Trans-life cycle impacts of ocean acidification on the green sea urchin *Strongylocentrotus droebachiensis*

Doctoral thesis

Narimane Dorey

Department of Biological and Environmental Sciences

Faculty of Science, University of Gothenburg

Gothenburg, Sweden

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UNIVERSITY OF GOTHENBURG

Cover illustration by N. Dorey: Stylized life-cycle of the green sea urchin *Strongylocentrotus droebachiensis*

Abstract: This thesis studies the impacts of ocean acidification on an ecologically and economically important invertebrate of the Nordic waters: the green sea urchin *Strongylocentrotus droebachiensis*. Acidification affects the different life stages and transitions composing the life-cycle.

Paper I establishes the robustness of the larval stage to a broad range of acidification (-1.5 pH_T units) covering present, projected near-future variability and beyond. Development of normal, although showing morphological plasticity, swimming larvae was possible as low as $pH_T \geq 7.0$. Acidification increased mortality and respiration and decreased growth rate.

Paper II focuses on the impacts of a decreased pH (-0.4 and -0.8 units) on the transitions phases between the larval and juvenile stages and on juveniles' survival. Lowered pH induced both direct effects of (e.g. juvenile spine amount) and carry-over effects (e.g. increased settlement rates).

Paper III deals with juvenile and adult stages. While adult fecundity was reduced after a 4-months exposure to low pH (-0.4 units), it was not affected anymore after 16-months. On the other hand, juveniles experienced a 95% mortality when grown at low pH since fertilization.

Paper IV is a meta-analysis based on the available experimental data available on echinoderms in 2010 revealing differing sensitivities of the stages and processes studied to near-future predictions.

Paper V reveals, from *in vivo* measurements on *S. droebachiensis* pluteus larvae, that the extracellular compartment surrounding the calcifying cells conforms to the surrounding seawater with respect to pH. Under ocean acidification, maintaining constant intracellular pH for calcium precipitation probably causes enhanced metabolic costs.

Populärvetenskaplig Sammanfattning

Denna avhandling fokuserar på havsförsurningens effekter på sjöborrens (*Strongylocentrotus droebachiensis)* livscykel.

Havsförsurning beror på människans utsläpp av koldioxid (CO_2): koldioxid i gasform löses upp i havsvattnet och förändrar karbonaternas kemi, som i sin tur skapar en ökning av vätejonskoncentrationen (H^+) i haven. När H^+ koncentrationen ökar, så ökar också mängden koldioxid i atmosfären, vilket ökar havets surhetsgrad (d.v.s. sänker pH-värdet). Enligt modellerna, så minskar pH-värdet (som för närvarande ligger på ~ 8,0–8,1 enheter) med 0,3 och 0,4 enheter mot slutet av 2000-talet. Detta innebär en fördubbling av vätejonskoncentrationen. De här förändringarna har en direkt påverkan på marina arter och "havsförsurning" har en betydande inverkan på de marina ekosystemen och eventuellt på dess tjänster.

Den gröna sjöborren (*Strongylocentrotus droebachiensis*) är ett ryggradslöst djur som lever i vattnet i den kalltempererade zonen på norra halvklotet. Den spelar en central ekologisk roll i steniga marina ekosystem, bland annat i nordöstra Atlanten. De vuxna djuren kan reglera överflöd av kelp, brunalger som fungerar som reservoarer av biodiversitet. När de gröna sjöborrarna finns i hög densitet, så kan de förstöra hela kelpskogar och skapa ett område som mest liknar marina öknar. Dessutom har den gröna sjöborren en livscykel som består av flera stadier (larv, juvenil, vuxen), som binds samman genom olika övergångsprocesser (ägg och spermier släppande, kolonisering, metamorfos, etc.).

Den gröna sjöborren förefaller vara relativt robust när den utsätts för pH-sänkning i modellerna (**artikel I och III**). Det juvenila stadiet, liksom övergångarna mot det, verkar ha påverkats mycket av havsförsurningen

(**artikel II**). Effekterna av ett lägre pH-värde under larvstadiet är främst icke-dödliga: havsförsurning orsakar en försenad tillväxt, morfologiska förändringar och en ökad metabolism (**artikel I**) - det senare kan möjligen vara på grund av ökade kostnader för att underhålla förkalkningen (**artikel V**). Effekterna av försurning hos tagghudingar beror i allmänhet på vilka funktioner och livsstadier som studeras, liksom vilken experimentell design som använts (**artikel III och IV**). Ändå visar den här avhandlingen att även subtila icke-dödliga effekter - exempelvis fördröjd tillväxt - kan ha en negativ och signifikant påverkan på överlevnaden hos framtida populationer av sjöborrar (**artikel I och IV**).

Résumé

Cette thèse s'intéresse aux effets de l'acidification des océans sur le cycle de vie de l'oursin vert : *Strongylocentrotus droebachiensis*.

L'acidification des océans est une conséquence des émissions humaines de dioxyde de carbone (CO_2) : le CO_2 gazeux se dissout dans l'eau de mer et altère la chimie des carbonates, créant une augmentation de la concentration d'ions hydrogène (H^+) dans les océans. Par conséquent, l'accroissement du taux de CO_2 dans l'atmosphère augmente l'acidité (diminution du pH) des océans, via la hausse de la concentration en H^+. D'après les modèles, le pH (actuellement $\approx$ 8,0-8,1 unités) pourrait diminuer de 0,3 à 0,4 unités d'ici la fin du XXI$^{\text{ème}}$ siècle. Ceci représente un doublement de la concentration en H^+. Les êtres vivants marins sont directement soumis à ces changements, et cette « acidification des océans » a des répercussions importantes sur les écosystèmes marins.

L'oursin vert (*Strongylocentrotus droebachiensis*) est un invertébré des eaux de la zone tempérée froide de l'hémisphère nord. Il joue un rôle écologique central au cœur des écosystèmes marins rocheux, y compris dans les eaux nordiques de l'Atlantique Nord-Est. Adulte, il peut réguler l'abondance des kelps, des algues marines représentant d'importants réservoirs de biodiversité. En effet, en forte densité, ils sont capables de détruire des forêts entières de kelps, laissant derrière eux des déserts. L'oursin vert possède par ailleurs un cycle de vie composé de plusieurs stades (larve, juvénile, adulte) reliés par différents processus de transition (ponte, métamorphose, etc.).

L'oursin vert semble relativement résistant aux diminutions de pH prévues par les modèles (**Papier I and III**). Cependant, le stade juvénile et les

transitions pour y parvenir semblent très affectés par une acidification du pH (**Papier II**). Le stade larvaire est toutefois touché de façon sub-létale : une diminution du pH entraîne un délai de croissance, une modification de la morphologie larvaire ainsi qu'une augmentation du métabolisme (**Papier I**) – celle-ci étant possiblement liée à la hausse d'énergie nécessaire à la calcification (**Papier V**). Les effets de l'acidification sur les échinodermes sont globalement dépendants des fonctions et des stades de vie étudiés, mais aussi du modèle expérimental (**Papier III and IV**). Néanmoins, cette thèse démontre que même de subtils effets sub-létaux – tel qu'un délai de croissance – peuvent affecter négativement et significativement la survie des populations de l'oursin vert dans le futur (**Papier I and IV**).

Contents

List of papers

This doctoral thesis is based on the following manuscripts/publications:

Paper I: <u>Dorey N</u>, Lançon P, Thorndyke M & Dupont S (2013) Assessing physiological tipping point of sea urchin larvae exposed to a broad range of pH. *Global Change Biology*

Paper II: <u>Dorey N</u>*, Butera E* & Dupont S (manuscript) Ocean acidification affects transition between life-history stages in sea urchin. * *both authors contributed equally to the work.*

Paper III: Dupont S, <u>Dorey N</u>, Stumpp M, Melzner F & Thorndyke MC (2012) Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology*: **160**, 1835-43

Paper IV: Dupont S, <u>Dorey N</u> & Thorndyke MC (2010) What meta-analysis can tell us about vulnerability of marine biodiversity to ocean acidification? *Estuarine, Coastal and Shelf Science*: **89**, 182–185

Paper V: Stumpp M, Hu MY, Melzner F, Gutowska MA, <u>Dorey N</u>, Himmerkus N, Holtmann WC, Dupont S, Thorndyke MC & Bleich M (2012) Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification. *Proceedings of the National Academy of Sciences of the United States of America*, 109, 18192–7

Related articles not included in the thesis:

<u>Dorey N</u>, Melzner F, Martin S, Oberhänsli F, Teyssié J-L, Bustamante P, Gattuso J-P & Lacoue-Labarthe T (2012) Ocean acidification and temperature rise: effects on calcification during early development of the cuttlefish *Sepia officinalis*. *Marine Biology*, **160**, 2007-2022.

Preface

> **"That we develop from a single cell, the fertilized egg, just one tenth of a millimeter in diameter – smaller than a full stop – is amazing."**

— Lewis Wolpert, *Developmental Biology – A very short Introduction.*

In the marine environment, 70% of the invertebrates living on the bottom possess a larval stage that lives and feed in the plankton (i.e. planktotrophic larvae; Thorson 1950). These microscopic, often beautiful and very fragile-looking larvae develop through several life transitions, including some complete morphological reorganization into mature macroscopic adults. They do so in an environment that is naturally variable, in cohabitation with other organisms. This "Struggle for existence" (Darwin 1859) involves finding enough food while not being eaten by others and coping or adapting to the environment chemical and physical conditions for e.g. climate. A first aspect of my thesis refers to understanding how organisms achieve this "existence", with a particular focus on development.

> **"[...] we are awfully lucky to be here - and by 'we' I mean every living thing. To attain any kind of life in this universe of ours appears to be quite an achievement."**

— Bill Bryson, *A Short History of Nearly Everything*

A second aspect of this thesis concerns our influence, as humans, on natural systems. Originating from Africa, about 200 000 years ago (McDougall *et al.* 2005), the recent *Homo sapiens* species has a heavy

footprint on the planet earth's ecosphere (atmosphere, geosphere/lithosphere, hydrosphere and biosphere). Via the use of natural resources (e.g. food, water, wood, fossil energies), humans modify atmospheric composition, landscapes and ecosystems. Since the industrialization (18th – 19th century), the efficiency in using natural resource has progressed, in parallel to the human population's exponential growth. Of the anthropogenic CO_2, one fourth is incorporated in the oceans, buffering the effect of this green-house gas in the atmosphere (Sabine *et al.* 2004). Ocean acidification (abbreviated OA in this thesis) is a consequence of this incorporation: the CO_2 dissolution in seawater produces H^+ ions and acidifies the seas, affecting marine organisms and modifying valuable ecosystems.
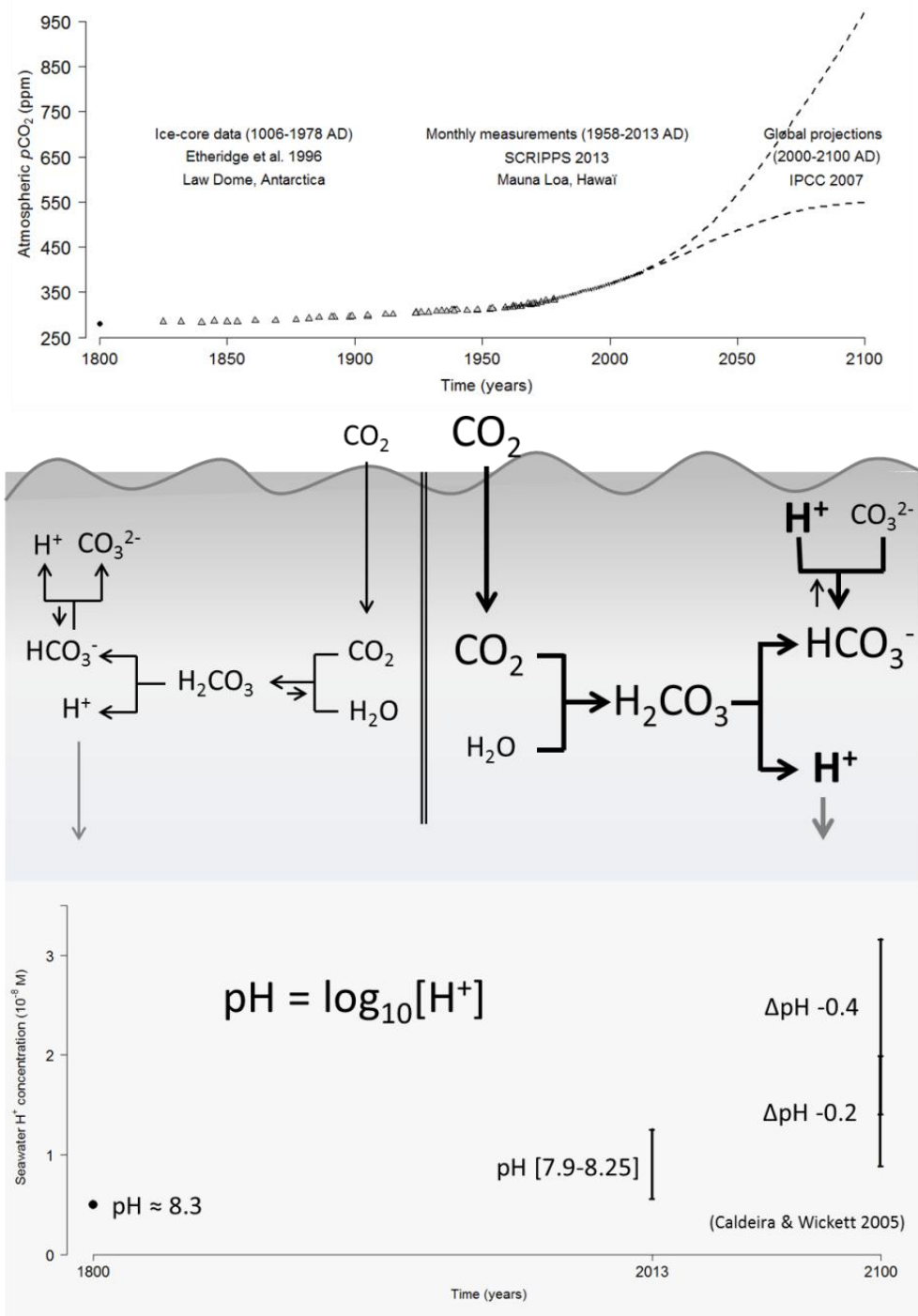
This thesis deals with understanding the impacts of OA on an important regulator of the North Atlantic ecosystems, the green sea urchin *Strongylocentrotus droebachiensis*: How is the life-cycle of this organism affected by OA? Is it sensitive/robust to the projected changes? The answers to these question hopefully provide insight for generalization to other organisms and, ultimately, for realistic predictions on the future changes in oceanic ecosystems. I will first present background information on OA, *S. droebachiensis* and a snapshot of the effects of OA on echinoderms. In a second time, I will introduce each problematic this thesis has focused on, give a summary and discuss the results exposed in the papers composing it.

