale *S. droebacheinsis*. Cependant, Uthicke *et al.* (2014) n'ont pas observé une diminution de développement des gonades après 10 semaines d'exposition à un pH_{EM-T} 7,9. Par contre, une diminution de la quantité de gamètes émis par les mâles, mais pas par les femelles, à l'issue d'une exposition de 6 semaines au même pH a été mise en évidence (Uthicke *et al.*, 2012). De même, lorsque l'AO est associée à une augmentation de 3 °C, le développement des gonades est retardé chez les mâles (Uthicke *et al.*, 2014). Toutefois, cet effet devrait être vérifié à plus long terme. En effet, une acclimatation de 16 mois permet à *S. droebacheinsis* de reconstituer l'énergie nécessaire à la reproduction (Dupont *et al.*, 2013). Cette hypothèse est en accord avec les données obtenues par Kurihara *et al.* (2013) où seul un retard de développement a été observé, sans conséquence sur le nombre de gamètes produits, chez l'espèce tempérée *Hemicentrotus pulcherrimus*.

L'étude de l'impact de l'AO sur le stade adulte des oursins bioérodeurs, autres qu'*E. mathaei*, est beaucoup moins complète (Table 18). La régulation du pH extracellulaire n'a été étudiée que chez un seul autre oursin bioérodeur de récif : *Eucidaris tribuloides* (Collard *et al.*, 2014). Chez ce dernier, le pH_{LC} n'est pas affecté par une diminution du pH_{EM-T} à 7,7. Par contre, cette observation n'a pas été corrélée à une augmentation de bicarbonates. Ceci serait expliqué par le fait que cet oursin cidaroïde a un pH_{LC} naturellement plus bas que les euéchinoïdes réguliers et la diminution du pH_{EM} attendue en 2100 a donc un impact relativement négligeable dans son cas. En accord, Ries *et al.* (2009) n'ont pas observé d'effet sur la calcification chez cet espèce à $pH_{EM-T} \geq 7,65$. Chez *Echinometra viridis*, la calcification était diminuée par une diminution de 0,2 unité du pH_{EM-T} mais cette réduction était compensée par une augmentation de 10 °C de la température (Courtney *et al.*, 2013). Enfin, chez *Phyllacanthus imperialis*, seuls les piquants primaires sont affectés par l'AO (Dery *et al.*, 2014). Les résultats obtenus jusqu'à présent indiquent que les effets négatifs de l'AO sont compensés par une augmentation de la température. Cependant davantage d'études portant sur l'interaction OA-réchauffement climatique sont nécessaires pour tirer une conclusion générale.

L'effet de l'AO sur le développement embryonnaire et larvaire n'a été étudié que chez un seul échinide bioérodeur, *E. mathaei*. Le taux de fécondation n'est pas modifié par l'AO, une caractéristique générale chez les échinides (Byrne, 2011). Au stade larvaire, l'effet principal est un retard de développement. Il a été suggéré qu'un tel retard pourrait avoir des conséquences sérieuses sur la population d'oursins (Dupont *et al.*, 2010). Il induirait une augmentation du temps passé dans le milieu pélagique et donc les risques de prédation et la probabilité d'être transporté loin des sites de recrutement. Cependant, une diminution de la taille des larves diminue aussi le risque de prédation visuel. Chez les échinides, on retrouve un mode de reproduction asexuée où des larves bourgeonnent en plusieurs larves plus petites. Ce processus est notamment associé à une pression accrue de prédation, démontrant qu'une réduction rapide de la taille constitue un moyen de défense contre les prédateurs (Vaughn, 2010 ; Vaughn & Strathmann, 2008). Récemment, une augmentation de ce mode de reproduction a également été observé chez des larves de *Strongylocentrotus purpuratus* lorsque la pCO_2 est plus élevée (Chan *et al.*, 2013). De plus, la plupart des échinides, dont *E. mathaei*, sont reconnus comme des espèces à stratégies r qui produisent un grand nombre de larves. Le stade critique serait plutôt post-métamorphique pour lequel un taux de mortalité très important, de l'ordre de 70-80 % dans les 24 premières heures après fixation sur le substrat, est rapporté (Gosselin & Qian, 1997 ; Rowley, 1990). En outre, le retard de développement des larves ne se traduit pas forcément par une plus longue durée du stade larvaire. En effet, Wangensteen *et al.* (2013) ont montré, chez l'espèce tempérée *Arbacia lixula,* que la métamorphose avait lieu au même moment indépendamment du pH de l'eau de mer. Le taux de métamorphose et la taille des juvéniles précoces étaient néanmoins plus faibles. De même, le stade juvénile chez *E. mathaei* semble plus sensible à l'OA puisque celle-ci entraîne une diminution de la croissance sans effet sur la survie (Shiramaya & Thornton, 2005). Cette dernière constatation semble pouvoir être généralisée à l'ensemble des échinides tropicaux (Table 18 ; Albright *et al.*, 2012 ; Challener *et al.*, 2014). Cependant, il faudrait tester l'effet d'une acclimatation à long terme des parents. En effet, Dupont *et al.* (2013) ont montré, chez *S. droebacheinsis,* qu'une préexposition des larves à des conditions acidifiées augmente la mortalité des juvéniles mais que les survivants grandissent plus vite. Cependant, il faut rester prudent face à cette dernière observation. En effet, Il est possible que les juvéniles qui grandissent plus vite à plus bas pH soit également ceux qui survivent, résultant en un biais de la vitesse moyenne de croissance. La réduction de la croissance des juvéniles pourrait être influencée par la nature de la nourriture proposée aux oursins (calcaire ou non, *chapitre 2* et *3*). En effet, les algues calcaires

permettrait d'annihiler au moins partiellement les effets de l'OA sur la croissance, fournissant peut-être le bicarbonate utilisé dans la régulation acide-base chez les euéchinoïdes réguliers (Asnaghi *et al.*, 2013 ; Holtmann *et al.*, 2013 ; *chapitre 2, 3 et 4*).

De plus, l'effet de la température sur les stades pré-métamorphiques doit également être pris en compte puisque cette variable régule le succès de reproduction, le développement larvaire, la métamorphose et la croissance chez les échinides (pour une synthèse, Byrne, 2011). Une augmentation de la température inférieure à 6 °C au cours du 21ème siècle n'aura pas d'effet net sur la fécondation et le développement larvaire des oursins bioérodeurs tropicaux *Diadema savignyi* (Rupp, 1973), *Echinometra lucunter* (Sewell & Young, 1999) et *E. mathaei* (Kurihara, 2008 ; Rupp, 1973). Chez de nombreux échinides, une augmentation de température inférieure à 4 °C entraîne une augmentation du taux de fécondation et une croissance larvaire plus rapide (Byrne, 2011 ; Hoegh-Guldberg & Pearse, 1995). L'effet d'interaction entre le pH et la température n'a été étudié chez aucun bioérodeur récifal. Chez l'oursin tropical, *Tripneustes gratilla*, une élévation de la température entraîne une augmentation de la croissance des larves contrebalançant les effets de l'acidification prévue en 2100 (Sheppard Brennand *et al.*, 2010).

Le présent travail est le seul qui se soit, jusqu'à présent, intéressé à l'impact de l'AO à long terme sur la bioérosion par un échinide (*chapitre 4*). Le taux de bioérosion a presque triplé suite à une exposition de 13 mois à un pH_{EM-T} moyen 7,65 (dont 6 mois de diminution progressive). De même, le broutage de l'oursin *Amblypneustes pallidus* était plus élevé après 11 jours d'exposition à pH 7.92 (Burnell *et al.*, 2013). Par contre, aucune variation du taux de nutrition n'a été observée chez *E. tribuloides* à pH_{EM-T} 7,7 pendant 1 mois (Collard *et al.*, 2014). Cependant, dans cette étude, les oursins étaient nourris de granulés artificiels spécialement formulés pour augmenter la croissance somatique. Une diminution du broutage a été observée chez *T. gratilla* par Borell *et al.* (2013) mais uniquement lorsque des algues *Ulva lactuca* étaient cultivées à 4000 ppm, un niveau de pCO_2 bien plus élevé que celui attendu en 2100. Ainsi l'apport énergétique supplémentaire nécessaire à la régulation acide-base pourrait provenir d'une plus grande quantité de nourriture ingérée. Celle-ci devrait aller de pair avec un taux de respiration accrue des oursins, un paramètre pourtant non significativement affecté dans notre étude. Cependant, cette augmentation pourrait être négligeable en comparaison de la variabilité interindividuelle. En effet, McClanahan & Kurtis (1991) ont observé une diminution de 20 % du taux de respiration chez *E. mathaei* lorsque celui-ci était privé de nourriture durant 2 jours. McPherson (1968) a observé une diminution comparable de 22 % du taux de respiration d'*E. tribuloides* non nourris. L'augmentation du taux de nutrition

liée à l'élévation de la pCO_2 était d'environ 20 % dans l'étude de Burnell *et al.* (2013), ce qui correspondait à une faible augmentation du taux de respiration (4 %).

Ainsi, une acclimatation à long terme et des conditions de nutrition naturelles permettent à *E. mathaei* de résister à l'acidification des océans. Pour les autres bioérodeurs tropicaux étudiés jusqu'à présent, il est difficile de conclure quant à leur devenir dans un océan acidifié bien qu'aucun effet létal n'ait été observé. Parmi l'ensemble de ces espèces, seuls *E. mathaei* et *E. viridis* sont connus comme des bioérodeurs non négligeables des récifs coralliens dans la littérature (Table 19). De nombreuses autres espèces appartenant principalement au genre *Echinostrephus*, *Echinothrix* et *Diadema* sont également reconnues comme telles. Néanmoins, l'impact du changement global sur ces espèces et la bioérosion associée n'ont malheureusement pas été étudiés à l'heure actuelle malgré leur importance écologique dans de nombreux récifs coralliens. La seule donnée disponible pour le moment est qu'on retrouve à proximité d'évent à CO_2 situés dans un récif corallien en Papouasie-Nouvelle-Guinée une densité plus importante d'échinides bioérodeurs, à savoir *E. tribuloides*, *Echinometra sp.*, *Echinostrephus sp.*, et plus particulièrement, *Diadema savignyi* et *Echinothrix spp.* (Fabricius *et al.*, 2014). Il est donc probable que ces oursins bioérodeurs de récif résistent bien à l'AO. Si on se base sur les résultats obtenus dans le cadre du présent travail, leur effet bioérosif devrait augmenter en conséquence.

Table 19 : Principaux échinides bioérodeurs des récifs.

Espèce	Localité	Densité (ind m^{-2})	Taux de bioérosion (kg m^{-2} an^{-1})	Référence
Diadema mexicanum	Panama	2-150	0.1-10.4	Glynn, 1988
	Panama	1.5-48	1-3.65	Eakin, 1996
	Mexique	1-6.8	0.2-3.3	Herrera-Escalante *et al.*, 2005
Diadema savingnyi	Polynésie française	4.87	3.87	Bak, 1990
	Zanzibar	0.4-1.65	0.06-0.35	Bronstein & Loya, 2014
Diadema setosum	Golfe d'Aqaba	0.1-6.4	0.01-0.7	Mokady *et al.*, 1996
	China	0.35-3.99	0.12-0.66	Dumont et al., 2013
	Zanzibar	5.72-6.38	1.21-3.22	Bronstein & Loya, 2014
Echinometra lucunter	Iles Vlerges	100	3.9	Ogden, 1977
Echinometra mathaei	Iles Marshall	2-7	0.08-0.33	Russo, 1980
	Golfe arabique	30	9,9-15,3	Downing & El-Zahr, 1987
	Kenya		3.8	McClanahan & Muthiga, 1988
	Polynésie française	7.4	0.4	Bak, 1990
	Kenya	0.03-5.6	0.05-0.9	McClanahan & Kurtis, 1991
	Golfe d'Aqaba	3.7-10.5	0.5-0.9	Mokady *et al.*, 1996
	Réunion	3.8 – 73.6	0.4-8.3	Conand *et al.*, 1997
	Polynésie française	7.12 -10.10	0.6-7.5	Peyrot-Clausade *et al.*, 2000
	Kenya	56.35	0.86	Careiro-Silva & McClanahan, 2001
	Réunion	0.09-14	1.6-4.3	Chazottes *et al.*, 2002
	Australie	12	0.4	Johansson *et al.*, 2010
	Zanzibar	1.26-20.28	0.21-4.22	Bronstein & Loya, 2014
	Récif artificiel (condition contrôle)	8	0-0.33	Chapitre 4
Echinometra viridis	Puerto Rico	0.77-62	0.114-4.14	Griffin *et al.*, 2003
Echinostrephus aciculatus	Iles Marshall	0.86	0.04-0.07	Russo, 1980
	Barbade	23	9.69	Hunter, 1977
	Iles Vierges	9	4.6	Ogden, 1977
	Barbade	23	5.9	Stearn & Scoffin, 1977
	Barbade	23	5.29	Scoffin *et al.*, 1980
	Curacao	12	2.9	Bak *et al.*, 1984
Echinotrhix diadema	Polynésie française	0.6	0.8	Bak, 1990
	Zanzibar	0.18-1.41	0.1-3.46	Bronstein & Loya, 2014
Eucidaris galapagensis	Galapagos	4.6-30.8	3.3-22.3	Glynn, 1988
	Galapagos	-	1.9	Glynn & Wellington, 1983
	Galapagos	30.8	22.3	Glynn, 1988
	Golfe de Californie	0.2	0.1	Reyes-Bonilla & Calderon-Aguilera, 1999

1.2. Les autres bioérodeurs

De nombreux autres phylums contribuent à la bioérosion des récifs coralliens : les microorganismes (principalement les cyanobactéries et chlorophytes), les macroorganismes endolithes (polychètes, siponcles, mollusques bivalves et éponges perforantes) et les brouteurs (poissons Scaridae et Acanthuridae, échinides, mollusques gastéropodes). A titre d'exemple, une étude menée à l'île de Moorea, en Polynésie française, a mesuré des taux de bioérosion de 0,2 et 0,09 kg $CaC0_3$ m^{-2} an^{-1} attribuables respectivement aux microorganismes et macroorganismes endolithes sur des blocs de squelette de coraux morts après 2 ans d'exposition dans le milieu naturel. Les brouteurs, principalement les poissons scaridés et oursins, étaient les agents dominants de la bioérosion, responsables de 89 % de la bioerosion, soit 2,3 kg $CaCO_3$ m^{-2} an^{-1} (Chazottes *et al.*, 1995). Reaka-Kudla *et al.* (1996) ont mesuré des taux de bioérosion plus élevés par des macroorganismes endolithes et des brouteurs (principalement l'oursin corallivore *Eucidaris thouarsii*), respectivement 2.6 kg $CaC0_3$ m^{-2} an^{-1} et 22.8 kg $CaC0_3$ m^{-2} an^{-1}, aux Galápagos. Dans certains récifs, la bioérosion attribuée aux éponges du genre *Cliona* est très importante, pouvant atteindre 23 kg $CaC0_3$ m^{-2} an^{-1} (Neumann, 1966). L'estimation du taux de bioérosion des polychètes varie entre 0,1 et 4,8 kg $CaC0_3$ m^{-2} an^{-1} (Andersson & Gledhill, 2013). Les poissons perroquets peuvent également effectuer une bioérosion très importante (par exemple 6,5 kg $CaC0_3$ m^{-2} an^{-1} – Bellwood, 1996 ; 9,1 kg $CaC0_3$ m^{-2} an^{-1} – Kiene, 1988). Ainsi, la dominance et le taux de bioérosion de chacun des différents groupes varient en fonction du récif. L'impact du changement global sur la capacité bioérosive de chacun d'eux peut donc avoir un impact non négligeable, spécifiquement, sur le bilan de masse du récif.

