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Evaluation des contaminations en éléments traces et leurs effets sur les stades larvaires et adulte de *Paracentrotus lividus*

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Préface

Ce doctorat a été réalisé au sein de l'Unité d'Appui et de Recherche 3514 **STELLA MARE** (UAR CNRS/Università di Corsica ; Sustainable TEchnologies for LittoraL Aquaculture and MArine REsearch), grâce à l'obtention d'un contrat doctoral financé par l'Università di Corsica Pasquale Paoli et la Collectivité de Corse. La recherche en écologie est rattachée à l'Unité Mixte de Recherche 6134 SPE (UMR CNRS/Università di Corsica ; Science Pour l'Environnement) et s'inscrit dans le projet structurant « Gestion et valorisation des Eaux en Méditerranée » qui concerne notamment la connaissance, l'exploitation durable et la préservation de la ressource en eau et les écosystèmes associés.

Ces travaux de recherche ont été effectués dans le cadre d'une convention de cotutelle de thèse internationale entre l'**UMR 6134 SPE** (**Università di Corsica**) et le **laboratoire d'Océanologie Biologique** (**Université de Liège**).

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Les résultats présentés s'inscrivent dans les thématiques développées au sein de l'UMR SPE 6134 et font partie intégrante du **programme « Oursin »** de la plateforme STELLA MARE dont l'objectif est de contribuer à la connaissance de la biologie et l'écologie de l'oursin et à la gestion durable de ses populations sur le littoral de la Corse.

Ce doctorat a bénéficié du soutien financier de la **Collectivité de Corse**, de la **Fondation de la Mer**, du **programme de recherche MISTRAL** (Mediterranean Integrated STudies at Regional And Local Scales), ainsi que de l'**Université de Liège** et de l'**Università di Corsica**.

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A mes grands-parents
A mes parents

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« *La reconnaissance est la mémoire du cœur.* »

Hans Christian Anderse

Résumé

Les éléments traces font partis des contaminants les plus répandus dans l'écosystème marin. Au-dessus de seuils critiques, tous les éléments traces présentent un danger potentiel pouvant entraîner des perturbations à l'échelle cellulaire et individuelle, mais aussi au niveau de la population ou de l'écosystème. En Méditerranée, la Corse a longtemps été considérée comme une zone subissant de faibles pressions anthropiques. De par ses caractéristiques écologiques, sa haute tolérance aux contaminants et sa capacité à bioaccumuler des éléments traces, l'oursin violet *Paracentrotus lividus* (Lamarck, 1816) est reconnu en tant que bioindicateur. L'objectif de ce travail de thèse a consisté à mieux comprendre les variations des contaminations en éléments traces en Corse ainsi que leurs effets chez un organisme emblématique, *Paracentrotus lividus*.

Une évaluation de la contamination en éléments traces classiques et émergents a été menée sur les gonades d'oursins. Dans ce cadre, plusieurs prélèvements en mer ont été effectués afin de déterminer les principaux éléments traces susceptibles d'être bioaccumulés par l'oursin adulte et les variations spatio-temporelles de ces contaminants. Ces travaux ont mis en évidence des régions en Corse présentant des concentrations en éléments traces plus élevées dues aux fonds géochimiques associés aux pressions anthropiques. Des variations saisonnières ont également été décelées avec des concentrations plus faibles durant la saison estivale. Ces variations sont notamment liées au cycle de reproduction de l'oursin et démontrent l'importance des facteurs biotiques dans l'évaluation des niveaux de contamination. Les analyses biochimiques des oursins adultes suggèrent que le stress oxydatif induit par les contaminations mesurées n'a pas d'impact direct sur leur santé.

Les bioessais menés sur les stades larvaires de l'oursin ont permis d'évaluer les effets de différentes contaminations en éléments traces. Ces contaminations ont induit des malformations ainsi qu'un ralentissement de la croissance, voire une inhibition du développement larvaire. En parallèle, des recherches sur les réponses au stress des différents stades larvaires ont permis de souligner la grande capacité des larves à répondre au stress oxydatif, mais également l'importance des géniteurs dans les études utilisant les larves d'oursin. Ces expérimentations innovantes sont les premières à s'intéresser aux effets d'une combinaison aussi importante d'éléments traces sur l'ensemble du cycle larvaire de l'oursin. Le présent travail pourrait renforcer d'autres outils de diagnostic et de suivi de la qualité des eaux côtières en vue d'améliorer la qualité écologique des eaux marines.

Mots clés : Eléments traces ; *Paracentrotus lividus* ; Bioindicateur ; Bioaccumulation ; Bioessai

Abstract

Trace elements are among the most common contaminants in the marine ecosystem. Beyond critical thresholds, all trace elements present a potential danger that can lead to disturbances at the cellular and individual level, but also at the population or ecosystem level. In the Mediterranean Sea, Corsica Island has long been considered an area of low anthropic pressure. Due to its ecological characteristics, its high tolerance to contaminants and its ability to bioaccumulate trace elements, the sea urchin *Paracentrotus lividus* (Lamarck, 1816) is recognized as a bioindicator. The aim of this thesis was to better understand the variations of trace element contamination in Corsica and their effects on an emblematic organism, *Paracentrotus lividus*.

An assessment of the contamination of classical and emerging trace elements was conducted on sea urchin gonads. In this context, several samples were collected in order to determine the main trace elements susceptible to be bioaccumulated by the adult sea urchin and the spatio-temporal variations of these contaminants. This research has highlighted areas in Corsica with higher trace element concentrations due to geochemical backgrounds associated with anthropogenic pressures. Seasonal variations were also identified with lower concentrations during the summer season. These variations are mainly due to the reproduction cycle of the sea urchin and show the importance of biotic factors in the evaluation of contamination levels. Biochemical analyses of adult sea urchins suggest that the oxidative stress induced by the measured contaminations has no direct impact on their health. Bioassays performed on larval stages of sea urchins were used to evaluate the effects of several trace element contaminations. These contaminations induced malformations and a slowing of the development. In addition, research on the stress responses of the different larval stages has highlighted the high capacity of larvae to respond to oxidative stress, but also the importance of spawners in studies using sea urchin larvae. These innovative experiments are the first to investigate the effects of such important combinations of trace elements on the entire larval cycle of the sea urchin. This present work could strengthen other tools for diagnosing and monitoring coastal water quality in order to improve the ecological quality of marine waters.

Keywords: Trace elements; *Paracentrotus lividus*; Bioindicator; Bioaccumulation; Bioassay

Riassuntu

L'elementi tracce fanu parte di i cuntaminanti i più sparsi ind'è l'ecusistema marinu. Aldilà di sogli critichi, tutti l'elementi traccei prisentanu un periculu pussibule capace à pruvucà disturbi à livellu cellulare è individuale ma dinù à quellu di a pupulazione o di l'ecusistema. In u Mediterraniu, A Corsica, da assai, hè stata cunsiderata cum'è un locu spostu à debule pressioni antrupugeniche. Per via di e so caratteristiche ecologiche, a so alta capacità à accittà cuntaminanti è a so faciulità à bioammansà elementi tracce, u zinu viulettu *Paracentrotus lividus* (Lamarck, 1816) hè ricunnisciutu cum'è biotestimone. U scopu di issu travagliu di tesi hè statu di pruvà à capì megliu e variazione di e cuntaminazione in elementi tracce in Corsica è i so effetti annant'à un urganisimu riprisentativu, *Paracentrotus lividus.*

Un'evaluazione di a cuntaminazione in elementi tracce classichi è emergenti hè stata operata annant'à gonadi di zini. Ind'è issa logica, numarosi prilevi sò stati fatti in mare da definisce l'elementi tracce principali capaci à esse bioammansati da u zinu adultu è e varazioni spaziu-timpurale di issi cuntaminanti. Isse ricerche anu palisatu u fattu chì certe regione di Corsica prisentanu cuncentrazioni d'elementi tracce più alte pè via di i so fondi geochimichi assuciati à presioni antrupugeniche. Sicondu e stagioni, variazione sò state scuparte cun cuncentrazioni più debule d'estate. Isse variazioni sò pè u più in leia cù u ciculu di ripruduzione di u zinu è accertanu l'impurtanza di fattori biotichi ind'è l'evaluazione di i livelli di cuntaminazione. L'analisi biochimiche di i zini adulti suggeriscenu chì u frasturnà ussidativu arricatu da e cuntaminazione misurate ùn hà nisun impattu direttu annant'à a so salute.

E bioprove fatte nant'à u zinu à l'età di larve anu permessu di misurà l'effetti di e sfarente cuntaminazione in elementi tracce. Isse cuntaminazioni anu inghjennatu malfurmazioni è un attimpà di a crescita o puru un inibizione di u sviluppu di a larva. In issu mentre e ricerche annant'à e risposte à u frasturnà di sfarente età larvare anu permessu di sottulineà a capacità maiò di e larve à risponde à u frasturnà ossidativu ma dinù l'impurtanza di i genitori in i studii chì apprudavanu larve di zinu. Isse sperimentazioni nuvatrice sò e prime à primurà si d'un assestu cusì impurtante d'elementi tracce annant'à u ciculu larvare di u zinu. Issu travagliu puderebbe rinfurzà altri attrazzi di diagnosticu è di seguitu di a qualità di l'acque custiere pè migliurà a qualità ecologica di l'acque marine.

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Liste des abréviations

•OH	Radical hydroxyle
ADN	Acide désoxyribonucléique
BAF	Facteur de bioaccumulation
BMF	Facteur de bioamplification
BSAF	Facteur d'accumulation biote-sédiment
CAT	Catalase
CE50	Concentration Efficace Médiane
CNRS	Centre National de la Recherche Scientifique
CRM	Certified Reference Material
DGT	Diffusion Gradient in Thin Films
LD	Limite de détection (Detection limit)
DRC ICP-MS	Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology
DW	Dry weight (Poids sec)
e.g.	*exempli gratia* (par exemple)
EC50	Median Effect Concentration
EMBIO	Ecologie Marine et Biodiversité
ERO	Espèce Réactives de l'Oxygène
FAO	Food and Agriculture Organization of the United Nations (Organisation des Nations unies pour l'alimentation et l'agriculture)
GEM	Gestion et valorisation des Eaux en Méditerranée
GFW	Gonad Fresh weight (Poids humide des gonades)
GPX	Glutathion peroxydases
GST	Glutathion-s-transférase
H_2O_2	Peroxyde d'hydrogène
HNO_3	Acide nitrique
i.e.	*id est* (c'est à dire)
IPCC	Intergovernmental Panel on Climate Change
LC50	Median lethal concentration (Concentration létale médiane)
MDA	Malondialdéhyde
MIO	Institut Méditerranéen d'Océanologie
MISTRAL	Mediterranean Integrated STudies at Regional And Local Scales
ppm	Parties par million
r	Coefficient de corrélation
ROS	Reactive Oxygen Species
S.E.	Standard Error (Erreur standard)
SCUBA	Self Contained Undewater Breathing Apparatus (Scaphandre autonome)
SOD	Superoxyde dismutase
SPE	Science Pour l'Environnement
STARESO	Station de Recherches Sous-marines et Océanographiques
STELLA MARE	Sustainable TEchnologies for LittoraL Aquaculture and MArine REsearch

SW	Seawater
TE	Trace Element (Element trace)
TFW	Total fresh weight (Poids frais total)
UAR	Unité d'Appui et de Recherche
UMR	Unité Mixte de Recherche
UNCLOS	United Nations Convention on the Law of the Sea
WW	Wet weight (Poids humide)

Eléments traces

Ag	Argent
Al	Aluminium
As	Arsenic
Ba	Baryum
Be	Béryllium
Bi	Bismuth
Cd	Cadmium
Co	Cobalt
Cr	Chrome
Cu	Cuivre
Fe	Fer
Hg	Mercure
Li	Lithium
Mn	Manganèse
Mo	Molybdène
Ni	Nickel
Pb	Plomb
Sb	Antimoine
Se	Sélénium
Sn	Etain
Sr	Strontium
U	Uranium
V	Vanadium
Zn	Zinc

CHAPITRE 1

INTRODUCTION GÉNÉRALE

1. Les écosystèmes marins

1.1. Importance des milieux marins

Le milieu marin couvre plus de 70 % de la surface totale de la Terre et joue un rôle essentiel sur la qualité et le maintien de la vie (Lorey, 2003 ; Barbier, 2017). Ses écosystèmes constituent les milieux les plus diversifiés au regard du biote qu'ils abritent, représentant ainsi un important réservoir de la biodiversité mondiale (Sala & Knowlton, 2006). En parallèle, ces derniers jouent un rôle important dans la régulation du climat en absorbant 93 % de la chaleur produite par les gaz à effet de serre et contribuent à l'équilibre climatique *via* les courants océaniques et les interactions avec l'atmosphère (Georges & Le Maho, 2003). Ils constituent également d'importants puits de carbone grâce à plusieurs organismes marins comme le phytoplancton et à plusieurs écosystèmes côtiers comme les herbiers marins (*Nellemann et al.*, 2009 ; Basu & Mackey, 2018 ; Monnier *et al.,* 2021).

Ils fournissent également de nombreuses ressources alimentaires, pharmaceutiques, minérales et énergétiques et sont le siège de nombreux espaces de loisirs et voies de transport ce qui font d'eux une source économique et sociale importante (Cicin-Sain *et al.*, 2002 ; Remoundou *et al.*, 2009 ; Pakalniete *et al.*, 2017). Ainsi, l'Organisation des Nations Unies pour l'alimentation et l'agriculture estime que la pêche et l'aquaculture assure les moyens de subsistance de 10 à 12 % de la population mondiale (FAO, 2022). La convention des nations unies sur le droit de la mer évalue la valeur combinée des ressources marines et de leurs utilisations aux alentours de 7 milliards de dollars par an (UNCLOS, 2002).

1.2. Menaces sur les écosystèmes marins

La dépendance de l'homme vis-à-vis des milieux marins et le mode de vie des populations entraînent des pressions importantes dans les écosystèmes marins (Harvell *et al.*, 2004 ; Pyke, 2004). Ainsi, malgré l'importance de ces derniers, ils sont aujourd'hui menacés par plusieurs pressions anthropiques telles que la surexploitation des ressources halieutiques, la pollution, la dégradation des habitats marins, l'invasion d'espèces exotiques et les manifestations du changement climatique. (Pitcher, 2001 ; Scheffer & Carpenter, 2003 ;

Fig. 1). Ces pressions sont la résultante d'une démographie et d'une activité humaine en pleine expansion dont les besoins en ressources renouvelables (pêche) et non renouvelables (minérales, énergétiques) ne cessent de s'accroître (Amar, 2010).

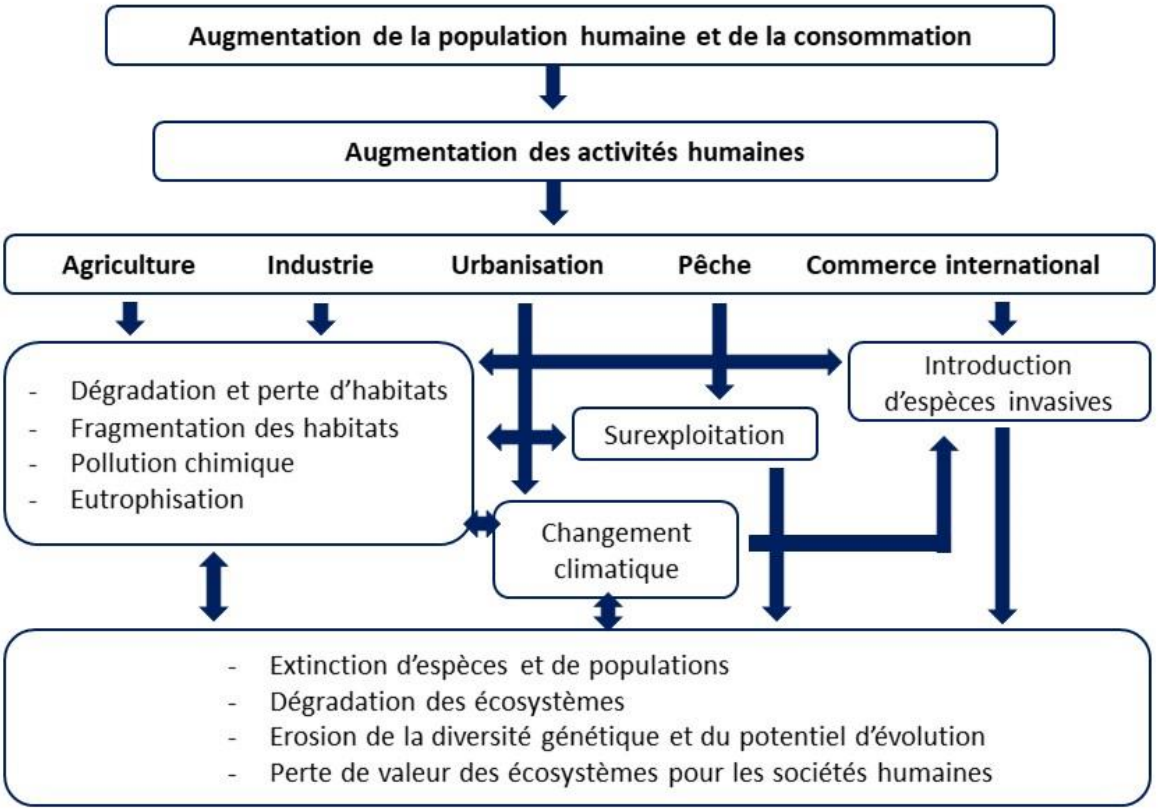


Figure 1. Principales pressions ayant une incidence sur les écosystèmes marins (modifié d'après Groom *et al.*, 2006).

Selon Neumann *et al.* (2015), la population humaine devrait augmenter de 50 à 122 % au cours de la période 2030-2060 le long des écosystèmes côtiers du monde entier. À titre d'exemple, la ville de Cancún a déjà multiplié sa population par 5 000 en l'espace de 47 ans (Häder *et al.,* 2020). À mesure que les populations humaines continuent de croître et de migrer vers les côtes, la demande d'espaces et de ressources océaniques augmente, ce qui amplifie les pressions individuelles et cumulatives exercées par toute une série d'activités humaines (Halpern *et al.,* 2008, Halpern *et al.,* 2015 ; Fig. 2). La pêche est le principal facteur qui menace la biodiversité des poissons marins avec la disparition de plusieurs populations d'espèces locales dans le monde en raison de la surexploitation (Dulvy *et al.,* 2003). Selon plusieurs auteurs, le changement climatique d'une part et la pollution d'autre part comptent parmi les plus grandes menaces qui pèsent sur les milieux marins (*e.g.* Scheffer *et al.,* 2001 ; Folke *et al.,*

2004). En effet, depuis le début de la période industrielle, la hausse continue des teneurs en gaz à effet de serre a causé un important déséquilibre du cycle naturel du carbone et par conséquent du système climatique mondial (IPCC, 2013). Cette hausse pourrait conduire à des changements globaux ou régionaux majeurs tels que l'augmentation des températures, une hausse du niveau marin, une acidification des océans ou encore des évènements extrêmes plus fréquents (*i.e.* inondations, sécheresses, tempêtes, IPCC, 2013, 2019) qui auraient un impact considérable sur les écosystèmes marins et leur biodiversité.

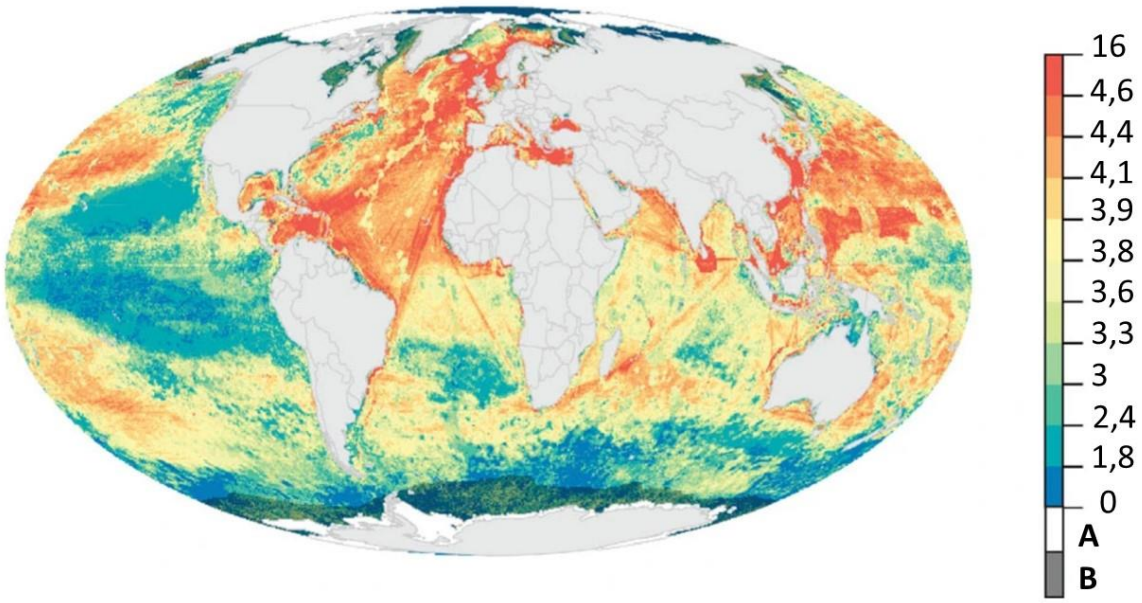


Figure 2. Impacts anthropiques cumulés sur les écosystèmes marins en 2013 (A : Couverture glaciaire permanente ; B : Couverture glaciaire saisonnière). Les indices d'impact sont basés sur l'ensemble de 19 facteurs de stress anthropiques. Plus l'indice est élevé et plus les impacts cumulés sont élevés (modifié d'après Halpern *et al.,* 2015).