Background

Ocean acidification

pCO_2 rise in the atmosphere

Earth's atmospheric layer is constituted of ≈0.04 % of carbon dioxide gas (CO_2), representing a partial pressure pCO_2 of ≈400 ppm (Scripps CO_2 Program, June 2013). Since pre-industrial era (1760's), pCO_2 has risen by ≈100 ppm, as a consequence of human-caused CO_2 emissions (Etheridge *et al.* 1996). Following the currently high human activities, different models project an increase of CO_2 concentrations, reaching **500 to 1000 ppm** by the end of the 21st century (IPCC 2007; **Fig. 1**, *upper panel*). Such a production of CO_2, a greenhouse gas, could increase mean surface Earth's temperatures between 2.0°C to 4.5°C from 1990 to 2100 (IPCC 2007). Consequently, similar trends are expected for surface ocean temperature due to the warming of the oceanic mixed surface layer (Levitus *et al.* 2005). Only considered recently (Haugan & Drange 1996; Auerbach *et al.* 1997; Brewer 1997), the rise in atmospheric pCO_2 (atm. pCO_2) is also driving changes in ocean seawater chemistry.

CO$_2$ CO$_2$

H$^+$ CO$_3^{2-}$

H$^+$ CO$_3^{2-}$

HCO$_3^-$

HCO$_3^-$ ← H$_2$CO$_3$ ⇌ CO$_2$ / H$_2$O

H$^+$

CO$_2$ / H$_2$O → H$_2$CO$_3$ → HCO$_3^-$

H$^+$

Seawater H$^+$ concentration (10^{-8} M)

3
2
1
0

$$pH = \log_{10}[H^+]$$

ΔpH -0.4

ΔpH -0.2

pH [7.9-8.25]

pH ≈ 8.3

(Caldeira & Wickett 2005)

1800   2013   2100
Time (years)

Fig. 1 From increasing atmospheric CO$_2$ partial pressure (atm. pCO$_2$; parts per million: ppm) to increasing ocean acidity (seawater [H$^+$]; 10^{-8} M).

Fig. 1 legend (continued) *Upper panel*: Data for past atm. pCO$_2$ from Etheridge *et al.* (1996), current records from Scripps CO$_2$ Program (June 2013) and two projections from the IPCC (2007). The point at 1800 represents the mean of the data points before 1800 AD (1006 to 1796: 280±3 ppm; N=27). *Middle panel*: Chemical reactions following the dissolution of CO$_2$ in the seawater lead to a production of H$^+$ ions. With more CO$_2$ in the atmosphere, the production of H$^+$ is increased. *Lower panel*: Indicative H$^+$ concentrations as calculated from estimates of average pH values for 1800's (Caldeira & Wickett 2005), today's pH range (Raven *et al.* 2005) and projected decrease for 2100 (-0.2 to -0.4 pH units from today's range: Caldeira & Wickett 2005). A decrease of pH from 8.1 to 7.7, as projected for global surface oceans, corresponds to a doubling of the concentration of H$^+$.

$$CO_2 + H_2O \rightarrow OA$$
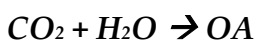
Oceans, as active carbon sinks, limit atmospheric pCO$_2$ rise by incorporating CO$_2$. According to Sabine *et al.* (2004), oceans are responsible for the uptake of 30% of anthropogenic CO$_2$ emissions produced since 1800. The atm. pCO$_2$ rise increases the incorporation of CO$_2$ in the oceans, causing major shifts in seawater carbonate chemistry (Caldeira & Wickett 2003; Feely *et al.* 2004; Sabine *et al.* 2004).

Briefly (**Fig. 1**, *middle panel*), gaseous CO$_2$, once dissolved in seawater (CO$_{2\,(aq)}$; 0.3% of the seawater Total Inorganic Carbon: TIC), forms H$_2$CO$_3$ (carbonic acid). This latter immediately dissociate into HCO$_3^-$ (bicarbonate ions; 87.1% TIC), producing hydrogen ions H$^+$. Bicarbonate ions transform into carbonate ions (CO$_3^{2-}$: 12.6% TIC), depending on H$^+$ availability.

The higher pCO$_2$ in the surface ocean, the higher the hydrogen ion concentration (**Fig. 1**, *lower panel*) and, as a consequence, the lower the seawater pH (pH = -log$_{10}$[H$^+$]). This phenomenon is referred to as Ocean

Acidification (OA), even though ocean surface is overall alkaline (pH>7). Current global surface pH of the oceans (≈8.1) has fallen by ≈0.1 pH units since pre-industrial era (Caldeira & Wickett 2005), representing a 25% increase in H$^+$ concentration. Depending on the anthropogenic emission scenarios, models project a global surface ocean **pH decrease of 0.2 to 0.4 units for 2100** (Caldeira & Wickett 2005), an acidification event unprecedented for the last 300 million years (Hönisch *et al.* 2012).

OA and calcification

With an increase of H$^+$ concentration, the concentration of CO_3^{-2} decreases, in favor of HCO_3^-. Because CO_3^{2-} is one of the carbon forms used during the calcification processes of marine organisms, calcification was therefore predicted to be one of the first processes to be impacted by OA (e.g. Orr *et al.* 2005; Kleypas *et al.* 2006). From a purely chemical point of view, calcification is the production of calcium carbonate ($CaCO_3$) structures according to the following equation:

CO_3^{2-} $_{(aq)}$ + Ca^{2+} $_{(aq)}$ ↔ $CaCO_3$ $_{(s)}$
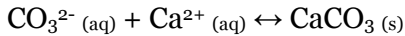
Chemical conditions driving calcification processes can be expressed by the saturation state (Ω). This coefficient is specific to the calcium carbonate polymorph formed, i.e. calcite or aragonite:

$\Omega = [CO_3^{2-}] \, [Ca^{2+}] \, / \, K^*_{sp}$

where K^*_{sp} (depending on temperature, pressure, salinity and calcium carbonate polymorph) is the apparent stoichiometric solubility product. $[CO_3^{2-}]$ and $[Ca^{2+}]$ are the *in situ* calcium and carbonate concentration, respectively. The precipitation of $CaCO_3$ is favored when Ω>1. For Ω<1, the

seawater is corrosive to $CaCO_3$ structures. Generally, surface tropical waters are supersaturated ($\Omega > 1$) for every carbonate minerals while deeper waters (higher pressure) or higher latitudes (lower temperature) tend towards under-saturation ($\Omega < 1$).

In the context of OA, the CO_3^{2-} converted to HCO_3^- is projected to lower the saturation state. Invertebrates calcifying marine organisms have therefore been a considerable driving force of the research effort (Doney *et al.* 2009). However, **biocalcification is not calcification**. Some calcifiers live in environments experiencing CO_2-rich waters (e.g. mussels and barnacles in Kiel fjord, Germany: Thomsen *et al.* 2010) or even under-saturated waters (e.g. mussels in the Mariana Volcanic Arc: Ω from 0.61 to 0.01; Tunnicliffe *et al.* 2009). Results of perturbation experiments show that calcification responses are species-specific (e.g. Ries *et al.* 2009; Dorey *et al.* 2012), even within taxonomic groups. For instance, sea urchin calcification was increased by 4.5 time in adults of *Arbacia punctulata*, while it was decreased by half in adults of *Eucidaris tribuloides* (60 days exposure to $\Omega = 0.5$: Dupont *et al.* 2010b).

Calcification sites can be protected from direct contact to the seawater (Simkiss & Wilbur 1989), and thus not directly subject to its corrosive chemistry (e.g. Addadi *et al.* 2006). As an illustration, echinoderm larval endoskeleton formation was described by Killian & Wilt (2008) and Matranga *et al.* (2011). In summary, larval spicules develop inside the larvae and is a calcite structure made of calcium carbonate ($CaCO_3$) combined to magnesium-rich calcite ($MgCO_3$: 5-15%) embed in a matrix of proteins. **Primary mesenchyme cells (PMCs)** are the cells building the spicules, they import calcium from the seawater through calcium channels and precipitate it in the syncytium (i.e. fusion of cells) thanks to favorable

conditions (high saturation state), probably maintained by ion pumps and channels. Skeleton is therefore built inside the cellular covering of the organism.

Specific mechanisms of biocalcification still need understanding. Apprehending the relation between biocalcification processes and environmental conditions may be a key to understand the broad range of calcification responses encountered in acidified conditions.

Strongylocentrotus droebachiensis

Strongylocentrotus droebachiensis (Müller 1776), commonly known as the green sea urchin, is a marine calcifying invertebrate belonging to the Echinoidea class. It has a circum-boreal distribution (**Fig. 2**; Scheibling & Hatcher 2007), ranging from temperate waters (Atlantic and Pacific oceans) to Arctic waters and distributed as south as England in Europe and Puget Sound (Washington State, USA) in the Pacific. It is a Nordic urchin that tolerates cold temperatures (range≈-1°C to 18-20°C; optimum≈9°C to 13°C). Echinoderms are usually not tolerant to low salinity, nevertheless, *S. droebachiensis* is found in the relatively euryhaline waters of the Puget Sound (down to a salinity of 27) or in the variable salinity of the Saint-Laurent estuarine.

The adult green sea urchin lives on rocky substrates, mostly in the intertidal zone down to 30 meters but can be found as deep as 1200 meters. Principally herbivorous, it grazes on macro algae (mainly laminarian kelps), but can secondarily feed on small animals or detritus when algae are not available. Adults can move to search for new food resource using their tube

feet (or podias) or stay in a burrow into rocks dug with their spines. The adult average diameter is 5 cm (50-80 g), but it can reach sizes of 8-9cm. It is a relatively fast growing urchin and reaches maturity at diameters of 2-3 cm, around 3 years-old (Raymond & Scheibling 1987). Although it is called "green" this is not characteristic on the whole body and colors of the skeletal plates can range from greenish to brown-pink. Spines are numerous, green to violet-red and usually white tipped. The specific morphological characteristics of this species are: (*i*) adults show full radial symmetry; (*ii*) the anus is situated outside the apical system; (*iii*) jaws lack the lateral teeth; (*iv*) there is more than three pore plates per ambulacral plate and (*v*) primary and secondary spines have similar length (Müller, 1776).

Fig. 2 Worldwide distribution of *Strongylocentrotus droebachiensis*. Image source from freely licensed media *Wikimedia Commons*.

The sea urchin (**Fig. 3**), and especially the species *S. droebachiensis*, is a good model to study the impact of future changes of the carbonate system in the ocean. Urchins possess an endoskeleton as adult ($CaCO_3$ tests and spines covered by a thin epidermal tissue) and are commercially and ecologically important.
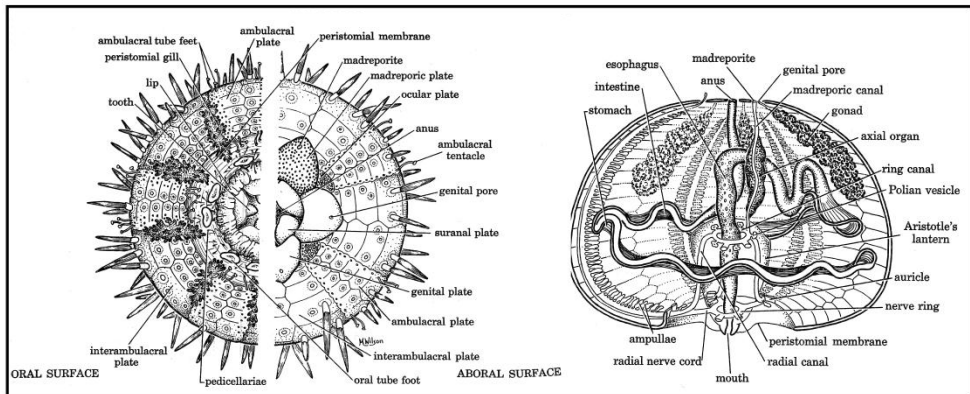
Fig. 3 A sea urchin (*Arbacia punctulata*) morphology and anatomy. Left: Oral and aboral surfaces of the test, with spines partly removed to show detailed structure. Right: Vertical section of the animal to show internal organs. From W.M. Reid in *Selected Invertebrate Types*, edited by F.A. Brown, Jr (John Wiley and Sons, New York, 1950).

