

# Table of contents

<i>Acknowledgments</i>	<i>i</i>
<i>Résumé</i>	<i>vii</i>
<i>Abstract</i>	<i>ix</i>
<b>Chapter I – General Introduction</b>	<b>1</b>
1. Anthropogenic CO <sub>2</sub> emissions and ocean acidification	1
1.1. Anthropogenic CO <sub>2</sub> emissions	1
1.2. Ocean acidification	2
1.3. Assessing the effects of OA	15
2. Impact of OA on marine calcifiers	16
2.1. General aspects	17
2.2. Adult echinoderms	21
<b>Chapter II - Aim of the thesis</b>	<b>39</b>
<b>Chapter III</b>	<b>41</b>
<b>Skeletal integrity of a marine keystone predator (<i>Asterias rubens</i>) threatened by ocean acidification</b>	
1. Introduction	43
2. Materials and Methods	45
2.1. Seawater acidification experiments	45
2.2. Preparation of ambulacral plates and spines	46
2.3. Scanning electron microscopy (SEM)	47
2.4. Mechanical tests	48
2.5. Sample preparation and analysis of magnesium content	49
2.6. Statistical analysis	50
3. Results	50
3.1. Corrosion	50
3.2. Mechanical properties of ambulacral plates	52
3.3. Nanoindentation	56
3.4. Magnesium content of the skeleton	56
4. Discussion	56
4.1. Corrosion	57
4.2. Mechanical properties	58
4.3. Biomechanical and ecological impacts	59
5. Conclusions	61
<i>Supplementary information</i>	62

**Chapter IV** 73**Skeletal properties of the sea urchin *Echinometra spp.* from ocean acidification experiments in mesocosms and a CO<sub>2</sub> vent**

1. Introduction	75
2. Materials and Methods	79
2.2. Sample origins	79
2.3. Ossicle samplings and preparation for mechanical tests	80
2.4. Corrosion analyses	80
2.5. Mechanical tests	81
2.6. Statistical analyses	82
3. Results	84
3.1. Corrosion	84
3.2. Mechanical and morphological properties of the ambital plates	85
3.3. Mechanical and morphological properties of the spines	89
3.4. Nanoindentation	92
4. Discussion	94
4.1. Ocean acidification impact	94
4.2. Field vs mesocosm sea urchins	95
4.3. Importance of <i>Echinometra spp.</i>	96
5. Conclusion	97
<i>Supplementary information</i>	98

**Chapter V** 109**Are control of extracellular acid-base balance and regulation of skeleton genes linked to resistance to ocean acidification in adult sea urchins?**

1. Introduction	111
2. Materials and Methods	113
2.1. Sampling and physico-chemical measurements	113
2.2. Ossicle sampling and preparation for mechanical tests	115
2.3. Mechanical test methods	115
2.4. Nanoindentation	117
2.5. RNA Extractions	117
2.6. Synthesis of cDNA and preliminary amplifications (PCRs)	118
2.7. Real Time qPCR Comparative Assays	119
2.8. Statistical analyses	120
2.9. Weibull analysis	120
3. Results	121
3.1. Carbonate chemistry of seawater and acid-base physiology of the sea urchin	121
3.2. Mechanical properties	123
3.3. Nanoindentation	126
3.4. Gene expression analysis, Real Time qPCR comparative assays	129
4. Discussion	131
4.1. Acid-base physiology	131
4.2. Impact on the skeleton	132
4.3. Impact on gene expression	132
4.4. Ecological impacts	133

5.	Conclusion	134
	<i>Supplementary Information</i>	135
<b>Chapter VI</b>		<b>147</b>
<b>Acid-base physiology and skeleton properties of two echinoderms in the caldera of the active volcano of Deception Island, Antarctica</b>		
1.	Introduction	149
2.	Materials and Methods	151
2.1.	Sampling	151
2.2.	Physico-chemical measurements in seawater and in the coelomic fluid	153
2.3.	Metals analyses	154
2.4.	Ossicle sampling and preparation for mechanical tests	154
2.5.	Statistical analyses	156
2.6.	Weibull analysis	156
3.	Results	157
3.1.	Physico-chemical parameters measurements in seawater	157
3.2.	Acid-base physiology of the coelomic fluid and morphometry of <i>O. validus</i> and <i>S. neumayeri</i>	159
3.3.	Morphometry and mechanical properties of the skeleton	160
3.4.	Metals concentration in three compartments of <i>O. validus</i> and <i>S. neumayeri</i>	164
3.5.	Relations between pH <sub>e</sub> , pH <sub>sw</sub> , metals concentration in the integument and mechanical properties of the skeleton	167
4.	Discussion	168
4.1.	Sampling stations	168
4.2.	Acid-base physiologies of the two species	168
4.3.	Mechanical properties of the skeleton of echinoderms of Deception Island	170
4.4.	Metals contamination in the two species	172
5.	Conclusion	173
	<i>Supplementary information</i>	174
<b>Chapter VII - General discussion</b>		<b>191</b>
1.	Methodological aspects	191
1.1.	Long-term laboratory experiments vs. CO <sub>2</sub> vents	191
1.2.	Skeleton investigations	194
1.3.	Weibull analysis	200
2.	Biological characteristics modulating the impact of OA on the echinoderm skeleton	201
2.1.	Acid-base physiology	203
2.2.	Habitat	204
2.3.	Food	206
3.	Conclusions and perspectives	207
<b>Appendix</b>		<b>209</b>
<b>Bibliography</b>		<b>211</b>