En parallèle, les activités anthropiques sont responsables de l'introduction dans le milieu marin d'un grand nombre de substances avec environ 80 % des pollutions marines d'origine terrestre et anthropique (Amar, 2010) menaçant ainsi les organismes marins. Ces pollutions peuvent provenir de plusieurs sources telles que les activités industrielles, les rejets d'eaux usées et les déversements de pétrole (Häder *et al.,* 2020). Ainsi, une connaissance approfondie du fonctionnement de ces écosystèmes marins et une approche bien planifiée de leur gestion sont essentielles pour atteindre la durabilité au vu de l'importance écologique, économique et sociale de ces ressources (Hughes *et al.,* 2005 ; Salomidi *et al.,* 2012)

1.3. La Méditerranée : un milieu d'étude

Le bassin méditerranéen est une mer semi-fermée couvrant une surface d'environ 2,5 millions de km^2 et située entre l'Europe, l'Afrique et l'Asie (Coll *et al.,* 2010). Elle communique avec l'océan Atlantique *via* le détroit de Gibraltar, la mer de Marmara et la mer Noire à travers le détroit de Bosphore et la mer Rouge et l'Océan Indien par le canal de Suez. Alors qu'elle ne représente que 0,8 % de la superficie marine mondiale (Defant, 1961), cette dernière regroupe 4 à 18 % de la biodiversité marine connue avec un fort taux d'endémisme (25 % des espèces qui y vivent ; Bianchi & Morri, 2000 ; Coll *et al.,* 2010). La Méditerranée est ainsi considérée comme un haut lieu de biodiversité compte tenu du nombre et de la diversité d'espèces abritées (Myers *et al.,* 2000).

Cette dernière a également une haute importance économique de par son trafic maritime présentant 28% du trafic pétrolier mondial en mer mais également de par l'exploitation de ses ressources et du tourisme élevé sur le littoral méditerranéen (Bianchi & Morri, 2000 ; Laubier, 2003). Ces activités génèrent des pressions sur les écosystèmes qui entraînent la surexploitation et la destruction des habitats marins conduisant à des changements de la biodiversité en Méditerranée (Coll *et al.,* 2010). De plus, le changement climatique induit une accélération de l'invasion d'espèces exotiques à la fois par les espèces venues de Mer Rouge et par celles provenant des basses latitudes de l'Atlantique (Ben Rais Lasram & Mouillot, 2009). Ce dernier est également à l'origine de disparitions de populations locales et de réduction des niches écologiques entrainant une diminution de la diversité génétique, la perte de fonctions des écosystèmes et l'augmentation du risque de déclin des populations (Galil, 2007 ; Edelist *et al.,* 2013). En parallèle, la croissance des activités industrielles, agricoles et urbaines depuis le début des années 1960 génère une augmentation des teneurs de contaminants tels que les éléments traces (*e.g.* Bethoux *et al.*, 1990 ; Saliot, 2005). La réponse de la Méditerranée aux perturbations environnementales est plus rapide que dans les grands océans (Augier, 2010). En effet, le temps de séjour des eaux profondes est d'environ 10 à 15 ans pour le bassin occidental et 60 ans pour le bassin oriental tandis que les temps de résidence des eaux océaniques varient entre 500 et 1000 ans (Bethoux & Gentili, 1999). Ainsi, les spécificités et les menaces subies par la Méditerranée en font une unité écologique

vulnérable et un site privilégié pour l'étude des pressions et des changements exercés par l'Homme sur l'environnement (Bethoux *et al.*, 1999 ; Turley, 1999 ; Richir & Gobert, 2016).

2. Les éléments traces

2.1. Définition et typologie

Les éléments traces sont décrits comme des éléments présents à de faibles niveaux dans les différents compartiments de l'environnement (Baize, 2009 ; Chojnacka, 2018). En fonction des différents domaines scientifiques (*e.g.* géochimie, médecine, océanologie), ces derniers ont fait l'objet de plusieurs définitions. Conventionnellement, un élément trace est défini comme un élément chimique dont la concentration moyenne est inférieure à 100 parties par million (ppm ; McNaught & Wilkinson, 1997 ; Górecki & Chojnacka, 2018). Toutefois, les éléments à l'état de traces dans les matériaux biologiques ne sont pas nécessairement à l'état de traces dans les environnements terrestres. C'est le cas de l'aluminium et du fer, considérés comme des éléments fondamentaux en géochimie, mais qui seront considérés comme des éléments traces dans les organismes (Navratil & Minarik, 2002 ; Shaheen *et al.*, 2013). Longtemps désignés sous le terme de « métaux lourds », cette appellation a été revue et est de moins en moins utilisée dans la communauté scientifique de par son aspect restrictif. En effet, s'il est toujours possible de retrouver cette désignation, la masse volumique de certains éléments n'est pas assez élevée (< 5 g m^{-3}) pour être qualifiée de « lourds » comme l'aluminium et le nickel tandis que d'autres ne sont pas des « métaux » comme l'arsenic et le sélénium (Richir & Gobert, 2016).

Il existe pour un organisme et un élément trace donné, une concentration optimale permettant la croissance et le développement normal de cet organisme. Au-dessous du seuil optimal, l'élément trace peut devenir facteur limitant et entrainer des phénomènes de carence tandis qu'au-dessus de ce seuil optimal, une toxicité potentielle plus ou moins aiguë peut être observée (Amiard, 2011). Ces éléments traces sont considérés comme des « éléments essentiels » pour la cellule vivante, dans un intervalle de concentration défini. Ces derniers jouent un rôle dans de nombreux processus biochimiques, biologiques et physiologiques (Vincevica-Gaile *et al.,* 2013). L'essentialité est une caractéristique qui évolue

selon les connaissances et la sensibilité des auteurs (Amiard, 2011). En revanche, des éléments traces tels que le plomb ou le cadmium, pour lesquels il n'est reconnu, en l'état actuel des connaissances, aucun rôle bénéfique dans les processus biologiques sont nommés « éléments non-essentiels » (Amiard, 2011 ; Richir & Gobert, 2016). Ces derniers pourraient au contraire représenter un danger pour la santé des organismes quel que soit leur concentration (Miquel, 2001 ; Nordberg *et al.*, 2007). Au-dessus de seuils critiques, tous les éléments traces présentent un danger potentiel pouvant entraîner des perturbations à l'échelle cellulaire et individuelle mais également au niveau de la population ou de l'écosystème.

Les éléments traces peuvent aussi être classés en fonction de leur apparition comme contaminants dans l'environnement. Dans cette optique, on distingue les éléments traces « classiques » et les éléments traces « émergents ». Les éléments traces classiques font l'objet d'une importante documentation scientifique (*e.g.* cadmium, chrome, cuivre, fer, nickel, zinc). Les éléments traces émergents désignent, quant à eux, des éléments provenant de nouveaux usages et mesurés depuis peu dans l'environnement ou des éléments déjà présents dans l'industrie mais dont les effets néfastes et la toxicité viennent seulement d'être découverts (*e.g.* béryllium, molybdène, sélénium, vanadium ; Daughton, 2005 ; Richir & Gobert, 2016).

2.2. Sources des éléments traces

Les éléments traces peuvent être de sources naturelles ou anthropiques (Baize, 2009). Leur dispersion naturelle peut être liée à divers phénomènes géologiques tels que l'érosion et les éruptions volcaniques. L'activité volcanique est l'une des principales sources naturelles d'éléments traces dans le milieu aquatique. Les éruptions volcaniques constituent une source non négligeable avec 800 à 1 400 tonnes de cadmium, 18 800 à 27 000 tonnes de cuivre, 3 200 à 4 200 tonnes de plomb et 1 000 tonnes de mercure libéré en moyenne chaque année dans le monde (Miquel, 2011). Des évènements comme les incendies de forêt peuvent également favoriser la mobilité des éléments traces par le déplacement des poussières et des particules de cendres par le vent (Cordeiro *et al.,* 2002). Cependant, même si les dépositions atmosphériques d'éléments provenant de l'activité volcanique et de feux de forêt amènent des éléments traces dans les environnements aquatiques (Callender, 2003), l'érosion des sols et le ruissellement terrestre sont considérés comme des sources significatives des éléments

traces dans le milieu marin (Konieczka *et al.,* 2018). Ainsi, les eaux de ruissellement provoquent le lessivage du sol, riche en éléments traces, induisant une augmentation des concentrations dans le milieu.

En plus de ces sources naturelles, il existe une grande diversité de sources anthropiques (Konieczka *et al.,* 2018). En effet, suite au développement des activités industrielles, agricoles et domestiques, les activités humaines constituent aujourd'hui une importante source de dispersion des éléments traces dans l'environnement (Baize, 2009). Parmi les innombrables sources anthropiques, les activités industrielles constituent une des principales causes de contamination. L'industrie minière et de raffinerie s'est fortement développée ces dernières décennies suite à l'explosion de la demande en minerai augmentant en même temps la production d'éléments traces (Kesle, 2007 ; Occelli *et al.*, 2013). De plus, du fait de la large gamme d'éléments traces contenus dans les gaz et dans les particules de cendres et de poussières, les divers processus de combustion pour la production d'énergie font également partie des plus grandes sources anthropiques (Burmistrz *et al.,* 2016 ; Konieczka *et al.,* 2018). Les activités agricoles et urbaines contribuent à une grande part de dispersion des éléments traces (Tanaka, 2006). En effet, le développement de l'agriculture intensive s'est accompagné d'une multitude d'effets liés à la contamination croissante de la biosphère. Ainsi, bien que l'application d'engrais, de pesticides et d'herbicides a longtemps été considérée comme l'une des mesures les plus importantes pour augmenter les rendements des cultures, ces produits contiennent généralement plusieurs éléments tels que l'arsenic, le cuivre, le cadmium, le fer, le plomb et le zinc. (He *et al.,* 2005 ; Zhao *et al.,* 2014 ; Zhou *et al.,* 2015). Le traitement des déchets et des eaux usées constitue également un enjeu de taille. Chaque jour dans le monde, des tonnes d'eaux usées et de déchets contaminés sont produits. Jusqu'à la fin du XX^{ème} siècle, la plupart d'entre eux n'étaient pas traités et ont conduit à la contamination de nombreuses écosystèmes (Szynkowska *et al.*, 2018). Du fait même de la multiplicité des sources de rejets, les éléments traces revêtent une importance capitale dans la contamination des écosystèmes.

2.3. Spéciation des éléments traces

Les éléments traces peuvent exister sous diverses formes dans l'environnement : sous différents états d'oxydation ; ils peuvent être réduits ou oxydés selon les conditions, sous forme d'ions libres et de nanoparticules, ou interagir fortement avec d'autres groupes réactifs qui les lient et les stabilisent comme, par exemple, des complexes inorganiques et organiques (Donat & Bruland, 1995 ; Tseng *et al.*, 2015). Une espèce chimique peut être définie comme une forme spécifique d'un élément. Ainsi, la spéciation chimique qualifie la distribution d'un élément donné au sein des différentes espèces chimiques qu'il peut présenter dans un environnement donné (Templeton *et al.*, 2000). La mobilité, la biodisponibilité, le stockage et la toxicité des éléments traces dépendent de leur spéciation chimique. La biodisponibilité est également tributaire des conditions environnementales et des caractéristiques physiologiques et biologiques des organismes (Chapman, 2008). Elle désigne la capacité d'une quantité d'un élément présent dans le milieu à être absorbé par un organisme vivant (Fairweather-Tait, 1992). C'est un critère essentiel dans l'analyse des risques écologiques dans la mesure où c'est la concentration en éléments traces biodisponibles qui entraînera des effets biologiques sur les organismes. Ainsi, l'importance de considérer la partie d'une substance réellement mobile, disponible et réactive pour établir un lien entre une concentration et un effet sur l'organisme a été soulignée dans de nombreuses publications (Maćkiewicz *et al.*, 2018).

2.4. Impacts sur les organismes marins

Bien que présents en faibles quantités, les éléments traces peuvent avoir des effets significatifs sur les organismes vivants (Szynkowska *et al.,* 2018). Tout d'abord, il est important de faire la distinction entre toxicité aiguë et chronique. La toxicité aiguë est généralement considérée comme une exposition unique ou multiple survenant dans un court laps de temps et ses effets biologiques sont souvent différents de ceux résultant d'une exposition chronique, où il existe une exposition à de faibles concentrations de l'agent toxique sur une longue période (Bonner & Bridges, 1983).

La réponse biologique d'un organisme aux éléments traces peut être très variée, puisque certains éléments ont des fonctions essentielles dans l'organisme tandis que d'autres n'en ont pas. Certains éléments traces comme le zinc, connus pour être essentiels à la vie, produiront des défauts métaboliques importants en cas de carence et des effets toxiques qu'à des doses relativement élevées. D'autres éléments comme le chrome, peuvent également avoir des propriétés essentielles mais produire une toxicité à des doses modérées. Et à contrario, les éléments traces non-essentiels comme le cadmium peuvent provoquer des effets toxiques à des doses relativement faibles (Bonner & Bridges, 1983).

Parmi les effets toxiques des éléments traces sur les organismes marins, on observe une diminution de la croissance, des malformations, l'altération de la reproduction, l'endommagement des branchies et une altération de la réparation des tissus. (Viarengo, 1989). L'évaluation des risques liés aux éléments traces doit également tenir compte des effets au niveau moléculaire. Ainsi, les mécanismes moléculaires de la cytotoxicité des éléments traces comprennent des dommages aux membranes plasmatiques suite à la liaison aux protéines et aux phospholipides, l'inhibition des transports transmembranaires des acides aminés, la peroxydation lipidique, la diminution du glutathion réduit et l'inhibition de certaines enzymes telles que l'ADN[1] polymérase, Ca^{2+}ATPase (pompe calcium), Na^{+}-K^{+} ATPase (pompe sodium-potassium ; Viarengo, 1989 ; Fernandes & Henriques, 1991). La modification des fonctions enzymatiques est souvent la principale cause de toxicité des éléments traces. Ces derniers sont également capables d'altérer la synthèse et la réparation de l'ADN provoquant ainsi des dommages importants aux organismes (Depledge *et al.,* 1997 ; Bal & Kasprzak, 2002). En raison des menaces que représentent les éléments traces dans l'environnement, ces derniers doivent être surveillés en permanence, depuis leurs sources d'émission jusqu'à leur dépôt final dans les écosystèmes marins (Richir & Gobert, 2014).

[1] ADN : Acide désoxyribonucléique

3. La surveillance du milieu marin

3.1. La biosurveillance (ou biomonitoring)

Le développement industriel et économique moderne a entraîné une forte charge de polluants dans l'environnement impactant les écosystèmes et les organismes vivants (Romano *et al.,* 2004 ; Al-Sarawi *et al.,* 2015). Bien que les termes pollution et contamination soient utilisés de manière interchangeable dans la littérature, une certaine distinction dans leur signification doit être faite. La contamination est définie comme la concentration d'un élément présent dans l'environnement variant par rapport au niveau de fond sans pour autant causer de dommages aux organismes (Szynkowska *et al.,* 2018). Le mot pollution est approprié lorsque des effets nocifs peuvent être constatés. Selon Holdgate (1979), la pollution est définie comme étant « *[…] l'introduction par l'homme dans l'environnement de substances ou d'énergie susceptibles de provoquer des risques pour la santé humaine, des dommages aux ressources vivantes et aux systèmes écologiques, des atteintes à la structure, ou des interférences avec les utilisations légitimes de l'environnement.* ».

De nombreuses méthodes sont utilisées pour juger du degré de contamination dans les écosystèmes marins. Une des approches les plus communes consiste à prendre des mesures des caractéristiques physiques telles que la température et le pH et à déterminer la répartition des contaminants dans la colonne d'eau ou le sédiment *via* la surveillance chimique (Burger, 2006 ; Amiard, 2011). Cependant, la mesure des concentrations en éléments traces dans la colonne d'eau ne permet pas une évaluation des contaminations intégratives dans le temps due à ses fortes fluctuations causées par plusieurs facteurs tels que l'hydrodynamisme ou le ruissellement de l'eau (Jahan & Strezov, 2019). De plus, les techniques analytiques ne permettent pas d'évaluer les effets des contaminants sur les organismes vivants ou sur l'état de santé de l'écosystème. La biodisponibilité et les effets toxicologiques peuvent en effet varier selon leur forme chimique, l'existence d'interactions entre contaminants ou entre les contaminants et les autres éléments abiotiques et/ou biotiques du milieu ou bien encore en fonction de l'organisme considéré, de son stade de développement ou de son état physiologique (Lagadic *et al.,* 1998 ; Chen *et al.,* 2008). Ainsi, l'étude des contaminants sur les organismes pourrait refléter avec plus de fiabilité le risque potentiel et les dangers pour

l'écosystème (D'Adamo *et al.,* 1997 ; Liang *et al.,* 2009). De nombreuses espèces ont d'importantes capacités de bioaccumulation des contaminants facilitant leur mise en évidence et leur quantification (Zenker *et al.,* 2014). La bioaccumulation se définit comme un processus dans lequel la concentration chimique dans un organisme atteint un niveau supérieur à celui de son milieu de vie et/ou de son régime alimentaire (Gobas *et al.,* 2009). Elle peut être évaluer par différents facteurs tels que le facteur de bioconcentration (BCF) qui est le rapport des concentrations d'un contaminant dans l'organisme et dans l'eau, déterminée dans une expérience contrôlée en laboratoire dans laquelle les organismes d'essai sont exposés à un produit chimique dans l'eau. Il y a également le facteur de bioaccumulation (BAF) qui est le rapport des concentrations d'un contaminant dans l'organisme et dans l'eau, déterminé à partir de données de terrain dans lesquelles des organismes échantillonnés sont exposés à un produit chimique dans l'eau et dans leur régime alimentaire (Gobas *et al.,* 2009). La bioamplification ou biomagnification est une augmentation de la concentration d'un contaminant dans un organisme d'un niveau trophique inférieur à un niveau trophique supérieur au sein du même réseau trophique en raison de la bioaccumulation provenant de l'alimentation (Gobas *et al.,* 2009). Cette dernière peut être exprimée par le facteur de bioamplification (BMF ; Ciesielski *et al.,* 2006 ; Gobas *et al.,* 2009). Enfin le facteur d'accumulation biote-sédiment (BSAF), rapport entre la concentration d'un contaminant dans le biote et celle dans le sédiment, permet d'estimer l'accumulation des contaminants dans l'organisme à travers le sédiment (Kwok *et al.,* 2013).

Aujourd'hui, la surveillance environnementale comprend l'échantillonnage de l'air, de l'eau, du sol et du biote afin d'évaluer la qualité de l'environnement. Selon Ellenberg *et al.* (1992), la biosurveillance correspond à l'interprétation des informations contenues dans les écosystèmes afin d'évaluer un espace ou un domaine donné. Elle peut être effectuée à de nombreuses fins telles que l'établissement de valeurs de références, des effets anthropiques ou d'aide à la décision (Artiola *et al.,* 2004 ; Wiersma, 2004). Cette dernière peut être active, impliquant la contamination d'organismes dans des conditions de laboratoire *via* des essais biologiques (ou bioessais), ou passive, consistant à récolter des informations sur la qualité de l'environnement par l'étude d'organismes déjà présents naturellement dans l'écosystème (Markert, 2007, 2008 ; Wiÿlkomirski, 2013).