Adult urchins are fed on by some large fishes (e.g. wolf-eel, cod), sea star, crabs, seabirds and mammals (including sea otters in the Pacific and humans). *S. droebachiensis* is indeed an edible species, and a commercially important one. The sea urchin roe is one of the highest paid invertebrate seafood (~6.3 US$ per kilogram of urchin; FAO 2010). Landings as well as aquaculture are not negligible: captures weighted more than 97 000 tons in 2008, of which more than a third are sea urchins of the genus *Strongylocentrotus* and nearly 40 000 tons from the Chilean sea urchin (*Loxxechinus albus*), making echinoderm a more important capture load than mussel by 10 000 tons (FAO 2010). While a delicacy in a number of countries where it is consumed (e.g. Japan, France, Chile), it is also considered a pest in other parts of the world. When predators regulating their abundance are lacking, sea urchin are able to eat whole kelp forests and transform these highly productive and diverse ecosystem to underwater deserts (e.g. Mann 1977; Steneck *et al.* 2002). Urchins in high density form feeding fronts along the kelp beds and graze the seaweeds, until they are

completely destroyed, creating so called barrens (e.g. Dayton 1975; Gagnon *et al.* 2004), with low productivity, habitat complexity and biodiversity. *S. droebachiensis* has a pivotal ecological role in determining the structure of rocky subtidal communities, including in North-East Atlantic waters (Norderhaug & Christie 2009).

Lastly, sea urchin is also a historically studied developmental model (**Fig. 4**). From the end of the 19th century, sea urchin has been heavily studied in embryology and cell biology. This interest was driven by some key advantages: sea urchins are easily accessible (in the tidal area and within only a few meters depth), external fertilization is effortless to perform *in vitro* and development can be clearly followed experimentally. Fertilization, cleavage and early development were first described on sea urchins, 165 years ago (Derbès 1847; Dufossé 1847; von Baer 1847). The complex life cycle of urchins is now well known: it is composed of six different life-history stages (i.e. sperm/egg, zygote, blastula/gastrula, pluteus larvae, juvenile, adult) and transitions from one stage to another generally require major morphological and ecological changes (see the *Introduction* of **Paper II** for a detailed description). Thanks to more than a century of research on this model, many tools for biologist have been developed. For instance, *Strongylocentrotus purpuratus* was the first marine bilaterian which genome was sequenced and partially annotated (Sodergren *et al.* 2006), highlighting an unexpected proximity to human genome (70% similarity).
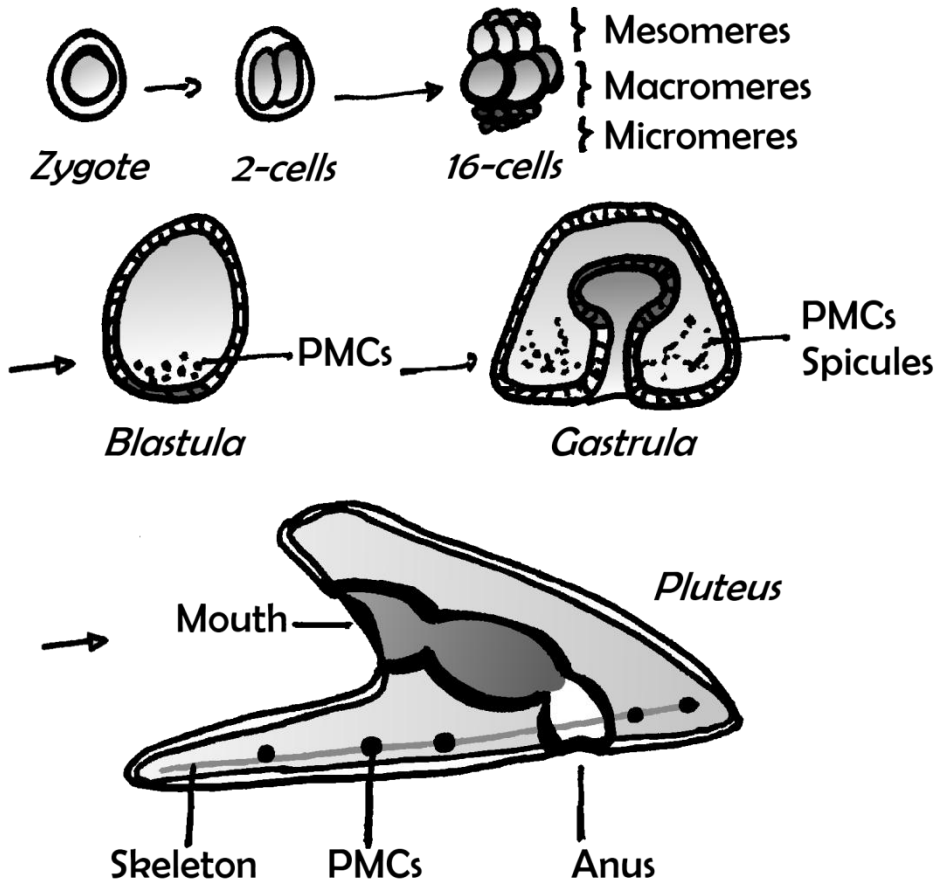
Fig. 4 Typical sea urchin embryogenesis and larval development until early *pluteus* stage (*stages are indicated in italic*). The fertilized egg (*zygote*) undergoes several cell divisions. The fourth unequal cleavage forms three types of cells (mesomeres, macromeres and micromeres). Reaching the 128-cell stage (*blastula*), the cells are arranged in one layer surround a cavity, the blastocoel. The micromeres induce invagination (or gastrulation) and produce primary mesenchyme cells (PMCs), that will later build the spicules/skeleton. The *pluteus* stage develops pairs of arms supported by the skeleton.

Echinoderms – a snapshot of OA research

The number of publications investigating the effect of manipulated pH/pCO$_2$ ("perturbation experiments") on echinoderms has been rising exponentially since 2004 (~20 additional publications per year since January 2011, R^2=0.98). The echinoderm literature represents nearly a fourth of the perturbation experiment literature on invertebrates (N=298, March 2013) and is an example of the knowledge but also of the gaps left in the field of OA. Some of the studies have already been described in reviews of the literature by Dupont *et al.* (2010b; litterature until 2009) and Dupont & Thorndyke (2013; litterature until November 2012). An update of the literature (June 2013) revealed that, out of the now 82 articles published concerning OA perturbation experiments on echinoderms, the most studied parameter is still growth (N=46), mortality/abnormality coming second (N=40). Physiological-related parameters (e.g. cellular processes, metabolism, biochemistry, immunity) were investigated in 31 studies and molecular-related parameters in 12 studies.

Growth: Overall, seawater acidification reduced somatic, calcified and reproductive growth as well as regeneration. Reduced pH generally decreased larval size or/and growth rates in feeding larvae (Kurihara & Shirayama 2004; Kurihara *et al.* 2004; Dupont *et al.* 2008; Clark *et al.* 2009; O'Donnell *et al.* 2009, 2010; Sheppard Brennand *et al.* 2010; Yu *et al.* 2011, 2013; Martin *et al.* 2011; Stumpp *et al.* 2011b; Catarino *et al.* 2011; Sunday *et al.* 2011; Chan *et al.* 2011; Uthicke *et al.* 2012; Doo *et al.* 2012; Gonzalez-Bernat *et al.* 2012, 2013; Matson *et al.* 2012; Byrne *et al.* 2013a, 2013b; Pespeni *et al.* 2013; Kelly *et al.* 2013; Padilla-Gamiño *et al.* 2013; Dorey *et al.* 2013 but see Moulin *et al.* 2011). Such effects have relevant

consequences on the design of data analyzes. For instance, Martin *et al.* (2011) showed that calcium accumulation in urchin larvae was not affected by pH when standardized by developmental speed. This developmental delay/size reduction was mostly true for pluteus larval stages. Echinoderm embryos were unaffected until gastrula stage (Ericson *et al.* 2010; Foo *et al.* 2012; Nguyen *et al.* 2012; Byrne *et al.* 2013b but see significant impacts on cleavage in Ericson *et al.* 2012) similarly to sea star lecitotrophic larvae (Dupont *et al.* 2010a). Juvenile growth response to reduced pH was mixed, two studies on sea stars displayed a positive impact of low pH (Gooding *et al.* 2009; Dupont *et al.* 2010a) while the studies on sea urchins displayed negative impacts (Shirayama & Thornton 2005; Byrne *et al.* 2011; Albright *et al.* 2012; Wolfe *et al.* 2013). Regarding adults, somatic/calcified growth (Stumpp *et al.* 2012b; Appelhans *et al.* 2012; Uthicke *et al.* 2012) as well as brittlestar regeneration (Wood *et al.* 2010, 2011) seemed mostly negatively impacted. However, Wood *et al.* (2008) reported increased regeneration and calcification rates in brittlestars (see also Findlay *et al.* 2011) while Schram *et al.* (2011) did not report any significant effects of lowered pH on neither the growth nor the regeneration of the sea star *Luidia clathrata*. The only two studies on adult urchin calcification on the other side display dissimilar calcification responses to acidification (negative: Courtney *et al.* 2013; parabolic: Ries *et al.* 2009). Gonadal growth has been studied solely in urchins and the response to lowered pH was mostly negative (Siikavuopio *et al.* 2007; Stumpp *et al.* 2012b: exposure time≤56 days; Dupont *et al.* 2012: exposure time=4 months but no difference after 16 months). However, Moulin *et al.* (2011) demonstrated that adults had similar gonadal maturation index regardless of the tide pool they were sampled from (i.e. cyclic decrease by -0.35 or -0.75 pH unit).

Survival: The effect of a seawater acidification on survival is relative to the stage considered. pH had no significant effects on adult sea urchins' survival (Dashfield *et al.* 2008; Dupont *et al.* 2012; Stumpp *et al.* 2012b; Dupont & Thorndyke 2012; Courtney *et al.* 2013) and Miles *et al.* (2007) observed mortality of *Psammechinus miliaris* only for pH as low as 6.6 (8-days exposure) and 6.15 (100% after 7-days exposure). Sea urchin juveniles survival however was either strongly diminished by lowered pH (Shirayama & Thornton 2005; Dupont *et al.* 2012) or unaffected (Albright *et al.* 2012; Wolfe *et al.* 2013), alike juvenile sea star survival (Dupont *et al.* 2010a). Under moderated acidification (pH>7.6), neither cleavage nor development until gastrulation were affected (Byrne *et al.* 2009; Ericson *et al.* 2010, 2012; Nguyen *et al.* 2012), except from one study (reduced cleavage: Foo *et al.* 2012). Under the same conditions, larval survival was not directly affected by pH (echinoplutei: Clark *et al.* 2009; Martin *et al.* 2011; Stumpp *et al.* 2011b; Chan *et al.* 2011; Gonzalez-Bernat *et al.* 2012; sea star larvae: Dupont *et al.* 2010a; Nguyen *et al.* 2012), to three exceptions (sea star larvae: Byrne *et al.* 2013a; Gonzalez-Bernat *et al.* 2013; brittlestar larvae: Dupont *et al.* 2008). Effects on fertilization varied, seven studies displaying no influence of pH (Byrne *et al.* 2009, 2010a, 2010b, 2013a; Ericson *et al.* 2010, 2012; Martin *et al.* 2011; Gonzalez-Bernat *et al.* 2013) while six studies showed that lowered pH decreased fertilization success (Kurihara & Shirayama 2004; Kurihara *et al.* 2004; Carr *et al.* 2006; Havenhand *et al.* 2008; Moulin *et al.* 2011; Reuter *et al.* 2011; Gonzalez-Bernat *et al.* 2012). The responses were however modulated by sperm concentration, combination with temperature and the pH range considered.

Physiology: The physiological processes most investigated in echinoderm literature concerned acid-base regulation (N=10), metabolic rates (respiration, excretion; N=15) and feeding (N=5).

Firstly, low pH induces acidosis of the coelomic fluids of adult echinoderms (urchins: Miles *et al.* 2007; Spicer *et al.* 2011; Spicer & Widdicombe 2012; Catarino *et al.* 2012; sea stars: Appelhans *et al.* 2012). Nevertheless, by accumulating bicarbonate ions in the coelomic fluid, some urchins are able to fully compensate the acidosis (Stumpp *et al.* 2012b; Dupont & Thorndyke 2012). Analyzing responses of 11 species, Collard *et al.* (2013) demonstrated that, while most sea urchins have large acidosis compensation abilities, this was not the case for holoturians nor sea stars. Furthermore, Calosi *et al.* (2013) showed that acidosis compensation abilities of two urchins correlated with the respective species distribution within natural CO_2 gradients. Regarding pluteus larvae, although extracellular fluids are uncompensated, the intracellular fluids of the calcifying PMCs are able to reach full compensation (Stumpp *et al.* 2012a).

Secondly, low pH generally increased metabolism in adult echinoderms (brittlestar respiration: Wood *et al.* 2008, 2010, 2011; urchin respiration: Catarino *et al.* 2012; urchin ammonia excretion: Stumpp *et al.* 2012b), with one exception (McElroy *et al.* 2012: decreased respiration rates of a sea star). However, Christensen *et al.* (2011; brittlestars) and Uthicke *et al.* (2012; urchins) showed that the direction of the respiration response depended on the pH decrease organisms were exposed to. Concerning larval metabolism, the four studies all involved Strongylocentrotus: Kelly *et al.* (2013) did not report any effect of pH on respiration and Padilla-Gamiño *et al.* (2013) reported a metabolic depression at high temperature only. However, larval respiration rates relative to larval size were increased by lowering pH in both Stumpp *et al.* (2011b) and Dorey *et al.* (2013). Place &

Smith (2012) reported no effect of pH on the progression of urchin embryonic developmental program (mitotic and DNA synthesis), suggesting that the negative effects on cleavage could be a consequence of cellular energetics disruption. As for feeding rates, they were either negatively impacted (adults: Siikavuopio *et al.* 2007; Stumpp *et al.* 2012b; Appelhans *et al.* 2012) or not affected (juveniles: Gooding *et al.* 2009; larvae: Stumpp *et al.* 2011b) by a decrease in pH.