Jusqu'à présent, très peu d'études ont porté sur la relation entre taux de bioérosion et impact du changement global chez ces bioérodeurs. La croissance et l'assimilation des nutriments des cyanobactéries et chlorophytes sont favorisées par l'augmentation de la pCO_2 (Fu *et al.*, 2007 ; Hutchins *et al.*, 2007 ; Levitan *et al.*, 2007 ; Tribollet *et al.*, 2009). Tribollet *et al.* (2009) ont exposé des blocs de coraux du genre *Porites* durant 3 mois afin d'estimer la perte de carbonates associée à ces microorganismes (ici principalement le chlorophyte *Ostreobium*). Le taux de dissolution du substrat mesuré à pCO_2 élevée (750 ppm) était 48 % plus important que sous condition actuelle (400 ppm) (respectivement 0.46 kg CaC03 m^{-2} an^{-1} et 0.31 kg CaC03 m^{-2} an^{-1}). Reyes-Nivia *et al.* (2013) ont étudié l'effet de l'exposition de squelettes de coraux scléractiniaires *Porites cylindrica* et *Isopora cuneata* à pCO_2 et température actuelles (400 ppm – 24 °C) et prévus en 2100 (610 ppm – 28 °C). Ils ont observé un accroissement de la biomasse et de la bioérosion des microorganismes

bioérodeurs endolithes variant entre 46 % et 89 % selon l'espèce lorsque la pCO_2 était augmentée de 210 ppm et la température de 4 °C. Cette différence d'augmentation du taux de bioérosion en fonction de l'espèce de corail considérée a démontré que l'architecture du squelette, sa porosité, sa densité et sa minéralogie influencent également la réponse de la bioérosion à l'AO. Dans le présent travail, nous avons montré que la dureté et la rigidité du squelette de deux des espèces de coraux étudiées ne varient pas en fonction du pH de l'eau de mer (*Chapitre 4*). Une autre étude récente a montré que la composition chimique et la structure cristalline du squelette n'étaient pas modifiées chez des coraux scléractiniaires solitaires *Balanophyllia europaea* à proximité d'évents à CO_2 en Méditerranée (Goffredo *et al.*, 2014). Dans cette même étude, la dureté du squelette ne variait pas en fonction de la diminution du pH mais le squelette devenait moins rigide et la porosité du squelette augmentait. De même, Crook *et al.* (2013) ont observé que des coraux *Porites astreoides* soumis naturellement à un flux d'eau souterraine à bas pH dans la péninsule du Yucatan ont un taux de calcification réduit et une densité plus faible que des colonies non soumises à ces diminutions du pH. Ainsi l'effet spécifique de l'AO sur les propriétés mécaniques et la porosité du squelette des coraux pourraient également influencer la réponse de la bioérosion à ce stress, ainsi qu'à l'érosion mécanique par l'effet des vagues et des tempêtes.

L'éponge *Cliona orientalis* est un des bioérodeurs les plus importants de certains récifs, notamment sur la Grande Barrière de Corail en Australie où elle attaque et tue les coraux vivants. Cette éponge perfore le substrat calcaire par un mécanisme à la fois chimique et mécanique, libérant ainsi du calcaire particulaire et des éléments dissous. Une exposition expérimentale de cette éponge à l'AO a montré une augmentation de la bioérosion totale lorsque le pH de l'eau de mer était diminué à des valeurs comparables à celles attendues en 2100, à savoir une augmentation de 9 %, 16 % et 31 % respectivement sous les scénarios d'émission RCP 4.5, 6 et 8.5 (Wisshak *et al.*, 2012). Cette capacité bioérosive plus élevée est expliquée par une plus grande croissance des éponges. Celle-ci est liée à un plus grand apport de produits photosynthétiques provenant des symbiontes autotrophes *Symbiodinium*, ces derniers étant favorisés par l'élévation de la pCO_2. De plus, une diminution du gradient de pH entre le site de dissolution et l'eau de mer environnante réduit les coûts métaboliques requis pour la dissolution chimique du substrat. Cependant, une augmentation de la température de 2 à 4 °C semble limiter la bioérosion puisque les éponges souffrent de ce stress qui entraîne une augmentation du métabolisme et une mortalité accrue (Wisshak *et al.*, 2013). De plus, l'apport énergétique et/ou la biomasse des endosymbiontes seraient réduits lorsque la température augmente (Fang *et al.*, 2013, 2014).

Toutefois, Rutzler (2002) a montré des exemples d'accélération de la bioérosion par les éponges sur les récifs suite à des épisodes de blanchiment massif au cours des années 90, certainement liée à une forte mortalité des coraux et donc à une invasion facilitée par les éponges.

L'impact du changement global sur la bioérosion des autres organismes présentés au début de cette section n'a pour l'instant pas été étudié. Néanmoins, on peut en partie prédire l'évolution future de ce processus en fonction de la sensibilité de ces bioérodeurs. En effet, on sait que la dissolution et l'érosion du substrat sont probablement facilitées par le changement global (Andersson & Gledhill, 2013). Ainsi, si les bioérodeurs y résistent, il est probable que leur bioérosion soit augmentée, d'autant plus si la nutrition (liée aux coûts métaboliques de la résistance aux stress) et/ou la biomasse de ces organismes s'accroissent également. Les espèces qui secrètent un squelette extracellulaire en carbonate de calcium sont sensibles à l'AO. En effet on observe généralement une diminution de la calcification associée à une dissolution de la coquille, un processus utilisée pour maintenir la balance acide-base interne, et une grande sensibilité des larves chez les mollusques gastéropodes et bivalves (par exemple, Gazeau *et al.*, 2007 ; Kroeker *et al.*, 2013 ; Kurihara *et al.*, 2007 ; Michaelidis *et al.*, 2005 ; Shirayama & Thornton, 2005). Des études menées à proximité d'évents volcaniques ont montré une réduction du recrutement des gastéropodes et bivalves alors que plusieurs espèces de polychètes étaient capable de survivre dans de tel environnement (Cigliano *et al.*, 2010 ; Ricevuto *et al.*, 2012). L'hypercapnie a également un effet sur le métabolisme, la croissance et la reproduction des poissons (Ishimatsu & Kita, 1999 ; Ishimatsu *et al.*, 2004 ; 2008; Kikkawa *et al.*, 2003, 2004 ; Pörtner et al., 2004). Cependant, la plupart des études menées à des valeurs de pCO_2 comprises entre 3000 et 6000 ppm montrent que la croissance somatique, les performances de nage et le métabolisme ne sont pas affectés après une acclimatation à long terme chez les poissons adultes (Fivelstad *et al.*, 1998, 2003 ; Foss *et al.*, 2003 ; Kroeker *et al.*, 2013 ; Melzner *et al.*, 2009). Les larves de poissons de récifs sont, par contre, plus sensibles à l'AO. Plusieurs études ont montré une altération du comportement chez celles-ci : attirance olfactive vers les prédateurs, non détection des habitats potentiels, ... (Devine & Munday, 2013 ; Munday *et al.*, 2009a ; Munday *et al.*, 2010). Cependant, leur croissance augmente, réduisant la durée du stade larvaire et conférant une plus grande vitesse de nage et donc une fuite plus facile face aux prédateurs (Munday *et al.*, 2009b). La résistance des poissons est possible notamment grâce à un système respiratoire et circulatoire bien développé qui permet un transport actif du CO_2 métabolique, l'accumulation de bicarbonates pour tamponner la diminution du pH et un efflux des protons efficaces au niveau des

branchies (Heisler, 1986). L'absence d'un système de régulation ionique et acide-base efficace chez les mollusques expliquerait en partie leur sensibilité à l'AO. Les siponcles sembleraient ne compenser que partiellement la diminution de leur pH extracellulaire, entraînant une diminution du métabolisme et de la synthèse des protéines (Reipschläger & Pörtner, 1996 ; Pörtner *et al.*, 2004 ; Pörtner, 2008).

Globalement, il est plus que probable que la bioérosion, et l'érosion en général, des récifs augmente au cours du siècle puisque les principaux agents de celle-ci (oursins, poissons perroquets et éponges) sont résistants au changement global attendu et que leur capacité bioérosive augmente ou est facilitée, notamment par l'action des microorganismes endolithes, suite à la diminution du pH de l'eau de mer.

2. Les changements globaux climatiques en milieu récifal tropical

2.1. Impact sur la calcification des récifs coralliens

La compréhension des mécanismes de contrôle de la biominéralisation chez les coraux hermatypiques et les algues corallines encroûtantes (ACE) est encore incomplète à l'heure actuelle. Cependant, une diminution de la croissance du squelette de ces organismes, du moins chez la plupart des espèces de ces deux groupes, est une conséquence indéniable de l'AO (Kroeker *et al.*, 2013). En moyenne, un doublement de la pCO_2 atmosphérique résulte en une diminution d'environ 10-50 % des taux de calcification chez les coraux (Kleypas & Langdon, 2006). Les ACE tropicales étudiées jusqu'à présent montrent une diminution du taux de recrutement de plus de 75 % (Kuffner *et al.*, 2008), un taux de calcification réduit de 20 à 50 % (Anthony *et al.*, 2008 ; Semesi *et al.*, 2009) et une mortalité accrue (Diaz-Pulido *et al.*, 2012).

La réponse de la calcification des coraux à l'OA varie selon les études et les espèces (Chan & Connolly, 2012). Certaines espèces de coraux semblent plus résistantes au pH prédit pour 2100 (*Porites* massifs - Fabricius *et al.*, 2011 ; *Stylophora pistillata* - Venn *et al.*, 2012). Dans une étude menée simultanément au présent travail, le taux de calcification des coraux n'était pas modifié pour les espèces d'*Acroporidae* étudiées (*Acropora muricata* et *Acropora digitifera*) et doublait pour les espèces de *Pocilloporidae* (*Seriatopora hystrix* et *Pocillopora damicornis*) (J. Leblud, comm. pers.). Ce résultat pourrait s'expliquer par différentes caractéristiques de notre design expérimental : la diminution du pH s'est faite progressivement, des variations journalières de pH et luminosité ont été incluses, l'alcalinité totale a été maintenue à des valeurs normales dans les deux conditions de pH et l'exposition à l'AO s'est faite à long terme. La résistance à l'AO serait également associée à la capacité de régulation du pH dans l'espace sous-calicoblastique où a lieu la calcification (Holcomb *et al.*, 2014). De plus, les coraux seraient capables d'utiliser les ions HCO_3^- dont la concentration augmente sous AO (Comeau *et al.*, 2013a).

Pour les espèces coralliennes résistantes à l'AO, l'augmentation de la température et les effets indirects sont également à prendre en compte. L'analyse de carottes de squelette de coraux sur la Grande Barrière de Corail couvrant les années 1990 à 2005 a indiqué une diminution de 14 % du taux de calcification. Cette réduction a été associée à des épisodes de blanchiment causés par des augmentations de température survenues au cours de cette période (De'Ath *et al.*, 2009). De plus, le stade larvaire des Scléractiniaires est plus sensible puisque l'AO affecte la production de gamètes et le recrutement des larves planula (Albright *et al.*, 2008, Jokiel *et al.*, 2008) soit directement, soit indirectement via l'effet négatif sur les ACE qui facilitent notamment le

recrutement du corail (Doropoulos *et al.*, 2012 ; Heyward & Negri, 1999 ; Webster *et al.*, 2013). Enfin, l'impact de stress locaux comme le développement côtier, l'eutrophisation, la pollution et la surpêche représentent des menaces supplémentaires qui augmentent parallèlement aux changements climatiques et qui peuvent fortement compromettre la persistance des récifs coralliens (Table 20 ; Ateweberhan *et al.*, 2013 ; Hoegh-Guldberg, 2013). Ces différents facteurs sont détaillés dans les sections suivantes.

Table 20 : Résumé des effets de l'acidification des océans, du réchauffement climatique et des facteurs locaux (synthétisé d'après Ateweberhan *et al.*, 2013)

Facteurs	Effets
AO	- Résistance de certaines espèces - Diminution du taux de calcification - Sensibilité accrue face aux maladies et à la bioérosion - impact négatif sur la reproduction, le développement et le recrutement
Réchauffement climatique	- Blanchiment du corail : taux de calcification réduit, sensibilité accrue face aux maladies et à la bioérosion, impact négatif sur la reproduction, le développement et le recrutement - Diminution du taux de calcification
Maladies	- Diminution du taux de calcification - Sensibilité accrue face au blanchiment - Impact négatif sur la reproduction, le développement et le recrutement
Eutrophisation	- Favorise le recouvrement des coraux par les macroalgues ➔ augmentation de l'activité microbienne et donc des maladies - Augmentation de la bioérosion
Surpêche	- Réduction des poissons algivores ➔ favorise le recouvrement des coraux par les macroalgues ➔ augmentation de l'activité microbienne et donc des maladies - Augmentation de la bioérosion (diminution des prédateurs d'oursins par exemple) - Pratiques destructives : dommages physiques
Sédimentation et turbidité accrues	- Augmentation de l'activité microbienne et donc des maladies - Diminution du taux de calcification

2.2. Impact sur la croissance des algues non calcifiées

La croissance des macroalgues non calcifiées, contrairement à leurs équivalentes calcifiées, répond positivement à l'OA (Gao *et al.*, 1991, 1993 ; Koch *et al.*, 2013 ; Kroeker *et al.*, 2013 ; Küble *et al.*, 1999 ; Porzio *et al.*, 2011). Les coraux et macroalgues étant en compétition constante pour l'espace et la lumière (McCook *et al.*, 2001), l'acidification des océans favorisera donc le

développement des macroalgues. De plus, celles-ci facilitent le développement de maladies coralliennes en augmentant l'activité microbienne via la libération de composés dissous (Smith *et al.*, 2006). Elles produisent également des molécules « antifouling » qui empêchent le recrutement du corail. Ces différents effets favorisent donc le basculement de l'écosystème corallien vers un écosystème dominé par les macroalgues (Hoegh-Guldberg *et al.*, 2007 ; Hugues *et al.*, 2003). De plus, l'exposition à une pCO_2 élevée résulte en une diminution significative des concentrations en protéines dans les algues et en une augmentation de la production du diméthylsulfoniopropionate reconnu comme un métabolite secondaire qui agit comme un répulsif pour les brouteurs, notamment les oursins (Borell *et al.*, 2013 ; Kerrison *et al.*, 2012). De manière similaire, plusieurs études ont montré que la croissance des phanérogames marines augmentait significativement dans des conditions de pCO_2 élevée (Palacios & Zimmerman, 2007). Les herbiers pourraient potentiellement s'étendre sur les récifs coralliens. Cependant, des études indiquent que les zones récifales à proximité pourraient également bénéficier des herbiers en bonne santé. En effet, certaines ACE calcifient plus vite à proximité des herbiers marins, en raison de la diminution du CO_2 environnant assimilé par les phanérogames marines (Semesi *et al.*, 2009 ; Unsworth *et al.*, 2012). Toutefois, on observe une diminution de substances phénoliques produites par ces plantes supérieures lorsque le pH_{EM} diminue, résultant en des taux de broutage plus élevés comme cela a été observé à proximité d'évents volcaniques (Arnold *et al.*, 2012). Donc l'AO pourrait induire une augmentation de la productivité des algues non calcifiées au détriment des phanérogames marines à la fois de manière directe et indirecte (réduction de la pression de broutage) alors que le développement des herbiers serait limité par leur broutage en augmentation.

Le réchauffement de l'eau de mer de surface est également à prendre en compte. Celui-ci pourrait réduire la photosynthèse comme cela a été démontré chez les plantes terrestres lorsque l'on dépasse leur seuil de tolérance thermique. Toutefois, chez ces dernières, cette réduction était partiellement contrebalancée par l'augmentation de la pCO_2, augmentant l'optimum thermique de photosynthèse et de croissance. L'interaction température-CO_2 n'a pas encore été étudiée chez les phanérogames et algues marines. En se basant sur les résultats obtenus en milieu terrestre, l'élévation de la pCO_2 pourrait masquer les effets délétères de l'augmentation de la température (Koch *et al.*, 2013).

3. Les facteurs influençant la résistance et la résilience des récifs coralliens en 2100

3.1. La bioérosion

Les bioérodeurs principaux des récifs coralliens apparaissent robustes face aux changements climatiques globaux et l'AO entraîne une augmentation de leur capacité bioérosive. Ainsi, la bioérosion peut être augmentée de manière directe par l'AO mais peut également être favorisée par différents facteurs répartis entre (a) les conditions provoquant la mort des tissus du corail et (b) les autres conditions qui fournissent un avantage de croissance aux bioérodeurs par rapport aux organismes hermatypiques.

Mis à part quelques espèces qui envahissent le corail vivant, la grande majorité des bioérodeurs attaquent le squelette mort (Glynn, 1997). De ce fait, les perturbations naturelles ou anthropiques qui conduisent à la perte de tissus coralliens vivants vont accroître les risques d'invasion par les bioérodeurs et donc induire des taux de perte de $CaCO_3$ plus élevés. Ces menaces devraient s'accroitre aux cours du 21ème siècle. Ainsi, une augmentation de la sédimentation due à l'érosion des sols résultant des pratiques culturales en conjugaison avec les changements de régime pluviométrique liés aux changements climatiques globaux, aura un effet négatif direct sur la croissance des coraux. Elle entraîne également une augmentation des maladies comme « la maladie de la bande noire » associée à une infection par les cyanobactéries, elles-mêmes favorisées par l'élévation de la pCO_2, la sédimentation, la turbidité et un enrichissement en nutriments (Kuta & Richardson, 2002 ; Voss & Richardson, 2006). De plus, l'augmentation de la bioérosion pourrait, en retour, augmenter la sédimentation et l'affaiblissement de la structure du récif face aux vagues et courants. Néanmoins, il semble que certains coraux puissent bénéficier d'une rupture facilitée par la bioérosion (Glynn, 1997). En effet, un mode commun de reproduction de nombreuses espèces de coraux ramifiés est la fragmentation asexuée (Highsmith, 1982 ; Tunnicliffe, 1979). Il a été avancé que la propagation par ce moyen, qui résulte en une dispersion locale, est avantageuse pour les populations qui sont bien adaptées aux paramètres environnementaux locaux. D'autre part, les cavités produites par la bioérosion augmentent la complexité de l'habitat (sa structure tridimensionnelle à diverses échelles) et donc la diversité et la biomasse des organismes associés au récif. Ces cavités facilitent la cimentation interne et le renforcement du substrat. Cependant, une bioérosion trop importante et la sédimentation accrue associée pourraient aussi profondément modifier ces microenvironnements et résulter en une réduction de la complexité et de la diversité du récif (Andersson & Gledhill, 2013). Une telle

diminution de biodiversité serait renforcée par l'impact négatif de l'AO sur de nombreux organismes récifaux (Fabricius *et al.*, 2014). Enfin, les épisodes de blanchiment massif dus à un accroissement de la température et l'eutrophisation font également partie des effets délétères de l'activité humaine qui pourraient diminuer la calcification, entraîner la mortalité des coraux et modifier dès lors le bilan accrétion-érosion à la faveur de la bioérosion.