3.2. Bioindicateurs

Afin d'évaluer les niveaux de contaminants disponibles dans l'écosystème, des organismes peuvent être utilisés comme indicateurs biologiques ou bioindicateurs. Le terme de bioindicateur a été défini et employé de façon très diverse selon le contexte (Heink & Kowarik, 2010). Pour éviter les confusions dues à différentes interprétations de ce terme, la définition de Blandin (1986) sera utilisée dans ce manuscrit : « *un indicateur biologique (ou bioindicateur) est un organisme ou un ensemble d'organismes qui permet, par référence à des variables biochimiques, cytologiques, physiologiques, écologiques ou éthologiques, de façon pratique et sûre, de caractériser l'état d'un écosystème ou d'un éco-complexe et de mettre en évidence le plus tôt possible leurs changements, naturels ou provoqués* ».

Pour être considérée comme un bon bioindicateur, l'espèce sélectionnée doit répondre à un certain nombre de critères (Cossa, 1989 ; Rainbow & Phillips, 1993 ; Ramade, 2007) :

- Être abondante dans l'ensemble de l'aire étudiée et avoir une distribution géographique étendue afin de favoriser les comparaisons entre zones distinctes ;
- Être sédentaire afin de représenter la zone d'étude ;
- Avoir une durée de vie suffisamment longue ;
- Présenter une corrélation entre la teneur moyenne d'un contaminant dans ses tissus et celle mesurée dans le biotope ou dans l'alimentation quelles que soient la localisation et les conditions environnementales ;
- Fournir des tissus en quantité nécessaire pour les analyses ;
- Être facile à échantillonner et résistance aux manipulations causés par les études en laboratoires et *in situ ;*
- Être tolérante aux variations environnementales ; les effets de ces variations sur l'organisme doivent être connus.

En réalité, la liste de ces critères est si contraignante qu'en pratique, aucune espèce ne combine toutes ces qualités (Cossa, 1989). Ainsi, le choix d'un bioindicateur varie en fonction des conditions du milieu, des contraintes de l'étude et des objectifs à atteindre (Cossa, 1989 ; Richir & Gobert, 2016). La fiabilité d'un bioindicateur peut être contrôlée par de nombreux

facteurs. Parmi ces facteurs, on observe des facteurs intrinsèques comme les taux d'accumulation et d'excrétion, l'état physiologique des individus, le niveau trophique des organismes ou encore les interactions physiotoxicologiques des contaminants et des facteurs extrinsèques tels que la température, le pH ou la salinité. (Ramade, 2007).

Dans les écosystèmes marins, plusieurs espèces constituent d'excellents indicateurs de contaminations utilisables pour la biosurveillance grâce à leur fort potentiel de bioaccumulation. Elles appartiennent à de nombreux groupes taxonomiques dont les macrophytes, les mollusques, les bivalves, les gastéropodes, les polychètes, les crustacés et les échinodermes. (Richir & Gobert, 2016). Parmi les échinodermes, les oursins, et plus particulièrement *Paracentrotus lividus* (Lamarck, 1816) sont des candidats intéressants pour la bioindication des contaminations et ont largement été utilisé dans les études écotoxicologiques (Salvo *et al.,* 2014. Guendouzi *et al.,* 2017 ; Rouane-Hacene *et al.,* 2018).

3.3. Biomarqueurs

L'évaluation des niveaux de contaminations dans un écosystème ne permet pas d'estimer leurs effets sur les organismes, les populations et les communautés qui y sont présentes. La mesure de certains paramètres, regroupés sous le terme de « biomarqueurs » peut contribuer à la compréhension de ces effets. Un biomarqueur peut être défini comme un changement moléculaire, biochimique, cellulaire, physiologique ou comportemental, qui peut être mesuré dans différents compartiments de l'organisme mettant en évidence l'exposition et/ou les effets d'un ou plusieurs xénobiotiques (Depledge *et al.,* 1995 ; Lagadic *et al.,* 1998). Dans ce manuscrit, nous nous concentrerons sur les biomarqueurs phénotypiques et les biomarqueurs biochimiques.

3.3.1. Les biomarqueurs phénotypiques

Les contaminations peuvent conduire à de nombreux effets sur les organismes marins (Carballeira *et al.,* 2012). Afin d'évaluer et de comprendre ces effets, de nombreuses techniques basées sur l'observation morphologique ont été développées. Dans ce but, le développement embryo-larvaire de plusieurs espèces d'oursins a largement été utilisé depuis

les années 1950 (*e.g.* Lallier, 1956 ; Marin *et al.,* 1987 ; His *et al.,* 1999 ; Pétinay *et al.,* 2009). Les variations de différents paramètres tels que le développement des larves, leurs mesures biométriques, leur morphologie (Fig. 3) et leur taux de mortalité permettent d'estimer les éventuels impacts des xénobiotiques. Ces biomarqueurs morphologiques sont faciles à observer en laboratoire et, la plupart du temps, de moindre coût. Toutefois, en raison de plusieurs facteurs tels que les conditions hydrodynamiques, l'observation de ces biomarqueurs est plus difficile dans le cadre d'études *in situ*.

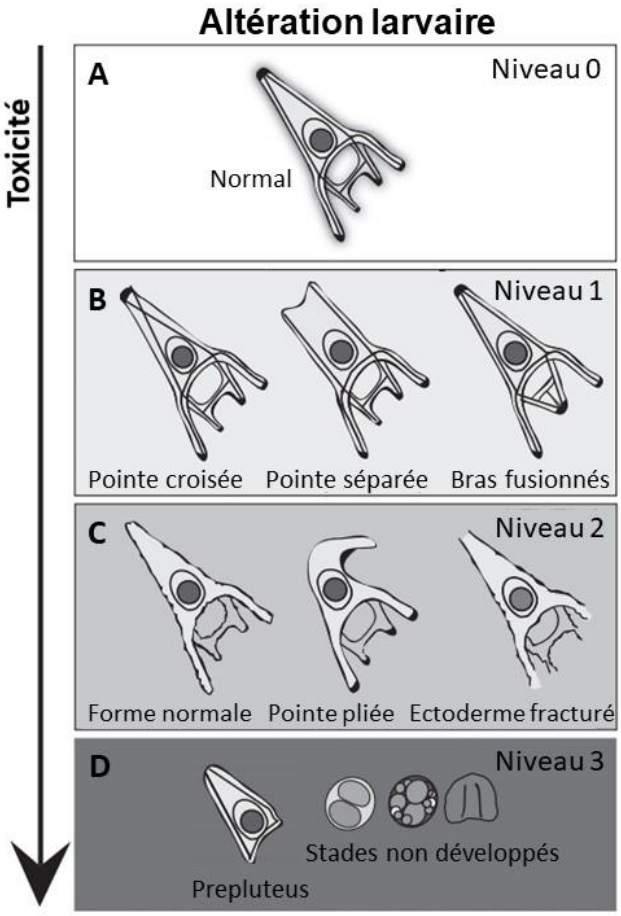
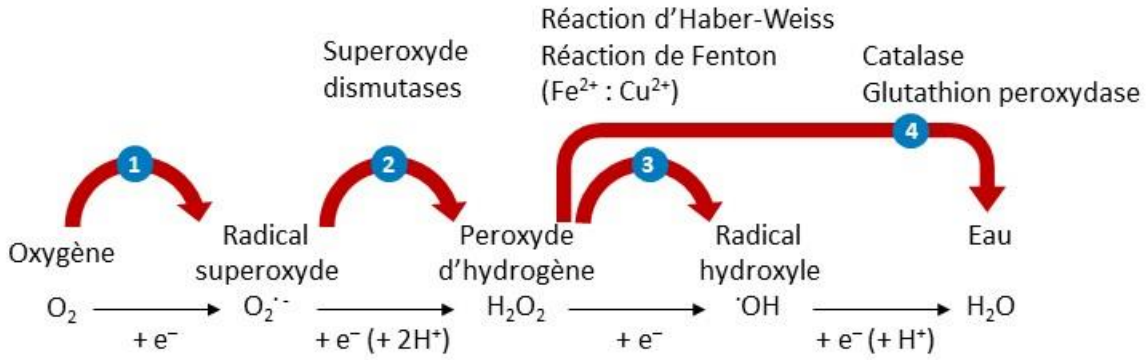


Figure 3. Classification des malformations larvaires chez l'oursin *P. lividus* en fonction du degré d'altération, afin d'établir la gravité de la toxicité. A : développement normal, B : localisation incorrecte des tiges squelettiques, C : tiges squelettiques incomplètes ou absentes, D : développement bloqué (modifié d'après Carbaillera *et al.,* 2012).

3.3.2. Les biomarqueurs biochimiques

Utilisé comme source d'énergie par son rôle au sein de la chaîne respiratoire mitochondriale, l'oxygène est indispensable aux organismes aérobies. Lors du métabolisme normal, la réduction tétravalente de l'oxygène en eau se fait en plusieurs étapes successives qui donnent naissance à des intermédiaires potentiellement réduits (Fig. 4). Les radicaux libres très réactifs, dérivés de l'oxygène, sont regroupés sous le terme d'Espèce Réactives de l'Oxygène (ERO) ou Reactive Oxygen Species (ROS). Ce sont des molécules produites physiologiquement en continu par les organismes aérobies (Sies, 1997 ; Mittler *et al.,* 2004 ; Del Río *et al.,* 2006 ; Navrot *et al.,* 2007). Elles interviennent dans de nombreux processus tels que la défense immunitaire, l'apoptose ou la régulation des gènes. Ces molécules peuvent aussi être le résultat de facteurs externes tels que les rayonnements UV, les changements thermiques et l'exposition aux contaminants (Lesser, 2006 ; Valavanidis *et al.,* 2006). L'interaction des radicaux libres avec les lipides, l'ADN ou les protéines peut conduire à leur altération et à la perte de leur activité au sein de la cellule (Halliwell & Gutteridge, 2015). En tant que producteur d'ERO, les contaminants tels que les éléments traces pourraient générer des réponses sévères et entraîner des dommages dans les organismes marins (Nieto *et al.,* 2010).

L'évaluation de certaines molécules permet d'estimer l'impact du stress oxydant sur l'organisme. Parmi ces molécules, on retrouve le peroxyde d'hydrogène (H_2O_2) obtenu à partir de l'anion superoxyde par dismutation spontanée ou par l'enzyme superoxyde dismutase (Fig. 4). Ce dernier peut être toxique et donner naissance, *via* des réactions de Fenton et d'Haber-Weiss, à la plus délétère des espèces radicalaires du stress oxydant, le radical hydroxyle •OH (Fig. 4). Les teneurs en malondialdéhyde (MDA) reflètent quant à eux, l'état de peroxydation des membranes cellulaires (Damiens *et al.,* 2004). En effet, le processus de peroxydation des lipides implique une chaîne de réaction conduisant à la décomposition des acides gras polyinsaturés et à la formation d'une grande variété de composés comme les radicaux lipidiques alcoxyles, des cétones, des alcanes, des époxydes et des aldéhydes, dont le MDA (Viarengo *et al.,* 1990 ; Valavanidis *et al.,* 2006 ; Almeida *et al.,* 2007).



Réduction tétravalente de l'oxygène		$O_2 + 4\,e^- + 4\,H^+ \rightarrow 2\,H_2O$
$\mathbf{O_2^{\cdot -}}$	Anion superoxyde	**1** $O_2 + 1\,e^- \rightarrow O_2^{\cdot -}$
$\mathbf{H_2O_2}$	Peroxyde d'hydrogène	**2** $O_2^{\cdot -} + O_2^{\cdot -} (+ 2\,H+) \rightarrow H_2O_2 + O_2$
$\mathbf{\cdot OH}$	Radical hydroxyle	**3** $H_2O_2 + Fe^{2+} \rightarrow {\cdot}OH + Fe^{3+} + OH^-$ **4** $H_2O_2 + H_2O_2 \rightarrow 2\,H_2O + O_2$ **5** $H_2O_2 + 2\,GSH \rightarrow 2\,H_2O + GSSG$

Figure 4. Exemple d'origine des espèces réactives de l'oxygène *via* la réduction tétravalente de l'oxygène en eau (modifié d'après Migdal & Serres, 2011).

La production d'ERO est régulée par des systèmes antioxydants. Les antioxydants sont des substances qui inhibent ou ralentissent l'oxydation d'un substrat (Mittler, 2002 ; Miller *et al.,* 2010 ; Migdal & Serres, 2011). Ils sont présents sous de nombreuses formes et peuvent intervenir en prévention de la formation des radicaux libres aussi bien que pour participer à leur élimination. De par leur grande valeur de diagnostics des contaminations environnementales, ces derniers peuvent être utilisés comme des biomarqueurs du stress oxydatif induit par des contaminants (Regoli *et al.,* 2002 ; Vlahogianni & Valavanidis 2007 ; Valavanidis *et al.,* 2006). Les enzymes antioxydantes telles que la superoxyde dismutase (SOD), la catalase (CAT), la glutathion peroxydases (GPX) et glutathion-s-transférase (GST) font partis des principaux biomarqueurs de défense (Mittler, 2002 ; Miller *et al.,* 2010). La SOD est une métalloenzyme capable de catalyser la conversion de l'ion superoxyde en molécule de peroxyde d'hydrogène et d'oxygène. Il s'agit de l'une des premières lignes de défense contre les ERO (Li *et al.,* 2009 ; Fig. 4). La CAT, quant à elle, cible le H_2O_2 afin de prévenir la peroxydation des molécules biologiques (Roméo *et al.,* 2000). Cette dernière, à l'aide de

l'atome de fer, va catalyser la transformation de $2H_2O_2$ en eau ($2H_2O$) et en oxygène (O_2 ; Ko *et al.,* 2000 ; Fig. 4). La GPX permet également de réduire le H_2O_2 grâce à l'oxydation d'un substrat réducteur, le glutathion (Ursini *et al.,* 1995 ; Fig. 4). Enfin, la GST est une enzyme de désintoxication dont la fonction est de conjuguer le glutathion à des composés électrophiles par formation d'un pont thioéther (Foureman, 1989). Les produits sont ensuite métabolisés en acide mercapturique puis excrétés dans la bile ou l'urine (Roméo & Giambérini, 2008).

Les mécanismes de défense antioxydants sont cruciaux pour que les organismes aérobies combattent les oxyradicaux tout en adoptant l'oxygène comme accepteur d'électrons pour une production d'énergie efficace. A l'heure actuelle, le stress oxydatif est défini comme un déséquilibre entre la production de pro-oxydant et la capacité de défense antioxydante d'un organisme (Sies, 1997 ; Durackova *et al.,* 2008 ; Limón-Pacheco & Gonsebatt, 2009).

4. L'oursin *Paracentrotus lividus*

4.1. Ecologie

Paracentrotus lividus, communément appelé oursin violet ou oursin comestible, appartient à l'embranchement des échinodermes. Réparti dans toute la mer Méditerranée, ce dernier est également présent au niveau de la Manche et au Nord-Est de l'Atlantique, de l'Ecosse jusque sur les côtes du Maroc, y compris en Irlande, aux Canaries et aux Açores (Harvey, 1956 ; Tejada *et al.,* 2013). Particulièrement abondant dans les régions où la température de l'eau varie entre 10 et 15 °C en hiver et 18 et 25 °C en été, son aire de répartition naturelle est délimitée par l'isotherme 8 °C en hiver et 28 °C en été (Boudouresque & Verlaque, 2013). En mer Méditerranée, *P. lividus* vit dans l'étage infralittoral, notamment dans ses hauts niveaux (entre 0 et 30 m de profondeur ; Mortensen, 1927 ; Nédélec, 1982), affectionnant particulièrement la zone intertidal (Grosjean, 2001). Sa distribution est très vaste puisqu'il occupe aussi bien les peuplements photophiles de substrat rocheux, qui lui assurent une protection contre les vagues et la prédation (Verlaque, 1987), que les herbiers à Posidonia oceanica (Linnaeus) Delile, 1813 et à Zostera marina (Linnaeus, 1753) Verlaque, 1987 jouant un rôle primordial dans la dynamique des peuplements superficiels (Lawrence, 1975 ; Verlaque, 1987 ; Azzolina, 1988). Certains individus peuplent également les lagunes

méditerranéennes comme l'étang de Thau (San Martin, 1987) ou encore l'étang d'Urbino (Fernandez & Caltagirone, 1998), principalement sur des substrats vaseux ou sur du sable grossier (Fernandez *et al.,* 2006, 2012). Au sein de son aire géographique, les densités de l'oursin violet sont principalement comprises entre quelques individus et une douzaine d'individus par mètre carré. Ce dernier présente souvent des mouvements migratoires quotidiens à petite échelle entre les abris qui offrent des refuges contre les prédateurs et les zones qui constituent une source de nourriture (Barnes & Crook, 2001). Son déplacement net sur 24 h est compris entre 0 et 260 cm, soit 50 cm en moyenne (Shepherd & Boudouresque, 1979 ; Dance, 1987).

4.2. Morphologie, anatomie et physiologie

Paracentrotus lividus est un oursin régulier possédant un squelette dermique (test), discontinu, composé de plaques juxtaposées qui portent des épines (Fischer *et al.,* 1987). Le diamètre des individus les plus gros peut atteindre 7,5 cm (*e.g.* Boudouresque *et al.,* 1989 ; Lozano *et al.,* 1995). C'est une espèce benthique possédant une symétrie pentaradiée dont la forme globuleuse comprend une face aborale et une face orale orientée vers le substrat. La face orale porte l'appareil buccal entouré par une membrane, le péristome, et renfermant la lanterne d'Aristote formée par cinq mâchoires pyramidales. La face aborale, quant à elle, comprend la madréporite, assurant la communication entre le milieu extérieur et le système aquifère, et l'anus, entouré par une membrane nommée périprocte. Ce pôle contient également les cinq pores génitaux qui permettent l'émission des gamètes et les pores aquifères qui assurent la sortie de l'eau. *P. lividus* est pourvu d'un système nerveux rudimentaire diffus, associé à l'épiderme (Fig. 5).

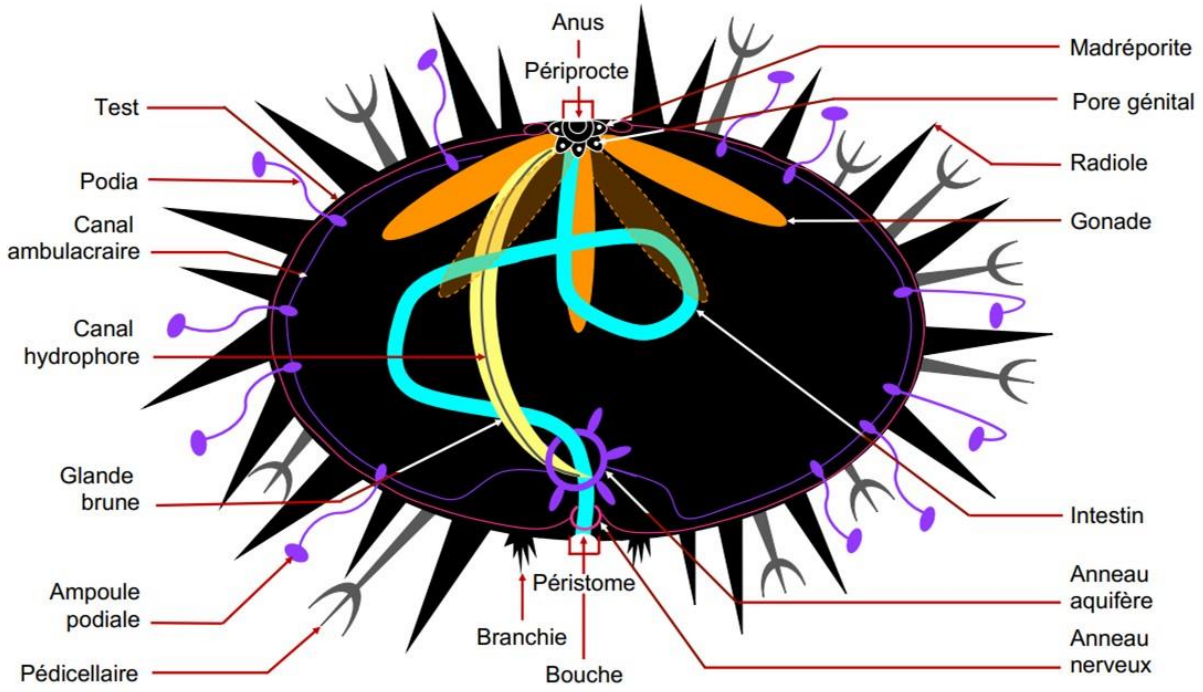


Figure 5. Schéma simplifié représentant la morphologie et l'anatomie interne de *P. lividus* (d'après Duchaud, 2018).