Finally, studies on sperm, organisms immunity and behavior are not yet common. Under acidified conditions, sperm motility as well as swimming speed were reduced in all (Havenhand *et al.* 2008; Morita *et al.* 2010; Schlegel *et al.* 2012) but one studies (Caldwell *et al.* 2011). Immune disruption due to reduced pH was demonstrated in the sea star *Asterias rubens* (decreased coelomocyte number and phagocyte capacity: Hernroth *et al.* 2011) as well as in one of the two species studied by Dupont & Thorndyke (2012; increased coelomocyte number and decreased vibratile cell number). Behavioral studies investigated the impact of acidification on sand dollar larval swimming behavior (no effect: Chan *et al.* 2011), brittlestar turn-over time (negative effect when combined with increased temperature: Wood *et al.* 2010) and sea star righting behavior (no effect: Schram *et al.* 2011). Unexpectedly, low pH also induced high-frequency budding in *S. prupuratus* larvae (Chan *et al.* 2012).

Molecular-related processes: Seven studies investigated gene expression patterns on urchin embryos and larvae. Overall, they show down- and/or up-regulation of genes related to biomineralization, development, metabolism, ion regulation/transport, acid base balance, cell signaling/stress response and apoptosis (Todgham & Hofmann 2009; O'Donnell *et al.* 2010; Martin *et al.* 2011; Stumpp *et al.* 2011a; Hammond &

Hofmann 2012; Kurihara *et al.* 2012; Evans *et al.* 2013). Hernroth *et al.* (2011) – the only study of this section not focusing on sea urchins-specifically targeted the expression of heat-shock proteins: mid-term exposure (1 week) of adult sea stars to low pH increased the proteins' expression although this effect disappeared in long-term exposure (6 months). On the contrary, low pH decreased heat-shock protein expression in response to acute (1-hour) thermal stress in urchin larvae (O'Donnell *et al.* 2009). Three studies aimed at quantifying adaptive potential of Strongylocentrotus larvae to OA. While Pespeni *et al.* (2013) used population genomics (changes in allele frequency), Sunday *et al.* (2011) and Kelly *et al.* (2013) investigated heritability and simulated evolutionary response. These three studies demonstrated a large potential for adaptation at relatively fast rates, with selection likely to happen on genes coding for biomineralization, lipid metabolism and ion homeostasis.

Multiple stressors: OA will act with concomitant changes such as warming, deoxygenation, eutrophication, over-fishing or pollution. It is therefore of high relevance to understand how these factors will interact. From the 19 papers that studied the interaction between temperature and OA in echinoderms, it is difficult to bring out clear trends. Five of these studies did not find any significant interactive effects of both factors (Byrne *et al.* 2009, 2013b; Wood *et al.* 2011; Foo *et al.* 2012; Nguyen *et al.* 2012), including two studies which also found no effect of neither temperature nor pH (Byrne *et al.* 2010b, 2010a). Although interactive effects are often complex, seven studies displayed additive effects of both factors (Gooding *et al.* 2009; Wood *et al.* 2011; McElroy *et al.* 2012; Ericson *et al.* 2012; Byrne *et al.* 2013a; Wolfe *et al.* 2013; Padilla-Gamiño *et al.* 2013) while eight displayed antagonistic effects (Gooding *et al.* 2009; Sheppard Brennand *et*

al. 2010; Wood *et al.* 2010; Christensen *et al.* 2011; Byrne *et al.* 2011; Caldwell *et al.* 2011; Catarino *et al.* 2012; Courtney *et al.* 2013). The effects largely dependent on the investigated process. For example, in the juveniles of the sea star *Pisaster ochraceus*, temperature (+3°C) and CO_2 (+400 ppm) acted in synergy on the growth rate but in antagonism on the calcification (Gooding *et al.* 2009). Interestingly, Foo *et al.* (2012) pointed out that the larval genotypes that did well in reduced pH also did well in increased temperature.

Ecology: Finally, acidification may impact ecological interactions and echinoderm-mediated impacts on the environment/community. For instance, in environment inhabited by burrowing echinoderms, pH could modify sediment pH profiles, communities (Dashfield *et al.* 2008) or biogeochemical cycles (Wood *et al.* 2009; Widdicombe *et al.* 2012). Moreover, Asnaghi *et al.* (2013) showed that test robustness and jaw size of the sea urchin *Paracentrotus lividus* relied on the diet (coralline vs. non-coralline algae), highlighting the dependence of pH effects on the survival of other trophic levels.
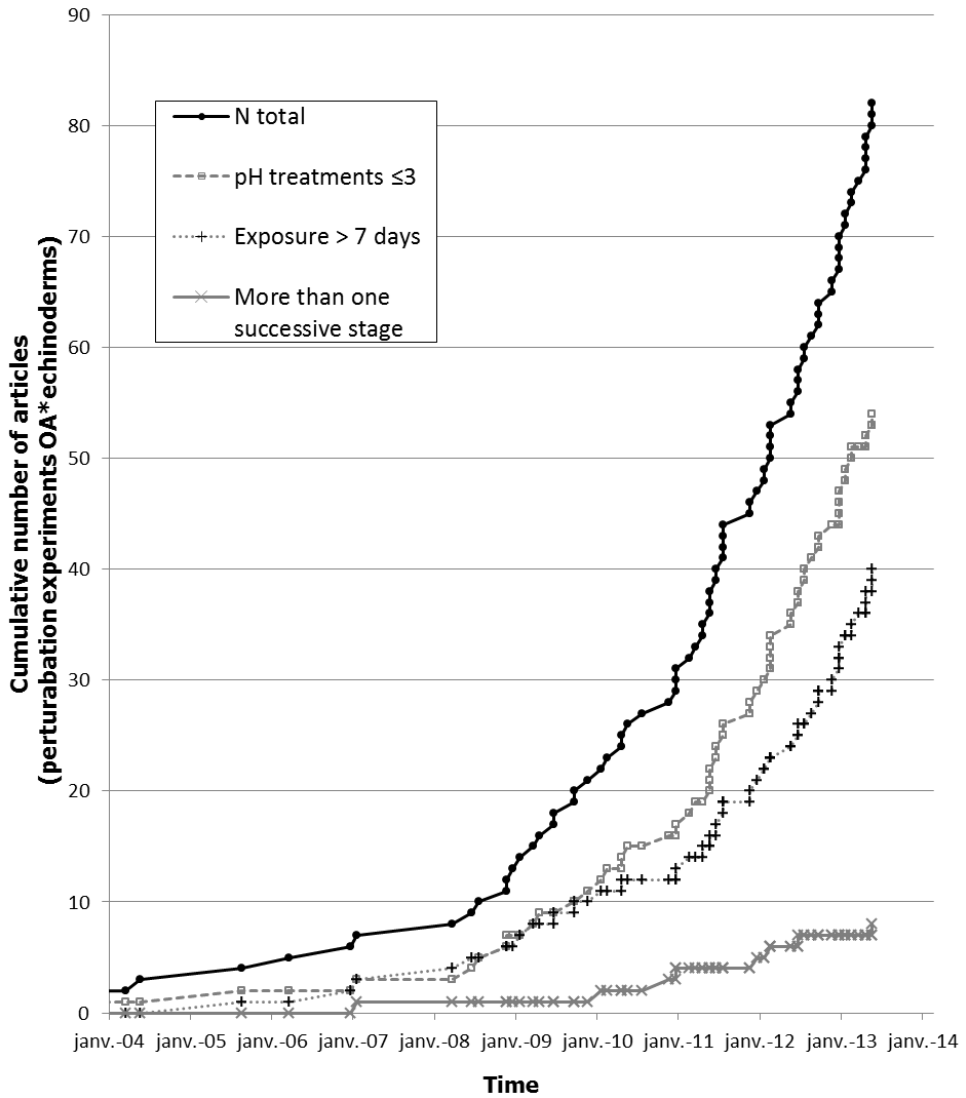
Fig. 5 Cumulative number of articles investigating the effect of manipulated pH/pCO$_2$ ("perturbation experiments") on echinoderms, from January 2004 (only one article relevant published before in 1993) until 17th June 2013 (N=82 articles).

However, the echinoderm literature is also an example of the gaps left in the field of OA:

- Studies are short-term: half of the studies are not longer than seven days (mean ± SD: 28 ± 42 days).

- Studies do not consider environmental variation: One study out of four compares a control pH (mean pH≈8.06) vs. one lower pH level (pH treatments=2) and 66% of the studies are made on two low-pH levels or less and a control (pH treatments ≤3).

- Studies do not consider the whole life-cycle: Pre-larval and larval stages are represented in 66% of the studies and adults in 34%. On the other hand, the juvenile stage, a critical bottleneck to population recruitment (Gosselin & Qian 1997), was studied in only eight of the 82 articles. Only eight studies consider two or more subsequent life-stages. Six studies examine maternal or carry-over effects.

- Studies on multiple stressors effects are scarce: One fourth of the studies were designed to investigate the impact of an interaction between pH and temperature, the only studied stressor.

- 70% of the studies investigated mortality or/and growth (half of those investigated both parameters together). However, some subjects represent major knowledge gaps: 35% of the studies investigated physiological-related parameters. Only 17% of the studies investigated molecular parameters, including evolution abilities. Finally, four articles investigated ecological interactions and echinoderm-mediated impacts on the environment/community (e.g. nutrient fluxes, nematode communities).

- Although responses are species-specific, "<u>pet organisms</u>" are over-represented. These animals, often chosen for their tolerance to laboratory conditions, may not be representative of the biological diversity:

  - 80% of the studies are on the Echinoid Class (sea urchins), one of the five Echinoderm Classes alive today. Crinoids (N=1) and Holoturians (N=2), biologically different from urchins, are nearly absent from this literature;

  - One third (26/82) of the studies have investigated impacts of OA on the sea urchin genus *Strongylocentrotus*. Concerning the genus *Strongylocentrotus*, eight studies have been published so far on the species *S. droebachiensis* (adult stages: Siikavuopio *et al.* 2007, Spicer *et al.* 2011, Stumpp *et al.* 2012b, Dupont & Thorndyke 2012; adults, larval and juvenile stages: Dupont *et al.* 2012; larval stages: Stumpp *et al.* 2012a, Dorey *et al.* 2013; fertilization, subm.: Bögner *et al.* 2013). Fourteen studies concerning other species of the same genus have been published: 14 on *S. purpuratus* (embryonic or larval stages: Todgham & Hofmann 2009, Yu *et al.* 2011, Stumpp *et al.* 2011b, 2011a, Place & Smith 2012, Hammond & Hofmann 2012, Chan *et al.* 2012, Matson *et al.* 2012, Evans *et al.* 2013, Padilla-Gamiño *et al.* 2013, Pespeni *et al.* 2013; larval and juvenile stages: LaVigne *et al.* 2013; sperm: Bay *et al.* 1993) three on *S. franciscanus* (larval stages: O'Donnell *et al.* 2009; Sunday *et al.* 2011; fertilization: Reuter *et al.* 2011) and one on *S. fragilis* (adults: Taylor *et al.* 2013).

From the information available today, it is still difficult to make realistic projections on the future of marine ecosystems or even populations in an acidified world. In order to improve our projection power, **this thesis investigates four under-estimated parameters**:

(i) Response to a shift of the natural pH range (**Paper I**),

(ii) Response from possible bottleneck stages such as the post-metamorphic stage, maternal/carry-over effects, as well as acclimation potential (**Papers II and III**),

(iii) Sub-lethal cascading effects on the overall survival of a population (**Papers I to IV**),

(iv) Mechanistic approach of the physiology allowing generalizations (**Paper I, Paper V**).

In the next section, I will describe why each point represents crucial knowledge and summarize the results of my thesis.

An alternative view on OA – examples from a sea urchin

Environmental pH and larval physiological robustness

pH variation can be broad in coastal environments, a consequence of variable biological activity such as photosynthesis and respiration, themselves depending on combinations of abiotic (e.g. diurnal, seasonal cycles) and biotic (e.g. communities successions) factors. As a result, the pH range experienced by organisms in the field is broader than the average ocean surface pH ($\approx$8.1). For example, in Gullmar Fjord, where Kristineberg marine station is situated, **pH can vary from 8.7 to 7.6** throughout the years (see Fig. 1b in **Paper I**). A shift of this current envelope by 0.4 pH units (projection for 2100), would therefore submit organisms to annual pH variation ranging from 8.3 to 7.2. Nevertheless, experiments investigating the effects of OA on marine organisms have traditionally compared a "control" (pH$\approx$8.1) with a "future scenario" (pH$\approx$7.7), ignoring spatial and temporal variability in pH (e.g. seasonal variations in an upwelling zone: Evans *et al.* 2013; daily variations in tide pools: Moulin *et al.* 2011; differences in habitat and ecoregions: McElhany & Busch 2012). This can lead to inappropriate controls or "future scenarios" that are within today's range of variability. For instance, Thomsen *et al.* (2010) revealed that a third of the pCO$_2$ values measured in the Kiel Fjord (April 2008 to April 2009) were above the projected values for 2100 global oceans (>840 µatm). It is therefore crucial to work on broad ranges of pH in laboratory-based experiments (McElhany & Busch 2012). In **Paper I**, we showed that larval development of the sea urchin is robust to seawater acidification down to

7.0 although, within this range, lowered pH decreased survival and growth rates as well as larval symmetry. For the same body size, larval respiration rates linearly increased with lowering pH (+9% per 0.1 pH units), suggesting disturbances in larval metabolism and energy allocation.