3.2. Relation algivores-algues-coraux

De nombreux algivores affectent positivement la croissance des coraux en empêchant le recouvrement de ceux-ci par les algues (Carpenter, 1990 ; Hughes *et al.*, 1987 ; Lirman, 2001 ; McClanahan *et al.*, 2001a, 2001b ; McManus *et al.*, 2000 ; Smith *et al.*, 2001 ; Sotka & Hay, 2009). Comme nous l'avons vu précédemment, les macroalgues non calcifiées sont favorisées par les changements climatiques globaux. Cependant, la croissance de celles-ci pourrait être contrecarrée par une pression trophique accrue des algivores qui semblent robustes face aux changementx climatiques globaux. La productivité nette des algues non calcifiées, et donc, leur biomasse sur le récif dépendra de la balance entre l'accroissement de leur vitesse de croissance et de l'accroissement du taux de broutage.

Un autre effet bénéfique de l'action des herbivores sur les récifs coralliens est la favorisation de la croissance des ACE par leur activité. En effet, des mortalités massives de l'oursin *Diadema antillarum* dans les années 80-90 dans les Caraïbes ont abouti à un changement dramatique de la communauté algale benthique d'un écosystème dominé par les coraux et ACE à un écosystème dominé par les macroalgues (Carpenter, 1990 ; Hughes, 1994 ; Hugues *et al.*, 1987 ; Steneck, 1994). Ainsi, la réduction du taux de calcification des ACE causée par l'AO pourrait en partie être compensée par l'effet favorisant des algivores.

Toutefois, l'eutrophisation est également un facteur important qui joue fortement sur cet équilibre coraux-algues-algivores. L'augmentation de la concentration en macronutriments, N et P en particulier, entraîne souvent une diminution de la croissance, de la reproduction et du recrutement des coraux (Koop *et al.*, 2001). Outre ces effets négatifs directs sur les coraux, les apports en nutriments peuvent également causer des changements dans la structure des communautés algales (Fig. 1). Par exemple, sur le récif de la Saline (Ile de la Réunion), une eutrophisation accrue a favorisé le remplacement des algues formant un gazon algal (« turf algaeé » en anglais) par les macroalgues (Chazottes *et al.*, 2002). Ce changement qualitatif dans la couverture algale a entraîné une réduction de la bioerosion associée aux algivores. Enfin, la

surpêche peut, d'une part, faciliter la croissance des macroalgues via la diminution de la densité des poissons algivores. D'autre part, elle peut également mener à une augmentation incontrôlée de la biomasse des oursins qui, en absence de leurs prédateurs, réduisent très nettement la biomasse des macroalges (phénomène connu comme « urchin barrens » en anglais). Dans un tel cas, les échinides affectent même les ACE (pour rappel, ces dernières facilitent le recrutement du corail, Fig. 55).

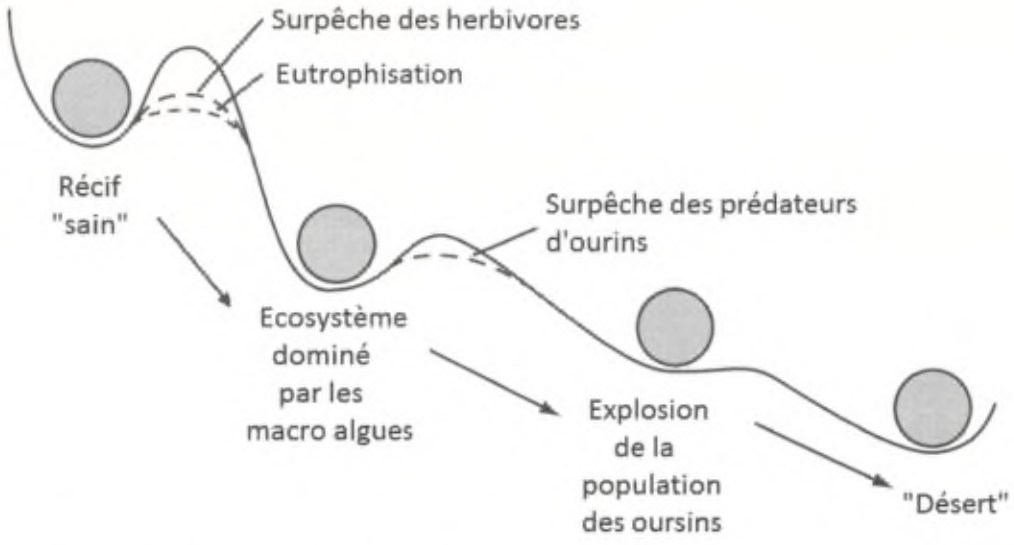

Figure 55 : Diagramme montrant comment la surpêche et l'eutrophisation peuvent modifier un écosystème. Les lignes pleines et pointillées représentent respectivement la résilience des récifs coralliens face à un basculement d'un état à un autre et la modification de cette résilience face aux changements liés à l'activité humaine (modifié d'après Bellwood *et al.*, 2004).

4. Devenir des récifs coralliens en 2100 : conclusion et perspectives

Ainsi une augmentation modérée de la bioérosion et/ou du taux de broutage peut favoriser ou non les récifs coralliens de différentes manières :

Facteurs défavorisants	Facteurs favorisants
• Diminution de la résistance du récif face aux vagues et courants marins (y compris les évènements climatiques extrêmes)	• Augmentation de la reproduction asexuée • Augmentation de la complexité et de la diversité du récif
• Augmentation de la sédimentation ➔ développement de maladies et diminution de la croissance des coraux	• Diminution de la croissance des macroalgues qui sont en compétition avec les coraux
• Perte de $CaCO_3$ qui pourrait devenir supérieure à la bioaccrétion du récif ➔ érosion et régression du récif à long terme	• Avantage aux ACE face aux macroalgues ➔ recrutement du corail facilité, du moins si les ACE survivent à l'AO

Il est probable que la calcification des coraux soit diminuée dans un contexte de changement global, ne fut-ce qu'à cause des coûts métaboliques associés au maintien du pH au niveau du site de calcification. Dès lors, l'avenir des récifs coralliens tropicaux dépend du taux de bioaccrétion (en majeure partie par la calcification des coraux) qui doit rester supérieur au taux d'érosion du substrat récifal (comme la bioérosion) pour que le récif subsiste à long terme. Si cette condition est remplie, l'augmentation de la bioérosion et/ou du broutage des algues pourrait dès lors favoriser la survie des récifs coralliens.

Par exemple, pour le récif modèle utilisé dans le présent travail (arrière récif de La Saline à l'ile de la Réunion), le taux de calcification net moyen est d'environ 12,4 kg m^{-2} an^{-1} et le taux de bioérosion s'élève à 8,3 kg m^{-2} an^{-1} (Conand et al., 1997). Si l'AO résulte en une réduction de 20 % de la croissance des coraux alors que le taux de bioérosion des oursins triple, cela conduira inévitablement à terme à l'érosion totale de cette partie du récif (basculement vers un écosystème à fonds sableux). Par contre, si le taux de bioérosion est plus faible comme par exemple au niveau du platier du même récif (0,4 kg m^{-2} an^{-1}), la calcification nette du récif restera positive et le broutage des algues par les oursins pourra favoriser la résistance du récif face aux changements

environnementaux, du moins si la croissance de ces algues n'augmente pas dans des proportions non régulables par les brouteurs.

Cependant, ce modèle est incomplet et de nombreux autres facteurs doivent être pris en considération. L'acclimatation/adaptation des organismes marins est une possibilité non négligeable qui peut changer bien des mécanismes identifiés jusqu'ici à court terme. En effet, de manière générale, une acclimatation lors d'expériences à long terme des oursins entraîne la disparition de la plupart des effets délétères de l'AO observés à court terme. Ces résultats sont en accord avec des observations *in situ* où des oursins soumis naturellement à une diminution du pH_{EM} s'adaptent aux conditions locales et ne sont pas affectés par rapport à leurs congénères vivants dans des régions plus stables. Il ne faut pas négliger non plus, les possibilités d'adaptation génotypique. Par exemple, Pespeni et al. (2013) ont montré chez *S. purpuratus* une fréquence plus importante d'allèles associés à une exposition à bas pH le long d'un gradient de conditions pH s'étendant de l'Oregon à la Basse Californie le long de la côte ouest des Etats-Unis. Ces allèles représentatifs des milieux à bas pH sont impliqués notamment dans le transport d'ions, la biominéralisation et, de manière générale, dans les voies fonctionnelles importantes pour le maintien de l'homéostasie. Chez les Scléractiniaires, l'acclimatation/adaptation doit également être envisagée. Les épisodes de blanchiment massif pourraient résulter en la sélection de symbiontes plus résistants aux changements climatiques globaux, en particuliers, d'autres clades de zooxanthelles (hypothèse du blanchiment adaptatif, Buddemeier *et al.*, 2004 ; Fautin & Buddemeier, 2004 ; Jones *et al.*, 2008). Les microalgues endolithes, favorisées par l'augmentation de la pCO_2, pourraient également servir de source alternative de photosynthétats lors de ces épisodes de blanchiment (Fine & Loya, 2002), favorisant ainsi la survie des colonies coralliennes en transition. Ainsi, certaines espèces de coraux ou populations pourraient être plus résistantes que d'autres aux changements climatiques globaux, induisant une diminution de la biodiversité mais pas forcément une disparition à long terme des coraux scléractiniaires.

Néanmoins, à l'échelle locale, d'autres facteurs pourraient influencer cet équilibre subtil et faire pencher la balance dans un sens ou dans l'autre. Ainsi, la prédiction du devenir des récifs coralliens tropicaux ne peut dès lors être généralisée à l'échelle du globe et nécessite de prendre en compte l'ensemble de ces facteurs locaux, à long terme, afin d'inclure l'acclimatation/adaptation des organismes. On pourrait imaginer l'usage de modèles mécanistiques prenant en compte ces différents facteurs dans un contexte de changements globaux (effets sur le taux de calcification, le taux de bioérosion/broutage et le taux de croissance

des algues en fonction des facteurs locaux) afin d'affiner nos prédictions et de déterminer le scénario où la calcification nette de l'écosystème atteindra une valeur nulle ou négative (Fig. 56). Ces prévisions permettront de mettre en place spécifiquement les méthodes de conservation locales, autres qu'un abattement massif de la production de gaz à effet de serre à l'échelle mondiale, très hypothétique. L'estimation présente et passée du taux de calcification nette des récifs coralliens varie entre 0,5 et 22 kg CaCO3 m^{-2} an^{-1} (voir Andersson & Gledhill, 2013 pour une synthèse). Ainsi, les récifs présentant déjà à l'heure actuelle un faible taux de calcification nette seront certainement d'autant plus sensibles aux changements anthropiques.

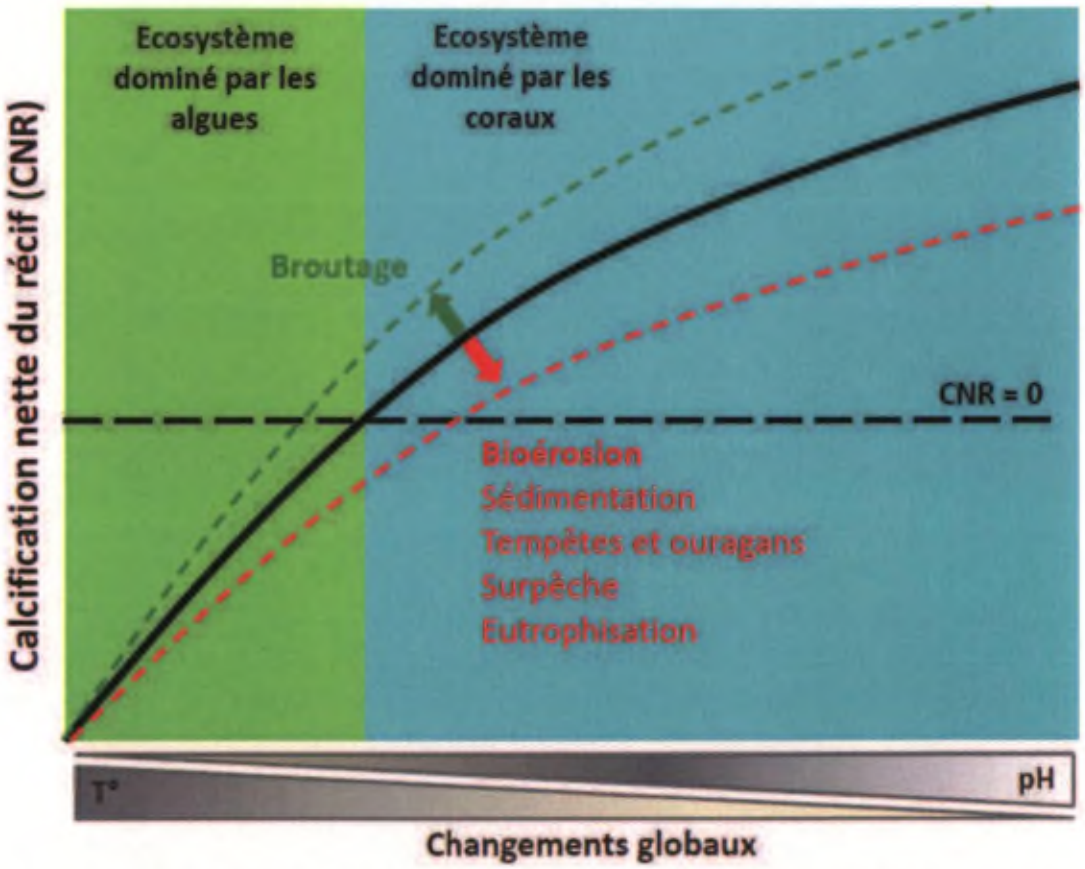

Figure 56 : Diagramme représentant l'impact de la bioérosion et des autres facteurs (représenté par des triangles et des flèches) qui peuvent influencer la calcification nette d'un récif (production – perte de $CaCO_3$, CNR, représentée par la courbe). La base des triangles correspond à la valeur maximale du paramètre.

Le présent travail a démontré que les mésocosmes récifaux artificiels étaient des outils très utiles dans l'étude des effets de l'AO. La possibilité d'inclure les relations écosystèmiques et des variations naturelles des paramètres physico-chimiques à long terme ont permis de mettre en évidence des effets indirects mais également une capacité d'acclimatation des différents organismes maintenus dans des conditions plus proches du milieu naturel, minimisant les facteurs confondants et le stress expérimental. Le besoin d'études prenant en compte ces considérations est urgent. Afin d'apporter des données numériques aux prévisions basées sur des modèles

mécanistiques, des études à long terme devraient également prendre en compte les nombreuses interactions qui prennent place entre les différents facteurs d'origine anthropique affectant la survie des récifs coralliens (température, pH, sédimentation, concentration en nutriments, pollution, densité des bioérodeurs/brouteurs, ...). Les mésocosmes artificiels ont l'avantage de pouvoir être répliqués et d'étudier ces différents paramètres sans impacter le milieu naturel. De plus, d'autres niveaux d'AO (notamment à celui prévu en 2300, et un pH plus élevé qu'actuellement correspondant à la période pré-industrielle) devraient être testés afin de mieux déterminer la relation entre le pH_{EM} et la régulation acide-base du pH extracellulaire des oursins, et déterminer à partir de quelle valeur cette régulation ne sera plus effective et où celle-ci aura un effet sur les autres processus physiologiques (croissance, reproduction). Enfin, d'avantages d'études devraient être menées sur les oursins tropicaux qui ne représentent à l'heure actuelle qu'une trop faible fraction des études réalisées jusqu'ici dans le domaine de l'effet des changements climatiques globaux.

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ANNEXE 1

Moulin L., Catarino A., Claessens T. & Dubois Ph. (2011) Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck 1816). *Marine Pollution Bulletin*, **62**, 48-54.