Il possède une cavité interne ou cavité cœlomique comportant notamment l'appareil masticateur, le tube digestif, les appareils ambulacraire et génital, la glande brune et le liquide cœlomique (Fig. 5). Ce derniers est en majeur partie composé par de l'eau de mer. Le système aquifère parfois qualifié d'appareil ambulacraire, permet la circulation de l'eau dans la cavité de l'oursin assurant ainsi les fonctions de locomotion, de défense et de respiration (Fig. 5). Cette dernière fonction est également réalisée par cinq petites branchies situées au niveau du péristome. Le système digestif débute par une cavité buccale portant la lanterne d'Aristote et se poursuit avec un œsophage vertical puis incurvé, un estomac en forme de boucle, un intestin également enroulé et un rectum vertical s'ouvrant sur l'anus (Fig. 5). L'oursin est dépourvu de système excréteur différencié et l'élimination des déchets s'effectue au niveau de l'épiderme par des cellules spécialisées, les amibocytes. La glande brune présente dans le système hémal pourrait également jouer un rôle en accumulant les déchets issus de la phagocytose (Fig. 5).

4.3. Alimentation

Les préférences alimentaires dépendent de la taille. Ainsi, les juvéniles (~ 1 mm de diamètre) broutent des macrophytes encroûtantes et endolithiques, par la suite, les oursins (~ 3 à 7 mm) se nourrissent de macroalgues filamenteuses puis (~ 7 à 10 mm) de macrophytes grossièrement ramifiées. Le régime alimentaire des adultes est atteint à partir d'environ 10 mm de diamètre (Verlaque & Nédélec, 1983 ; Verlaque, 1987). Un certain nombre d'espèces sont préférées comme *Rissoella verruculosa*, *Cymodocea nodosa*, *Cystoseira amentacea*, *Padina pavonica* et *Undaria pinnatifida*. D'autres sont fortement évitées comme *Asparagopsis armata*, *Gelidium spinosum*, *Anadyomene stellata*, *Caulerpa prolifera* et *Flabellia petiolata*. Le choix alimentaire dépend de l'abondance relative des aliments disponibles (Lawrence, 1975 ; Verlaque & Nédélec 1983 ; Frantzis *et al.*, 1988). Le contraste entre les espèces « préférées » et « évitées » est plus net pour les petits individus (20 à 25 mm) que pour les grands individus (>35 mm ; Traer, 1980 ; Nédélec, 1982 ; Verlaque, 1987). Bien que les macrophytes constituent la principale ressource alimentaire, *P. lividus* est une espèce opportuniste et peut devenir omnivore dans des conditions de ressources limitées (Mazzella *et al.,* 1992). Ce dernier joue un rôle majeur dans l'abondance et la répartition des communautés benthiques influencées à la fois par ses préférences alimentaires et son abondance (Sala *et al.,* 1998 ; Barnes *et al.,* 2002). En broutant les macrophytes endolithiques, les oursins arrachent les particules de roche et deviennent des agents d'érosion biologique (Torunski, 1979 ; Schneider & Torunski, 1983). En effet, la croissance constante de leurs dents (1 à 1,5 mm par semaine) nécessite que *P. lividus* broute le substrat pour les éroder (Boudouresque & Verlaque, 2013).

4.4. Cycle de reproduction

Paracentrotus lividus est une espèce gonochorique et ovipare ne présentant pas de dimorphisme sexuel (Neefs, 1937 ; Byrne, 1990 ; Zhang & Shear, 2007). L'appareil reproducteur des oursins est constitué de cinq gonades reliées aux cinq pores génitaux par des canaux excréteurs. L'émission des gamètes s'effectue par les pores génitaux visibles sur la partie aborale. Pendant la période de reproduction, le volume des gonades augmente en raison de l'augmentation du nombre et de la taille des cellules germinales et de la croissance

des cellules somatiques qui stockent d'importantes réserves nutritives nécessaires à la gamétogénèse (Walker *et al.,* 2013).

Le cycle de reproduction des oursins est un processus bien étudié depuis de nombreuses années (*e.g.* Spirlet *et al.,* 1998 ; Guettaf *et al.*, 2000 ; Ouchene *et al.,* 2021). L'indice gonadosomatique ou indice gonadique (IG), qui est le rapport de la masse des gonades à la masse totale de l'oursin, est largement étudié pour évaluer les changements saisonniers dans le cycle gamétogène et a produit de nombreuses études dans la littérature (*e.g.* Sánchez-España *et al.,* 2004 ; Shpigel *et al.,* 2004 ; Keshavarz *et al.,* 2017). Possédant un cycle de reproduction annuel, *P. lividus* présente un seul événement de ponte selon certains auteurs (Byrne, 1990 ; Lozano *et al.,* 1995 ; Spirlet *et al.,* 1998 ; De La Uz *et al.,* 2018), tandis que d'autres soutiennent l'hypothèse selon laquelle deux pontes peuvent survenir en une année (Crapp & Willis, 1975 ; Pedrotti, 1993 ; Ouchene *et al.,* 2021).

Divers facteurs environnementaux tels que la température (Byrne, 1990, Lozano *et al.,* 1995), la photopériode (Byrne, 1990 ; Lozano *et al.,* 1995 ; Sphigel *et al.*, 2004), les conditions hydrodynamiques (Guettaf *et al.,* 2000) et la disponibilité trophique (Lozano *et al.,* 1995 ; Guettaf *et al.,* 2000) influencent le cycle reproducteur (Duchaud *et al.,* 2021). Au sein d'une même région, le nombre de ponte annuel peut varier entre des sites et des habitats différents (Lozano *et al.,* 1995 ; Guettaf *et al.,* 2000 ; Sánchez-España *et al.,* 2004). L'analyse histologique permet de suivre l'évolution des cellules germinales et du tissu de réserve au cours du processus de gamétogenèse (Fig. 6). Ce dernier est constitué de différentes phases se succédant selon un ordre précis (Spirlet *et al.,* 1998) qui constituent le cycle reproducteur de l'organisme (Fuji, 1960 ; Fig. 6). L'étude histologique des gonades est très utilisée pour l'étude de la reproduction des populations d'oursins (Byrne, 1990 ; Guettaf *et al.,* 2000 ; De La Huz *et al.,* 2018) et permet de préciser les informations données par l'indice gonadique.

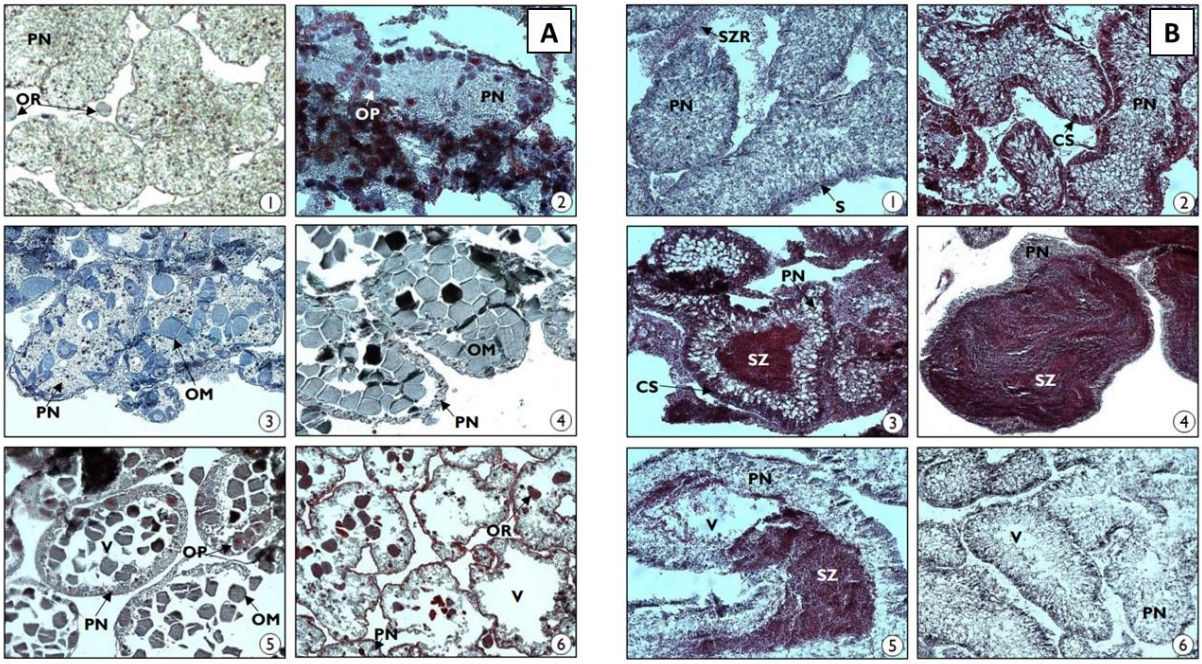


Figure 6. Micrographie réalisé sous microscope optique de coupes histologiques de gonades femelles (A) et mâles (B) de *P. lividus* au stade 1 : récupération (× 50), stade 2 : croissance (× 200), stade 3 : pré-mature (× 100), stade 4 : mature (× 100), stade 5 : début de la ponte (× 100), et stade 6 : fin de la ponte (× 100). PN : phagocytes nutritifs, OR : ovocytes résiduels, OP : ovocytes primaires, OM : ovocytes mûrs, V : vide dans la lumière de l'acinus, S : spermatocytes, SZR : spermatozoïdes résiduels, CS : colonnettes de spermatocytes, SZ : spermatozoïdes (© STELLA MARE).

4.5. Cycle de développement

Avec en moyenne un million d'œufs par femelle, l'abondance des œufs dépend généralement de la taille des gonades. Les spermatozoïdes, libérés dans l'eau, nagent vers les ovocytes guidés par chimiotaxie (Kaupp *et al.,* 2006). Les pronucléi se rencontrent, fusionnent et la première mitose est déclenchée. Le premier clivage a lieu environ 90 minutes après la fécondation (Fig. 7). L'axe animal-végétal est déjà établi dans les œufs non fécondés par une distribution asymétrique des composants cellulaires (Romancino *et al.,* 2001 ; Romancino *et al.,* 2004). À la fin de la période de clivage (Fig. 7), chaque blastomère porte un cil mobile battant pour générer un courant et se déplacer dans le milieu. L'invagination de l'endoderme permet l'obtention de la gastrula qui acquiert une forme conique à symétrie bilatérale possédant deux sacs cœlomiques (droit et gauche). L'extrémité de l'intestin fusionne avec le futur ectoderme (Fig. 7). Pendant ce temps, les cellules mésenchymateuses primaires entourent l'intestin, fusionnent pour former un syncytium et commencent la spiculogenèse

qui forme la larve pluteus. Le développement embryonnaire représentatif de l'oursin est illustré dans la Fig. 7.

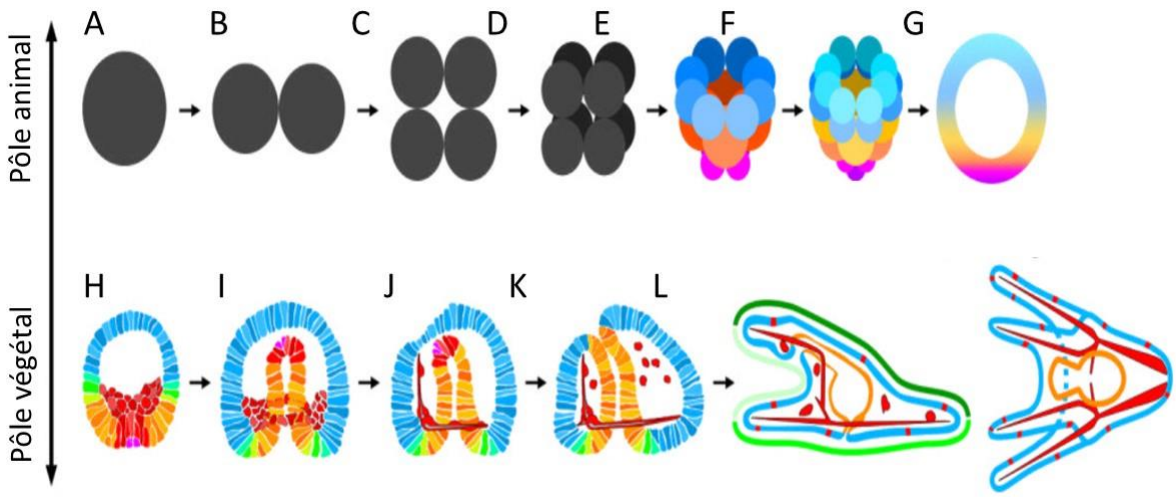


Figure 7. Schéma du développement classique d'un oursin (*P. lividus*), de la fécondation à la larve pluteus. A-F : période de clivage, G : blastulation, H-I : gastrulation, J-K : fusion de l'intestin avec le futur ectoderme, L : spiculogénèse ; bleu : mésomères, orange : macromères, rose-violet : micromères, rouge : cellules mésenchymateuses primaires, vert clair : oral/ventral, vert foncé : aboral/dorsal (modifié d'après Banks, 2014).

Des larves pélagiques font suite au développement embryonnaire et sont appelées *echinoplutei* (McEdward & Miner, 2001). Quatre bras sont formés, deux bras antéro-latéraux et deux bras post-oraux (Fig. 8A). Les deux paires de bras sont séparés par une bande de cellules différenciées formant la bande ciliaire qui permet d'amener la nourriture à la bouche et contribue aux mouvements de la larve. Les larves sont autonomes, nagent et se nourrissent au sein du zooplancton et leur morphogenèse se poursuit, notamment au niveau du cytosquelette. En fonction du nombre de bras, on distingue trois stades larvaires : le stade « 4 bras », le stade « 6 bras » et le stade « 8 bras ». Le premier stade acquiert la capacité de se nourrir lors de l'ouverture de la bouche et de la formation du système digestif. Le stade « 6 bras » apparaît lors de l'acquisition d'une troisième paire de bras par la larve : les bras postéro-dorsaux (Fig. 8B). Les sacs cœlomiques se divisent en trois, donnant 2 antérieurs, 2 intermédiaires et 2 postérieurs. Le sac intermédiaire gauche devient ensuite l'hydrocœle, futur système aquifère de l'adulte. Enfin, le stade « 8 bras » est caractérisé par l'apparition des bras pré-oraux (Fig. 8C). Parallèlement, l'épiderme au contact de l'hydrocœle s'invagine et ébauche une poche destinée à englober l'hydrocœle. Cette structure nommée rudiment

devient alors pentagonal et est porteur de quelques radioles et de cinq podias ; à ce stade, la larve est dite compétente (Hinegardner, 1969). La larve va alors migrer vers le fond à la recherche d'un substrat afin d'initier sa métamorphose qui représente une étape clé du cycle de développement (Hadfield & Paul, 2001). Les tissus larvaires ne participant pas à la métamorphose sont absorbés par le rudiment (Chino *et al.,* 1994). Une fois métamorphosée, la post-larve de quelques microns n'a pas encore de tube digestif et survit grâce aux ressources acquises au cours de ses phases larvaires (Magesky *et al.,* 2016 ; Fig. 8D). Après de nouveaux changements au niveau de son système interne avec l'ouverture de la bouche et de l'anus et la formation de son système digestif, cette dernière devient un juvénile indépendant de 0,5 à 1 mm (Fig. 8E).

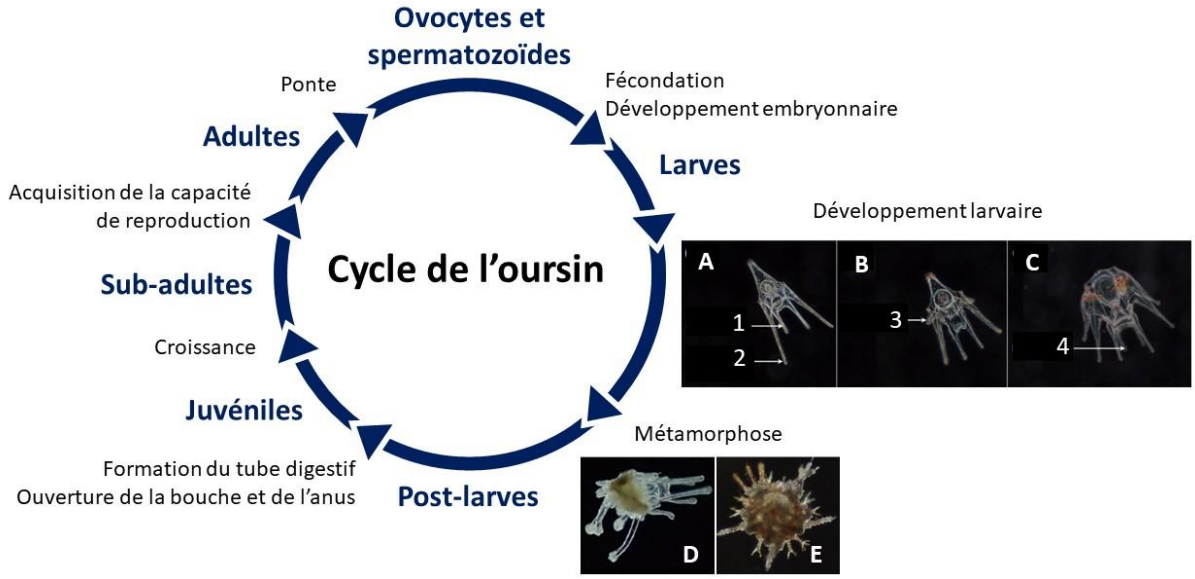


Figure 8. Schéma des différents stades du cycle de développement de *P. lividus*. A-C : stade larvaire, grossissement × 40 (A : stade 4 bras, B : stade 6 bras, C : stade 8 bras, 1 : bras antéro-latéral, 2 : bras post-oral, 3 : bras postéro-dorsal, 4 : bras pré-oral) ; D : stade post-larvaire, grossissement × 40 ; E : stade juvénile, grossissement × 20.

4.6. Statut de bioindicateur

Emblématique dans les régions méditerranéennes, *P. lividus* est une espèce d'importance économique et écologique. Ce dernier a une haute valeur commerciale (Cook & Kelly, 2007 ; Fernández-Boán *et al.*, 2013 ; Bertocci *et al.,* 2014 ; Sun & Chiang, 2015) et représente une ressource complémentaire pour la pêche artisanale. La qualité gustative de ses gonades en fait également une espèce appréciée et ciblée par la pêche récréative

(Stefansson *et al.,* 2017). Organisme opportuniste, l'oursin joue un rôle clé dans les écosystèmes marins, contrôlant par son activité de broutage la dynamique, la structure et la composition des algues et des herbiers marins (Tomas *et al.,* 2004). Il est également un élément crucial du réseau trophique en tant que proies de plusieurs organismes marins tels *Diplodus sargus* (Linnaeus, 1758), *Labrus merula* (Linnaeus, 1758), *Maja crispata* (Risso, 1827) et *Trunculariopsis trunculus* (Linnaeus, 1758 ; Tertschnig, 1989 ; Hereu *et al.*, 2005). Ainsi, des changements dans leur survie ou leur croissance peuvent entraîner des changements à l'échelle de l'écosystème (Dupont *et al.*, 2010).

L'oursin violet est un organisme reconnu pour son rôle de bioindicateur (Warnau *et al.,* 1998 ; Geraci *et al.,* 2004). En effet, en raison de sa large répartition, son abondance naturelle, sa longévité, sa relative sédentarité, sa facilité d'échantillonnage, sa disponibilité toute l'année, son excellente capacité d'accumulation, sa bonne tolérance aux contaminants et son comportement benthique, les oursins possèdent les caractéristiques recherchées chez un bioindicateur (Pancucci *et al.,* 1993 ; Soualili *et al.,* 2008). De plus, *P. lividus* montre une grande sensibilité à une variété de contaminant ainsi qu'un large éventail de réponses adaptatives aux conditions environnementales telles que la température, la nourriture et l'action des vagues (Bayed *et al.*, 2005). Cette espèce est sensible aux polluants tels que les éléments traces (Gharred *et al.,* 2016 ; Guendouzi *et al.*, 2017 ; Bonaventura *et al.,* 2018), les pesticides (Levert *et al.,* 2018), les perturbateurs endocriniens (Bošnjak *et al.,* 2011 ; Tato *et al.,* 2018), les antibiotiques (Gharred *et al.,* 2016), les hydrocarbures aromatiques polycycliques (Bellas, 2008 ; Rocha *et al.,* 2018) ainsi que les agents physiques (*e.g.* rayons X, rayons ultra-violets) (Matranga *et al.,* 2010 ; Bonaventura *et al.,* 2011). Son régime alimentaire peut également fournir des renseignements précieux sur les transferts trophiques de différents contaminants (Warnau *et al.,* 1996 ; Den Besten *et al.,* 2001).