A mechanistic understanding of energy allocation under control and acidified conditions, along with an estimation of the range of plasticity available for this process, will permit to generalize particular biological responses to a range of organisms. An **energy budget** (**Fig. 6**) is a balance sheet of energy income versus expenditure. For heterotrophic organisms, energy is found in food (C: consumption) and spent in various processes such as the synthesis of new tissue blocks (S: somatic and calcified growth, lipidic/reproductive storage) and loss/maintenance (M: respiration and waste production). M is obligatory and varies as a function of the physiological conditions (e.g. physical activity increases respiration).

$$C = M + S$$
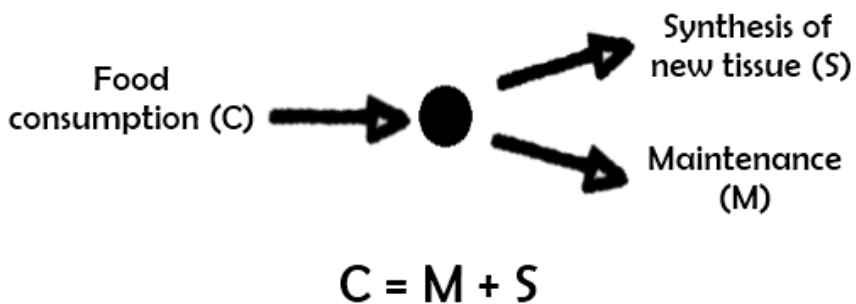
Fig. 6 Schematic energy budget of an animal.

Hypothetically, when exposed to acidification, the costs to run the same basic metabolic activities (M) will be increased and, for a constant energy input (C), **the energy left for the production of new tissue will be diminished** (S=C-M; **Fig. 7**). For example, Stumpp *et al.* (2011b) calculated that the larvae of the sea urchin *S. prupuratus* grown in moderately low pH ($\approx$7.7) invest 40% less energy in somatic growth than in the control pH ($\approx$8.1). Similarly, a significant diminution of the reproductive output was noted for the adults exposed to low pH ($\approx$7.7) for four months (**Paper III**, but not after 16 months). Food consumption as well has its importance in this energy budget: Thomsen *et al.* (2013) showed that the mass of the shell of *Mytilus edulis* juveniles collected on settlement panels was seven fold higher in the eutrophicated part (i.e. high food availability and pH$\approx$7.76) than in the outer part of the Kiel Fjord (Germany; low food availability pH$\approx$7.93). From laboratory experiments, the authors showed that the growth of the mussels was more dependent on the food supply than on the pCO$_2$. While the organisms have a certain tolerance range ("plasticity") in this process, the loss of available energy can create sub-lethal but irreversible damages. Drastically, if the maintenance costs outweigh the energy input, a **physiological "tipping point"** will be reached and the organism will not be able to survive (**Fig. 7**). In this thesis (**Paper I**), I showed that lowering pH decreased *S. droebachiensis* larval growth (S) while linearly increasing respiration (M) until a tipping point (pH$\approx$7.0), below which larvae were not able to develop anymore.
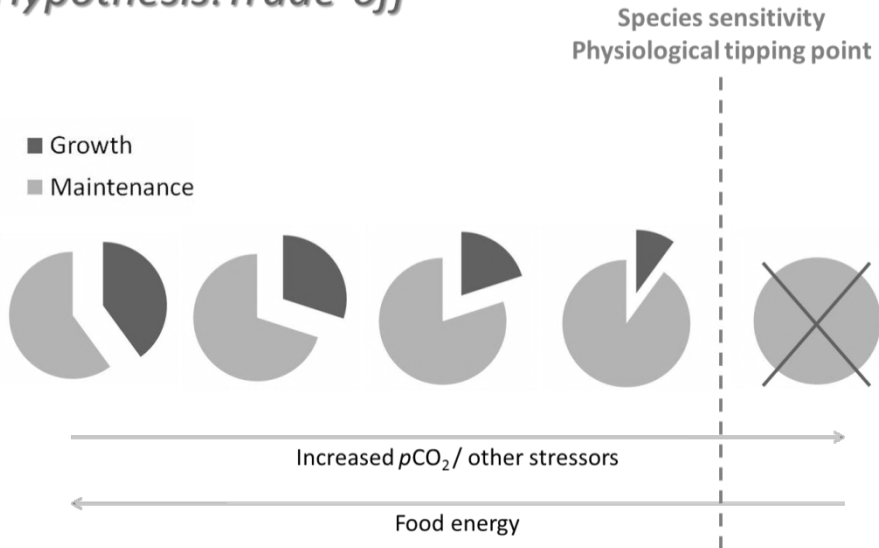
Fig. 7 Trade-off between the energy needed for maintenance (M) and the energy invested in growth/synthesis of new tissues (S). The more stressors (or/and the less food available), the less energy left for growth. When energy inputs are overcome by maintenance costs, the system reaches its tipping point.

In **Paper V**, we propose a model to explain **why the maintenance costs increase in low pH**. Although calcification of urchin larvae does not seem to be negatively impacted by low pH (e.g. Martin *et al.* 2011), maintaining it could be more costly in lowered seawater pH. We showed that the syncytia of cells building the larval skeleton (**PMCs**) is directly exposed to seawater pH. Since the pH/Ω inside the PMCs must be maintained constant to favor carbonate precipitation (see: *Background*), seawater acidification may stimulate membrane pumps in order to regulate the intracellular conditions. Such pumps depend, directly or indirectly, on energy consumption and their excitation could increase the organism's maintenance costs (see Fig. 6 in **Paper V**).

The circle of life

Complex life-cycle and bottlenecks

Most of the benthic marine invertebrate have indirect, also called **complex life-cycles** (Thorson 1950; *e.g*. **Fig. 8**). They are composed of different life stages (*e.g*. larvae, juvenile, adult) that follow each other in time. Each stage is characterized by a different morphology and a different ecology (habitat, food resource, predators). For example, most of the benthic species develop via a planktotrophic larval stage. Heavy morphological reorganization, ontogenetic and ecological shifts are often needed to reach a subsequent stage (*e.g*. larval settlement towards juvenile metamorphosis: see *Introduction* of **Paper II**). Every life stage differs in form and function and the effects of a stressor may depend on the considered stage. It is therefore imperative to investigate the effect of ocean acidification at every of the life-stages.

It is important to investigate where is (are) the **bottleneck stage**(s) in the life-cycle when exposed to acidified waters. It has often been proposed that early life-history stages such as eggs and larvae may indeed be most sensitive to future environmental changes, including OA (e.g. Pörtner & Farrell 2008; Kurihara 2008). This hypothesis was supported by the fact that early development of calcifying marine organisms (*i*) is when the skeleton is primarily built-up (but see **Paper V**); (*ii*) is allegedly physiologically more sensitive to environmental disturbance than the adult stage (but see **Paper I**) and (*iii*) suffers extremely high mortality rates (especially due to predation; *e.g*. Lamare & Barker 1999). For similar reasons, the juvenile stage could as well represent a bottleneck. Firstly,

juvenile biomineralization is a different process from larval spicule formation (Killian & Wilt 2008).
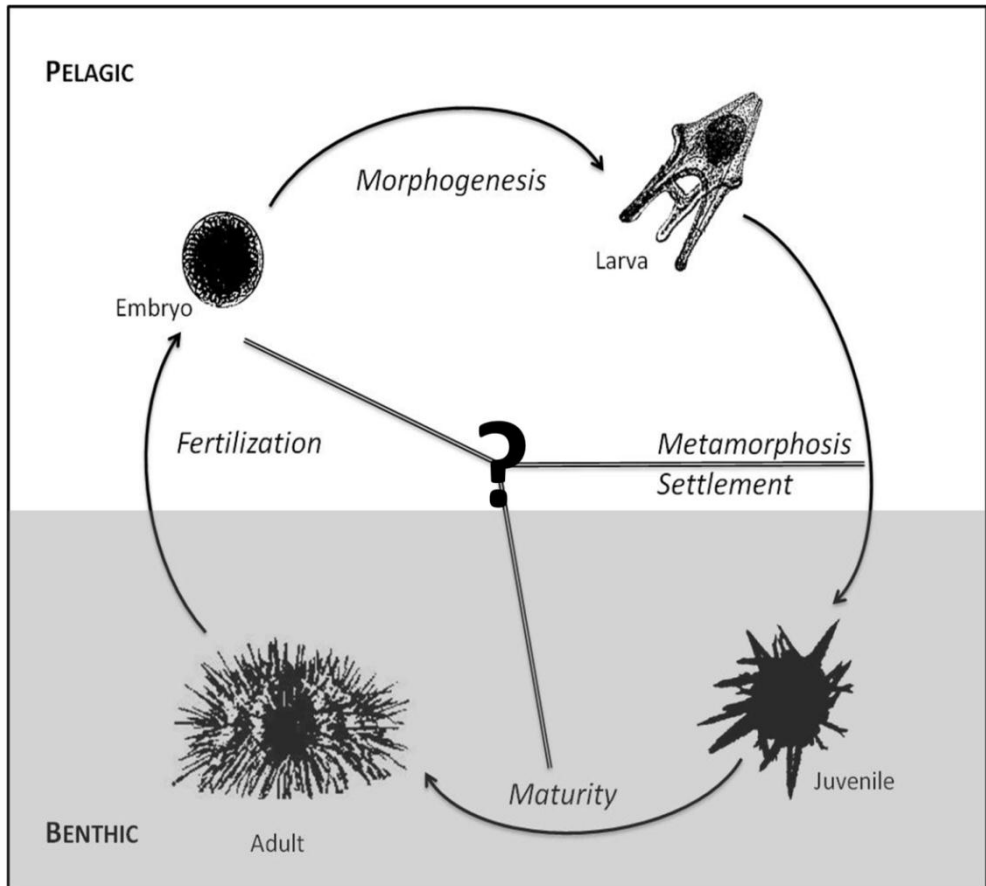
Fig. 8 Schematic life cycle of the sea urchin. Benthic adults live on the substrate and release eggs and sperm in the water, where fertilization occurs. Pelagic embryos develop into echinopluteus larvae (few weeks) that feed in the water column for some more weeks before acquiring the ability to metamorphose (i.e. competence) and transform into a benthic juvenile that will settle on the adequate substrate. The last major ontogeny shift occurs when juveniles sexually mature to become adults.

Secondly, the early post-settlement mortality is high in benthic invertebrates possessing planktonic larval stages (>90%: Gosselin & Qian 1997) and determines the recruitment of individuals into adult populations (Hunt & Scheibling 1997). Studying settling larvae and young juveniles comes with logistical constraints and, as a result, experiments investigating the effect of OA on this transition phase are scarce.

The results from **Paper II**, indicate that settling larvae and juveniles of *S. droebachiensis* are indeed sensitive to acidification. Few individuals that were grown as larvae in pH 7.3 metamorphosed during the course of our experiment (vs. normal development for larvae until pH 7.0: see **Paper I**) and none of the juveniles (<one-month old) exposed to a pH of 7.3 grew spines (vs. 46% at 7.7 and 96% at 8.1). In **Paper III**, juvenile mortality (three-months old) was not visibly affected by the pH, except when both larvae and juvenile were exposed to low pH (7.7: +45-65% mortality compared to the other conditions). It was however not possible to distinguish if the differences in body size were a result of higher individual growth rates or mortality of the smallest individuals, as death by dissolution in acidified conditions may be consequent (see also Byrne *et al.* 2011). Although it is still early to determine bottleneck stages, the meta-analysis presented in **Paper IV** (n=23 articles published by February 2010) encourages to consider stages differential sensitivities: while adult echinoderms appeared resistant to OA (see Fig. 2 in **Paper IV**: effect size>1 for calcification, growth or survival), juveniles, embryos/larvae and gametes seemed negatively impacted (effect size<1).

Carry-over effects

OA experiments have traditionally focused on only one life-history stage. However, the different life-stages uniting the whole life-cycle are a **continuum**: although they can possess various degrees of autonomy, they are linked to each other. The consequences of an environmental change, leading to a disturbance in one stage can "**carry over**" into following stages and be detrimental by altering the performance and selection of subsequent stages (Podolsky & Moran 2006). The most documented carry-over effect is the maternal effect: egg quality and subsequent offspring fitness are dependent on diet quality, life style, temperature or O_2 concentrations experienced by the mothers (review by Bernardo 1996). Carry-over effects are also observed in marine animals from eggs/sperm to larvae (*e.g.* Marshall *et al.* 2002), from embryo to larva or juvenile (*e.g.* Giménez & Anger 2009) as well as from larva to juvenile or adults (reviewed by Pechenik 2006). For instance, experiences during the pelagic period can determine phenotypic traits (*e.g.* larval size) or post-settlement probability and performance, and for competent larvae, delays in metamorphosis can reduce juvenile performance (Emlet & Sadro 2006; Hamilton *et al.* 2008).

Byrne *et al.* (2008) showed that, in the sea urchin *Tripneustes gratilla*, the larval lipid reserves fuel the development of the juvenile for a short post-settlement period. Therefore, in **Paper II**, we hypothesized that the energy limitations larvae experienced in low pH would carry over until the juvenile stage, making the latest more vulnerable to OA. The larval exposure to low pH did however not impact the growth rates of the rudiment (i.e. the embryonic juvenile). Nevertheless, settlement of the competent larvae was speeded-up when they had grown at low pH (albeit delayed larval growth at 7.3; see Fig. 5 in **Paper II**). This "positive effect" of

low pH could be a result of a negative carry-over effect: the "desperate larvae hypothesis" proposed by Marshall & Keough (2003) postulates that a larva in unfavorable water column conditions (e.g. low food concentration) would haste settlement, as an alternative to a risky or costly planktonic life. Another positive carry-over effect was found in **Paper III** where the average size of the three-month old juveniles was significantly higher when both larvae and juveniles had been exposed to the same pH. The origin of this effect may however be a result of size dependent mortality (30-95%) or a real carry-over effect. Nevertheless, studying **two or more subsequent life stages** can lead to substantially different conclusions than the study of one stage in isolation.