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Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck 1816)

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ABSTRACT

The effect of pH ranging from 8.0 to 6.8 (total scale – pH_T) on fertilization, cleavage and larval development until pluteus stage was assessed in an intertidal temperate sea urchin. Gametes were obtained from adults collected in two contrasting tide pools, one showing a significant nocturnal pH decrease (lowest pH_T = 7.4) and another where pH was more stable (lowest pH_T = 7.8). The highest pH_T at which significant effects on fertilization and cleavage were recorded was 7.6. On the contrary, larval development was only affected below pH_T 7.4, a value equal or lower than that reported for several subtidal species. This suggests that sea urchins inhabiting stressful intertidal environments produce offspring that may better resist future ocean acidification. Moreover, at pH_T 7.4, the fertilization rate of gametes whose progenitors came from the tide pool with higher pH decrease was significantly higher, indicating a possible acclimatization or adaptation of gametes to pH stress.

1. Introduction

The continuous increment of anthropogenic carbon dioxide emissions is inducing changes in seawater carbon chemistry, lowering its pH. This phenomenon is known as ocean acidification. Since the industrial revolution, the average surface seawater pH has already been reduced by approximately 0.1 units. Expected surface pH reductions are of around 0.4 units by 2100 and 0.77 units by 2300 (Caldeira and Wickett, 2003, 2005; IPCC, 2007). Some particular environments already present lower pH such as upwelling zones (Feely et al., 2008), coastal areas (Wootton et al., 2008), the deep-sea (Park, 1966; Millero, 1996) and volcanic carbon dioxide vents (Hall-Spencer et al., 2008). Tide pools also undergo significant variations of their physicochemical conditions, including pH (due to pCO_2 fluctuations). Truchot and Duhamel-Jouve (1980) reported in rocky tidal pools (Roscoff, Brittany, France) a night increment of pCO_2, accompanied by a decrease of pH until 7.29. This is caused by the community respiration and absence of photosynthesis. During daytime, the tendency is inverted. These daily fluctuations depend on season, tide duration and algal cover (Truchot and Duhamel-Jouve, 1980; Morris and Taylor, 1983; Denny and Gaines, 2007). The same authors also reported an increase of total alkalinity at night which was associated with calcium carbonate dissolution due to low pH. Thus, intertidal pools offer an interesting model where organisms are exposed to a succession of pH fluctuations and may possibly present adaptations (linked to genetic variability at a population level) and/or acclimatization (due to phenotypic plasticity at an individual level) processes to face them.

Early development stages of marine invertebrates (fertilization, embryogenesis and larval development) are generally the most sensitive life phases to environmental stresses (Pörtner and Farrell, 2008; Melzner et al., 2009; Dupont et al., 2010). Recruitment success depends on the survival of the embryos and larvae (López et al., 1998) and, consequently, any decrease in embryo and larval survival or delay in development can reduce population long-term viability (Morgan, 1995). Sea urchins are key species in many coastal ecosystems, being important grazers, and the sustainability of their populations is vital (Paine, 1966; Harrold and Pearse, 1987; Leblanc et al., 2005). Several studies showed that fertilization and early development stages of sea urchins can be negatively impacted by ocean acidification which causes a decrease of fertilization and cleavage rates and/or a reduction of the pluteus larva size (Kurihara and Shirayama, 2004; Havenhand et al., 2008; Clark et al., 2009). A down regulation of genes involved in calcification, cellular stress response, metabolism and apoptosis were reported in *Strongylocentrotus* larvae raised in low pH seawater (Todgham and Hofmann, 2009; O'Donnell et al., 2010). However, larvae of *Strongylocentrotus droebachiensis* raised at lower pH (7.9 and 7.7) were more successful in reaching metamorphosis than those raised at control pH (8.0), although it took them longer to reach this stage (Dupont and Thorndyke, 2008). It is noteworthy that a slower development can result in higher plantktonic mortality due to increased predation exposure and desynchronization with algal

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blooms, decreasing recruitment success (Morgan, 1995; Elkin and Marshall, 2007). Nonetheless, in other studies, no effect of ocean acidification was observed on fertilization and embryogenesis (Byrne et al., 2009a,b, 2010). These facts suggest that actually the response of early life-history stages appears to be highly species-specific and differs even in closely related taxa (Dupont and Thorndyke, 2008, 2009; Clark et al., 2009). This fact emphasizes the need for a survey of specific effects of acidification as broad as possible and to understand the origin of the observed differences of sensitivity to ocean acidification between species. It is also essential not to disregard the fact that long-term exposure of adults to lower pH can affect gonad growth (Siikavuopio et al., 2007; Kurihara, 2008), reproductive success and future larval performance (Dupont and Thorndyke, 2008; Kurihara, 2008).

In this work, we studied the sea urchin *Paracentrotus lividus*, an important grazer species (Bulleri et al., 1999) with a broad distribution and that can be found in the whole Mediterranean and North Atlantic coasts of Europe (from Morocco to Scotland), inhabiting intertidal rock pools, seagrass meadows and shallow subtidal shores (Boudouresque and Verlaque, 2001). Furthermore, this species shows a high gene flow over extended distances (Duran et al., 2004; Calderón et al., 2008). The aim of this study was to understand if pH extreme oscillations to which *P. lividus* adults from rock tide pools are submitted could have an influence on fertilization, embryonic and larval development of their progeny. The strategy was to compare the effect of pH on the progeny of individuals collected from the same shore, i.e. same population, but from distinct tide pools: one where night pH was significantly reduced and the other where this decline was not so important.

2. Materials and methods

2.1. Study site and measure of physicochemical parameters

Observations were done in two tide pools, distant of around 2 m, in Aber, Crozon peninsula (48°14′N; 04°27′W, southern Brittany, France), in April 2009, i.e., during the spawning period of *Paracentrotus lividus* populations in this region (Mercier and Hamel, 2009). Intertidal adult individuals occurred in tide pools and showed a sedentary behavior in self burrowed holes. They are thus partially protected from wave action and never get emersed during low tide. Previous tagging experiments (data not shown) confirmed this population sedentary behavior. New recruits are found every year in this population (Catarino and Dubois, personal observation).

The physicochemical parameters of two tide pools were measured every half an hour starting at pool individualization (ebb tide) until its cover (rising tide) during two night and two day low tides: temperature, salinity and pH_{NIST} (National Institute of Standards and Technology), also known as NBS (previous National Bureau of Standards, now NIST) scale. The temperature and pH_{NIST} were measured using a 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor) calibrated with pH_{NIST} buffers 4 and 7 (Merck CertiPUR®, Darmstadt, Germany). Even though pH variation within each pool never exceeded 0.1 units, a pH cycle measurement was always done on the same spot. The salinity was measured using a conductivity meter pH/Cond 340i WTW (USA). Sea water samples were collected at the beginning and end of each low tide and immediately filtered (0.22 μm) in order to determine total alkalinity (TA). This was carried out by a potentiometric titration with HCl 0.1 M using a Titrino 718 STAT Metrohm (Switzerland), and calculated using the Gran function (Gran, 1952). Our measurements had a deviation of 0.65% of the standard certified material provided by Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control laboratory. Aragonite and calcite saturation values (Ω_{ar} and Ω_{cal}, respectively) and pCO_2 were determined from TA, pH_{NIST} and salinity data using the software CO2SYS (Pierrot et al., 2006) and by using the dissociation constants from Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and KSO_4 using Dickson (1990).

2.2. Gonad maturity

Ten *P. lividus* individuals with a minimum diameter of 30 mm were collected from each tide pool, transported to the laboratory in tide pool water and dissected on the same day. A piece of gonad was removed and fixed in Bouin's fluid. The gonads were then dehydrated, embedded in paraffin, cut in 7 μm sections (Leica RM 2155 microtome) and stained with Masson's trichrome. The gonad maturity was estimated on a scale of 1–8 based on morphological characteristics according to the method of Spirlet et al. (1998).

2.3. Fertilization and larval development experiments

Thirty individuals were collected from each tide pool and kept at pH_{NIST} 8.13, 12.7 °C and 32.1 PSU until the beginning of the experiment 14 days later. All experiments were conducted in a temperature controlled room at 14 °C and in filtered seawater (0.22 μm) from the study site. The pH of the seawater was adjusted by bubbling CO_2 (Air Liquide) until the required pH was obtained.

The pH_{NIST}, the electromotive force (e.m.f) and the temperature of the seawater were measured at the start and at the end of each experiment with the same pH meter as previously described. These values and sequential measurements of the e.m.f. of the cell using standard buffers of known pH, 2-aminopyridine/HCl (AMP) and tris/HCl (TRIS) were applied on the calculation of the pH expressed in total scale (pH_T) (DOE, 1994; Del Valls and Dickson, 1998; Dickson et al., 2007). The salinity was measured using a conductivity meter pH/Cond 340i WTW (USA). The TA, pCO_2, Ω_{ar} and Ω_{cal} were determined in each vial as described in Section 2.1.

To induce spawning, ca. 1 ml of 0.5 M KCl was injected into the perivisceral cavity of individual sea urchins. Gametes of 5 males and 5 females from each tide pool, selected according to their gamete quantity and quality (shape), were collected in control pH seawater. Gametes of the same sex were gently mixed in order to have a homogeneous batch and to avoid individual variations.

The Lowest Observed Effect Concentration (LOEC; results in nominal pH), i.e. the highest pH at which the considered end point significantly differed from that at control pH (see e.g. Rand, 1995), was calculated for the fertilization rate, cleavage rate, larval morphology and rod size. The LOEC was also determined for other sea urchin species from contrasting environments, based on literature data (Byrne et al., 2009a,b, 2010; Clark et al., 2009; Havenhand et al., 2008; Kurihara et al., 2004; Kurihara and Shirayama, 2004; O'Donnell et al., 2010). Only works in which pH was manipulated by CO_2 addition and for which data for determining a LOEC were available were included.

2.3.1. Fertilization and cleavage

Fertilization was conducted in Petri dishes by mixing diluted sperm and eggs at selected pH_T (control 8.0, 7.6, 7.4 and 6.8). Three replicates by pH for each tide pool were produced. Embryos were randomly sampled from each treatment at different times (10, 20, 30, 60, 90 and 120 min) and fixed in Bouin's fluid and 200 embryos were observed in each replicate using an optical microscope. Fertilization was defined as the presence of an elevated normal fertilization membrane (15 min after gamete mixing) and cleavage as the presence of minimum 2 blastomeres (1 h after gamete mixing). At pH_T 6.8, embryos presenting a very thin membrane not clearly visible or with a fertilization membrane not surrounding completely the embryo were observed. They were counted as

abnormal and they were considered as neither "fertilized" nor "cleaved".

2.3.2. Larval development

Eggs were suspended as a homogeneous layer in a Petri dish containing control seawater and a drop of diluted sperm was added. Twenty minutes later, the presence of the fertilization membrane was checked. Fertilization rate was always higher than 95%. Two hours after insemination, three replicates of ca. 500 embryos were transferred into 125 ml vials at each selected pH_T (control 8.0, 7.8, 7.6, 7.4, 7.2, 7.0 and 6.8). Vials were completely filled to avoid air spaces and thus preventing CO_2 exchanges. After 72 h of incubation, the echinopluteus stage was reached and the 4 arms were visible in control larvae with an optical microscope. The larval development was stopped at this moment (before the feeding stage). Some larvae were fixed in Bouin's fluid without acetic acid (for skeleton preservation) and the remaining ones in ethanol 70%. One hundred pluteus larvae fixed in Bouin's fluid were observed in each replicate using an optical microscope and the number of normal and abnormal larvae was counted. The criteria of abnormality were: absence of one or several arms, absence of stomach or totally abnormal bowl-like shape (adapted from Warnau and Pagano, 1994). Larvae preserved in ethanol 70% were observed in phase contrast inverted microscopy and photographed using a digital camera (QImaging, Micropublisher, software Qcapture). Sixty larvae per vial were measured from the apical extremity to the extremity of an anal arm (skeletal rod) using the software ImageJ (Fig. 1).

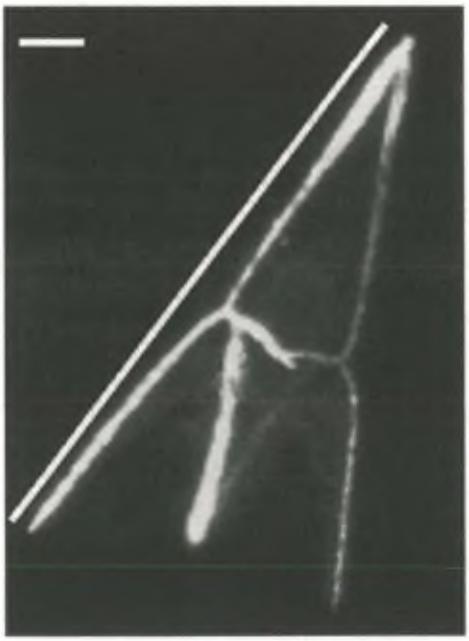

Fig. 1. Pluteus larvae observed in phase contrast inverted microscopy. The long bar indicates the morphometric measurement of skeletal rod. Small bar = 50 μm (upper left corner).

2.4. Data analysis

Differences of pH, salinity and temperature during night and day tidal cycle between tide pools at the end of the low tide as well as comparison of gonad maturity between sea urchins of the two tide pools were investigated using a one way ANOVA (fixed factor tide pool). The fertilization rate was analyzed using a repeated measurement ANOVA (fixed factors nominal pH and tide pool and repeated factor time). All mean multiple comparisons were performed using Tukey tests. The analysis of the cleavage rate was carried out using a two way ANOVA (fixed factors tide pool and nominal pH) followed by a post hoc Dunnett test. To test for possible differences in pH condition in the vials between the beginning and the end of the experiment after 72 h, a repeated measurement ANOVA was done. The size of the calcareous rods of pluteus larvae was analyzed for each tide pool separately using a model III nested ANOVA (fixed factor nominal pH and random factor replicate nested in factor nominal pH) followed by a Tukey test on the pH variable. The analysis of the percentage of normal larvae (arcsin transformed) was performed using a two way ANOVA (fixed factors tide pool and nominal pH) followed by a post hoc Dunnett test. All test were conducted according to Zar (2005) using the software Systat 9 (Systat Software Inc.). The level of significance α was set at 0.05.

3. Results

3.1. Tide pools parameters

The pH_{NIST} of coastal seawater was 8.14. At the end of the night low tides, tide pools 1 (TP1) and 2 (TP2) had a pH_{NIST} of, respectively, 7.8 and 7.4, i.e. a pH decrease of respectively, 0.34 and 0.74 (Table 1, Fig. 2a). There was a significant difference of pH drop between the two tide pools during the night tidal cycle ($p_{Tukey} = 0.021$), but not of temperature and salinity ($p_{ANOVA} \geqslant 0.178$). During the day tidal cycle, the tendency was inverted with the pH increasing up to 8.83 in TP1 and 8.63 in TP2 (Table 1, Fig. 2b). However, the pH increase was not significantly different between the two tide pools as well as the temperature and the salinity ($p_{ANOVA} \geqslant 0.333$).

3.2. Gonad maturity

There was no significant difference in gonad maturity stage between individuals from both tide pools ($p_{ANOVA} = 0.331$). Most individuals were in premature stage (stage V according to Spirlet et al., 1998), i.e., immediately before the mature stage and spawning; ova and spermatozoa were accumulated in the center of the gonad acini.

3.3. Fertilization and larval development experiments

The initial experimental water conditions (pH_T, carbonate and pCO_2 parameters) are presented in Table 2. These conditions were

Table 1
Sea water conditions at the beginning (sea) and at the end of representative night and day tidal cycles in the two tide pools.

Tidal cycle	Location	Salinity (psu)	Temperature (°C)	pH_{NIST}	TA (μmol/kg)	DIC (μmol/kg)	pCO_2 (μatm)	Ω_{cal}	Ω_{ar}
Night	Sea	34.7	13.0	8.14	2321	2070	313	4.25	2.72
Night	Tide pool 1	34.7	12.3	7.80	2315	2215	759	2.11	1.35
Night	Tide pool 2	34.7	11.4	7.40	2397	2429	2084	0.89	0.57
Day	Sea	35.0	11.5	8.21	2322	2101	346	3.79	2.42
Day	Tide pool 1	35.4	15.0	8.83	2244	1579	51	10.55	6.78
Day	Tide pool 2	35.0	14.7	8.63	1935	1482	84	6.87	4.41

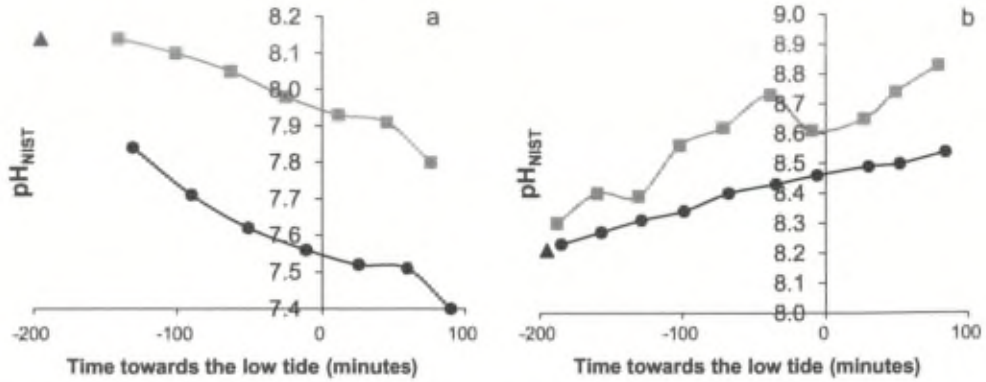

Fig. 2. Evolution of pH in tide pool 1 (grey squares) and 2 (black points) according to time towards the low tide during representative night (a) and day (b) tidal cycles in April. The pH of seawater at the beginning of the tidal cycle is represented by the black triangle.