Ainsi, depuis de nombreuses années, les oursins adultes sont largement étudiés pour évaluer les niveaux de contamination en éléments traces dans les écosystèmes côtiers (*e.g.* Guendouzi *et al.,* 2017 ; Rouhane-Hacene *et al.,* 2018). Bien que cela permette de déterminer le degré et la nature de la contamination, peu d'indication concernant les conséquences biologiques n'ont été apportée (Chapman & Long, 1983). Les bioessais permettent de détecter ces effets en mesurant les réponses biologiques des organismes marins (Fernandez & Beiras, 2001). Les

gamètes, embryons et larves d'échinodermes sont parmi les outils biologiques les plus largement utilisés pour l'évaluation de la qualité des eaux côtières (Daby, 2006). Cela est notamment dû à leur disponibilité, leur accessibilité et leur réactivité, mais également au faible coût de la plupart des bioessais. De plus, les embryons et larves d'oursins sont faciles à manipuler et se développent rapidement avec une relative synchronie. Ils sont faciles à élever dans des conditions de laboratoire et permettent ainsi une réplicabilité élevée. Ces derniers sont également transparents et donc adaptés à la détection microscopique des effets des contaminants sur le développement (Kobayashi & Okamura, 2005 ; Zito *et al.,* 2005 ; Bellas, 2008 ; Bosnjak *et al.,* 2011 ; Bonaventura *et al.,* 2011). Enfin, les oursins étant beaucoup utilisés dans les recherches sur de nombreux processus génériques tels que la fécondation, la division cellulaire et l'embryogenèse, leur développement est aujourd'hui bien connu ce qui est un atout considérable pour comprendre les effets des contaminants.

Ainsi, en tant qu'espèce clé bien représentée dans l'écosystème marin planctonique et benthique aux stades larvaires et adulte, respectivement, et étant très sensible aux variations des conditions ambiantes, les oursins peuvent être utilisés pour évaluer les niveaux de contamination et leur effet sur les organismes (Kobayashi, 1971).

5. Objectifs de la thèse

La surexploitation des ressources, la dégradation, la fragmentation et les pertes d'habitats sont autant de facteurs responsables de l'érosion de la biodiversité marine. Exacerbée par la pollution des écosystèmes côtiers, l'anthropisation menace de détruire l'équilibre fragile des écosystèmes marins et de la biodiversité qu'ils renferment. Parmi les contaminants, les éléments traces sont considérés comme des polluants préoccupants nécessitant une surveillance accrue. C'est pourquoi cette thèse s'articule autour de deux axes principaux :

(i) Évaluer les niveaux de contamination en éléments traces dans le milieu marin grâce à l'oursin *P. lividus* ;

(ii) Définir les effets de ces contaminants sur cet organisme clé de l'écosystème méditerranéen.

Ces axes correspondent aux deux chapitres présentés dans ce manuscrit. Les travaux réalisés dans ces chapitres ont été rédigés en anglais sous la forme d'articles scientifiques publiés ou en cours de publications.

Afin de répondre aux objectifs du premier axe, trois études complémentaires ont été réalisées sur les oursins adultes et constituent le **premier chapitre** (Fig. 9).

- La première vise à évaluer les niveaux de contaminations en Corse afin d'effectuer une comparaison à l'échelle de la Méditerranée mais aussi à identifier les éventuelles sources de dispersion des éléments traces ;
- La deuxième étude permet de comprendre les variations spatio-temporelles des niveaux de contamination dans les gonades d'oursins. Elle contribue également à déterminer les principaux éléments traces susceptibles d'être bioaccumulés et l'importance des facteurs biotiques et abiotiques dans les études écotoxicologiques ;
- Enfin, la troisième évalue les niveaux de contamination en éléments traces grâce à l'utilisation de différentes matrices (oursin, macroalgues, colonne d'eau et sédiment). Cette dernière vise également à estimer l'efficacité de plusieurs indices utilisés dans l'évaluation des contaminations. Ce travail permet également de commencer à

répondre aux objectifs du deuxième axe en évaluant les effets de ces contaminations sur sur le stress oxydatif de l'oursin adulte.

Le **deuxième chapitre** porte sur les effets des contaminations sur les stades embryolarvaires des oursins (Fig. 9).

- L'objectif de la première étude est de déterminer les effets d'une exposition chronique à un mélange d'éléments traces sur le développement embryolarvaire de *P. lividus* ;
- La seconde permet, quant à elle, d'évaluer la capacité des stades larvaires à répondre au stress induit par ces contaminations.

  Ce chapitre a également pour vocation de comprendre dans quelle mesure la contamination des géniteurs affecte le développement des larves dans les zones contaminées.

Enfin, l'ensemble des résultats est discuté dans la partie **synthèse et perspectives**, dans laquelle nous résumons les avancées scientifiques de ce travail concernant la compréhension des contaminations en éléments traces chez l'oursin ainsi que les perspectives de recherche à développer.

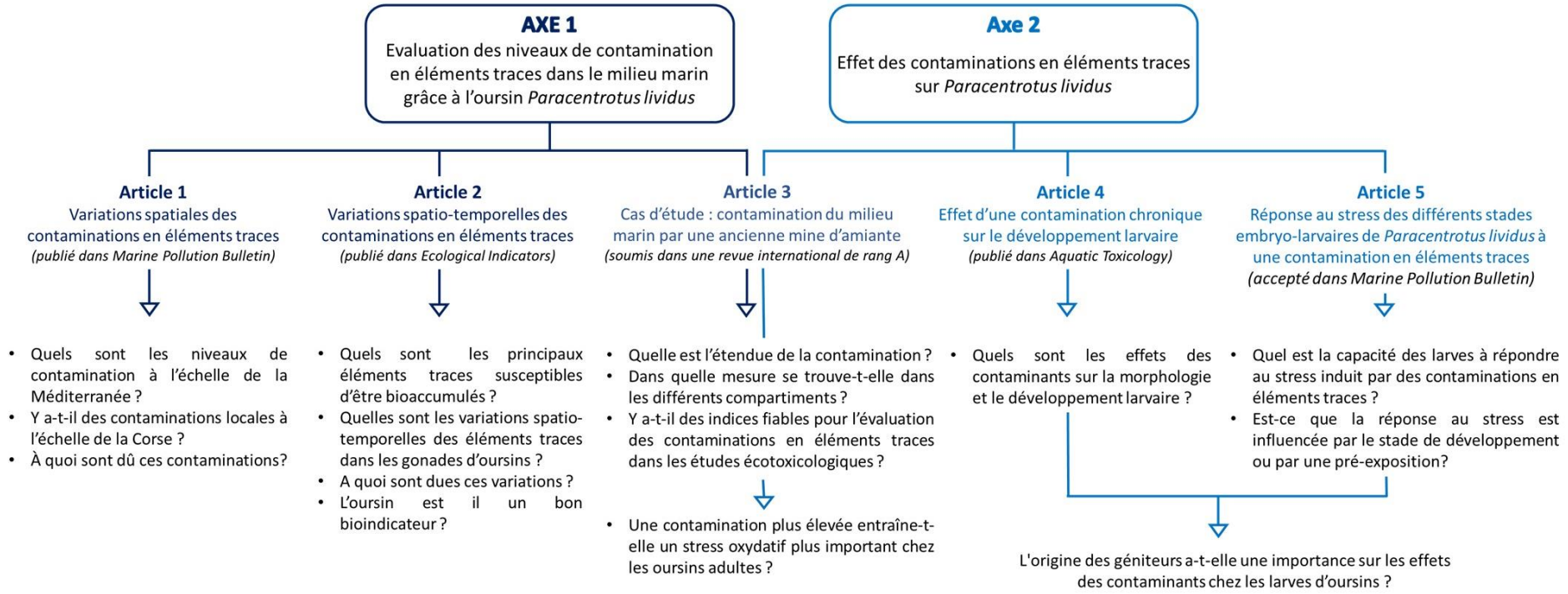


Figure 9. Objectifs et problématiques de la thèse.

CHAPITRE 2

CARACTERISATION ET VARIATIONS SPATIO-TEMPORELLES DES CONCENTRATIONS EN ELEMENTS TRACES CHEZ L'OURSIN *PARACENTROTUS LIVIDUS*

Plan et objectifs du chapitre

Le chapitre 2 s'appuie sur l'utilisation de l'oursin violet *P. lividus* pour évaluer les contaminations en éléments traces dans le milieu marin. Il est composé de trois articles scientifiques.

- **L'article 1** étudie les variations spatiales des concentrations en éléments traces à l'échelle de la Corse en comparaison avec le reste de la Méditerranée ;

- **L'article 2** aborde les variations saisonnières des concentrations en éléments traces chez *P. lividus* et l'utilisation de ce dernier comme bioindicateur ;

- **L'article 3** vise à évaluer la contamination et ses effets sur *P. lividus* à l'aide de plusieurs approches : indices de pollution, facteurs d'accumulation et outils biochimiques.

Pour un confort de lecture, ces publications sont précédées d'un résumé en français. Dans un souci de mise en forme, l'ensemble des textes des articles publiés et acceptés ont été mis au format de la thèse.

Article 1

Spatial variations in trace element concentrations of the sea urchin, *Paracentrotus lividus*, a first reference study in the Mediterranean Sea

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Contexte et résumé de l'article 1 « Variations spatiales des concentrations en éléments traces chez l'oursin, *Paracentrotus lividus*, un première étude de référence en mer Méditerranée »

La croissance des activités industrielles, agricoles et urbaines génère l'introduction d'une quantité considérable de produits chimiques dans l'écosystème marin. Ces substances présentent des propriétés toxiques susceptibles de causer des dommages multiples au niveau des organismes, des populations et des écosystèmes. En raison de ses côtes fortement peuplées, la mer Méditerranée est soumise à de nombreuses pressions anthropiques parmi lesquelles figure la pollution par les éléments traces. Au cœur de celle-ci, la Corse est un atelier idéal pour suivre les concentrations des contaminants dans le cadre du développement des zones côtières. Afin d'obtenir des informations sur la qualité environnementale des eaux marines autour de l'île et d'identifier les zones de contamination locale, des prélèvements d'oursins ont été réalisés en hiver dans les quatre prud'homies corses. Deux sites ont été définis dans chaque prud'homie divergeant par leurs caractéristiques écologiques et leur degré d'anthropisation supposé : un site référence, choisi pour sa distance de toute source de contamination et supposé avoir un bon état écologique, et un site à proximité de sources anthropiques identifiées (émissaires de stations d'épuration, ports de commerce et ancienne mine d'amiante) et donc supposé contaminé. Les gonades ont été prélevées et des analyses à l'ICP-MS ont été effectuées dans le but de déterminer les éléments traces classiques et émergents susceptibles d'être bio-accumulés par l'oursin *Paracentrotus lividus*. Dans un ordre croissant, les concentrations en éléments traces dans les gonades suivent la séquence suivante : Zn > Fe > As > Al > Cu > V > Mn > Se > Cr > U > Ni > Co > Mo > Li > Ba > Cd > Pb > Sb > Ag > Sn. L'indice « Trace Element Pollution Index » (TEPI) a ensuite été calculé afin de déterminer les niveaux de contamination autour de la Corse en hiver. Un des niveaux de contamination les plus élevé est située proche de l'ancienne mine d'amiante de Canari avec des éléments traces (cobalt, chrome et nickel) mesurés en fortes quantités, caractéristiques du site et indiqué par un indice « Trace Element Spatial Variation Index » (TESVI) élevé. Enfin, une comparaison des niveaux de contamination à l'échelle méditerranéenne a décrit des niveaux de contaminations en Corse équivalant à d'autres sites anthropisés de la Méditerranée.

1. Introduction

Human activities have caused a considerable increase of potentially toxic discharges into the marine environment. This has led to a decline in species diversity and negative consequences for human health (Salomidi *et al.*, 2012; Belabed *et al.*, 2013). The presence of trace elements in marine ecosystems is growing. There are two potential sources of emissions: anthropogenic and naturally occurring (Serrano *et al.*, 2011). Due to their toxicity, persistence, and ability to accumulate in marine organisms, trace elements are considered a major source of pollution in the marine environment (Bonanno and Di Martino, 2017).

Bioindicators are commonly used to monitor marine environment quality. They allow evaluation of the levels of biologically available contaminants in the environment (Campanella *et al.*, 2001). The concentrations found indicate the type, nature, and exposure time of these contaminants to organisms in the study area (Morrison *et al.*, 2017). Based on this method, many classification tools have been developed to characterize the overall quality of water bodies and the health status of coastal ecosystems (Lopez y Royo *et al.*, 2009; Richir and Gobert, 2014). It turns out that several species have specific criteria used to assess the contamination status of marine ecosystems which makes them good bioindicators (Zhou *et al.*, 2008; Richir and Gobert, 2016). Among them, the sea urchin *Paracentrotus lividus* (Lamarck, 1816) is considered as a sentinel species. It has often been used as a tool to assess marine pollution of coastal ecosystems (Gharred *et al.*, 2015; Guendouzi *et al.*, 2017; Soualili *et al.*, 2008; Warnau *et al.*, 1998). This marine invertebrate is a relevant biological model for the study of environmental pollution. It is recognized as a bioindicator for several reasons: its ecological relevance, benthic and relatively sedimentary lifestyle, rapid response, and high sensitivity to many types of contaminants (Ruocco *et al.*, 2016; Salvo *et al.*, 2016; Scanu *et al.*, 2015). These qualities allow the comparison of marine trace elements contamination on both the local and the international scale. The Corsica Island is often considered a pristine region (largely due to its low industrialization rate); the quality of its waters and its low fishing pressure give it a reference site status in the Mediterranean (Galgani *et al.*, 2006; Gobert *et al.*, 2017). However, there is very little data available on pollution levels in this region.

Therefore, the aim of this study was to: (i) assess 20 trace elements contaminating the Corsican coastline using *P. lividus* as a bioindicator; (ii) track observed contamination levels to possible sources; (iii) obtain background levels on the local scale and complete existing databases; and (iv) perform intraspecific comparisons with previous worldwide studies (industrialized and non-industrialized areas).

2. Material and methods

This study was carried out along the Corsican coastline (western Mediterranean Sea) in four coastal areas (Ajaccio, Bonifacio, Calvi, and Saint-Florent) between February and March 2017. In each area, two stations were sampled (Fig. 1). In view of the possible sources of contamination, the main characteristics used for choosing the sampling zones were differences in types and levels of contamination assumed.

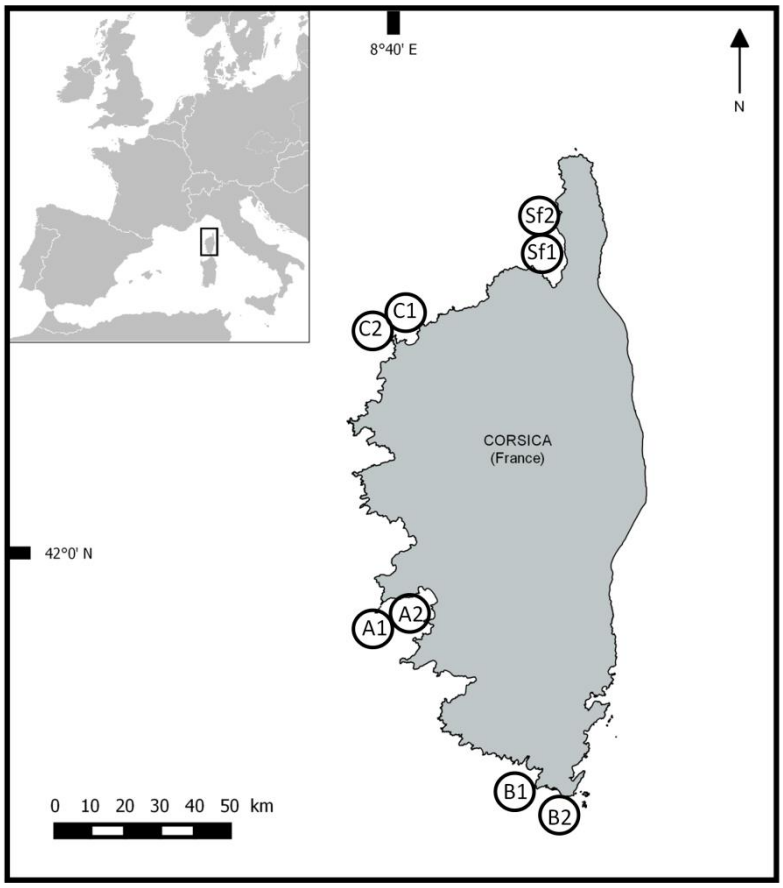

Figure 1. Location of study coastal areas in Corsica (NW Mediterranean, France), showing the eight sampling stations of *P. lividus* (A: Ajaccio; B: Bonifacio, C: Calvi; Sf: Saint-Florent), 1 reference site, 2 impacted site.

The sampling was designed to characterize the coastline in order to compare local values with previously reported data from other Mediterranean areas (Guendouzi *et al.*, 2017; Soualili *et al.*, 2008; Storelli *et al.*, 2001; Warnau *et al.*, 1998). The analysis of this information has contributed to the ranking of sampling sites with regard to the environmental quality. The sampling points were localized to cover several environmental parameters and anthropogenic pressures (wastewater treatment plant, commercial harbor, marina, and a former asbestos mine; Fig. 1).

Divers collected specimens of *P. lividus* from natural populations, with a minimum of fifteen individuals taken at each station, resulting in a total of 120 individuals collected. Until analysis, the gonads were stored at −20 °C. Gonads were cleaned with ultrapure water before analysis. The samples were mineralized in Teflon digestion vessels, in a closed microwave digestion LabStation (Ethos D. Milestone Inc.), using nitric acid and hydrogen peroxide as reagents (Suprapur® grade, Merck).

Analyses of 20 trace elements (Ag, Al, As, Ba, Cd, Co, Cr, Cu, Fe, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, U, V, and Zn) were determined by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, PerkinElmer®), according to the method described by Richir and Gobert (2014). In order to check the purity of the chemicals used, a number of chemical blanks were run; there was no evidence of any contamination in these blanks. Analytical quality control was achieved using Certified Reference Materials (CRM), DOLT-5: Dogfish Liver; DORM-4: Fish protein; NIST 1566b: Oyster; and NIST 2976: Mussel tissue. A high level of agreement was obtained on the certified values for all the trace elements by the analysis of the CRM (global mean recovery was 92 ± 14%). Noticing that no certified values were reported for Ba, Bi and Sb. For each trace element, detection limit (LD) and quantification limit (LQ) were calculated, depending on their specific blank distribution (Currie, 1999). The unit used to present our results is expressed in milligrams of element per kilogram of dry weight (mg kg^{-1} DW).

The level of trace elements contamination for each site was calculated using the Trace Element Pollution Index (TEPI) developed by Richir and Gobert (2014). As recommended for the calculation of the TEPI, the data were standardized by mean normalization. A high TEPI

value indicates a potentially polluted site. Unlike the Metal Pollution Index (MPI), the TEPI allows a reliable comparison of study sites, regardless of the type of trace element dosed or the biological model used (Richir and Gobert, 2014). TEPI values were calculated using the following formula:

$$TEPI = (Cf_1 * Cf_2 \ldots Cf_n)^{1/n}$$

where, Cf_n is the mean normalized concentration of the trace elements in each site or station and n is the number of trace elements examined. A 3-level water quality scale was established, using the quartile method and TEPI values. Based on TEPI values, the quartiles were calculated to determine the class limits of the 3-level water quality scale. The three levels of the quality scale were defined based on the values of the upper and lower quartiles: (i) low contamination level (LCL, TEPI values below the first quartile mean), (ii) medium contamination level (MCL, TEPI values between the 1st and 3rd quartile means), and (iii) high contamination level (HCL, TEPI values above the 3rd quartile means) (Morrison *et al.*, 2017; Richir *et al.*, 2015).

Contamination levels for each station were then determined based on the TEPI values. The calculation of the Trace Element Spatial Variation Index (TESVI) makes it possible to compare trace element according to the overall spatial variability of their environmental levels according to Richir and Gobert (2014). For each TE, the TESVI was calculated following the formula:

$$TESVI = [(x_{max} / x_{min}) / (\Sigma(x_{max} / x_i) / n)] * SD$$

where, x_{max} and x_{min} are the maximum and minimum mean concentrations recorded among the n sites, x_i are the mean concentrations recorded in each of the n sites, and SD is the standard deviation of the mean ratio $\Sigma(x_{max} / x_i) / n$. For a given TE, the higher the value of the TESVI, the more its environmental levels will vary globally across the study area (for further details see Richir and Gobert, 2014). To better comply with the conditions of the application of parametric statistical tests, and to bring elemental concentrations within the same range, data were natural-log transformed (Gobert *et al.*, 2017). A one-way analysis of variance

(ANOVA) and post-hoc Tukey's Honestly Significant Difference (HSD) tests were used to assess the existence of significant differences between trace element concentrations for all stations.