Long-term exposure and acclimation potential

Time of exposure to a stressor is a well-known parameter modulating biological responses. Organisms have the ability to **adjust to changes** in their environment (*i.e.* acclimation). For example, the adult sea urchin *S. purpuratus* is extremely plastic and can go through major changes - including a drastic deformation of its morphology (e.g. shape of the skeleton) and modified behavior - in a matter of 8-20 weeks when exposed to a different habitat structure (Hernández & Russell 2010). Available studies on OA have largely ignored this acclimation potential despite the fact that studying long-term effects can result in contrasting conclusions. Out of the 23 papers published by February 2010 on echinoderms, **Paper IV** revealed a greater impact of low pH following long-term exposure (six months, one study) compared to short-term (<two weeks), albeit the bias by the low number of long-term studies. In **Paper III** we show that *S. droebachiensis* females exposed to low pH ($\approx$7.7) for four months produced

ca. five times less eggs than in the control, likely reflecting the increased energy costs needed for survival in a challenging new environment. The gonads of adult sea urchins allow a high degree of plasticity within energy allocation as they can serve as a transient nutrient source (Russell 1998). After 16 months exposure, pH did not influence *S. droebachiensis* females fecundity anymore, suggesting that adults were then fully acclimated to their new environmental conditions. While adults may need more than a few weeks to acclimate, the mean exposure time of adult echinoderms during perturbation experiments is still of 25±36 days (n=28 publications, as reviewed in June 2013).

Indirect ecological consequences

While the different stages of *S. droebachiensis* appear to be globally resistant to the direct effects of OA on survival, indirect effects of low pH disturbances may be highly significant. Changes in processes such as growth, morphometry, settlement or calcification due to elevated pCO_2 have consequences for fitness (i.e. the ability for an individual or a group of individuals to both survive and reproduce in a given environment).

Firstly, changes in growth rates can impact survival (see Fig. 3 in **Paper IV**). Combining the results found in **Paper I** and an oceanographic-biogeochemical model integrating pH natural variability (Artioli *et al.* 2013), Y. Artioli (in prep.) demonstrated that, in a future scenario (-0.4 pH units), mortality before the pluteus stage would have increased by 10-15%, compared to the present-day scenario ($\approx$5%). Larval mortality will as well be indirectly increased by predation under acidified conditions (see Fig. 9 in **Paper I**): the slower the larvae, the longer time spent in the water column, an environment where predation pressure is high (Lamare & Barker 1999).

Allometric alterations caused by reduced pH in plutei morphology may as well negatively affect fitness. Arm length is tightly linked to feeding (Hart 1991) and echinoplutei are known to modify their arm length and stomach volume in order to adapt to externally fluctuating conditions (Strathmann & Grünbaum 2006). For instance, in low food treatments, larvae grow longer arms to increase capture efficiency and smaller stomachs (Miner 2005). In contrast, when food is abundant, larvae benefit from an increase in the surface area of their stomach, and can minimize the energy invested in growing arms. This energetic trade-off (Miner 2005) allows larvae to invest energy in growing the future juvenile urchin. In **Paper I**,

where larvae were well-fed, we observed that declining pH produced a response similar to high-food conditions (small arms and large stomach). Small arms may constrain food capture but, on the other hand, this result could suggests that food capture is more efficient in acidified conditions. Data by M. Stumpp *et al.* (subm.) suggest that large *S. droebachiensis* larvae increase their swallowing frequency and clearance rates when raised in lower pH (7.2 and 7.6 vs. 8.0). This increased food capture might be permitted by the increase of the basal metabolism (e.g. strengthening of the feeding current by increasing beats of the ciliary bands). Besides, arm length and shape are involved into positioning in the water column, stability and swimming (Grünbaum & Strathmann 2003). Nevertheless, Chan *et al.* (2011) found no significant impact of altered morphology due to reduced pH (-0.4 pH unit) on swimming speeds of larvae in the sand dollar *Dendraster excentricus*. More investigation is nevertheless required on the effects of disturbed larval morphology on fitness.

In **Paper II**, we showed that settlement was faster when larvae had been grown at low pH. Although this "desperate larvae" strategy permits to escape unfavorable planktonic conditions, it limits the probability of finding an adequate substrate for juvenile growth and survival. Yet, at metamorphosis, larvae grown at pH 7.7 made more numerous and significantly bigger juveniles than in the control. However, during the following days, pH highly influenced the presence of spines: when nearly all the juveniles in pH 8.1 had spines, only half did in pH 7.7 . $CaCO_3$ structures in sea urchin juveniles have vital functions such as feeding and protection against the strong predators/bulldozing pressure (Scheibling & Robinson 2008). A lack of spines at low pH (see also Byrne *et al.* 2011 and Wolfe *et al.* 2013) could therefore have deadly consequences on the small early post-settlement survival.

Conclusions

In conclusion, *S. droebachiensis* appears to be quite robust to ocean acidification and the most sensitive stage may not be the larval stage but the juvenile stage. The green sea urchin is not at risk of disappearing due to direct effects of OA in a close future, but I have demonstrated that even subtle changes in organisms energy budget and sub-lethal effects on growth and fitness can affect the survival of populations (**Fig. 9**). Several studies highlighted the importance to examine as well organisms behavior and interactions (e.g. chemo-reception: Bibby *et al.* 2007; Munday *et al.* 2009; de la Haye *et al.* 2012; ecological interactions: Widdicombe *et al.* 2009, 2012; Asnaghi *et al.* 2013). For instance, I. Casties (in prep.) demonstrated that adult *S. droebachiensis* escape response to the crab *Cancer pagurus* was modified by low pH, possibly due to both disturbances of the crab cue emission and the urchins cue perception. In projections, we should not underestimate the power of synergistic/antagonistic effects of concomitant anthropogenic-driven changes such as desalination, hypoxia, pollution warming or over-fishing, just to name a few.

Selection and adaptation

Nevertheless, we cannot exclude the possibility that some species will be able to adapt to future environmental changes. Global changes are going to be driving selective factors, forcing population to select for only the fittest individuals in the new environment. OA will affect organisms in different ways, depending on the evolutionary rate at which the considered population can respond. Adaptation potential, built from the history of population, is still largely unknown and therefore neglected. A study by

Sunday *et al.* (2011) demonstrated that the sea urchin *S. franciscanus* may have a better adaptation potential than the mussel *Mytilus trossulus* in future high CO_2 conditions, thanks to a higher phenotypic and genetic variation for larval size. From a transcriptome-wide gene expression study on *S. droebachiensis* larvae, D. Runcie *et al.* (in prep.) drew similar conclusions: sea urchin display ample genetic variation in many molecular traits (e.g. metabolism, cell-cell interaction). However, this variation was not significantly visible in some other traits (e.g. cell-cycle, DNA replication), traits that may constrain organism's adaptation abilities. Studying and comparing population- and species-specific **phenotypic and genetic variability** to investigate adaptation potentials will be an insightful approach in order to better project the effects of OA on ecosystems persistence.

Fig. 9 (next page) Graphical summary of the thesis: Effects of OA on the life-cycle of *S. droebachiensis* - From the release of gametes to the maturation of a new adult. The papers to which the mortality/survival data (black, green or orange) and the results/concepts (blue) refer to are indicated by roman numbers (I-V).

3. Larval mortality

(I) pH effect vs. size

Number of larvae

Larval body length (µm)

— 8.1
— 7.7
— 7.3

2. Fertilization

No pH effect

1. Fecundity

(III) No pH effect
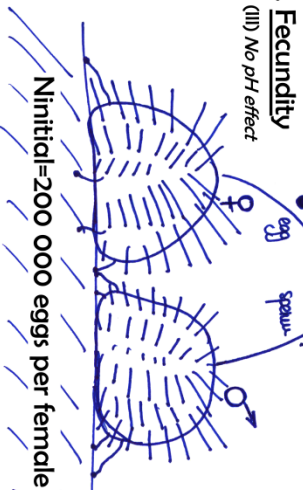
Ninitial=200 000 eggs per female

♀ egg

♂ sperm

Growth delay (I)

Larval body length (µm)

500

33
49
79

Time (d)

3bis. Additional predation due to planktonic predation

(I, IV) **+ 10% mortality per day**

Increased larval metabolic rates (I, V)
due to additional costs of acid-base
regulation inside the PMCs (V)

Competence (~500µm)

pediveliger period

Desperate larvae
hypothesis?

4. Competent larva to juvenile

*(II) Complex pH effects on mortality
and settlement rates*

8.1: 4%
7.7: 33% metamorphosed
7.3: 0% in 14 days

5. Juvenile mortality

(III) pH effect after 3 months

8.1: 50% mortality
7.7: 95% mortality

2 - 3 years

Nfinal?
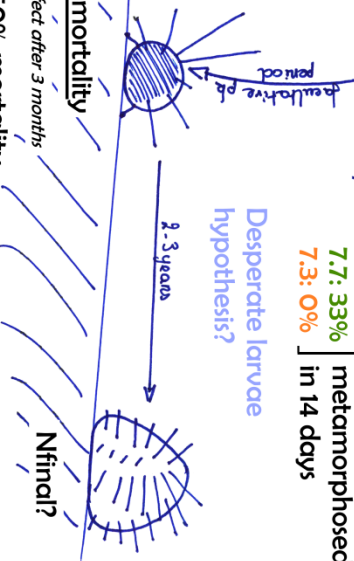
References

Addadi L, Joester D, Nudelman F, Weiner S (2006) Mollusk shell formation: a source of new concepts for understanding biomineralization processes. *Chemistry (Weinheim an der Bergstrasse, Germany)*, **12**, 980–7.

Albright R, Bland C, Gillette P, Serafy JE, Langdon C, Capo TR (2012) Juvenile growth of the tropical sea urchin *Lytechinus variegatus* exposed to near-future ocean acidification scenarios. *Journal of Experimental Marine Biology and Ecology*, **426-427**, 12–17.

Appelhans YS, Thomsen J, Pansch C, Melzner F, Wahl M (2012) Sour times: seawater acidification effects on growth, feeding behaviour and acid–base status of *Asterias rubens* and *Carcinus maenas*. *Marine Ecology Progress Series*, **459**, 85–98.

Artioli Y, Blackford JC, Nondal G, *et al.* (2013) Heterogeneity of impacts of high CO_2 on the North Western European Shelf. *Biogeosciences Discussions*, **10**, 9389–9413.

Asnaghi V, Chiantore M, Mangialajo L, Gazeau F, Francour P, Alliouane S, Gattuso J-P (2013) Cascading effects of ocean acidification in a rocky subtidal community. *PloS one*, **8**, e61978.

Auerbach DI, Caulfield JA, Adams EE, Herzog HJ (1997) Impacts of ocean CO_2 disposal on marine life : I . A toxicological assessment integrating constant-concentration laboratory assay data with variable-concentration field exposure. *Environmental Modeling and Assessment, 2*, 333–343.

von Baer KE (1847) Auszug aus einem Berichte des Akademikers v Baer, aus Triest vom I. (13) November 1845. *Bulletin de la Classe Physico-Mathématique de l'Académie Impériale des Sciences de St. Pétersbourg*, **5**, 231–239.

Bay S, Burgess R, Nacci D (1993) Status and applications of echinoid (Phylum Echinodermata) toxicity test methods. In: *Environmental Toxicology and Risk Assessment* (eds: Landis, G. W, Hughes JS, Lewis MA), pp281–302. Philadelphia,, American Society for Testing and Materials.

Bernardo J (1996) Maternal effects in animal ecology. *Integrative and Comparative Biology*, **36**, 83–105.

Bibby R, Cleall-Harding P, Rundle SD, Widdicombe S, Spicer JI (2007) Ocean acidification disrupts induced defences in the intertidal gastropod Littorina littorea. *Biology letters*, **3**, 699–701.

Bögner D, Bickmeyer U, Köhler a. (2013) Fertilization success of an arctic sea urchin species, *Strongylocentrotus droebachiensis* (O. F. Müller, 1776) under CO_2-induced ocean acidification. *Biogeosciences Discussions*, **10**, 8027–8064.

Brewer PG (1997) Ocean chemistry of the fossil fuel CO_2 signal: The haline signal of "business as usual". *Geophysical Research Letters*, **24**, 1367–1369.

Byrne M, Prowse T, Sewell MA, Dworjanyn SA, Williamson JE, Vaïtilingon D (2008) Maternal provisioning for larvae and larval provisioning for juveniles in the

toxopneustid sea urchin *Tripneustes gratilla*. *Marine Biology*, **155**, 473–482.

Byrne M, Ho MA, Selvakumaraswamy P, Nguyen HD, Dworjanyn SA, Davis AR (2009) Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. *Proceedings of the Royal Society B: Biological sciences*, **276**, 1883–8.

Byrne M, Soars NA, Ho MA, *et al.* (2010a) Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. *Marine Biology*, **157**, 2061–69.

Byrne M, Soars NA, Selvakumaraswamy P, Dworjanyn SA, Davis AR (2010b) Sea urchin fertilization in a warm, acidified and high pCO_2 ocean across a range of sperm densities. *Marine environmental research*, **69**, 234–9.

Byrne M, Ho MA, Wong E, *et al.* (2011) Unshelled abalone and corrupted urchins: development of marine calcifiers in a changing ocean. *Proceedings of the Royal Society B: Biological sciences*, **278**, 2376–83.

Byrne M, Gonzalez-Bernat MJ, Doo SS, Foo SA, Soars NA, Lamare M (2013a) Effects of ocean warming and acidification on embryos and non-calcifying larvae of the invasive sea star *Patiriella regularis*. *Marine Ecology Progress Series*, **473**, 235–246.