Table 2
Initial experimental seawater conditions (mean ± SD, $n = 3$) for fertilization, cleavage and larval development experiments (t_0) and after 72 h of incubation for larval development experiment (t_f) according to origin of genitors (tide pool 1 and 2). Initial and final conditions did not differ significantly (see text).

Tide pool	pH nominal	pH_T (t_0)	pH_T (t_f)	TA (t_0) (μmol/kg)	TA (t_f) (μmol/kg)	DIC (t_0) (μmol/kg)	DIC (t_f) (μmol/kg)	pCO_2 (t_0) (μatm)	pCO_2 (t_f) (μatm)	Ω_{cal} (t_0)	Ω_{cal} (t_f)	Ω_{ar} (t_0)	Ω_{ar} (t_f)
	6.8	6.80 ± 0.01	–	2208.37	–	2513.99	–	8108.88	–	0.22	–	0.14	–
1	7.0	7.07 ± 0.01	7.10 ± 0.00	2209.87	2214.66	2353.81	2347.57	4292.58	4050.29	0.41	0.44	0.26	0.28
	7.2	7.27 ± 0.02	7.24 ± 0.04	2215.62	2234.33	2282.28	2315.17	2686.36	2972.84	0.65	0.60	0.41	0.38
	7.4	7.47 ± 0.00	7.46 ± 0.06	2243.80	2251.68	2247.34	2258.04	1690.81	1743.88	1.03	1.02	0.66	0.65
	7.6	7.67 ± 0.00	7.60 ± 0.02	2242.98	2268.74	2186.78	2234.29	1037.37	1254.82	1.59	1.38	1.02	0.88
	7.8	7.83 ± 0.01	7.76 ± 0.03	2211.17	2233.37	2109.95	2152.73	697.22	829.64	2.14	1.89	1.36	1.20
	8.0 (Control)	8.14 ± 0.01	7.98 ± 0.02	2216.54	2390.59	1990.34	2224.33	310.66	507.99	3.98	3.18	2.53	2.02
	6.8	6.78 ± 0.01	–	2208.37	–	2523.25	–	8335.25	–	0.22	–	0.14	–
2	7.0	7.07 ± 0.01	7.14 ± 0.01	2209.87	2249.24	2353.90	2366.79	4294.44	3733.06	0.41	0.49	0.26	0.31
	7.2	7.29 ± 0.01	7.25 ± 0.02	2215.62	2235.18	2277.94	2311.67	2604.74	2885.64	0.67	0.62	0.42	0.39
	7.4	7.47 ± 0.00	7.38 ± 0.01	2243.80	2248.27	2247.17	2279.76	1688.46	2113.87	1.03	0.84	0.66	0.54
	7.6	7.67 ± 0.02	7.56 ± 0.02	2242.98	2274.66	2187.88	2250.91	1047.35	1373.17	1.58	1.28	1.01	0.82
	7.8	7.85 ± 0.02	7.73 ± 0.04	2211.17	2203.29	2102.50	2134.31	660.09	895.15	2.24	1.73	1.43	1.10
	8.0 (Control)	8.13 ± 0.00	8.00 ± 0.04	2216.54	2226.14	1995.12	2057.88	319.16	444.55	3.91	3.10	2.48	1.97

the same for fertilization, cleavage and larval development experiments.

3.3.1. Fertilization and cleavage

Fertilization rate significantly decreased with pH_T ($p_{pH} < 10^{-3}$) (Fig. 3). The observed LOEC was 7.6 for embryos from parents of both tide pools ($p_{Tukey} < 10^{-3}$). However, the fertilization rate at pH_T 7.4 of gametes from TP2 parents was significantly higher than that of gametes from TP1 parents ($p_{pH \times tide\ pool} = 0.026$; $p_{Tukey} = 0.03$). The amount of fertilized eggs significantly increased with time ($p_{time} < 10^{-3}$) (Fig. 3).

The cleavage rate was significantly lower at lower pH_T ($p_{pH} < 10^{-3}$) (Fig. 4). The LOEC was 7.6 for embryos from progenitors from both tide pools ($p_{Dunnett} \leq 0.011$). Contrary to the fertilization rate, the percentage of cleaved eggs did not differ according to origin of progenitors ($p_{pH \times tide\ pool} = 0.804$) (Fig. 4).

3.3.2. Larval development

The pH_T water conditions were not significantly different between the start and the end of the experiment ($p_{ANOVA} = 0.1566$) (Table 2).

Rod size was significantly smaller at lower pH_T ($p_{pH} < 10^{-3}$) (Fig. 5). The LOEC was 7.2 for larvae from genitors from both tide pools.

Lower pH also significantly affected the form of pluteus larvae ($p_{pH} < 10^{-3}$) (Fig. 6), the percentage of abnormal pluteus being higher at lower pH_T. The LOEC was 7.4 and there was no difference between larvae from parents of both tide pools ($p_{tide\ pool} = 0.721$). At pH_T 6.8 no embryo developed to the pluteus stage and all larvae had an abnormal morphology (bowl shape).

4. Discussion

In the present study, the most critical phases observed during early life stages of *Paracentrotus lividus* were fertilization and cleavage, as evidenced by the lowest observed effect concentration (LOEC). The LOEC pH_T was higher (7.6) for those stages than for larval viability (7.4) and larval size (7.2) (Table 3). This shows that the initial embryonic development of this species is particularly sensitive to acidification levels that could be reached already in 2100, according to IPCC predictions (IPCC, 2007). Gametes, zygote and early cleavage stages can be more vulnerable than cells during later ontogenetic stages as changes in surrounding pCO_2 cause a higher relative change in internal pCO_2, (Melzner et al., 2009). In line with these results, previous studies on *P. lividus* showed that an earlier exposure to acidified water (HCl) promoted more severe developmental defects than a post-hatching one (Pagano et al., 1985). Early life stages can present diverse defense mechanism against common natural stressors within a large variation range, but their vulnerability can increase while facing rapid anthropogenic environmental changes (Hamdou and Epel, 2007). Furthermore, the responses of different species are diverse and highly

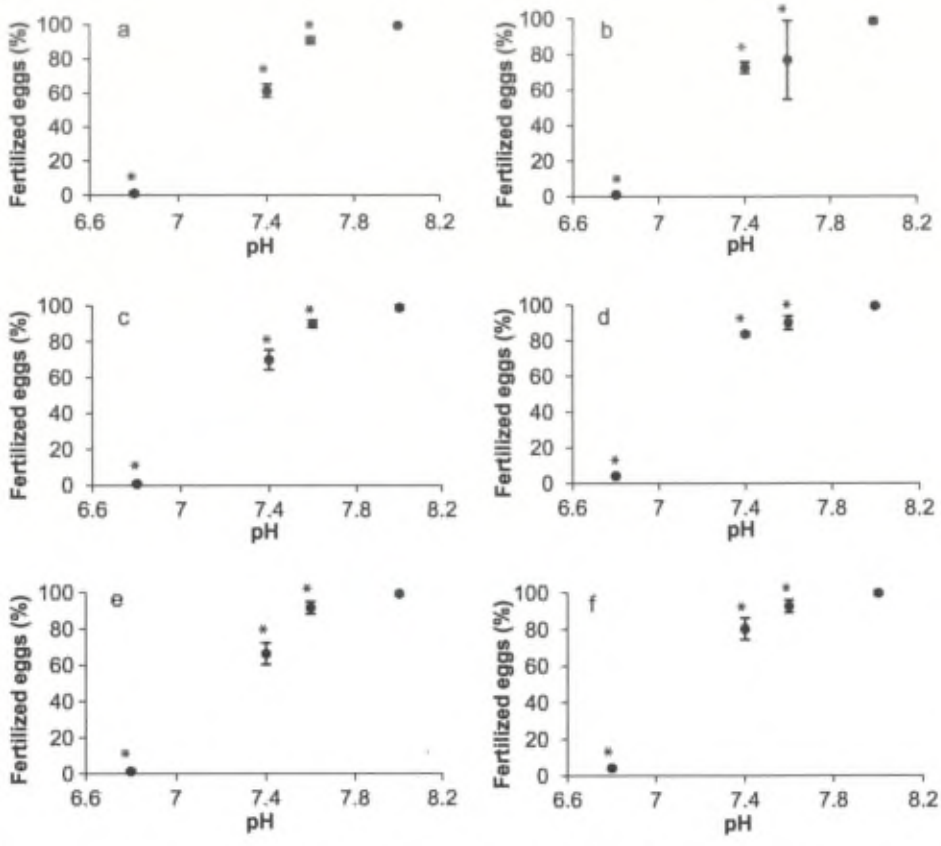

Fig. 3. Fertilization rate (%, mean ± SD, $n = 3$) at different nominal pH and at different time ($a - b = 10'$, $c - d = 60'$, $e - f = 120'$) for embryos from sea urchins of tide pool 1 ($a - c - e$) and tide pool 2 ($b - d - f$). *: Mean value significantly different from control ($p_{Tukey} < 0.05$).

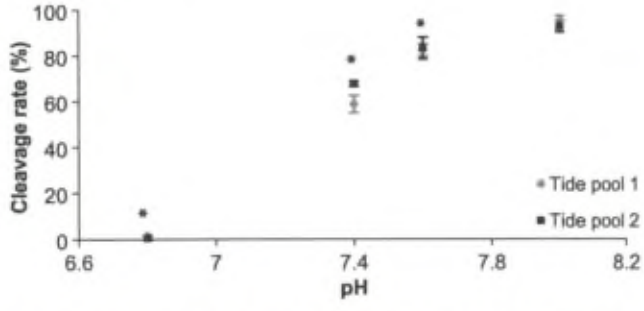

Fig. 4. Cleavage rate (%, mean ± SD, $n = 3$) at different nominal pH of embryos from progenitors of both tide pools. *: Mean value from both tide pools significantly different from control ($p_{Dunnett} < 0.05$).

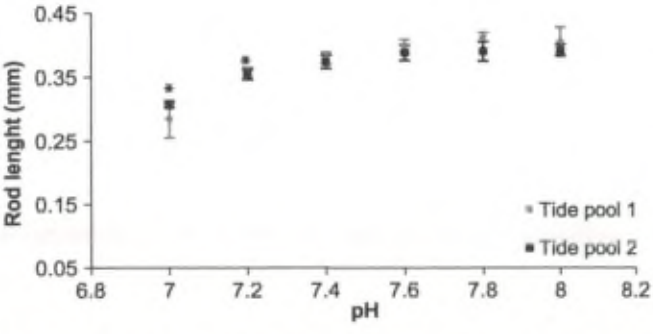

Fig. 5. Length (mean ± SD, $n = 3$) at different nominal pH of calcareous rod of pluteus larvae from sea urchins of both tide pools. *: Mean value from both tide pools significantly different from control ($p_{Tukey} < 0.05$).

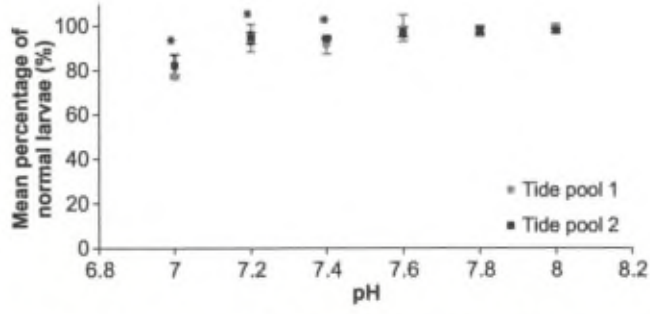

Fig. 6. Percentage (mean ± SD, $n = 3$) of normal pluteus larvae at different nominal pH in the offspring of sea urchins from the 2 tide pools. *: Mean value from both tide pools significantly different from control ($p_{Dunnett} < 0.05$).

specific (Table 3) and clear responses are hard to be established even within the same species. For instance, Havenhand et al. (2008) reported similar results to our study for the tropical species *Heliocidaris erythrogramma* where fertilization success was reduced at pH_{NIST} 7.7. However, for the same species, Byrne et al. (2009b) reported that fertilization success was not reduced at pH_{NIST} 7.6, probably due to methodological differences (see Byrne et al., 2009b) such as fertilization success measurement timing (Reuter et al., 2010). It is noteworthy that the methodology of the present study was analogous to that of Byrne et al., 2009b, i.e., the use of gamete pools and not of single female–male pairs, and that even so, at a similar pH, a reduction of fertilization success of *P. lividus* was already observed in our study.

In other cases, larval development was more impaired than fertilization and cleavage stages, such as for *Hemicentrotus pulcherri-*

Table 3
LOEC (in pH_{NIST} unit, except for *P. lividus* results where units are in pH_T) for embryonic and larval development of different species of echinoids: *P. lividus* collected from intertidal zone in temperate region, *S. neumayeri* collected from subtidal zone in Antarctic region, *P. huttoni* and *E. chloroticus* collected from subtidal zone in temperate region. Other species collected from subtidal zone in tropical region. ND: no data. NE: no pH effect observed (lowest pH tested between brackets).

Species	Fertilization rate	Cleavage rate	Larval morphology	Decreased rod size	References
Paracentrotus lividus	7.6	7.6	7.4	7.2	Present study (pH_T)
Sterechinus neumayeri	ND	ND	ND	7.6	Clark et al. (2009) (pH_{NIST})
Pseudechinus huttoni	ND	ND	ND	NE (7.7)	Clark et al. (2009) (pH_{NIST})
Evechinus chloroticus	ND	ND	ND	7.7	Clark et al. (2009) (pH_{NIST})
Tripneustes gratilla	ND	ND	ND	7.8	Clark et al. (2009) (pH_{NIST})
	NE (7.6)	ND	ND	ND	Byrne et al. (2010) (pH_{NIST})
Centrostephanus rodgersii	NE (7.6)	ND	ND	ND	Byrne et al. (2010) (pH_{NIST})
Heliocidaris tuberculata	7.6	ND	ND	ND	Byrne et al. (2010) (pH_{NIST})
Heliocidaris erythrogramma	NE (7.6)	NE (7.6)	ND	ND	Byrne et al. (2009a,b, 2010) (pH_{NIST})
	7.7	ND	ND	ND	Havenhand et al. (2008) (pH_{NIST})
Hemicentrotus pulcherrinus	6.83	6.8	7.4	7.8	Kurihara et al. (2004) Kurihara and Shirayama (2004) (pH_{NIST})
Echinometra mathaei	7.12	ND	7.4	7.8	Kurihara et al. (2004) (pH_{NIST})
Lytechinus pictus	ND	ND	ND	7.78	O'Donnell et al. (2010) (pH_{NIST})

nus (Kurihara et al., 2004; Kurihara and Shirayama, 2004) and for *Echinometra mathaei* (Kurihara et al., 2004) (Table 3). Nevertheless, concerning *P. lividus* larval development, the LOEC pH_T was near or lower than those of other species studied (Table 3). It is noteworthy that the impact in larvae development was not only a size reduction, but also an increment of abnormal larvae that will never develop further. Actually, the LOEC pH_T for the latter was higher than for the former effect. This indicates that contrary to what was observed in other sea urchins species, the impact of ocean acidification can have more severe effects than that of just a simple delay in development (see Dupont et al., 2010 for discussion).

The larvae from intertidal *P. lividus* seem to be more resistant to acidification than those of species collected from subtidal sites. This suggests that sea urchins living in the stressful intertidal zone may be adapted or acclimatized to pH stress. In their study, Clark et al. (2009) observed that the Antarctic sea urchin *Sterechinus neumayeri* larvae were the least affected by low pH compared to tropical and temperate sea urchin species. They postulate that, evolving in an environment with historically higher levels of CO_2, as a result of polar seawater temperatures and upwelling of CO_2-rich water, *S. neumayeri* would be adapted to higher CO_2 conditions and, therefore, may have greater capacity to acclimatize to lowered seawater pH. In this context, it is noteworthy that *P. lividus* is a widely distributed species, ranging from Scotland to Morocco through the whole Mediterranean, indicating that the adaptation potential of the species is very high, even if the embryo and larva development of this species appeared rather sensitive to pH in comparison with other species.