3. Results

The results of mean trace elements concentrations in *P. lividus* gonads are presented in Table 1. There is a very strong variation in the trace elements concentrations found according to the sampling stations. Significant differences between stations were found for a large number of trace elements (Table 1). The accumulated levels of trace elements followed the same sequence Zn > Fe > As > Al > Cu > V > Mn > Se > Cr > U > Ni > Co > Mo > Li > Ba > Cd > Pb > Sb > Ag > Sn. At all eight sampling stations, Zn was always the most abundant element; the lowest abundance was always observed for Sn, indicating that *P. lividus* has a higher tendency to accumulate essential trace elements such as Zn, Fe, Cu, and Mn.

For each station, the TEPI was calculated, and the values obtained showed a small range of variation. TEPI values ranged from 1.033 at Saint-Florent2 to 0.440 at Calvi1 (Table 2). Based on the TEPI, Saint-Florent2 was the most contaminated site with the highest levels of Co, Cr, Fe, and Ni. The high concentrations of these trace elements seem to influence the TEPI values obtained. Based on the TEPI values calculated for the eight stations studied, three class levels were defined from the quartiles (low, medium, and high contamination) (Table 2).

Three stations were classified as highly contaminated (Ajaccio2, Calvi2, Saint-Florent2), three as moderately contaminated (Bonifacio1, Bonifacio2, Saint-Florent1), and two as slightly contaminated (Ajaccio1, Calvi1) (Table 2). The results showed that some reference stations, chosen for their apparent non-anthropized zone status, are nevertheless impacted by trace elements contamination.

Table 1. Mean (± standard deviation; SD) trace elements concentrations (mg kg^{-1} DW) in the gonads of *P. lividus* in Corsica Island. ^{abcd} Dissimilar letters denote significant differences between groups ($p < 0.05$).

TEs	Ajaccio1	Ajaccio2	Bonifacio1	Bonifacio2	Saint-Florent1	Saint-Florent2	Calvi1	Calvi2
Ag	0.02 (±0.01)^{c}	0.12 (±0.02)^{ab}	0.03 (±0.01)^{c}	0.02 (±0.01)^{c}	0.27 (±0.11)^{a}	0.08 (±0.01)^{b}	0.09 (±0.01)^{b}	0.23 (±0.06)^{a}
Al	85.56 (±16.07)^{a}	38.67 (±7.65)^{abc}	24.17 (±9.73)^{cde}	28.47 (±6.59)^{cde}	13.29 (±2.72)^{bcd}	49.4 (±10.94)^{ab}	13.27 (±6.06)^{e}	10.08 (±1.88)^{e}
As	26.67 (±1.27)^{cd}	24.06 (±1.89)^{cd}	35.25 (±4.03)^{bc}	20.97 (±2.32)^{d}	59.41 (±5.43)^{a}	56.71 (±5.97)^{a}	33.58 (±2.24)^{bc}	45.60 (±5.15)^{ab}
Ba	0.47 (±0.07)^{a}	0.43 (±0.07)^{ab}	0.24 (±0.09)^{cd}	0.34 (±0.06)^{abc}	0.14 (±0.01)^{d}	0.18 (±0.02)^{cd}	0.25 (±0.06)^{bcd}	0.35 (±0.09)^{abc}
Cd	0.08 (±0.01)^{d}	0.13 (±0.01)^{bcd}	0.16 (±0.01)^{bc}	0.12 (±0.02)^{cd}	0.38 (±0.04)^{a}	0.48 (±0.09)^{a}	0.18 (±0.02)^{b}	0.35 (±0.03)^{a}
Co	0.14 (±0.01)^{cd}	0.25 (±0.03)^{bc}	0.21 (±0.03)^{bcd}	0.26 (±0.03)^{bc}	0.28 (±0.04)^{b}	1.36 (±0.17)^{a}	0.12 (±0.01)^{d}	0.30 (±0.04)^{b}
Cr	0.39 (±0.05)^{c}	0.58 (±0.08)^{bc}	0.56 (±0.09)^{bc}	0.68 (±0.12)^{bc}	0.98 (±0.17)^{b}	4.08 (±0.85)^{a}	0.37 (±0.05)^{c}	0.79 (±0.14)^{c}
Cu	4.56 (±0.17)^{a}	4.09 (±0.12)^{ab}	3.65 (±0.13)^{bc}	4.70 (±0.17)^{a}	3.54 (±0.11)^{bc}	3.46 (±0.17)^{c}	3.57 (±0.18)^{bc}	3.557(±0.14)^{bc}
Fe	115.08 (±15.62)^{a}	95.21 (±11.84)^{ab}	59.49 (±11.89)^{bc}	52.03 (±7.59)^{bc}	37.39 (±6.82)^{c}	237.88 (±45.96)^{a}	46.84 (±10.61)^{c}	38.79 (±5.18)^{c}
Li	0.32 (±0.01)^{a}	0.32 (±0.02)^{a}	0.32 (±0.02)^{a}	0.30 (±0.01)^{a}	0.29 (±0.01)^{a}	0.36 (±0.02)^{a}	0.34 (±0.02)^{a}	0.35 (±0.02)^{a}
Mn	3.04 (±0.28)^{ab}	2.50 (±0.19)^{abc}	1.66 (±0.19)^{d}	2.24 (±0.17)^{abcd}	1.69 (±0.13)^{cd}	3.48 (±0.49)^{a}	1.53 (±0.13)^{d}	1.93 (±0.15)^{bcd}
Mo	0.23 (±0.03)^{b}	0.32 (±0.02)^{ab}	0.33 (±0.04)^{ab}	0.37 (±0.04)^{a}	0.37 (±0.03)^{a}	0.42 (±0.05)^{a}	0.30 (±0.03)^{ab}	0.36 (±0.04)^{ab}
Ni	0.22 (±0.03)^{b}	0.46 (±0.07)^{b}	0.30 (±0.06)^{b}	0.44 (±0.08)^{b}	0.50 (±0.09)^{b}	5.25 (±1.34)^{a}	0.33 (±0.03)^{b}	0.39 (±0.06)^{b}
Pb	0.17 (±0.02)^{bc}	0.50 (±0.26)^{ab}	0.16 (±0.02)^{bc}	0.39 (±0.06)^{a}	0.11 (±0.01)^{c}	0.09 (±0.01)^{c}	0.12 (±0.01)^{c}	0.11 (±0.01)^{c}
Sb	0.16 (±0.02)^{ab}	0.15 (±0.03)^{abc}	0.34 (±0.10)^{a}	0.18 (±0.02)^{ab}	0.10 (±0.01)^{bc}	0.27 (±0.16)^{bc}	0.06 (±0.01)^{c}	0.06 (±0.01)^{c}
Se	1.78 (±0.10)^{a}	1.76 (±0.10)^{a}	2.19 (±0.22)^{a}	2.03 (±0.23)^{a}	1.96 (±0.16)^{a}	2.35 (±0.28)^{a}	1.62 (±0.11)^{a}	2.186 (±0.125)^{a}
Sn	0.01 (±0.01)^{b}	0.02 (±0.01)^{b}	0.05 (±0.01)^{a}	0.02 (±0.01)^{b}	0.01 (±0.01)^{c}	0.01 (±0.01)^{c}	0.01 (±0.01)^{c}	0.02 (±0.01)^{bc}
U	0.43 (±0.07)^{c}	0.67 (±0.08)^{bc}	0.94 (±0.24)^{bc}	0.74 (±0.11)^{bc}	1.75 (±0.29)^{a}	1.61 (±0.33)^{a}	0.64 (±0.12)^{bc}	1.11 (±0.16)^{ab}
V	1.93 (±0.27)^{b}	2.82 (±0.42)^{ab}	2.92 (±0.64)^{ab}	2.53 (±0.47)^{ab}	4.62 (±0.83)^{a}	5.05 (±0.75)^{a}	1.65 (±0.32)^{b}	3.18 (±0.49)^{ab}
Zn	201.82 (±54.30)^{a}	304.12 (±76.44)^{a}	195.16 (±42.68)^{a}	133.24 (±30.64)^{a}	192.98 (±48.40)^{a}	192.23 (±78.61)^{a}	115.90 (±25.25)^{a}	231.93 (±53.51)^{a}

Table 2. Trace Element Pollution Index (TEPI) of sea urchin *P. lividus* collected from eight stations in Corsica Island.

Stations	TEPI	Local quality scale
Ajaccio1	0.548	Low contamination level
Ajaccio2	0.701	High contamination level
Bonifacio1	0.622	Medium contamination level
Bonifacio2	0.590	Medium contamination level
Saint-Florent1	0.621	Medium contamination level
Saint-Florent2	1.033	High contamination level
Calvi1	0.440	Low contamination level
Calvi2	0.638	High contamination level

The TESVI values obtained ranged from 0.10 to 11.84 (Table 3). Ni was the element that showed the largest spatial variation between sites, with the highest value of TESVI (11.84). The station with the highest average concentration of Ni in *P. lividus* was Saint-Florent2 (5.25 ± 1.34 mg kg^{-1}). Following Ni, only five of the 20 trace elements examined obtained a TESVI value>5.0: Ag (9.49), Sn (8.51), Co (5.98), Al (5.55), and Cr (5.41). Li is the element with the lowest spatial variation between stations (0.10) (Table 3). TESVI values were listed in ascending order as: Li, Cu, Se, Mo, Mn, Zn, As, V, Ba, U, Pb, Fe, Cd, Sb, Cr, Al, Co, Sn, Ag, Ni. The ranking of trace elements in ascending order of their TESVI values also makes it possible to clearly highlight the trace element of key environmental concern.

Table 3. Trace Element Spatial Variation Index (TESVI) of 20 trace elements examined in *P. lividus* from eight stations in Corsica Island. The higher the TESVI value the greater the spatial variation of that element among the sampling locations.

TE	(x_{max}/x_{min})	$\sum (x_{max}/x_i)$ /n ± SD	TESVI	Station x_{max}
Ag	11.30	4.81 ± 4.04	9.49	SF1
Al	8.48	4.11 ± 2.69	5.55	A1
As	2.83	1.79 ± 0.67	1.06	SF1
Ba	3.38	1.83 ± 0.81	1.50	A1
Cd	5.73	2.84 ± 1.62	3.28	SF2
Co	11.37	5.97 ± 3.14	5.98	SF2
Cr	10.88	6.45 ± 3.21	5.41	SF2
Cu	1.36	1.22 ± 0.14	0.16	B2
Fe	6.36	3.96 ± 1.95	3.13	SF2
Li	1.26	1.12 ± 0.09	0.10	SF2
Mn	2.26	1.66 ± 0.46	0.63	SF2
Mo	1.79	1.29 ± 0.24	0.34	SF2
Ni	23.77	13.02 ± 6.49	11.84	SF2
Pb	5.14	3.31 ± 1.55	2.40	A2
Sb	5.77	2.89 ± 1.83	3.65	B1
Se	1.45	1.20 ± 0.15	0.19	SF2
Sn	11.20	5.28 ± 4.01	8.51	B1
U	4.08	2.16 ± 1.01	1.91	SF1
V	3.07	1.86 ± 0.70	1.16	SF2
Zn	2.62	1.68 ± 0.52	0.82	A2

4. Discussion

The results of this study are discussed and compared with those of previously published studies from the Mediterranean Sea. We compiled our data together with the re-calculated TEPI values for other locations around the world to obtain insight into the trace elements concentration ranges at the global scale, and to place our results in perspective (Fig. 2, Table 4). Trace elements concentrations in gonads are known to vary according to many biological parameters such as sex, age, or season (Hambidge *et al.*, 1986; Unuma *et al.*, 2007); these variables should be taken into account to give a more reliable image of the contamination rates in sea urchin gonads. It appears that no study on *P. lividus* has analyzed so many trace elements (20) in a Mediterranean region. For many of these studies (especially

those on emerging elements), the comparison with other sites is therefore impossible, but our data are essential and will serve as a reference state to follow the evolution of these elements in the Mediterranean ecosystems.

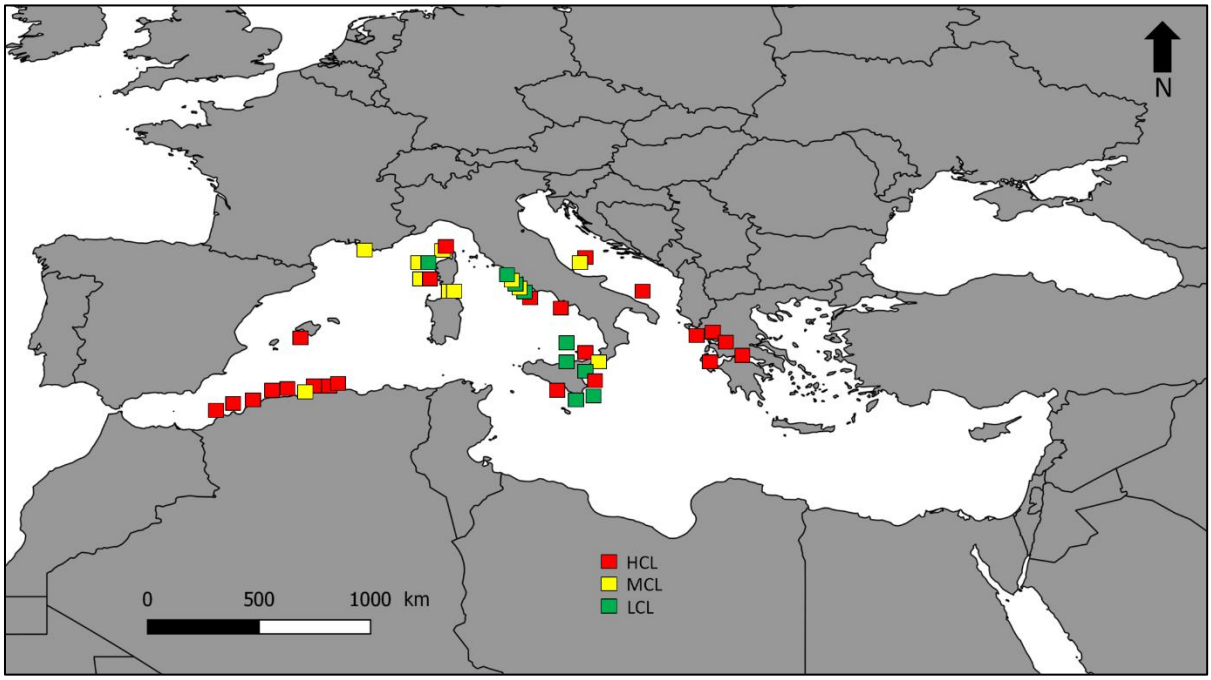

Figure 2. Map showing the visual interpretation of trace elements contamination based on the TEPI from various geographical locations. LCL is the low contamination level, MCL is the medium contamination level, and HCL is the high contamination level. Data from Algeria (Guendouzi *et al.*, 2017; Rouane-Hacene *et al.*, 2017; Soualili *et al.*, 2008), France (our study, Warnau *et al.*, 1998), Italy (Pinsino *et al.*, 2008; Salvo *et al.*, 2014; Scanu *et al.*, 2015; Storelli *et al.*, 2001; Warnau *et al.*, 1998), Greece (Portocali *et al.*, 1997; Strogyloudi *et al.*, 2014), and Spain (Deudero *et al.*, 2007).

The analysis of contaminants accumulated in sea urchin gonads allowed us to calculate a global pollution index. TEPI is used here as a tool that allows a reliable comparison of trace elements contamination, both locally and internationally, regardless of the number of trace elements used or the biological model (Wilkes *et al.*, 2017). Trace element contamination is widespread in the Mediterranean Sea with nearly half of the study sites showing high concentrations. The TEPI carried out revealed spatial differences in contamination. As previously described by Richir *et al.* (2015), trace elements contamination in the western Mediterranean overall displayed a north-to-south gradient, from the Italian coasts down through the insular Corsican coasts to the north African littoral. Based on the available data, the western (Algeria, Spain) and eastern (Greece) basins of the Mediterranean seem to be contamination hotspots with almost all stations classed in HCL sites. On the other hand, the

central area between continental French coasts, Corsica Island, continental Italian coasts, and Sicily Island presents a heterogeneity resulting in sites with varying degrees of contamination. The present study has shown that a region as pristine as Corsica can be subject to significant anthropogenic pressures that influence local trace elements contamination levels. Indeed, in Corsica we find a wide range of types of pressure and environmental conditions representative of the western Mediterranean region (Lafabrie *et al.*, 2008; Lopez y Royo *et al.*, 2009).

In Corsica, two stations (Ajaccio2 and Saint Florent2) are referenced as HCL sites with important concentrations in Zn, Fe, As, Al, and Cu. The calculated TEPI can be related to some contamination levels reported from other Mediterranean coasts [for example: Algeria (Guendouzi *et al.*, 2017), Greece (Strogyloudi *et al.*, 2014), Italy (Scanu *et al.*, 2015), and Spain (Deudero *et al.*, 2007)]. In our study, the observed concentrations and low TESVI define Zn as the dominant trace element both in these HCL sites and in the whole study area. Concentration magnitudes of Zn are of the same order as those observed elsewhere in the Mediterranean (Guendouzi *et al.*, 2017; Soualili *et al.*, 2008; Storelli *et al.*, 2001; Warnau *et al.*, 1998). Zn is an essential element for reproduction (Ahn *et al.*, 2009), explaining generally high concentrations in all, but especially female, gonads. Urban sewage is considered as a potential source of Zn contamination (Portocali *et al.*, 1997), which is in accordance with our results. Indeed, the highest concentrations of the trace elements were observed in Ajaccio2, the wastewater treatment plant of Ajaccio city.

Trace elements such as Fe, Al, As, and Cu, observed in high concentration in the present study are also representative of anthropogenic contamination, showing that HCL sites are influenced by domestic and/or industrial discharges. In contrast other studies (Guendouzi *et al.*, 2017; Rouane-Hacene *et al.*, 2017; Scanu *et al.*, 2015; Warnau *et al.*, 1998), our results show very low Pb concentration levels in Corsica, although they are quite common in industrial and harbor effluents (antifouling point, fuels, etc.).

Table 4. Comparison of calculated TEPI values from different studies published in the Mediterranean Sea in *P. lividus*. LCL is the low contamination level, MCL is the medium contamination level, and HCL is the high contamination level.

Country	Station	TEPI	Quality scale	Reference
Algeria	Algiers Beach	0.930	HCL	Soualili *et al.*, 2008
	Tamentfoust	0.771	HCL	
	Sidi-Fredj	0.611	MCL	
	Sidi Mejdoub	1.513	HCL	Guendouzi *et al.*, 2017
	Abdelmalek	1.261	HCL	
	Bateau Cassé	1.452	HCL	
	Oran	1.523	HCL	Rouane-Hacene *et al.*, 2017
	Ain Defla	0.916	HCL	
	Hadjaj	0.741	HCL	
France	Marseille	0.561	MCL	Warnau *et al.*, 1998
	Ajaccio 1	0.549	MCL	This study
	Ajaccio 2	0.701	HCL	
	Calvi 1	0.441	LCL	
	Calvi 2	0.638	MCL	
	Saint-Florent 1	0.621	MCL	
	Saint-Florent 2	1.034	HCL	
	Bonifacio 1	0.623	MCL	
	Bonifacio2	0.591	MCL	
Italy	Ischia Island	0.954	HCL	Warnau *et al.*, 1998
	Apulian coast	0.880	HCL	Storelli *et al.*, 2001
	Pianosa	0.732	HCL	Pinsino *et al.*, 2008
	Caprara	0.701	MCL	
	Syracuse	0.515	LCL	Salvo *et al.*, 2014
	Raguse	0.391	LCL	
	Messina	0.641	MCL	
	Milazzo	0.928	HCL	
	Priolo	1.663	HCL	
	Gela	0.749	HCL	
	Brolo	0.221	LCL	
	Catania	0.051	LCL	
	Filicudi	0.099	LCL	
	Civitavecchia 1	0.704	HCL	Scanu *et al.*, 2015
	Civitavecchia 2	0.218	LCL	
	Civitavecchia 3	0.070	LCL	
	Civitavecchia 4	0.688	MCL	
	Civitavecchia 5	0.225	LCL	
	Civitavecchia 6	0.527	MCL	
	Civitavecchia 7	0.605	MCL	
	Civitavecchia 8	0.159	LCL	
	Civitavecchia 9	0.451	LCL	
Greece	Akoli	1.992	HCL	Portocali *et al.*, 1997
	Patras	0.860	HCL	
	Agios Thomas	1.301	HCL	Strogyloudi *et al.*, 2014
	Koronisia	1.184	HCL	
	Mytikas	1.309	HCL	
Spain	Mallorca	0.843	HCL	Deudero *et al.*, 2007

The second HCL station in Corsica (Saint-Florent2) is close to a former asbestos mine. The trace elements contamination in sea urchin gonads from this site is characterized by similar Zn, Fe, As, and Al concentrations as in Ajaccio2. The difference between these two sites is the presence of three traces elements specific to the site indicated by high TESVI: Ni, Cr, and Co. These elements, as well as iron and manganese, associated with the amiantiferous serpentine deposit have been released at sea and continue to fuel the coastline through the leaching of tailings still present on the flanks of the mine (Andral *et al.*, 2004; Galgani *et al.*, 2006). Bouchoucha *et al.* (2012) reported heavier Ni and Cr levels in sea urchin gonads than those found in our study, probably due to the sample sites closer to the asbestos mine.