Byrne M, Ho M a, Koleits L, *et al.* (2013b) Vulnerability of the calcifying larval stage of the Antarctic sea urchin *Sterechinus neumayeri* to near-future ocean

acidification and warming. *Global change biology*, **19**, 2264–75.

Caldeira K, Wickett ME (2003) Oceanography: anthropogenic carbon and ocean pH. *Nature*, **425**, 365.

Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *Journal of Geophysical Research*, **110**, C09S04.

Caldwell GS, Fitzer SC, Gillespie CS, Pickavance G, Turnbull E, Bentley MG (2011) Ocean acidification takes sperm back in time. *Invertebrate Reproduction & Development*, **55**, 217–221.

Calosi P, Rastrick SPS, Graziano M, *et al.* (2013) Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid-base and ion-regulatory abilities. *Marine pollution bulletin*, doi: 10.1016/j.marpolbul.2012.11.040.

Carr RS, Biedenbach JM, Nipper M (2006) Influence of potentially confounding factors on sea urchin porewater toxicity tests. *Archives of environmental contamination and toxicology*, **51**, 573–9.

Catarino AI, Ridder C, Gonzalez M, Gallardo P, Dubois P (2011) Sea urchin *Arbacia dufresnei* (Blainville 1825) larvae response to ocean acidification. *Polar Biology*, **35**, 455-461.

Catarino AI, Bauwens M, Dubois P (2012) Acid-base balance and metabolic response of the sea urchin *Paracentrotus lividus* to different seawater pH and temperatures. *Environmental science and pollution research international*, **19**, 2344–53.

Chan KYK, Grünbaum D, O'Donnell MJ (2011) Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. *The Journal of experimental biology*, **214**, 3857–67.

Chan KYK, Grünbaum D, Arnberg M, Thorndyke MC, Dupont S (2012) Ocean acidification induces budding in larval sea urchins. *Marine Biology*, **160**, 2129-35.

Christensen AB, Nguyen HD, Byrne M (2011) Thermotolerance and the effects of hypercapnia on the metabolic rate of the ophiuroid *Ophionereis schayeri*: Inferences for survivorship in a changing ocean. *Journal of Experimental Marine Biology and Ecology*, **403**, 31–38.

Clark D, Lamare M, Barker M (2009) Response of sea urchin pluteus larvae (Echinodermata: Echinoidea) to reduced seawater pH: a comparison among a tropical, temperate, and a polar species. *Marine Biology*, **156**, 1125–1137.

Collard M, Laitat K, Moulin L, Catarino AI, Grosjean P, Dubois P (2013) Buffer capacity of the coelomic fluid in echinoderms. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, **166**, 199-206.

Courtney T, Westfield I, Ries JB (2013) CO_2-induced ocean acidification impairs calcification in the tropical urchin *Echinometra viridis*. *Journal of Experimental Marine Biology and Ecology*, **440**, 169–175.

Darwin C (1859) Chapter III: Struggle for existence. In: *On the origin of species* - London: John Murray.

Dashfield SL, Somerfield P, Widdicombe S, Austen M, Nimmo M (2008) Impacts of ocean acidification and burrowing urchins on within-sediment pH profiles and subtidal nematode communities. *Journal of Experimental Marine Biology and Ecology*, **365**, 46–52.

Dayton PK (1975) Experimental evaluation of ecological dominance in a rocky intertidal algal community. *Ecological Monographs*, **45**, 137–159.

Derbès A (1847) Observations sur le mécanisme et les phénomènes qui accompagnent la formation de l'embryon chez l'oursin comestible. *Annales de Sciences Naturelles. Serie 3: Zoologie et Biologie animale*, **8**, 80–98.

Doney SC, Fabry VJ, Feely RA, Kleypas JA (2009) Ocean acidification: The other CO_2 problem. *Annual Review of Marine Science*, **1**, 169–192.

Doo SS, Dworjanyn SA, Foo SA, Soars NA, Byrne M (2012) Impacts of ocean acidification on development of the meroplanktonic larval stage of the sea urchin *Centrostephanus rodgersii*. *ICES Journal of Marine Science*, **69**, 460–464.

Dorey N, Melzner F, Martin S, Oberhänsli F, Teyssié J-L, Bustamante P, Gattuso J-P & Lacoue-Labarthe T (2012) Ocean acidification and temperature rise: effects on calcification during early development of the cuttlefish *Sepia officinalis*. *Marine Biology*, **160**, 2007-22.

Dorey N, Lancon P, Thorndyke MC, Dupont S (2013) Assessing physiological tipping point of sea urchin larvae exposed to a broad range of pH. *Global Change Biology*, doi: 10.1111/gcb.12276.

Dufossé (1847) Observations sur le développement des oursins. *Annales de*

Sciences Naturelles. Serie 3: Zoologie et Biologie animale, **7**, 44–52.

Dupont S, Havenhand JN, Thorndyke W, Peck L, Thorndyke MC (2008) Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. *Marine Ecology Progress Series*, **373**, 285–294.

Dupont S, Lundve B, Thorndyke MC (2010a) Near future ocean acidification increases growth rate of the lecithotrophic larvae and juveniles of the sea star *Crossaster papposus*. *Journal of experimental zoology Part B Molecular and developmental evolution*, **314**, 382–389.

Dupont S, Ortega-Martínez O, Thorndyke MC (2010b) Impact of near-future ocean acidification on echinoderms. *Ecotoxicology*, **19**, 449–62.

Dupont S, Dorey N, Stumpp M, Melzner F, Thorndyke MC (2012) Long-term and trans-life-cycle effects of exposure to ocean acidification in the green sea urchin *Strongylocentrotus droebachiensis*. *Marine Biology*, **160**, 1835-43.

Dupont S, Thorndyke MC (2012) Relationship between CO_2-driven changes in extracellular acid–base balance and cellular immune response in two polar echinoderm species. *Journal of Experimental Marine Biology and Ecology*, **424-425**, 32–37.

Dupont S, Thorndyke M (2013) Direct impacts of near-future ocean acidification on sea urchins. In: *Climate change perspectives from the Atlantic: past, present and future* (eds Fernández-Palacios JM, de Nascimiento L, Hernández JC, Clemente

S, González A, Díaz-González JP) Servicio de Publicaciones de La Universidad de La Laguna, La Laguna, Spain. *In press*.

Emlet RB, Sadro SS (2006) Linking stages of life history: How larval quality translates into juvenile performance for an intertidal barnacle (*Balanus glandula*). *Integrative and comparative biology*, **46**, 334–46.

Ericson J, Lamare M, Morley S, Barker M (2010) The response of two ecologically important Antarctic invertebrates (*Sterechinus neumayeri* and *Parborlasia corrugatus*) to reduced seawater pH: effects on fertilisation and embryonic development. *Marine Biology*, **157**, 2689–2702.

Ericson J, Ho M a, Miskelly A, King CK, Virtue P, Tilbrook B, Byrne M (2012) Combined effects of two ocean change stressors, warming and acidification, on fertilization and early development of the Antarctic echinoid *Sterechinus neumayeri*. *Polar Biology*, **35**, 1027–1034.

Etheridge D, Steele L, Langenfelds R, Francey R, Barnola J-M, Morgan V (1996) Natural and anthropogenic changes in atmospheric CO_2 over the last 1000 years from air in Antarctic ice and firn. *Journal of Geophysical Research*, **101**, 4115–4128.

Evans TG, Chan F, Menge BA, Hofmann GE (2013) Transcriptomic responses to ocean acidification in larval sea urchins from a naturally variable pH environment. *Molecular ecology*, **22**, 1609–1625.

FAO (2010) *FAO Yearbook 2008 - Fishery and Aquaculture Statistics*. FAO FIPS, Roma, Italy.

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas JA, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. *Science*, **305**, 362–6.

Findlay HS, Wood HL, Kendall MA, Spicer JI, Twitchett RJ, Widdicombe S (2011) Comparing the impact of high CO_2 on calcium carbonate structures in different marine organisms. *Marine Biology Research*, **7**, 565–575.

Foo SA, Dworjanyn SA, Poore AGB, Byrne M (2012) Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: performance of early embryos. *PloS one*, **7**, e42497.

Gagnon P, Himmelman JH, Johnson LE (2004) Temporal variation in community interfaces: kelp-bed boundary dynamics adjacent to persistent urchin barrens. *Marine Biology*, **144**, 1191–1203.

Giménez L, Anger K (2009) Larval performance in an estuarine crab, *Chasmagnathus granulata*, is a consequence of both larval and embryonic experience. *Marine Ecology Progress Series*, **249**, 251–264.

Gonzalez-Bernat MJ, Lamare M, Uthicke S, Byrne M (2012) Fertilisation, embryogenesis and larval development in the tropical intertidal sand dollar Arachnoides placenta in response to reduced seawater pH. *Marine Biology*, **160**, 1927-41.

Gonzalez-Bernat MJ, Lamare M, Barker M (2013) Effects of reduced seawater pH on fertilisation, embryogenesis and larval development in the Antarctic seastar *Odontaster validus*. *Polar Biology*, **36**, 235–247.

Gooding RA, Harley CDG, Tang E (2009) Elevated water temperature and carbon dioxide concentration increase the growth of a keystone echinoderm. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 9316–9321.

Gosselin LA, Qian P-Y (1997) Juvenile mortality in benthic marine invertebrates. *Marine Ecology Progress Series*, **146**, 265–282.

Grünbaum D, Strathmann RR (2003) Form, performance and trade-offs in swimming and stability of armed larvae. *Journal of Marine Research*, **63**, 659–691.

Hamilton SL, Regetz J, Warner RR (2008) Postsettlement survival linked to larval life in a marine fish. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 1561–6.

Hammond LM, Hofmann GE (2012) Early developmental gene regulation in *Strongylocentrotus purpuratus* embryos in response to elevated CO_2 seawater conditions. *The Journal of experimental biology*, **215**, 2445–54.

Hart MW (1991) Particle captures and the method of suspension feeding by echinoderm larvae. *Biological Bulletin*, **180**, 12–27.

Haugan P, Drange H (1996) Effects of CO_2 on the ocean environment. *Energy Conversion and Management*, **37**, 1019–22.

Havenhand JN, Buttler FR, Thorndyke MC, Williamson JE (2008) Near-future levels of ocean acidification reduce fertilization success in a sea urchin. *Current Biology*, **18**, 651–652.

Hernández JC, Russell MP (2010) Substratum cavities affect growth-plasticity, allometry, movement and feeding rates in the sea urchin *Strongylocentrotus purpuratus*. *The Journal of experimental biology*, **213**, 520–5.

Hernroth B, Baden S, Thorndyke MC, Dupont S (2011) Immune suppression of the echinoderm *Asterias rubens* (L.) following long-term ocean acidification. *Aquatic toxicology*, **103**, 222–4.

Hönisch B, Ridgwell A, Schmidt DN, *et al.* (2012) The geological record of ocean acidification. *Science*, **335**, 1058–63.

Hunt H, Scheibling RE (1997) Role of early post-settlement mortality in recruitment of benthic marine invertebrates. *Marine Ecology Progress Series*, **155**, 269–301.

IPCC (2007) *Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change: "The Physical Science Basis"* (eds Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL). Cambridge University Press, Cambridge, UK.

Kelly MW, Padilla-Gamiño JL, Hofmann GE (2013) Natural variation, and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Global change biology*, **19**, 2536-46.

Killian CE, Wilt FH (2008) Molecular aspects of biomineralization of the echinoderm endoskeleton. *Chemical reviews*, **108**, 4463–74.

Kurihara H, Shimode S, Shirayama Y (2004) Sub-lethal effects of elevated concentration of CO_2 on planktonic copepods and sea urchins. *Journal of Oceanography*, **60**, 743–750.

Kurihara H, Shirayama Y (2004) Effects of increased atmospheric CO_2 on sea urchin early development. *Marine Ecology Progress Series*, **274**, 161–169.

Kurihara H (2008) Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. *Marine Ecology Progress Series*, **373**, 275–284.

Kurihara H, Takano Y, Kurokawa D, Akasaka K (2012) Ocean acidification reduces biomineralization-related gene expression in the sea urchin, *Hemicentrotus pulcherrimus*. *Marine Biology*, **159**, 2819–2826.

De la Haye KL, Spicer JI, Widdicombe S, Briffa M (2012) Reduced pH sea water disrupts chemo-responsive behaviour in an intertidal crustacean. *Journal of Experimental Marine Biology and Ecology*, **412**, 134–140.

Lamare M, Barker M (1999) In situ estimates of larval development and mortality in the New Zealand sea urchin *Evechinus chloroticus* (Echinodermata: Echinoidea). *Marine Ecology Progress Series*, **180**, 197-211.

LaVigne M, Hill TM, Sanford E, *et al.* (2013) The elemental composition of purple sea urchin (*Strongylocentrotus purpuratus*) calcite and potential effects of $p$$CO_2$ during early life stages. *Biogeosciences*, **10**, 3465–3477.

Levitus S, Antonov J, Boyer T (2005) Warming of the world ocean, 1955–2003. *Geophysical Research Letters*, **32**, L02604.

Mann KH (1977) Destruction of kelp-beds by sea-urchins: A cyclical phenomenon or irreversible degradation? *Helgoländer Wissenschaftliche Meeresuntersuchungen*, **30**, 455–467.

Marshall DJ, Styan A, Keough MJ (2002) Sperm environment affects offspring quality in broadcast spawning marine invertebrates. *Ecology Letters*, **5**, 173–176.

Marshall DJ, Keough MJ (2003) Variation in the dispersal potential of non-feeding invertebrate larvae : the desperate larva hypothesis and larval size. *Marine Ecology Progress Series*, **255**, 145–153.