The intertidal populations of *P. lividus* experience appreciable decrease of pH when compared to subtidal populations. The offspring of sea urchins from the tide pool with higher pH decrease (tide pool 2) showed a better resistance to acidification at pH_T 7.4 than that of sea urchins from the tide pool with low pH decrease (tide pool 1) in terms of fertilization rate, viz. a reduction of over 30% compared to about 20% for tide pool 2. A possible explanation could be a better resistance of male and/or female gametes to acidification. For instance, there could be a greater allocation of resources in reproduction by individuals subjected to lower pH. The eggs would then be stronger and more resistant to lower pH values. However, no significant differences were detected between cleavage rate of embryos according to progenitor origin. Moreover, gonad maturation also did not differ significantly between sea urchins of both tide pools. As a consequence, the better resistance to acidification of the offspring of sea urchins from tide pool 2 could be explained by a better performance of the spermatozoa. The impact of pH on the sperm of sea urchins has been widely documented by embryologists. A decrease in pH of seawater lowers the internal pH of sperm. This is accompanied by a decrease in sperm motility and in acrosome reaction (Christen et al., 1986). The regulation of internal pH of sperm is controlled by a Na^+/K^+-ATPase pump and Na^+/H^+ transporters (antiport) located on the plasma membrane of spermatozoa (Gatti and Christen, 1985). In spermatozoa already subjected to pH stress in the gonads, these transmembrane proteins may be more effective or expressed in greater number. This could be due to either acclimatization (phenotypic plasticity) or natural selection. As the fertilization rate of sea urchins can be affected by sperm motility (Havenhand et al., 2008), the fertilization rate of acclimatized/selected sperm would be higher. This fact remains to be confirmed and we think that pH stressed populations offer the possibility to better understand potential gonad and gamete adaptations. Endotrophic larval growth of *P. lividus* was only affected at pH levels predicted for 2300 (IPCC, 2007) or lower (Table 3) indicating the relative resistance of this stage. No information is currently available on the effect of pH on exotrophic larval development of this nor of other sea urchin species (for a review see Dupont et al., 2010). Further investigation should not only address this aspect, but also compare the development success of different populations, such as intertidal vs. subtidal, or with those from upwelling zones. Echinoid mechanisms of pH stress resistance and/or adaptation are currently poorly understood and little studied. Additional knowledge of the consequences of ocean acidification and the potential ability of organisms to cope with it will require such information.

Applying a widespread, practical and sensitive chronic toxicology test (the use of exposed sea urchin gametes, embryos and larvae to a stress) and relevant endpoints such as the LOEC offers the opportunity to clarify one species sensitivity to lower pH during distinct life stages, allowing inter-specific comparisons. Furthermore, this type of stress-response evaluation can allow the development of monitoring tools in areas highly vulnerable to ocean acidification, such as upwelling and cold water regions, in water bodies subject to industrial acid waste and in areas where acid dependent toxicants can be present.

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ANNEXE 2

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Buffer capacity of the coelomic fluid in echinoderms

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ABSTRACT

The increase in atmospheric CO_2 due to anthropogenic activity results in an acidification of the surface waters of the oceans. The impact of these chemical changes depends on the considered organisms. In particular, it depends on the ability of the organism to control the pH of its inner fluids. Among echinoderms, this ability seems to differ significantly according to species or taxa. In the present paper, we investigated the buffer capacity of the coelomic fluid in different echinoderm taxa as well as factors modifying this capacity. Euechinoidea (sea urchins except Cidaroidea) present a very high buffer capacity of the coelomic fluid (from 0.8 to 1.8 mmol kg^{-1} SW above that of seawater), while Cidaroidea (other sea urchins), starfish and holothurians have a significantly lower one (from −0.1 to 0.4 mmol kg^{-1} SW compared to seawater). We hypothesize that this is linked to the more efficient gas exchange structures present in the three last taxa, whereas Euechinoidea evolved specific buffer systems to compensate lower gas exchange abilities. The constituents of the buffer capacity and the factors influencing it were investigated in the sea urchin *Paracentrotus lividus* and the starfish *Asterias rubens*. Buffer capacity is primarily due to the bicarbonate buffer system of seawater (representing about 63% for sea urchins and 92% for starfish). It is also partly due to coelomocytes present in the coelomic fluid (around 8% for both) and, in *P. lividus* only, a compound of an apparent size larger than 3 kDa is involved (about 15%). Feeding increased the buffer capacity in *P. lividus* (to a difference with seawater of about 2.3 mmol kg^{-1} SW compared to unfed ones who showed a difference of about 0.5 mmol kg^{-1} SW) but not in *A. rubens* (difference with seawater of about 0.2 for both conditions). In *P. lividus*, decreased seawater pH induced an increase of the buffer capacity of individuals maintained at pH 7.7 to about twice that of the control individuals and, for those at pH 7.4, about three times. This allowed a partial compensation of the coelomic fluid pH for individuals maintained at pH 7.7 but not for those at pH 7.4.

1. Introduction

Anthropogenic emissions of carbon dioxide (CO_2) are inducing an important increase in the atmospheric CO_2 concentration which in turn modifies seawater chemistry of the oceans, lowering its pH and shifting the carbonate equilibrium (IPCC, 2007; Orr, 2011). These two effects are commonly regrouped under the name of ocean acidification. The pH decrease is predicted to reach 0.3–0.4 U by 2100 and 0.8 by 2300, following the IPCC "business-as-usual" IS92a scenario (Caldeira and Wickett, 2003, 2005; IPCC, 2007).

The increased CO_2 concentration in seawater impacts marine organisms directly as CO_2 enters the organisms by diffusion inducing hypercapnia (i.e. CO_2 accumulation in the internal fluids) and indirectly through acidosis (internal pH decrease). Hypercapnia and/or acidosis may affect different physiological processes such as calcification, nutrition and metabolism (Pörtner, 2008; Melzner et al., 2009). In order to avoid this, organisms need to maintain the homeostasis of their inner fluids through different processes such as respiration, circulation, ionic and acid–base regulation. The maintenance of intracellular pH is crucial to numerous enzymatic reactions (Pörtner, 2008). For that purpose, intracellular protons are transported into the extracellular fluids. Therefore, the buffer capacity of the latter is considered essential for the maintenance of intracellular pH. This buffer capacity depends on both the capability of substances present in the fluid to transfer free protons into a non-dissociated state and the available volume of extracellular fluid (Heisler, 1989). If extracellular pH decreases, additional energy will be required to transport, first, the protons from intra- to extracellular compartments and, second, from the extracellular compartment to the external medium. Consequently, less energy can be allocated to other processes such as growth or reproduction (Pörtner et al., 1998; Pörtner et al., 2000; Melzner et al., 2009).

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Metazoans have two buffer systems to compensate for fluctuations of the extracellular pH: a bicarbonate buffer system and a non-bicarbonate buffer system (Heisler, 1986). The former is the most commonly used as it is a low cost and fast system that may be used in order to buffer a too large concentration of protons. However, this system is more or less sustainable, depending on the species, as an increase in the proton concentration in the extracellular fluid will result in a higher concentration in CO_2 which will have to be eliminated later. The efficiency of this system will depend on the physiological bicarbonate accumulation potential of the species and its capacity to evacuate CO_2 (Heisler, 1986; Melzner et al., 2009). The second system, the non-bicarbonate buffer system, can also allow the regulation of the acid–base balance of the extracellular fluids by capturing the protons liberated by the hydration of CO_2. From non-bicarbonate substances, only those whose p*K* is close to that of the physiological pH of the organisms may play a role. This principally includes residues of polypeptide chains, e.g. histidine, cysteine and terminal NH_2 groups and phosphates (either inorganic secondary phosphate or miscellaneous organic phosphate compounds). Differences in the p*K* values associated to these different compounds allow a large range of pH to be buffered. However, this system includes the production of proteins and other compounds, which requires a high amount of energy (Heisler, 1986; Melzner et al., 2009). By combining both of these systems, some organisms are capable of partially or totally compensate the extracellular acidosis induced by seawater acidification. For instance, Gutowska et al. (2010) showed that the cuttlefish *Sepia officinalis* partially compensates its extracellular pH (pH_e) by accumulating bicarbonate ions in its internal fluid. This was also observed in the mollusk *Patella vulgata* where a passive dissolution of the shell contributes to a compensation of pH_e (Marchant et al., 2010).

Many echinoderms live in frequently changing environments such as intertidal and upwelling zones. For these organisms, variations in pH and temperature are a regular or frequent feature (Truchot and Duhamel-Jouve, 1980; Feely et al., 2008; Moulin et al., 2011). Therefore, it might be expected that these organisms have the capacity to bear these fluctuating parameters. Moreover, numerous echinoderm species occur under the saturation horizon for magnesium calcite (David et al., 2005; Hall-Spencer et al., 2008; Sewell and Hofmann, 2011), indicating a possible tolerance to corrosive seawaters. Finally, recent studies have delivered results suggesting that adult echinoderms may be able to cope with seawater pH decrease within the scope of ocean acidification (see Dupont et al., 2010 for review; Catarino et al., 2012; Dupont and Thorndyke, 2012; Stumpp et al., 2012). Spicer et al. (1988) showed that, during air exposure, the sea urchins *Echinus esculentus* and *Psammechinus miliaris* are able to compensate acidosis for a short period (several hours) as no extracellular pH decrease was observed. However, Miles et al. (2007) observed that *P. miliaris* was unable to regulate its acid–base balance if submitted to a low pH (7.4, 6.63 and 6.16) for about a week. These authors hypothesized that acidosis of the coelomic fluid induced a dissolution of the skeleton (source of bicarbonate ions), which resulted in a slight compensation of the coelomic fluid pH (pH_{CF}). However, other authors did not observe any evidence of skeleton dissolution in another sea urchin species (Burnett et al., 2002). On the contrary, two studies reported that the sea urchin, *Strongylocentrotus droebachiensis*, maintained at reduced seawater pH during several weeks, is able to fully or partly compensate the acidosis (Dupont and Thorndyke, 2012; Stumpp et al., 2012). Over similar time scales, acidosis of the coelomic fluid in the starfish *Asterias rubens* and *Leptasterias polaris* remained uncompensated (Appelhans et al., 2012; Dupont and Thorndyke, 2012).

The main circulatory medium of echinoderms is the coelomic fluid (CF), i.e. the fluid enclosed in the main body cavity, that, together with the water vascular system, ensures gas transportation (Farmanfarmaian, 1966). Although echinoderms are osmoconformers, their CF ionic composition slightly differs of that of seawater (Bialaszewicz, 1933; Binyon, 1972). Their pH_{CF} is usually 0.5 to 1.5 U lower than the pH of surrounding seawater because of a high pCO_2 of metabolic origin (Cole, 1940; Hyman, 1955; Farmanfarmaian, 1966). Echinoderms also have organic compounds present in their CF, mainly amino acids, reduced sugars, proteins, lipids and nitrogenous wastes. This composition is directly dependent on the nutritional state of the organism as the CF serves as a vessel for nutrient transport (Ferguson, 1964; Holland et al., 1967; Binyon, 1972). Finally, the CF of echinoderms contains circulating cells, i.e. coelomocytes, whose functions range from metabolite transport to immunity (Endean, 1966). Even though it was shown previously that the CF of echinoderms may exhibit a much higher buffer capacity than seawater (sea urchins CF exhibits the highest buffer capacity followed by sea stars and sea cucumbers) (Collip, 1920; Gellhorn, 1926; Sarch, 1931; Koller and Meyer, 1933; Meyer, 1935), nothing is known about the environmental and physiological factors influencing this property. The origin of the buffer capacity is unknown, and so is its nature. This increased buffer capacity could explain, for a part, the occurrence of echinoderms in low pH environments.

The goal of the present study was to investigate the factors influencing the CF buffer capacity of echinoderms and to get a first insight into the source of this capacity. For that purpose, we have scanned a range of species to assess this capacity in the phylum. We also studied the effects of nutrition on the CF buffer capacity of the sea urchin *Paracentrotus lividus* and the starfish *A. rubens* and how it is distributed among the CF constituents. The effect of reduced seawater pH on this property was investigated in *P. lividus*.

2. Materials and methods

2.1. pH, alkalinity and buffer capacity measurements

In order to measure pH and alkalinity (A_T), coelomic fluid (CF) was sampled from each individual using a syringe and was transferred to an Eppendorf tube. The pH and the electromotive force (emf) were measured immediately after sampling at the same temperature as the seawater hosting the animals using a Metrohm pH meter fitted with a microelectrode (826 pH mobile, microelectrode reference 6.0224.100; Metrohm, Switzerland). The electrode had been previously calibrated with CertiPUR® Buffer solutions pH 4.00 and 7.00 (Merck, Darmstadt, Germany). Taking into account the similar composition of CF and that of seawater (SW) (Stickle and Diehl, 1987), the pH measurements were converted in total scale according to DelValls and Dickson (1998) calibration method using standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS) (kindly provided by the Department of Astrophysics, Geophysics and Oceanography of the University of Liège, Belgium). The alkalinity of SW ($A_{T\text{-}SW}$) and the alkalinity of the CF ($A_{T\text{-}CF}$) were measured using a micromeasurement technique based on a potentiometric titration (Gran, 1952). The titration took place by first adding 5 μL of HCl 0.1 mol L^{-1} (Merck) and then 1 μL at a time to an initial sample of 0.5 mL until reaching a pH lower than 3.00.

In order to verify that the measured increase in $A_{T\text{-}CF}$ is efficient in the physiological range of pH, the CF of four *P. lividus* (3rd batch, for origin see Table 1) was titrated by adding 0.5 μL of HCl 0.1 mol L^{-1} at a time.

The method was tested by using total alkalinity standard certified material provided by Andrew G. Dickson's Oceanic Carbon Dioxide Quality Control Laboratory (batch number 94). The reproducibility of the method was also tested on the CF of *P. lividus* (first batch, Table 1): $A_{T\text{-}CF}$ was measured 3 times for three independent individuals.

In order to make the results comparable between individuals of different species and/or from different origins, all measures were converted into delta alkalinity (ΔA_T) which was calculated as follows:

$$\Delta A_T = A_{T\text{-}CF} - A_{T\text{-}SW}$$

Table 1
Origin of the different species of Echinodermata and parameters of the seawater in which they were maintained prior to analysis of the alkalinity of the coelomic fluid. $A_{T\text{-}SW}$: total alkalinity of the seawater; C_T = total carbon content; Ω Ca = saturation state of calcite; Ω Ar = saturation state of aragonite.

Species	Origin (provided by/collected at)	Date	pH_{SW}	Temperature (°C)	Salinity (PSU)	$A_{T\text{-}SW}$ (mmol kg^{-1} SW)	C_T (mmol kg^{-1} SW)	pCO_2 (μatm)	Ω Ca	Ω Ar
Euechinoidea										
Paracentrotus lividus—1st batch	Telgruc, Brittany, provided by the Marine Station of Roscoff, France	February 2011	8.12	10.6	34.2	3.104	2.831	458	5.21	3.31
P. lividus—2nd batch	Morgat, Brittany, provided by the Marine Station of Roscoff, France	April 2011	8.12	10.6	34.2	3.104	2.831	458	5.21	3.31
P. lividus—3rd batch	Crozon, Brittany, France	February 2012	8.05	11.1	31.6	3.411	3.176	619	4.90	3.10
Echinocardium cordatum	Wimereux, France	May 2011	8.12	10.6	34.2	3.104	2.831	458	5.21	3.31
Echinometra mathaei	Saint-Denis, La Réunion, France	May 2011	8.08	25.5	34.1	2.135	1.829	340	5.18	3.42
Tripneustes ventricosus	Discovery Bay, Jamaica	April 2012	8.06	27.9	39.7	2.479	2.082	395	6.48	4.33
Cidaroidea										
Phyllacanthus imperialis	Philippines (aquarium trade)	January 2011	8.08	25.5	34.1	2.135	1.829	340	5.18	3.42
Eucidaris tribuloides	Discovery Bay, Jamaica	April 2012	8.07	27.9	39.7	2.417	2.021	373	6.41	4.28
Asteroidea										
Asterias rubens	Knokke, Belgium	March 2011	8.12	10.6	34.2	3.104	2.831	458	5.21	3.31
Echinaster sepositus	Marine station of Roscoff, France	February 2011	8.23	16.5	35	3.247	2.810	351	8.10	5.22
Henricia oculata	Marine station of Roscoff, France	February 2011	8.23	16.5	35	3.247	2.810	351	8.10	5.22
Holothuroidea										
Holothuria forskali	Mediterranean Sea, provided by the Marine Laboratory of Mons, Belgium	July 2009 and April 2010	8.23	16.5	35	3.247	2.810	351	8.10	5.22
Holothuria tubulosa	Mediterranean Sea, provided by the Marine Laboratory of Mons, Belgium	July 2009 and April 2010	8.23	16.5	35	3.247	2.810	351	8.10	5.22

2.2. Buffer capacity in different echinoderm taxa

Alkalinity and pH of the CF were measured in different echinoderm taxa (Table 1): (1) Euechinoidea (*Echinometra mathaei* and *Tripneustes ventricosus*, both regular tropical sea urchins, $n = 10$ and 9, respectively; *P. lividus*—first batch, a regular temperate sea urchin, $n = 10$; *Echinocardium cordatum*, a temperate spatangoid, $n = 3$), (2) Cidaroidea (*Phyllacanthus imperialis* and *Eucidaris tribuloides*, both tropical cidaroids, $n = 6$ and 9, respectively), (3) Asteroidea (*A. rubens*, temperate carnivorous starfish, $n = 10$; *Henricia oculata* and *Echinaster sepositus*, both temperate microphagous starfish, $n = 2$ and 3, respectively), and (4) Holothuroidea (*Holothuria forskali* and *H. tubulosa*, both temperate deposit feeders, $n = 3$ and 2, respectively). The origin of the individuals and the parameters of the seawater in which they were maintained are summarized in Table 1. All specimens were fed prior to measurements. All species were sampled between April and June 2011 except *T. ventricosus* and *E. tribuloides*, which where sampled in April 2012. They were all held in aquarium for at least 2 weeks prior to measurement. For *P. lividus*, *E. cordatum* and *A. rubens*, the animals were sampled at low tide and brought back in aerated seawater to the laboratory within the day. *E. mathaei* were collected by hand and brought back to Belgium by air freight (no mortality occurred during transport). *P. imperialis* were bought from a reseller who had imported them from the Philippines. *T. ventricosus* and *E. tribuloides* were hand collected by snorkeling and diving and were immediately put within tanks containing natural seawater. *E. sepositus* and *H. oculata* were collected by the services of Marine Station of Roscoff. *H. forskali* and *H. tubulosa* were a courtesy of the Marine Laboratory of the University of Mons and were collected by scuba diving.