Five stations are classed as medium contamination levels (MCL) sites. With lower concentrations of Zn, Fe, As, Al and Cu, MCL sites have two marked typologies. While two of them, close to commercial harbor and leisure marina (Bonifacio2 and Calvi2), are clearly influenced by anthropogenic activities, three other stations (Saint-Florent1, Bonifacio1, Ajaccio1) are natural sites without an apparent source of contamination. However, each of these last stations is marked by a specific trace element (high TESVI): Ag for Saint-Florent1, Al for Ajaccio1, and Sn for Bonifacio1. The TEPI indicated other coastal MCL sites. For most of them, the sources of contamination are clearly established and include harbor activities and urban, agricultural, and industrial discharges. Natural sites with no defined contamination source are uncommon; only the Capara site, a protected marine area in Italy, is described (Pinsino *et al.*, 2008).

A single station, Calvi1, is specified as a Low Contamination Level (LCL) site due to the relatively clean homogenous status of the water body of the Calvi Bay area. All trace elements have a lower concentration here than in other sites and none of these have high TESVI. These LCL stations are usually distant from the source of contamination, with a surprising exception for Civitavecchia (Italy) or Catania (Sicily) sites which have low contaminations despite their close proximity to well-known harbor activities (Salvo *et al.*, 2014; Scanu *et al.*, 2015).

Because of its sedentary lifestyle, *P. lividus* has often been described as an indicator of local pollution (Warnau *et al.*, 1998; Bayed *et al.*, 2005). The assessment of trace element levels in sea urchin gonads clearly confirmed this hypothesis, showing local contaminations even

within a preserved area such as Corsica. This region is impacted by many sources of contamination (*e.g.* harbors, mines, and sewage effluents) representative of pressures found at the Mediterranean scale and may in the future serve as a new comparison area to monitor the spatial and temporal variations of contamination. A better understanding of these pressures will allow managers to act at the source and reduce the degradation or improve the ecological quality of water bodies (Blanfuné *et al.*, 2017). As proposed by Govers *et al.* (2014), from a research perspective, it could be interesting to study the importance of seasonality and physiological status for the use of sea urchins and other marine organisms as bioindicators for trace elements pollution.

Article 2

Seasonal change in trace element concentrations of *Paracentrotus lividus*: Its use as a bioindicator

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Ecological Indicators

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Contexte et résumé de l'article 2 « Variation saisonnière des concentrations en éléments traces chez *Paracentrotus lividus* : son utilisation comme bioindicateur »

Les bioindicateurs sont des organismes marins capables de fournir des informations qualitatives et quantitatives sur la qualité de son environnement et présentant un haut degré de sensibilité ou de tolérance à de nombreux types de contamination ou à ses effets. Les oursins sont considérés comme des organismes modèles privilégiés en écotoxicologie. Toutefois, la concentration en éléments traces dans les gonades est connue pour varier en fonction de différents facteurs biologiques (âge, sexe), physiologiques (reproduction) ou environnementaux (saison). Ces variables ont été prises en compte dans cette deuxième étude afin d'obtenir une image plus fiable des niveaux de contamination. Pour cela, d'autres prélèvements ont été réalisés au printemps, en été et en automne afin d'avoir un suivi saisonnier. Les résultats indiquent des différences significatives des teneurs en éléments traces entre les deux sexes. Ces dissimilitudes sont probablement associées au rôle de certains éléments traces essentiels dans la gamétogenèse. Il est également observé une corrélation négative entre les indices gonadosomatiques et les teneurs en éléments traces.

Deux populations de cellules composent l'épithélium germinal de la gonade de l'oursin : les cellules germinales et les cellules somatiques. Si l'accumulation des éléments traces se déroule dans les cellules somatiques, un phénomène de dilution sera observé lors de la gamétogenèse. Ce phénomène est constaté pour la majorité des sites étudiés. En effet, les niveaux de contamination mesurés sont plus faibles lors de la période de reproduction. Une variation temporelle des teneurs en éléments traces dans les gonades est ainsi constatée avec des niveaux de contamination plus faible durant la saison estivale et à l'inverse, plus élevé en automne et en hiver. Outre les facteurs physiologiques, il a également été souligné la nécessité de tenir compte des facteurs abiotiques tels que l'alimentation lors de l'interprétation des résultats. Cette étude a permis de vérifier l'efficacité de l'oursin *Paracentrotus lividus* en tant que bioindicateur des contaminations en éléments traces.

1. Introduction

The growth of industrial, agricultural and urban activities gives rise to the introduction of considerable amounts of chemicals in marine coastal ecosystems. These substances have toxic properties likely to cause extensive damage at the scale of organisms, populations and ecosystems (Nordberg *et al.*, 2007; Amiard, 2011). Furthermore, intensive human activities, particularly in coastal areas, have a major environmental impact on these productive zones (Papathanassiou and Gabrielides, 1999). The United Nations Environment Programme estimated that 650 million tons of sewage, 129,000 tons of mineral oil, 60,000 tons of mercury, 3,800 tons of lead and 36,000 tons of phosphates are dumped into the Mediterranean each year. In addition, 70 per cent of the wastewater dumped into the Mediterranean is untreated. These pressures make the Mediterranean a vulnerable ecological unit (Turley, 1999). Furthermore, as the water bodies renews with a few decades in the Mediterranean versus a few centuries for the ocean, this sea is a veritable laboratory for observing the pressures and changes that humans exert on the environment (Bethoux *et al.*, 1999).

Trace elements are among the most common contaminants in the marine ecosystem. Due to their toxicity, persistence and ability to accumulate in marine organisms, they are considered as serious pollutants in marine ecosystems (Bonanno and Di Martino, 2017). Trace elements are present in the different compartments of the environment at low concentrations (Baize, 2009). In the marine environment, they can remain in solution, be adsorbed on sedimentary particles, precipitate to the bottom, or be bioaccumulated or biomagnified by organisms and to reach concentrations that can be toxic (Warnau *et al.*, 1998). Above a certain threshold, all trace elements present a potential danger that can cause disturbances at cellular level, individual level, and also population or ecosystem levels (Amiard, 2011). They represent a potential danger for marine organisms (*e.g.* Allemand *et al.*, 1989; Walter *et al.*, 1989) and for human consumers of sea urchin gonads. As a result of the threats posed by trace elements in the environment, they must be continuously monitored from their emission sources to their final deposition in the oceans (Richir and Gobert, 2014).

In order to assess the levels of contaminants available in the ecosystem, organisms can be used as bioindicators. Postmetamorphic echinoids in general, and *Paracentrotus lividus* (Lamarck, 1816), in particular, are interesting candidates for the bioindication of trace elements contaminations and have already been used in the Mediterranean and elsewhere (*e.g.* Augier *et al.,* 1989; Guendouzi *et al.,* 2017; Ternengo *et al.,* 2018). Due to its wide distribution, abundance in several coastal ecosystems, ease of harvesting, longevity, relative sedentarity and high tolerance of pollutants, *P. lividus* is an organism that is recognized for its role as bioindicator (Warnau *et al.*, 1998; Geraci *et al.*, 2004. Salvo *et al.*, 2014). Its gonads and digestive tract are described as the organs accumulating the most trace elements (Augier *et al.,* 1989), thus, the study of their ecotoxicological properties is also of public health interest (Salvo *et al.*, 2015). Trace elements concentration in the gonads is known to vary according to different biological (age, gender), physiological (reproduction) or environmental (season) factors (Warnau *et al.,* 1998; Guendouzi *et al.,* 2017; Rocha *et al.*, 2019). The elementary constitution of the sea urchin reflects the composition of its environment and provides a basis for monitoring the patterns of change of contamination (Morrison *et al.*, 2017).

In the Mediterranean, numerous studies using *P. lividus* as a bioindicator have studied classical trace elements such as zinc or lead, but there has been little or no description of the contamination related to emerging trace elements (*e.g.* Rouane-Hacene *et al.,* 2017; Guendouzi *et al.,* 2017). Well-known throughout the Mediterranean region, *P. lividus* is a species of economic and ecological importance (Lawrence and Sammarco, 1982; Kelly, 2004). The sea urchin is of high commercial value and represents a complementary resource for artisanal fishing. The taste quality of its gonads also makes it a species appreciated and targeted by recreational fishing. In addition, as a primary consumer, it plays a key role in the structuring and functioning of benthic ecosystems and more particularly of macrophyte communities (Lawrence and Sammarco, 1982).

Corsica island is often considered as a 'pristine' region on account of its water quality and the low anthropic pressure (Lafabrie *et al.*, 2008; Gobert *et al.*, 2017; Marengo *et al.*, 2018). Nevertheless, according to recent studies, local contamination similar to that recorded in other anthropized areas in the Mediterranean can be found (Richir *et al.*, 2015), with areas classified on the basis of different levels of contamination as anthropized or preserved sites

(Ternengo *et al.*, 2018). This is a real asset that makes it a particularly suitable study area to identify contaminants and to monitor their dynamics according to anthropogenic pressures. The purpose of this study is (i) to monitor the spatio-temporal dynamics of 22 trace elements (classical and emerging) in sea urchin gonads collected along the Corsican coasts, and (ii) to evaluate the seasonal patterns of change in the pollution index characterizing each site to determine whether these variations are linked to the sea urchin's physiology or to contamination. This paper will assess the bio-indicator potential of sea urchins and compare this model with other bioindicators.

2. Material and methods

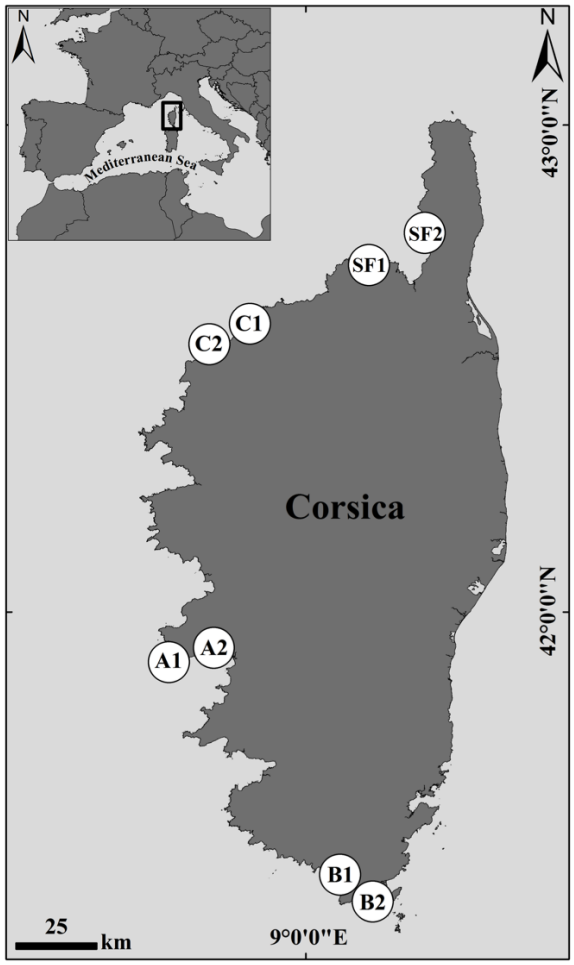
2.1. Sampling sites, collection and preparation of samples

Sea urchin samples were collected in May, August and November 2017, and in order to have a complete range of seasonal monitoring, the February 2017 data of Ternengo *et al.* (2018) were added. Sea urchins were collected in the Western Mediterranean Sea in four Corsican coastal areas between 1 and 5 m depth (Fig. 1). In each area, two sites were defined, diverging by their ecological characteristics and their degree of anthropization: (1) a reference site, chosen for its distance from any pollution source and supposed to have a good ecological status, and (2) a site close to identified anthropogenic sources (wastewater treatment plant, commercial harbour, marina and a former asbestos mine) supposedly impacted.

Thirty sea urchins were collected in each area, 15 per site for each season, resulting in a total of 480 individuals harvested in this study. After measuring height and weight, the sea urchins were dissected and the gender was determined. The sex ratio has been respected, to the extent possible, to avoid bias. The gonads were removed and weighed to calculate the gonadosomatic index of each individual. This index was calculated using the following formula:

$$(GFW/ TFW) *100$$

where GFW is the gonad fresh weight and TFW is total fresh weight. Gonads were cleaned with ultrapure water and stored at −20 °C.



Sampling site	Anthropogenic sources identified
Ajaccio 1 (A1)	Reference station
Ajaccio 2 (A2)	Wastewater treatment plant
Bonifacio 1 (B1)	Reference station
Bonifacio 2 (B2)	Commercial harbor / marina
Calvi 1 (C1)	Reference station
Calvi 2 (C2)	Wastewater treatment plant
Saint-Florent 1 (SF1)	Reference station
Saint-Florent 2 (SF2)	Former asbestos mine

Figure 1. Location of study coastal areas in Corsica (NW Mediterranean, France), showing the eight sampling stations of *P. lividus* and their characterization.

2.2. Trace elements analysis

Prior to the analysis, samples were lyophilized (CHRIST LCG Lyochamber Guard 121550 PMMA/Alpha 1-4 LD plus) and ground in an agate mortar. Approximately 0.2 g of each dried material was mineralized in a closed microwave digestion labstation (Ethos D Milestone Inc.), using nitric acid and hydrogen peroxide as reagents (suprapur grade, Merck). The trace element concentrations were determined by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, Perkin Elmer), according to the method described by Richir and Gobert (2014). A total of 22 trace elements were analyzed: silver (Ag), aluminium (Al), arsenic (As), barium (Ba), beryllium (Be), bismuth (Bi), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), lithium (Li), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), antimony (Sb), selenium (Se), tin (Sn), uranium (U),

vanadium (V), and zinc (Zn). In order to check the purity of the chemicals used, a large number of chemical blanks were run every 40 samples. Analytical quality control was achieved using Certified Reference Materials (CRM), DORM-4 (fish protein), NIST 1566b (oyster) and NIST 2976 (mussel tissue). For each trace element, detection limit (LD) and quantification limit (LQ) were calculated according to Currie (1999) and Grinzaid *et al.* (1977) depending on their specific blank distribution. The results are expressed in milligrams of element per kilogram of dry weight ± standard error (mg kg^{-1} DW ± SE). TEs with values generally below the detection limit were removed from the database. For the others, concentrations below the LD were replaced with a value of LD/2, as reported by Skrbić *et al.* (2010).

2.3. Data analysis

The data was log-transformed in order to meet the conditions of application of the parametric tests, to reduce the effect of outliers skewing the data distribution, and to bring elemental concentrations within the same range (Gobert *et al.,* 2017). Analyses were performed using XLSTAT software (Addinsoft, 2019). A multivariate analysis of variance (MANOVA) was applied to explore the influence of gender (2 levels), site (8 levels) and season (4 levels) factors to the observed differences in TE concentration. MANOVA was then followed by posteriori univariate ANOVA and post-hoc Tukey's honestly significant difference (HSD) tests. Pearson rank correlation tests were performed to investigate the relationship between the trace element levels (inter-element correlations) and the biological data (weight, size and gonadosomatic index). To determine the significance and strength of each relationship, the correlation coefficient was calculated together with p-values. A significant difference is considered as a p-value less than 0.05.

In order to compare the contamination levels of the different sites, the Trace Elements Pollution Index (TEPI) was calculated for each site. Developed by Richir and Gobert (2014), the TEPI is a modified version of the Metal Pollution Index (Usero *et al.*, 1996). It has the advantage of taking into account non-metallic trace elements to study As and Se. Moreover, unlike the Metal Pollution Index, the TEPI allows a reliable comparison of study sites, regardless of trace elements or the biological model used (Richir and Gobert, 2014). As recommended for the

calculation of the TEPI, the data were standardized by mean normalization (Richir and Gobert, 2014). TEPI values were calculated using the following formula:

$$TEPI = (Cf_1 * Cf_2 ... Cf_n)^{1/n}$$

where Cf_n is the mean normalized concentration of the trace element at each site or station and n is the number of trace elements examined. The higher the TEPI value, the more contaminated the site is. A 3-level water quality scale was established, using the method developed by Richir *et al.* (2015). The first level corresponds to the Low Contamination Level (LCL), the second level to a Medium Contamination Level (MCL) and the third level to a High Contamination Level (HCL).

3. Results

3.1. Biotic factors of the sea urchin

The gonadosomatic index ranged from 0.72 to 6.7 and shows different patterns according to the site considered (Fig. 2). Among the 20 trace elements measured, the concentration of 16 trace elements is found negatively correlated with gonadosomatic indices (Ag, Al, As, Ba, Cd, Co, Cr, Fe, Mn, Mo, Ni, Pb, Sb, Se, U, V) (p-value < 0.05) and only one trace element (Zn) is positively correlated with the gonadosomatic indices (r = 0.011, p = 0.020). There are significant positive correlations for 14 out of 20 trace elements with both the weight and the size of sea urchins. The larger the individuals, the more they tend to accumulate trace elements (Ag, As, Cd, Co, Cr, Fe, Li, Mo, Ni, Pb, Sb, Se, U, V) in the gonads (p-value < 0.05).

3.2. Trace element concentrations

The mean trace element concentrations measured in the gonads of *P. lividus*, at each site for all seasons, are presented in Fig. 2. Be and Bi observed concentrations are below the detection limit, so they have not been taken into account in the statistical analyses. Zn is the most abundant TE (175.454 ± 12.004 mg kg^{-1}) while the lowest abundance is observed for Sn (0.016 ± 0.001 mg kg^{-1}).

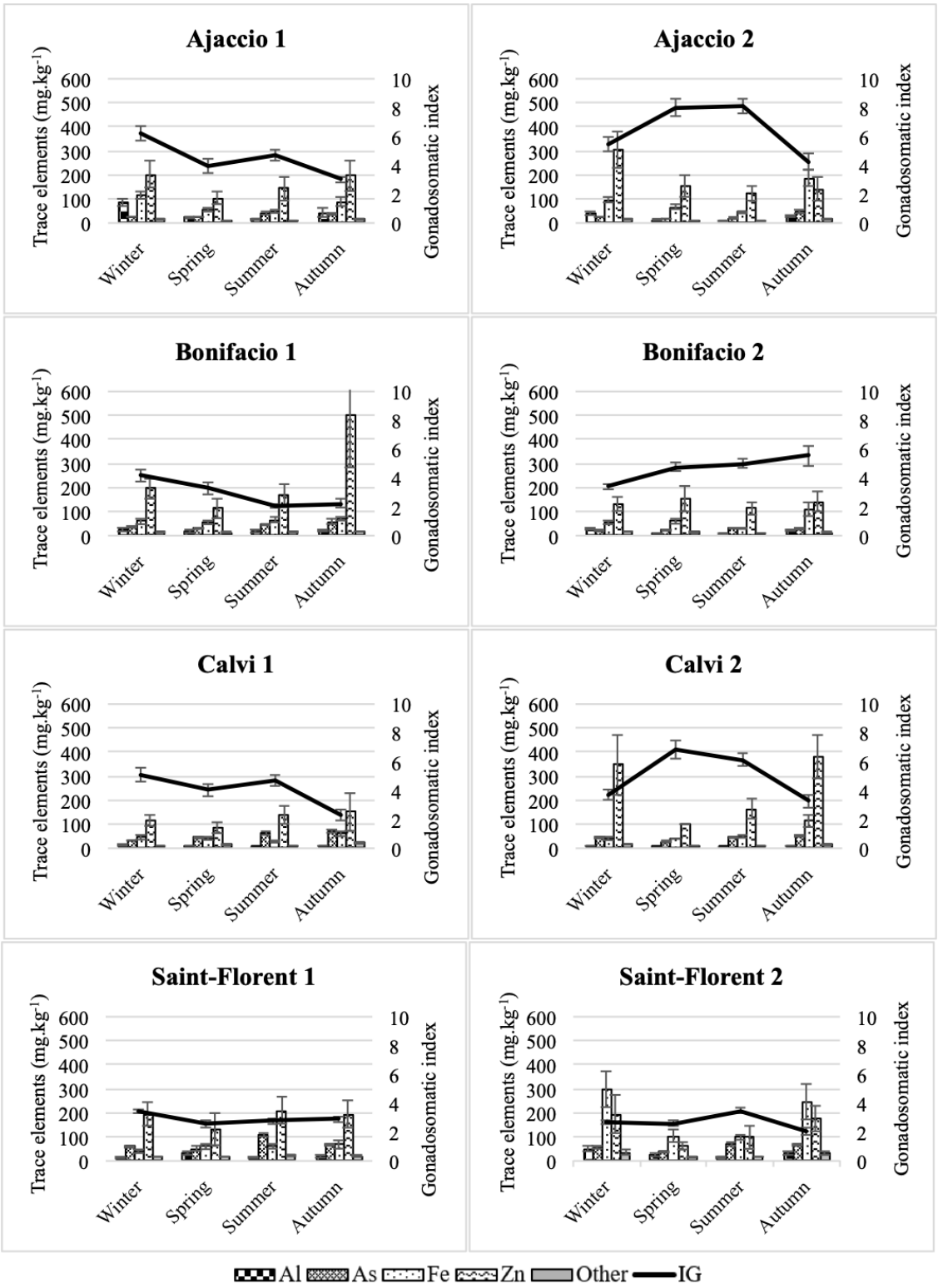


Figure 2. Variation in mean (mg kg^{-1} DW ± SE) trace elements concentrations in the gonads of *P. lividus* and gonadosomatic indices according to the season and on the eight sites (1: reference site; 2: impacted site).