Martin S, Richier S, Pedrotti M-L, *et al.* (2011) Early development and molecular plasticity in the Mediterranean sea urchin *Paracentrotus lividus* exposed to CO_2-driven acidification. *The Journal of experimental biology*, **214**, 1357–68.

Matranga V, Bonaventura R, Costa C, Pinsino A, Russo R, Zito F (2011) Chapter 8: Echinoderms as blueprints for biocalcification: regulation of skeletogenic genes and matrices. In: *Molecular Biomineralization, Progress in Molecular and Subcellular Biology* (eds: Müller WEG), pp225–248. Berlin, Heidelberg, Springer Berlin Heidelberg, Germany.

Matson PG, Yu PC, Sewell MA, Hofmann GE (2012) Development under elevated pCO$_2$ conditions does not affect lipid utilization and protein content in early life-history stages of the purple sea urchin, *Strongylocentrotus purpuratus*. *The Biological bulletin*, **223**, 312–27.

McDougall I, Brown F, Fleagle J (2005) Stratigraphic placement and age of modern humans from Kibish, Ethiopia. *Nature*, **433**, 733–736.

McElhany P, Busch SD (2012) Appropriate pCO$_2$ treatments in ocean acidification experiments. *Marine Biology*, **160**, 1807-1812.

McElroy DJ, Nguyen HD, Byrne M (2012) Respiratory response of the intertidal seastar *Parvulastra exigua* to contemporary and near-future pulses of warming and hypercapnia. *Journal of Experimental Marine Biology and Ecology*, **416-417**, 1–7.

Miles H, Widdicombe S, Spicer JI, Hall-Spencer JM (2007) Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin *Psammechinus miliaris*. *Marine pollution bulletin*, **54**, 89–96.

Miner BG (2005) Evolution of feeding structure plasticity in marine invertebrate larvae: a possible trade-off between arm length and stomach size. *Journal of Experimental Marine Biology and Ecology*, **315**, 117–125.

Morita M, Suwa R, Iguchi A, Nakamura M, Shimada K, Sakai K, Suzuki A (2010) Ocean acidification reduces sperm flagellar motility in broadcast spawning reef invertebrates. *Zygote*, **18**, 1–5.

Moulin L, Catarino AI, 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.

Müller F (1776) *Zoologiae Danicae prodromus, seu Animalium Daniae et Norvegiae Indigenarum characteres, nomina, et*

synonyma imprimis popularium. typis Hallageriis.

Munday PL, Dixson DL, Donelson JM, Jones GP, Pratchett MS, Devitsina G V, Døving KB (2009) Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 1848–52.

Nguyen HD, Doo SS, Soars NA, Byrne M (2012) Noncalcifying larvae in a changing ocean: warming, not acidification/hypercapnia, is the dominant stressor on development of the sea star Meridiastra calcar. *Global Change Biology*, **18**, 2466–76.

Norderhaug KM, Christie HC (2009) Sea urchin grazing and kelp re-vegetation in the NE Atlantic. *Marine Biology Research*, **5**, 515–528.

O'Donnell MJM, Hammond LM, Hofmann GE (2009) Predicted impact of ocean acidification on a marine invertebrate: elevated CO_2 alters response to thermal stress in sea urchin larvae. *Marine Biology*, **156**, 439–446.

O'Donnell MJ, Todgham AE, Sewell MA, *et al.* (2010) Ocean acidification alters skeletogenesis and gene expression in larval sea urchins. *Marine Ecology Progress Series*, **398**, 157–171.

Orr J, Fabry VJ, Aumont O, *et al.* (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature*, **437**, 681–686.

Padilla-Gamiño JL, Kelly MW, Evans TG, Hofmann GE (2013a) Temperature and CO_2 additively regulate physiology, morphology and genomic responses of

larval sea urchins, *Strongylocentrotus purpuratus*. *Proceedings of the Royal Society B: Biological sciences*, **280**, 20130155.

Pechenik JA (2006) Larval experience and latent effects-metamorphosis is not a new beginning. *Integrative and Comparative Biology*, **46**, 323–333.

Pespeni MH, Sanford E, Gaylord B, *et al.* (2013) Evolutionary change during experimental ocean acidification. *Proceedings of the National Academy of Sciences of the United States of America*, **110**, 6937–42.

Place SP, Smith BW (2012) Effects of seawater acidification on cell cycle control mechanisms in *Strongylocentrotus purpuratus* embryos. *PloS one*, **7**, e34068.

Podolsky RD, Moran AL (2006) Integrating function across marine life cycles. *Integrative and Comparative Biology*, **46**, 577–586.

Pörtner H-O, Farrell AP (2008) Physiology and Climate Change. *Science*, **322**, 690–692.

Raven JA, Caldeira K, Elderfields H, *et al.* (2005) *Ocean acidification due to increasing atmospheric carbon dioxide. Policy Document 12/05.* Royal Society, London.

Raymond EG, Scheibling RE (1987) Recruitment and growth of the sea urchin *Strongylocentrotus droebachiensis* (Muller) following mass mortalities off Nova Scotia, Canada. *Journal of Experimental Marine Biology and Ecology*, **108**, 31–54.

Reuter KE, Lotterhos KE, Crim RN, Thompson CA, Harley CDG (2011) Elevated *p*CO_2 increases sperm limitation and risk of

polyspermy in the red sea urchin *Strongylocentrotus franciscanus*. *Global Change Biology*, **17**, 163–171.

Ries JB, Cohen AL, McCorkle DC (2009) Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. *Geology*, **37**, 1131–34.

Russell MP (1998) Resource allocation plasticity in sea urchins: rapid, diet induced, phenotypic changes in the green sea urchin, *Strongylocentrotus droebachiensis* (Müller). *Journal of Experimental Marine Biology and Ecology*, **220**, 1–14.

Sabine CL, Feely RA, Gruber N, *et al.* (2004) The oceanic sink for anthropogenic CO_2. *Science*, **305**, 367–71.

Scheibling RE, Hatcher BG (2007) Chapter 18: Ecology of *Strongylocentrotus droebachiensis*. In: *Edible Sea Urchins: Biology and Ecology* pp353–392.

Scheibling RE, Robinson MC (2008) Settlement behaviour and early post-settlement predation of the sea urchin *Strongylocentrotus droebachiensis*. *Journal of Experimental Marine Biology and Ecology*, **365**, 59–66.

Schlegel P, Havenhand JN, Gillings MR, Williamson JE (2012) Individual variability in reproductive success determines winners and losers under ocean acidification: a case study with sea urchins. *PloS one*, **7**, e53118.

Schram JB, McClintock JB, Angus RA, Lawrence JM (2011) Regenerative capacity and biochemical composition of the sea star *Luidia clathrata* (Say) (Echinodermata: Asteroidea) under conditions of near-future ocean acidification. *Journal of*

Experimental Marine Biology and Ecology, **407**, 266–274.

Scripps CO_2 Program : SC, Keeling CD, Keeling RF http://scrippsco2.ucsd.edu/home/index.php.

Sheppard Brennand H, Soars NA, Dworjanyn SA, Davis AR, Byrne M (2010) Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. *PloS ONE*, **5**, e11372.

Shirayama Y, Thornton H (2005) Effect of increased atmospheric CO_2 on shallow water marine benthos. *Journal of Geophysical Research*, **110**, C09S08.

Siikavuopio SI, Mortensen A, Foss A (2007) Effects of carbon dioxide exposure on feed intake and gonad growth in green sea urchin, *Strongylocentrotus droebachiensis*. *Aquaculture*, **266**, 97–101.

Simkiss K, Wilbur KM (1989) *Biomineralization: Cell Biology and Mineral Deposition.* (Heinz A. Lowenstam and Stephen Weiner, Ed.). New York, Academic Press, San Diego, CA.

Sodergren E, Weinstock GM, Davidson EH, *et al.* (2006) The genome of the sea urchin *Strongylocentrotus purpuratus*. *Science (New York, N.Y.)*, **314**, 941–52.

Spicer JI, Widdicombe S (2012) Acute extracellular acid-base disturbance in the burrowing sea urchin Brissopsis lyrifera during exposure to a simulated CO_2 release. *The Science of the total environment*, **427-428**, 203–207.

Spicer JI, Widdicombe S, Needham HR, Berge JA (2011) Impact of CO_2-acidified seawater

on the extracellular acid–base balance of the northern sea urchin *Strongylocentrotus dröebachiensis*. *Journal of Experimental Marine Biology and Ecology*, **407**, 19–25.

Steneck RS, Graham MH, Bourque BJ, Corbett D, Erlandson JM, Estes JA, Tegner MJ (2002) Kelp forest ecosystems: biodiversity, stability, resilience and future. *Environmental Conservation*, **29**, 436–459.

Strathmann RR, Grünbaum D (2006) Good eaters, poor swimmers: compromises in larval form. *Integrative and Comparative Biology*, **46**, 312–322.

Stumpp M, Dupont S, Thorndyke MC, Melzner F (2011a) CO_2 induced seawater acidification impacts sea urchin larval development II: Gene expression patterns in pluteus larvae. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, **160**, 320–330.

Stumpp M, Wren J, Melzner F, Thorndyke MC, Dupont S (2011b) CO_2 induced seawater acidification impacts sea urchin larval development I: Elevated metabolic rates decrease scope for growth and induce developmental delay. *Comparative biochemistry and physiology. Part A, Molecular & integrative physiology*, **160**, 331–340.

Stumpp M, Hu MY, Melzner F, *et al.* (2012a) Acidified seawater impacts sea urchin larvae pH regulatory systems relevant for calcification. *Proceedings of the National Academy of Sciences of the United States of America*, **109**, 18192–7.

Stumpp M, Trübenbach K, Brennecke D, Hu MY, Melzner F (2012b) Resource allocation

and extracellular acid-base status in the sea urchin *Strongylocentrotus droebachiensis* in response to CO_2 induced seawater acidification. *Aquatic Toxicology*, **110-111**, 194–207.

Sunday JM, Crim RN, Harley CDG, Hart MW (2011) Quantifying rates of evolutionary adaptation in response to ocean acidification. *PLoS ONE*, **6**, e22881.

Taylor JR, Lovera C, Whaling PJ, Buck KR, Pane EF, Barry JP (2013) Physiological compensation for environmental acidification is limited in the deep-sea urchin *Strongylocentrotus fragilis*. *Biogeosciences Discussions*, **10**, 8313–8341.

Thomsen J, Casties I, Pansch C, Körtzinger A, Melzner F (2013) Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. *Global Change Biology*, **19**, 1017–1027.

Thomsen J, Gutowska MA, Saphörster J, *et al.* (2010) Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences*, **7**, 3879–91.

Thorson G (1950) Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, **25**, 1–45.

Todgham AE, Hofmann GE (2009) Transcriptomic response of sea urchin larvae *Strongylocentrotus purpuratus* to CO_2-driven seawater acidification. *The Journal of experimental biology*, **212**, 2579–94.

Tunnicliffe V, Davies KTA, Butterfield DA, Embley RW, Rose JM, Chadwick Jr WW (2009) Survival of mussels in extremely

acidic waters on a submarine volcano. *Nature Geoscience*, **2**, 344–348.

Uthicke S, Soars NA, Foo SA, Byrne M (2012) Effects of elevated pCO_2 and the effect of parent acclimation on development in the tropical Pacific sea urchin *Echinometra mathaei*. *Marine Biology*, **160**, 1913-26.

Widdicombe S, Beesley A, Berge JA, Dashfield SL, McNeill CL, Needham HR, Oxnevad S (2012) Impact of elevated levels of CO_2 on animal mediated ecosystem function: The modification of sediment nutrient fluxes by burrowing urchins. *Marine pollution bulletin*, doi: 10.1016/ j.marpolbul.2012.11.008.

Widdicombe S, Dashfield SL, McNeill CL, *et al.* (2009) Effects of CO_2 induced seawater acidification on infaunal diversity and sediment nutrient fluxes. *Marine Ecology Progress Series*, **379**, 59–75.

Wolfe K, Dworjanyn S a, Byrne M (2013) Effects of ocean warming and acidification on survival, growth and skeletal development in the early benthic juvenile sea urchin (*Heliocidaris erythrogramma*). *Global change biology*, **19**, 2698-2707.

Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. *Proceedings of the Royal Society B: Biological sciences*, **275**, 1767–73.

Wood HL, Widdicombe S, Spicer JI (2009) The influence of hypercapnia and the infaunal brittlestar *Amphiura filiformis* on sediment nutrient flux – will ocean acidification affect nutrient exchange? *Biogeosciences*, **6**, 2015–2024.

Wood HL, Spicer JI, Lowe DM, Widdicombe S (2010) Interaction of ocean acidification and temperature; the high cost of survival in the brittlestar *Ophiura ophiura*. *Marine Biology*, **157**, 2001–13.

Wood HL, Spicer JI, Kendall MA, Lowe DM, Widdicombe S (2011) Ocean warming and acidification; implications for the Arctic brittlestar *Ophiocten sericeum*. *Polar Biology*, **34**, 1033–1044.

Yu PC, Matson PG, Martz TR, Hofmann GE (2011) The ocean acidification seascape and its relationship to the performance of calcifying marine invertebrates: Laboratory experiments on the development of urchin larvae framed by environmentally-relevant pCO_2/pH. *Journal of Experimental Marine Biology and Ecology*, **400**, 288–295.

Yu PC, Sewell MA, Matson PG, Rivest EB, Kapsenberg L, Hofmann GE (2013) Growth attenuation with developmental schedule progression in embryos and early larvae of *Sterechinus neumayeri* raised under elevated CO_2. *PloS one*, **8**, e52448.

Preface:

Wolpert, L. (2011). Developmental Biology: A Very Short Introduction (Vol. 280). Oxford University Press, Oxford (UK).

Bryson, B. (2003). A Short History of Nearly Everything. Transworld Publishers, London (UK)