2.3. Effect of nutrition on buffer capacity

All the following experiments were run within a closed recirculating system. Nitrites were monitored and never rose above 0.3 mg/l.

The effect of the nutritional state of the individuals on the buffer capacity of the CF was investigated on 10 individuals of the sea urchin *P. lividus* (first batch) and the starfish *A. rubens* (Table 1), all of them maintained in the same system (separated by mesh cages) with salinity 34.2 ± 0.1 (mean ± SD, $n = 19$), temperature 10.6 ± 0.2 °C (mean ± SD, $n = 19$; both parameters measured with a WTW Multi 340i; WTW, Germany), pH_T 8.13 ± 0.04 (mean ± SD, $n = 19$; measured with a Metrohm pH meter 826 pH mobile, fitted with a combined glass electrode Metrohm 6.0228.010 and calibrated with CertiPUR® buffer solutions and converted to total scale as previously described) and A_T 3.14 ± 0.06 mmol kg^{-1} SW (mean ± SD, $n = 11$). The same individuals were used all along the experiment.

Sea urchins were fed *ad libitum* with Sea Urchin Diets (Zeigler Bros, Inc., USA). The mean size at the ambitus of the individuals was 43.6 ± 5.0 mm (ranging from 36 to 51.1 mm; $n = 10$). The sampling of 0.5 mL of CF represented about 2.5% of the total volume of the individual. Signs of activity were always observed (feeding, movement of spines and tube feet, movement of the individuals in the tank). At the end of the experiment, 2 individuals had died for the unfed condition and 1 for the fed condition.

At time 0, sea urchins were unfed and pH_{CF} and $A_{T\text{-}CF}$ were measured. Then sea urchins were fed, and after 1 week, the same parameters were measured. They were then segregated into two groups, one fed and one unfed, and further measurements took place after 2, 5 and 8 weeks.

Starfish were fed with mussels *Mytilus edulis* (collected in Knokke, Belgium) *ad libitum*. The mean longest arm length of the individuals was 64.4 ± 9.8 mm (ranging from 50.1 and 83.5 mm; $n = 10$). The sampling of 0.5 mL of CF represented about 18% of the total volume of the individual. No individual died during the course of the experiment, and signs of activity were always observed (feeding, movement of the tube feet, movement of the individuals in the tank).

For starfish, the time 0 measurement was done on fed individuals. They were then starved and after 1 week, the parameters were measured. Then, they were also segregated into two groups, one fed and

one unfed, and measurements took place after 2 weeks. Every time pH_{CF} and $A_{T\text{-}CF}$ were measured, pH of the seawater (pH_{SW}) and $A_{T\text{-}SW}$ were also measured.

2.4. Distribution of the buffer capacity in the coelomic fluid

The CF (3 mL) of 6 fed *P. lividus* and *A. rubens* (specimens of the nutrition experiment) was collected as previously described. Immediately after, 0.5 mL was subsampled and the $A_{T\text{-}CF}$ measured in the untreated fluid. The rest of the fluid was centrifuged at 4000g for 10 min at 10 °C. The supernatant was subsampled (0.5 mL) and the A_T measured. Finally, 2 mL of the leftover supernatant was ultrafiltrated using a centrifugal filter unit with a pore size of 3 kDa (Amicon Ultra 2 mL, Millipore, USA; 4000g, 20 min, 10 °C). After filtration, 0.5 mL of the ultrafiltrate was subsampled and the A_T once again measured. $A_{T\text{-}SW}$ was also measured by microtitration on the same day. Results are reported as percentage of the A_T of the untreated CF.

2.5. Seawater acidification effect on buffer capacity

Fed *P. lividus* (2nd batch, Table 1) were placed in 9 tanks maintained at nominal pH 8.0 (control) and at a temperature of 10.0 °C and a salinity of 32.1. The experimental pH, 7.7 and 7.4, were gradually reached (decrease of 0.2 or 0.1 U per day) and maintained by bubbling CO_2 (Air Liquide) through mixing columns (Cycloturbo, Dennerle, Germany). Three independent replicates were done for each treatment. The bubbling was managed by an automatic computer-controlled system Aquastar (iks ComputerSysteme GmbH, Karlsbad, Germany) calibrated every day against measures made using the Metrohm pH meter. Seawater samples were collected every day and immediately filtered through 0.22-μm filters (Millipore) and then maintained at 4 °C and in the dark until further analysis. Parameters of the tanks once the treatment conditions were reached are reported in Table 2. Water quality was assured by a renewal of at least 70% of the water every week. Nitrite concentration never rose above 0.3 mg/L except in one tank where the concentration reached 0.8 mg/L. After 6 days of exposure to the experimental pH, the $A_{T\text{-}CF}$ and pH_{CF} of the sea urchins were measured on 3 individuals in each tank.

2.6. Statistical analysis

$A_{T\text{-}CF}$ and pH_{CF} of the different echinoderm taxa were analyzed by one-way ANOVA and a Tukey test for the multiple comparisons. The differences in ΔA_T and ΔpH between the fed and unfed groups on the last sampling were analyzed with a Kruskal–Wallis test and the comparisons of ΔA_T between the measurements of each group taken separately were done using a Friedman test. The effect of the fractionation methods on $A_{T\text{-}CF}$ was investigated using a repeated measures one-way ANOVA (on raw A_T data), associated with a Tukey test in between untreated, centrifuged and filtered samples while the difference between filtered CF and seawater was assessed by a two-sample *t*-test. Finally, the impact of decreased pH_{SW} on pH_{CF} and $A_{T\text{-}CF}$ was analyzed with a nested ANOVA model III (tanks, the random factor, nested in pH_{SW} the fixed factor) followed by a Tukey test. A two-sample *t*-test was used to investigate the effect of the gender of the individuals on $A_{T\text{-}CF}$. All correlations were tested using a Pearson correlation associated to a Bonferroni probability. All data reported in the text and figures are mean ± standard deviation.

3. Results

3.1. Alkalinity measurements and buffer capacity

The average difference between measured and certified values of the alkalinity of the "Dickson" standard using our microtitration technique was 0.74 ± 0.61% ($n = 28$), indicating a good precision and reproducibility. The difference between replicated measures of the $A_{T\text{-}CF}$ of *P. lividus* ranged between 0.017 and 0.044 mmol kg^{-1} SW (i.e. 0.41–1.21% of the mean), indicating a good reproducibility of the microtitration method on CF samples. The progressive titration of *P. lividus* CF samples with 0.5 μL HCl 0.1 mol L^{-1} showed the typical sigmoid form of a buffer titration with the maximum buffer capacity at pH_{NIST} between 7.5 and 6.5 (Fig. S1), i.e. in the physiological range of pH_{CF}. Based on this result, alkalinity measurements will be considered as measures of the buffer capacity of the CF.

3.2. Buffer capacity of the CF in different echinoderm taxa

Euechinoidea (*E. mathaei*, *P. lividus*, *T. ventricosus* and *E. cordatum*) showed the highest buffer capacity above that of seawater ($\Delta A_T = A_{T\text{-}CF} - A_{T\text{-}SW}$), with differences between the two ranging from 1.800 ± 0.567 mmol kg^{-1} SW to 0.840 ± 0.345 mmol kg^{-1} SW. Other taxa (Asteroidea, Holothuroidea and Cidaroidea) showed lower values than that found for Euechinoidea with values varying between −0.136 ± 0.138 mmol kg^{-1} SW and 0.447 ± 0.138 mmol kg^{-1} SW (Fig. 1A and Table S1). However, the differences in $A_{T\text{-}CF}$ between taxa were not paralleled by those of the ΔpH ($pH_{SW} - pH_{CF}$) (Fig. 1B and Table S1) with values ranging from 0.30 ± 0.02 for the holothurian *H. forskali* to 1.13 ± 0.25 for the cidaroid *E. tribuloides* and all other species in between with no particular order. ΔA_T values did not correlate with ΔpH values of the same individuals (Pearson correlation test, $r = -0.062$, $p = 0.611$).

3.3. Effect of nutrition

The ΔA_T of fed *P. lividus* and fed *A. rubens* showed a clear and significant difference (two sample *t*-test, $t = 6.321$, $p < 10^{-3}$) with a value approximately 7 times greater for the former (1.510 ± 0.656 mmol kg^{-1} SW vs. 0.200 ± 0.209 mmol kg^{-1} SW; same data as in Fig. 1A).

ΔA_T of *P. lividus* and *A. rubens* according to nutritional status are presented in Fig. 2A and B, respectively. For *P. lividus*, ΔA_T increased in fed animals during the first 2 weeks (Friedman, $p \leq 0.046$) and then reached a plateau (Friedman, $p \geq 0.3$). In unfed individuals, ΔA_T fluctuated and remained low (Friedman, $p \geq 0.083$, except for 5-weeks point, which is significantly different from 0-week and 2-week points, $p = 0.046$; Fig. 2A). At the end of the experiment, ΔA_T significantly differed between fed and unfed sea urchins (2.343 ± 0.297 mmol kg^{-1} SW for fed individuals and 0.557 ± 0.167 mmol kg^{-1} SW for unfed ones; Kruskal–Wallis, $p = 0.034$). Contrary to ΔA_T, ΔpH did not significantly differ between fed and unfed individuals: 0.41 ± 0.04 for unfed individuals and 0.48 ± 0.07 for fed ones (Kruskal–Wallis, $p = 0.154$).

Table 2
Parameters (mean ± SD) of the seawater in the different treatments during the acidification experiment. For each treatment pH, $n = 1731$; salinity and temperature: $n = 27$, total alkalinity (A_T): $n = 9$, all other parameters: $n = 3$.

Nominal pH	pH (total scale)	Salinity (PSU)	Temperature (°C)	A_T (mmol kg^{-1} SW)	C_T (mmol kg^{-1} SW)	pCO_2 (μatm)	Ω Ca	Ω Ar
7.4	7.42 ± 0.11	32.1 ± 0.1	10.3 ± 0.2	2.989 ± 0.092	3.039 ± 0.040	2534 ± 102	1.1 ± 0.1	0.7 ± 0.0
7.7	7.70 ± 0.04	32.1 ± 0.1	10.3 ± 0.4	2.960 ± 0.096	2.905 ± 0.041	1293 ± 57	2.0 ± 0.1	1.3 ± 0.0
8.0	8.03 ± 0.05	32.1 ± 0.1	10.0 ± 0.3	3.019 ± 0.071	2.822 ± 0.020	576 ± 8	4.0 ± 0.0	2.5 ± 0.0

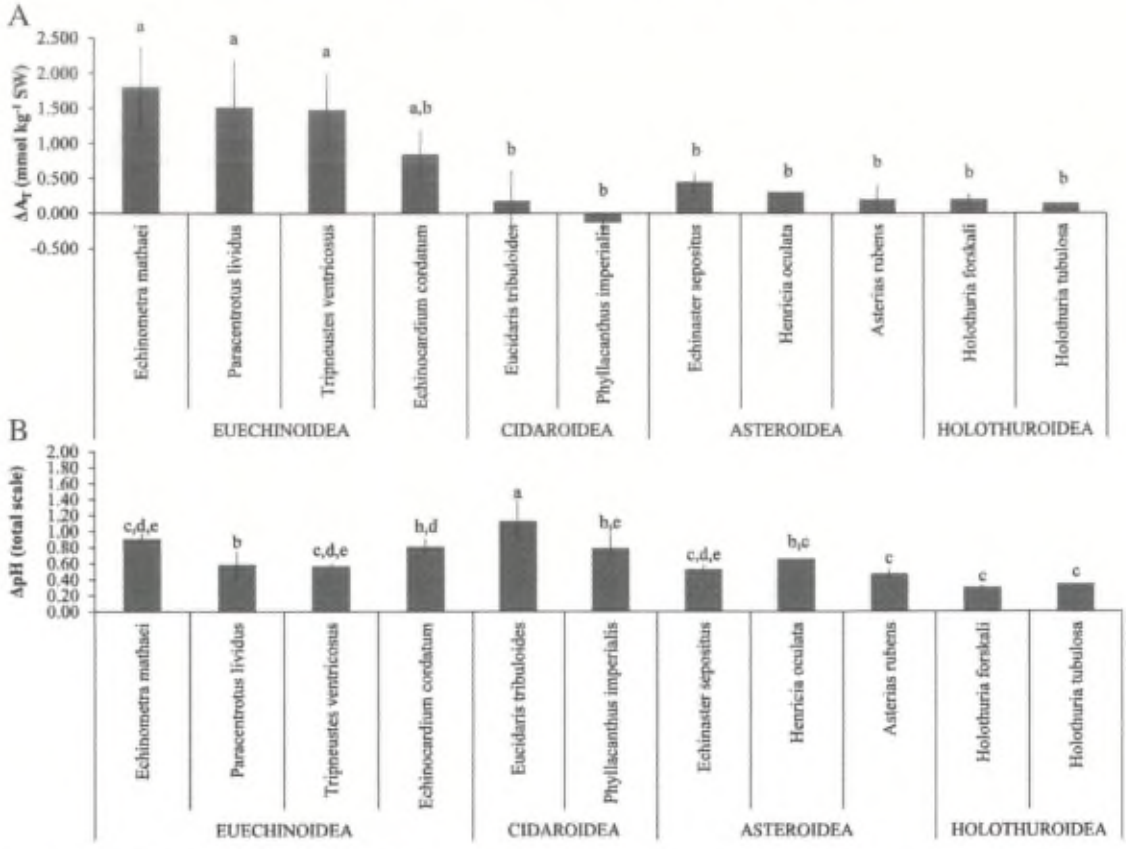

Fig. 1. ΔA_T ($A_{T\text{-}CF} - A_{T\text{-}SW}$) (A) and ΔpH ($pH_{SW} - pH_{CF}$) (B) for different species of the phylum Echinodermata (mean ± SD). Mean values sharing the same letter are not significantly different (one-way ANOVA associated to a Tukey test for multiple comparisons; $\alpha = 0.05$).

For *A. rubens*, there was no significant changes in ΔA_T over the experiment (Friedman, $p \geq 0.180$ in all cases for both groups), and after 2 weeks of experiment, there was no difference between the ΔA_T of the two groups (0.190 ± 0.374 mmol kg^{-1} SW for fed individuals and 0.256 ± 0.235 for unfed ones; Kruskal–Wallis, $p = 0.465$) (Fig. 2B) nor between the ΔpH of these (0.55 ± 0.08 for unfed individuals and 0.49 ± 0.13 for fed ones; Kruskal–Wallis, $p = 0.293$).

3.4. Distribution of the buffer capacity in the coelomic fluid

The role of different compartments of the CF in the buffer capacity was tested using different fractionation methods. The results for *P. lividus* and *A. rubens* are presented as the percentage of remaining alkalinity compared to that of the untreated fluid in Fig. 3. Centrifugation induced a significant decrease of 6–8% of $A_{T\text{-}CF}$ in both species (repeated measures ANOVA, $p_{Tukey} < 10^{-3}$ for both). Further, ultrafiltration at 3 kDa only significantly reduced $A_{T\text{-}CF}$ of sea urchins by about 22% compared to untreated fluid, but not that of starfish (repeated measures ANOVA, $p_{Tukey} < 10^{-3}$ for *P. lividus* and 0.470 for *A. rubens*). Moreover, after ultrafiltration, the difference between $A_{T\text{-}CF}$ and $A_{T\text{-}SW}$ (representing 63.16 ± 1.53% of the untreated fluid) was marginally not significant for sea urchins (two-sample *t*-test, $p = 0.051$) and highly not significant for starfish (91.32 ± 0.52% of the untreated fluid; two-sample *t*-test, $p = 0.474$).

3.5. Seawater acidification effect on the buffer capacity in sea urchins

P. lividus individuals were exposed to three different pH, i.e. 8.0, 7.7 and 7.4, for a week after a gradual decrease of the pH_{SW}. The pH_{CF} was significantly different for the sea urchins maintained at pH 7.4 (7.13 ± 0.37) compared to those from the treatments 7.7 (7.43 ± 0.26) and 8.0 (7.47 ± 0.07) (nested ANOVA, $p = 0.005$; p_{Tukey} between pH 7.4 and 7.7 = 0.011 and 7.4 and 8.0 = 0.006; Fig. 4A), whereas pH_{CF} of the sea urchins maintained at pH 7.7 was not significantly different from animals maintained at control conditions ($p_{Tukey} = 0.824$). ΔA_T of sea urchins showed a gradual increase with the decrease of pH_{SW} (Fig. 4B) with values ranging from 2.628 ± 1.101 mmol kg^{-1} SW for individuals maintained at pH 7.4, to 1.835 ± 0.796 for those at pH 7.7 and 0.805 ± 0.262 for the control ones. Only the two extreme conditions were significantly different (nested ANOVA, $p = 0.008$; $p_{Tukey} = 0.007$ between pH 7.4 and 8.0), while neither of them was different from the intermediate condition ($p_{Tukey} = 0.170$ between pH 7.4 and 7.7 and 0.076 between pH 7.7 and 8.0). No relationship was found between gender of the individuals and $A_{T\text{-}CF}$ (two-sample *t*-test, $p = 0.287$).

Our results also showed a high degree of correlation between ΔA_T or ΔpH and pH_{SW}. ΔA_T increased when pH_{SW} decreased, whereas ΔpH decreased (Pearson correlation, $r = -0.699$ and 0.441 with $p < 10^{-3}$ and 0.021 for ΔA_T and ΔpH, respectively).

4. Discussion

Among the studied echinoderms, the coelomic fluid (CF) of Euechinoidea exhibits a higher buffer capacity than that of cidaroids, starfish and holothurians, whose capacity did not differ between them. Sarch (1931) linked the higher buffer capacity to a higher content in carbon dioxide in the coelomic fluid. The CO_2 concentration of the coelomic fluid is the result of the CO_2 concentration in seawater and the metabolic CO_2 produced by respiration. The diffusion of CO_2 through the body wall of starfish and holothurians could be efficient enough to maintain an optimal pH_{CF} so that no extra buffer capacity is required and no mechanism of compensation has evolved in these

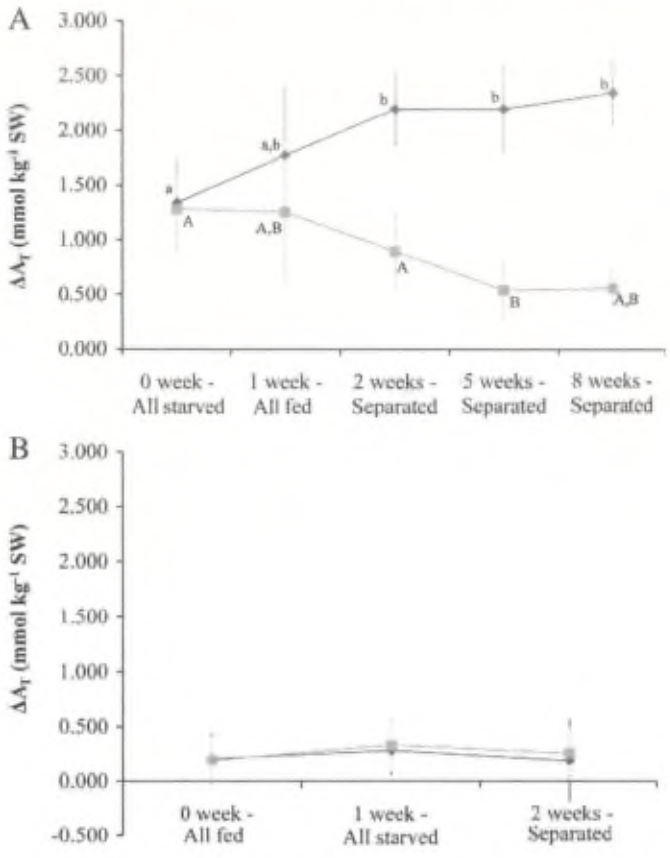

Fig. 2. ΔA_T (mean ± SD, $n = 5$ except for unfed urchins week 4 and fed urchins week 8 where $n = 4$ and unfed urchins week 8 where $n = 3$) in function of the nutritional state of *Paracentrotus lividus* (A) and *Asterias rubens* (B). (◆) Fed: individuals who were fed during the separation period; (■) Unfed: individuals who were not fed during the separation period.

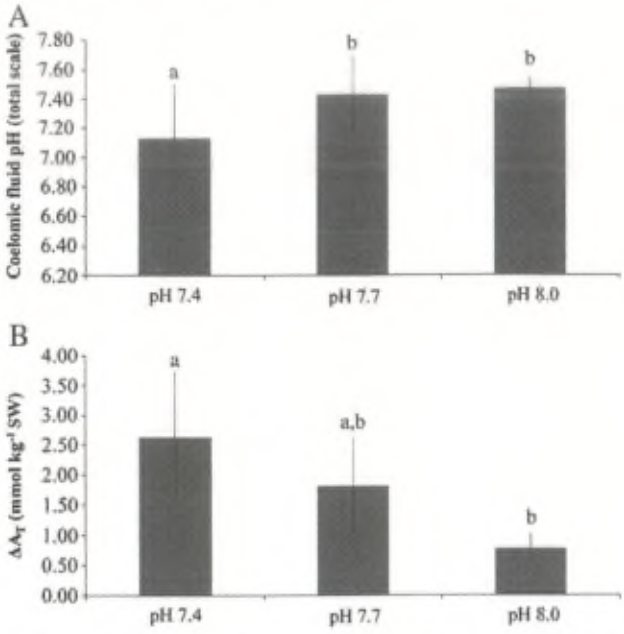

Fig. 4. *Paracentrotus lividus*: Coelomic fluid pH (total scale) (A) and ΔA_T (B) according to seawater pH (mean ± SD, $n = 9$ for each pH) at the end of the 7-day experiment. Mean values sharing the same letter are not significantly different ($\alpha = 0.05$).

two groups. Indeed, most species belonging to these two taxa are provided with structures specialized in gas exchanges (papulae for starfish and respiratory trees for holothurians) (Hyman, 1955; Ruppert et al., 2004). On the contrary, sea urchins have a heavily calcified skeleton that slows CO_2 diffusion and possess less developed structures for gas exchanges (which principally occur through the tube feet). In this context, it is noteworthy that the burrowing euechinoid *E. cordatum* is provided with specialized respiratory tube feet (Nichols, 1959; Smith, 1980) and showed the lowest CF buffer capacity among this taxon. A lower gas exchange capacity would probably induce a more pronounced acidosis of the CF in sea urchins than in the two other taxa if it was not compensated by a compound present in the CF. This is correlated to the reported values of CO_2 concentrations in the CF by Collip (1920) who measured a higher value in the CF of sea urchins than in that of starfish. In contrast, the cidaroids (a basal sister group of Euechinoidea) possess the so-called Stewart's organs which are absent in most euechinoids and are supposed to be involved in gas exchanges (De Ridder, 1988). These organs are located on the surface of the Aristotle lantern whose muscles are the major source of respiratory CO_2 freed in the CF (Prouho, 1888; De Ridder, 1988). These specialized organs could explain the difference observed between the two sea urchin taxa. It thus appears that among the echinoderms, an increased buffer capacity of the CF is linked to a lesser capacity of the species to evacuate CO_2 by means of diffusion through the body wall and/or through specialized gas exchange structures.

Removal of coelomocytes by centrifugation of the CF of sea urchins and starfish significantly reduced the buffer capacity of this fluid (by approximately 8%) as was already reported by Sarch (1931). This could be

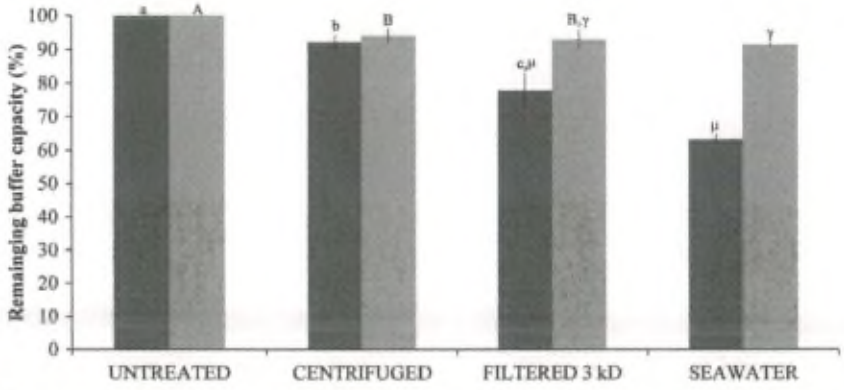

Fig. 3. Percentage of remaining buffer capacity (mean ± SD, $n = 6$) after centrifugation and following ultrafiltration of the coelomic fluid of *Paracentrotus lividus* (■) and *Asterias rubens* (■). The value of 100% was assigned to the value of the untreated fluid and all other samples were compared to the latter. The total alkalinity of seawater is also presented in terms of percentage of the untreated fluid. Comparisons were carried out on raw data for each species separately. Mean values sharing the same letter are not significantly different ($\alpha = 0.05$).

due to either passive transfer of amino nitrogen to the CF, adsorption of protons on the surface of the cells or by dissociation of H_2CO_3 inside the coelomocytes and release of the bicarbonate ion into the CF (as do the erythrocytes in the plasma-blood cell system in vertebrates) (Delaunay, 1926; Heisler, 1986). It could also be due to a contribution of the cell plasma of disrupted coelomocytes when reaching pH 5 and lower. However, the data from the slow titration (addition of 0.5 μL of acid at a time) show that the decrease in pH for the seawater and for the CF are very similar between pH 6 and 4 (Fig. S1). Also, Donnellon (1938) and Matranga et al. (2002) showed that sea urchin coelomocytes (including those of *P. lividus*) can be maintained at pH between 4.7 and 4.2 for up to 6 hours without disruption of the cellular membrane. In *A. rubens*, the buffer capacity of the centrifuged CF did not differ from that of seawater, indicating that aside from coelomocytes, the remaining buffer capacity can be explained by seawater components with the bicarbonate ion concentrations being similar. On the contrary, in *P. lividus*, the CF supernatant buffer capacity is significantly different from that of seawater indicating that one or several components confer an increased buffer capacity to the CF. We suggest that this component could be either proteinic (fraction above 3 kDa), metabolic end products as lactate (Spicer et al., 2011) and/or bicarbonate ions (fraction below 3 kDa). Indeed, ultrafiltration with a 3-kDa cutoff removed a significant part of the CF buffer capacity indicating that molecules larger than 3 kDa are involved. The ultrafiltrate alkalinity is not significantly different of that of seawater but the associated probability is marginal ($p = 0.051$), meaning that the involvement of small molecules cannot be excluded. This is further supported by the growing evidence that sea urchins are capable of accumulating bicarbonate ions into their CF as a response to reduced seawater pH (Spicer et al., 2011; Stumpp et al., 2012). The seawater components (i.e. mainly the carbonate system) account for ca. 63% of the buffering capacity of *P. lividus* CF and 92% of *A. rubens* CF. So it appears that the bicarbonate buffer system is the basis of the buffer capacity of the extracellular fluid of echinoderms, but coelomocytes and, in *P. lividus* and possibly other euechinoids, other buffering molecules account for a significant part of this capacity.

This is further supported by the fact that the buffer capacity of *P. lividus* increased with feeding, while that of *A. rubens* remained unchanged whatever the feeding state. This observation explains the results of Stumpp et al. (2012) in *S. droebachiensis* which showed that, at pH_{SW} 7.25, sea urchins that effectively fed could better compensate the pH_{CF} than those who did not. Holland et al. (1967) reported that the protein concentration of the CF in the euechinoid *Strongylocentrotus purpuratus* was higher in fed than in unfed specimens. Also, feeding leads to an increased metabolism which, in the case of sea urchins, would result in an increased CO_2 concentration in the CF. In order to cope with this, sea urchins could accumulate bicarbonate ions as a quick response to increase the buffer capacity of their inner fluid. On the contrary, Ferguson (1964) showed that the protein concentration of the CF of *Asterias forbesi* did not change with feeding or starvation. This further supports the link between protein content and buffer capacity of the CF. Moreover, the metabolism of starved sea urchins decreases (witnessed by a decrease of the respiration rate; Farmanfarmaian, 1966). In unfed conditions, *P. lividus* did not have a pH_{CF} different from that of fed individuals even though their buffer capacity was a fifth of that of fed individuals. This suggests that the production of metabolic CO_2 decreased leading to a decreased acidosis and concentration of bicarbonate ions.

Starvation could also induce a decrease in the number of coelomocytes. Holland et al. (1965) mentioned the fact that starved sea urchins (*S. purpuratus*; 8 days of starvation) showed a decreased number of coelomocytes; however, no value was given. Sea urchins (*S. purpuratus*) starved for 3 weeks had a level of red and white coelomocytes about half of that of the same individuals after refeeding (deduced from Fig. 4 in Boolootian and Lasker, 1964). Considering that our data show that coelomocytes contribute to about 8% of the total alkalinity measured in *P. lividus*, a decrease by half of the coelomocytes would correspond to about a loss of 4% of the alkalinity. When analyzing our data for the unfed individuals, the decrease after 1 week of starvation amounts to about 8%, which is twice what could be estimated only by loss of coelomocytes. After 4 and 8 weeks, this difference is around 16.5%, indicating the possible loss of other constituents as well.

As *P. lividus* frequently inhabits tide pools, this species faces tidal fluctuations of pH between 8.0 and 7.4 (Truchot and Duhamel-Jouve, 1980; Moulin et al., 2011). The fact that the buffer capacity has its range of action between pH 7.5 and 6.5 is particularly interesting as this range is that encountered for pH_{CF} during exposure to pH_{SW} 8.0, 7.7 and 7.4. This may allow a short term compensation of the pH_{CF} (for instance during the tides) as demonstrated for the sea urchins *P. miliaris*, *E. esculentus* and *P. lividus* during low tide (Spicer et al., 1988; Miles et al., 2007; Moulin L., personal observation).

When submitted to lowered seawater pH (pH_{SW}) for 7 days, the CF buffer capacity of *P. lividus* increased similarly at pH_{SW} 7.7 and 7.4 as compared to pH_{SW} 8.0. This suggests that either the accumulation of the buffering compound(s) is threshold controlled (e.g. by increased respiration rate, see Catarino et al., 2012) or that this accumulation reached a maximum at 7.7 and could not be further increased at lower pH. Whatever the case, it appeared that the increased buffer capacity is able to compensate pH_{CF} at pH_{SW} 7.7 but not at pH 7.4 (as also reported by Stumpp et al., 2012 in *S. droebachiensis*).

The nature of the buffering compounds accumulated in response to decreased pH_{SW} should be further investigated using fractionation methods. Stumpp et al. (2012) showed an increase of bicarbonate concentration in the CF. Furthermore, hypercapnia may lead to an anoxic state of the internal organs, which are already reported as hypoxic in normoxic seawater conditions as elimination of the CO_2 towards the CF will become slower due to the reduced gradient of concentration (Ellington, 1982; Shick, 1983; Bookbinder and Shick, 1986). An increased buffer capacity at reduced pH_{SW} may be due to an accumulation in the CF of the end products of this anaerobic metabolism, e.g. lactate and malate, which can act as proton acceptor as reported by Spicer et al. (2011) in *S. droebachiensis* (but not in another euechinoid, *P. miliaris*). This possibility is reinforced by a study of Bookbinder and Shick (1986) who showed that the buffer capacity of the CF increased during ovary (an anoxic internal organ) growth, in parallel with an increase in protein concentration in the CF. However, the increased buffer capacity was a characteristic of both males and females. As no study was conducted for male gonads, this may suggest either that the same phenomenon occurs for male gonads or that there is no direct effect of ovaries on the buffer capacity of the CF. This also raised the possibility that increased CF buffer capacity at low pH could be linked to an increased CF protein concentration (fraction above 3 kDa).

5. Conclusions

The primary buffer system of the coelomic fluid of echinoderms is, as for seawater, the bicarbonate buffer system. Coelomocytes and, in euechinoids, other unknown compounds also play a significant role. These compounds include molecules larger than 3 kDa like proteins and possibly small molecules like lactate and accumulated bicarbonate ions. The CF buffer capacity increases with feeding and when sea urchin face reduced seawater pH. Thus, at least, euechinoids appear to be able to regulate their extracellular pH to a certain point.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.cbpa.2013.06.002.

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