Trace element concentrations follow the sequence: Zn > Fe > As > Al > V > Cu > Se > Ni > Mn > Cr > U > Co > Mo > Cd > Ba > Li > Ag > Pb > Sb > Sn. There are 14 significant negative correlations (Ag - Sn, As - Cu, As - Sn, Cd - Cu, Cd - Sn, Zn - Cr, Zn - Fe, Zn - Mo, Zn - Ni, Zn - Pb, Zn - U, Zn - V). The highest positive inter-elemental relationships are U-V (r = 0.83), Co-Cr (r = 0.78), V - Cr (r = 0.71), Ni-Cr (r = 0.67) and U-Cr (r = 0.63) with p-value < 0.001. The MANOVA results showed significant differences (p-value < 0.001) in trace element concentrations between the genders, the seasons and the sites.

3.3. Temporal variations of trace elements

The concentration of all trace elements except Ag significantly vary among seasons variations (p-value < 0.0001) (Table 1). Noteworthy, trace element display similar fluctuation profiles that is a decrease during the spring and summer seasons. The result of TEPI (Fig. 3) is in accordance with such results, evidencing that sea urchins have higher trace element concentration during the legal harvesting season (*i.e.* autumn and winter).

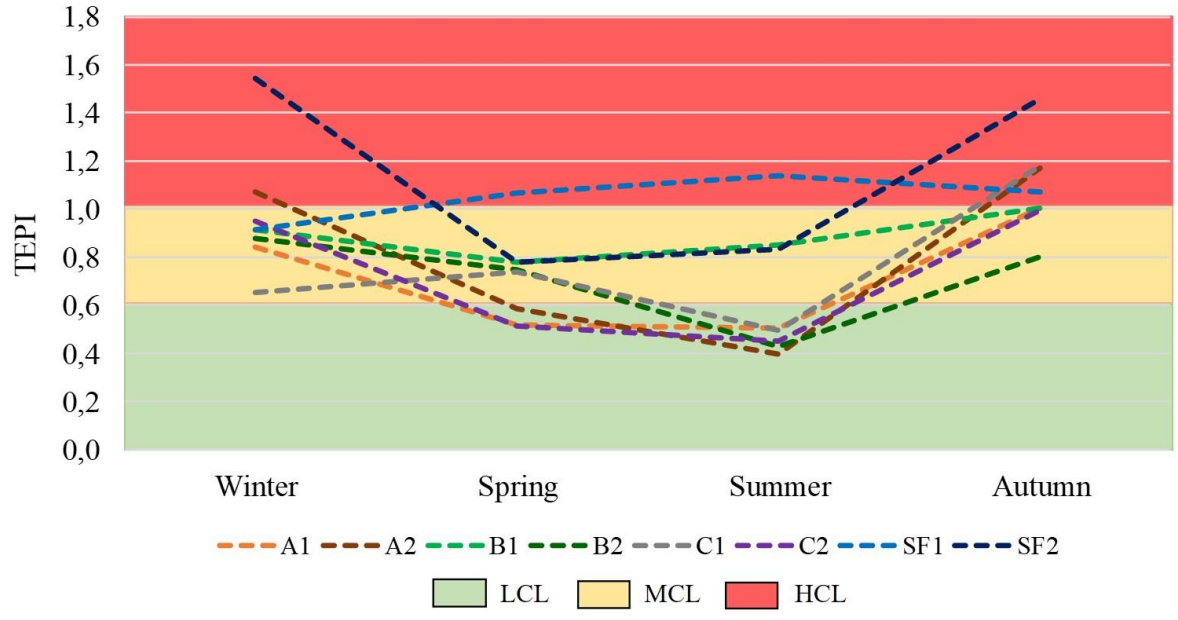


Figure 3. Trace Element Pollution Index (TEPI) variation of the eight sites (A: Ajaccio; B: Bonifacio; C: Calvi; SF: Saint-Florent; 1: reference site; 2: impacted site) according to the season (HCL: High Contamination Level, MCL: Medium Contamination Level, LCL: Low Contamination Level).

Over the eight values that are in HCL, six are in winter and concern four site of three areas (*i.e.* Saint-Florent 1, Saint-Florent 2, Ajaccio 2, Calvi 1). The trace element content is higher when gonadosomatic indices are low in autumn and winter. Most trace elements are probably stored in the gonads and are not expelled with the gametes.

Table 1. Mean (mg kg^{-1} DW ± SE) trace elements concentrations in the gonad of *P. lividus* according to the season. TE: Trace elements. ^{abcd} Dissimilar letters denote significant differences between groups (p-value <0.05). p-value: <0.05*; <0.01**; <0.001***

TE	Winter	Spring	Summer	Autumn	p-value
Ag	0.112 ± 0.018^{a}	0.159 ± 0.072^{a}	0.353 ± 0.101^{a}	0.187 ± 0.037^{a}	0.694
Al	32.888 ± 3.722^{a}	16.608 ± 1.757^{b}	9.637 ± 0.954^{c}	22.936 ± 2.802^{a}	<0.0001***
As	37.788 ± 1.845^{a}	32.083 ± 1.723^{a}	52.576 ± 3.061^{b}	53.253 ± 2.685^{b}	<0.0001***
Ba	0.305 ± 0.026^{a}	0.238 ± 0.034^{b}	0.116 ± 0.008^{c}	0.380 ± 0.092^{a}	<0.0001***
Cd	0.239 ± 0.019^{a}	0.240 ± 0.020^{a}	0.285 ± 0.022^{ab}	0.344 ± 0.027^{b}	0.0120*
Co	0.370 ± 0.042^{a}	0.295 ± 0.025^{a}	0.302 ± 0.024^{a}	0.504 ± 0.043^{b}	<0.0001***
Cr	1.204 ± 0.226^{a}	0.891 ± 0.101^{a}	0.850 ± 0.084^{a}	1.533 ± 0.193^{b}	<0.0001***
Cu	3.893 ± 0.068^{a}	2.949 ± 0.085^{b}	1.869 ± 0.041^{c}	2.731 ± 0.091^{b}	<0.0001***
Fe	92.708 ± 12.112^{a}	60.204 ± 4.489^{ab}	52.695 ± 3.371^{b}	117.273 ± 12.784^{c}	<0.0001***
Li	0.330 ± 0.008^{a}	0.239 ± 0.010^{b}	0.131 ± 0.004^{c}	0.324 ± 0.014^{a}	<0.0001***
Mn	2.264 ± 0.104^{a}	1.033 ± 0.060^{b}	0.568 ± 0.034^{c}	1.281 ± 0.101^{d}	<0.0001***
Mo	0.343 ± 0.014^{a}	0.311 ± 0.021^{a}	0.241 ± 0.022^{b}	0.346 ± 0.032^{a}	<0.0001***
Ni	1.735 ± 0.808^{a}	0.748 ± 0.157^{a}	0.549 ± 0.073^{a}	2.143 ± 0.335^{b}	<0.0001***
Pb	0.212 ± 0.036^{a}	0.131 ± 0.009^{b}	0.109 ± 0.008^{b}	0.223 ± 0.026^{a}	<0.0001***
Sb	0.169 ± 0.026^{a}	0.049 ± 0.006^{b}	0.036 ± 0.003^{c}	0.074 ± 0.004^{d}	<0.0001***
Se	1.990 ± 0.066^{a}	1.680 ± 0.057^{b}	1.627 ± 0.041^{b}	2.082 ± 0.069^{a}	<0.0001***
Sn	0.019 ± 0.002^{a}	0.016 ± 0.002^{a}	0.009 ± 0.001^{b}	0.020 ± 0.003^{a}	<0.0001***
U	0.991 ± 0.080^{a}	0.838 ± 0.067^{a}	0.872 ± 0.071^{a}	1.508 ± 0.125^{b}	<0.0001***
V	3.093 ± 0.217^{a}	3.133 ± 0.269^{a}	3.353 ± 0.289^{a}	5.108 ± 0.366^{b}	<0.0001***
Zn	210.167 ± 24.076^{a}	112.792 ± 14.137^{b}	144.450 ± 14.711^{bc}	234.408 ± 35.264^{ac}	<0.0001***

3.4. Spatial variations of trace elements

The trace element concentrations vary considerably depending on the sampling stations (Table 2). Zn is the only element that does not significantly vary spatially (p-value = 0.543). The annual TEPI reveals two stations with High Contamination Level: Saint-Florent 1 (TEPI = 1.127) and Saint-Florent 2 (TEPI = 1.222). Conversely, Bonifacio 2 (TEPI = 0.726) and Calvi 2 (TEPI = 0.738) have been classified as Low Contamination Level.

Table 2. Mean (mg kg^{-1} DW ± SE) trace elements concentrations (mg kg^{-1} DW) in *P. lividus* from eight stations in Corsica Island (A: Ajaccio; B: Bonifacio; C: Calvi; SF: Saint-Florent; 1: reference site; 2: impacted site). ^{abcd} Dissimilar letters denote significant differences between groups (p-value <0.05). p-value: <0.05*; <0.01**; <0.001***

TEs	A1	A2	B1	B2	C1	C2	SF1	SF2	p-value
Ag	0.043 ± 0.012^{a}	0.107 ± 0.015^{bc}	0.062 ± 0.008^{b}	0.015 ± 0.002^{d}	0.169 ± 0.054^{c}	0.146 ± 0.025^{c}	0.942 ± 0.234^{e}	0.138 ± 0.027^{bc}	<0.0001***
Al	41.522 ± 6.972^{a}	21.145 ± 2.824^{b}	19.512 ± 3.052^{b}	17.248 ± 2.354^{b}	9.102 ± 1.670^{c}	6.583 ± 0.746^{c}	19.105 ± 2.632^{b}	29.918 ± 4.144^{ab}	<0.0001***
As	31.895 ± 2.094^{a}	27.492 ± 3.123^{b}	42.410 ± 2.960^{c}	25.287 ± 1.427^{ab}	52.453 ± 3.243^{cd}	43.078 ± 2.578^{c}	71.095 ± 4.842^{e}	57.690 ± 2.935^{de}	<0.0001***
Ba	0.268 ± 0.036^{abc}	0.471 ± 0.182^{a}	0.223 ± 0.039^{abc}	0.247 ± 0.024^{ab}	0.189 ± 0.026^{bc}	0.212 ± 0.028^{abc}	0.304 ± 0.061^{abc}	0.161 ± 0.023^{c}	<0.0001***
Cd	0.123 ± 0.009^{a}	0.136 ± 0.016^{a}	0.246 ± 0.024^{b}	0.113 ± 0.008^{a}	0.339 ± 0.035^{cd}	0.314 ± 0.025^{bd}	0.491 ± 0.037^{e}	0.453 ± 0.039^{ce}	<0.0001***
Co	0.188 ± 0.014^{a}	0.216 ± 0.019^{a}	0.321 ± 0.026^{bc}	0.240 ± 0.020^{ac}	0.274 ± 0.033^{ac}	0.209 ± 0.017^{a}	0.395 ± 0.026^{b}	1.100 ± 0.078^{d}	<0.0001***
Cr	0.500 ± 0.052^{a}	0.505 ± 0.050^{a}	0.878 ± 0.088^{b}	0.688 ± 0.087^{ab}	0.854 ± 0.106^{ab}	0.657 ± 0.068^{ab}	1.301 ± 0.102^{c}	3.572 ± 0.515^{d}	<0.0001***
Cu	3.129 ± 0.170^{ab}	2.954 ± 0.143^{ab}	2.841 ± 0.107^{ab}	3.432 ± 0.166^{a}	2.536 ± 0.119^{b}	2.881 ± 0.118^{ab}	2.525 ± 0.120^{b}	2.586 ± 0.124^{b}	<0.0001***
Fe	76.417 ± 7.730^{ab}	98.113 ± 11.639^{a}	61.560 ± 5.072^{abc}	63.030 ± 8.361^{bc}	44.018 ± 4.256^{c}	60.570 ± 7.676^{bc}	55.663 ± 5.514^{bc}	186.388 ± 28.712^{d}	<0.0001***
Li	0.219 ± 0.017^{a}	0.250 ± 0.015^{abc}	0.250 ± 0.014^{abc}	0.272 ± 0.015^{bc}	0.258 ± 0.021^{abc}	0.307 ± 0.018^{c}	0.249 ± 0.015^{abc}	0.245 ± 0.021^{ab}	0.002**
Mn	1.463 ± 0.165^{ab}	1.305 ± 0.126^{ab}	1.149 ± 0.081^{ab}	1.263 ± 0.110^{ab}	0.965 ± 0.077^{a}	1.077 ± 0.097^{a}	1.019 ± 0.082^{a}	2.050 ± 0.243^{b}	<0.0001***
Mo	0.243 ± 0.034^{ab}	0.220 ± 0.020^{b}	0.371 ± 0.029^{c}	0.257 ± 0.026^{ab}	0.418 ± 0.052^{cd}	0.248 ± 0.018^{abd}	0.419 ± 0.039^{c}	0.306 ± 0.024^{acd}	<0.0001***
Ni	0.516 ± 0.112^{a}	0.729 ± 0.121^{ab}	0.492 ± 0.051^{ab}	0.583 ± 0.084^{ab}	0.983 ± 0.274^{ab}	0.510 ± 0.069^{a}	0.824 ± 0.088^{b}	5.712 ± 1.662^{c}	<0.0001***
Pb	0.191 ± 0.046^{abcd}	0.243 ± 0.070^{abc}	0.188 ± 0.013^{ab}	0.248 ± 0.029^{a}	0.129 ± 0.011^{cde}	0.110 ± 0.008^{de}	0.138 ± 0.013^{bcde}	0.102 ± 0.008^{e}	<0.0001***
Sb	0.077 ± 0.010^{abc}	0.072 ± 0.016^{ab}	0.135 ± 0.030^{c}	0.076 ± 0.011^{ab}	0.049 ± 0.004^{a}	0.051 ± 0.004^{a}	0.092 ± 0.013^{bc}	0.104 ± 0.043^{ab}	<0.0001***
Se	1.661 ± 0.066^{ab}	1.516 ± 0.071^{a}	2.039 ± 0.096^{bc}	1.827 ± 0.092^{abc}	1.831 ± 0.080^{bc}	1.799 ± 0.073^{abc}	2.001 ± 0.084^{bc}	2.086 ± 0.108^{c}	<0.0001***
Sn	0.011 ± 0.001^{a}	0.015 ± 0.001^{b}	0.036 ± 0.003^{c}	0.034 ± 0.006^{c}	0.007 ± 0.001^{d}	0.011 ± 0.003^{ad}	0.008 ± 0.001^{ad}	0.006 ± 0.001^{d}	<0.0001***
U	0.692 ± 0.100^{a}	0.652 ± 0.065^{a}	1.078 ±0.102^{bc}	0.660 ± 0.083^{a}	1.335 ± 0.170^{b}	0.818 ± 0.095^{ac}	1.784 ± 0.168^{d}	1.398 ± 0.137^{bd}	<0.0001***
V	2.570 ± 0.365^{a}	2.647 ± 0.265^{a}	4.294 ± 0.412^{b}	2.657 ± 0.405^{a}	3.775 ± 0.437^{ab}	2.483 ± 0.263^{a}	6.419 ± 0.526^{c}	4.531 ± 0.397^{bc}	<0.0001***
Zn	161.450 ± 25.331^{a}	179.567 ± 27.477^{a}	243.450 ± 59.087^{a}	134.083 ± 19.116^{a}	123.133 ± 23.404^{a}	248.133 ± 42.577^{a}	181.800 ± 28.013^{a}	132.017 ± 26.079^{a}	0.543

3.5. Variations of trace elements according to gender

The concentration of 11 out of the 20 trace elements are different between male and female (p-value < 0.05), the average concentrations of Fe, Cr, Mo, Ni, Pb, U and V being higher in male, although those of As, Cd, Se and Zn are higher in female (Table 3). Specifically, Zn and Fe are the two trace elements presenting the highest differences among gender, Zn being 5 times more concentrated in female and Fe 1.75 times more concentrated in males (p-value < 0.0001). The differences observed don't vary with the seasons and very little according to the sites.

Table 3. Mean concentrations (mg kg^{-1} DW ± SE) of the 20 trace elements in the male and female gonads of *P. lividus*. p-value: <0.05*; <0.01**; <0.001***

Trace elements	Males	Females	p-value
Ag	0.124 ± 0.021	0.260 ± 0.055	0.086
Al	22.983 ± 2.229	18.725 ± 1.605	0.123
As	36.082 ± 1.487	49.624 ± 1.828	<0.0001***
Ba	0.299 ± 0.057	0.231 ± 0.016	0.837
Cd	0.215 ± 0.013	0.322 ± 0.017	<0.0001***
Co	0.372 ± 0.028	0.365 ± 0.023	0.647
Cr	1.234 ± 0.136	1.036 ± 0.101	0.010**
Cu	2.950 ± 0.075	2.796 ± 0.065	0.085
Fe	106.575 ± 9.597	61.933 ± 3.994	<0.0001***
Li	0.266 ± 0.010	0.249 ± 0.008	0.341
Mn	1.250 ± 0.077	1.313 ± 0.064	0.652
Mo	0.370 ± 0.022	0.267 ± 0.012	<0.0001***
Ni	1.741 ± 0.490	0.968 ± 0.151	<0.0001***
Pb	0.204 ± 0.017	0.143 ± 0.016	<0.0001***
Sb	0.096 ± 0.016	0.072 ± 0.004	0.273
Se	1.542 ± 0.033	2.065 ± 0.043	<0.0001***
Sn	0.018 ± 0.002	0.015 ± 0.001	0.731
U	1.163 ± 0.084	0.972 ± 0.051	0.009**
V	4.088 ± 0.248	3.369 ± 0.184	<0.0001***
Zn	53.287 ± 8.275	264.223 ± 18.069	<0.0001***

4. Discussion

Measurements of 22 trace elements were conducted in 480 sea urchins. *P. lividus* has a greater tendency to accumulate essential trace elements such as Cu, Fe, Mn or Zn in contrast to non-essential trace elements (*e.g.* Storelli *et al.*, 2001; Guendouzi *et al.*, 2017). The difference of trace element concentration in sea urchin gonads between the two genders has been little explored (*e.g.* Bayed *et al.*, 2005; Soualili *et al.,* 2008). Zn is an essential element in gametogenesis (Unuma *et al.*, 2007), which explains the high content found in the gonads Unuma *et al.* (2007). Ovogenesis requires greater amounts of Zn than spermatogenesis, which is why concentrations are higher in females than in males. According to Unuma *et al.* (2007), the Major Yolk Protein (MYP) transports the assimilated Zn from the digestive tract to the gonads.

Although non-essential trace elements are expected to have no physiological role, we have evidence however, differential accumulation of those non-essential trace elements between male and female. They probably have a strong affinity with certain essential trace elements and are thus bioaccumulated with them. When there are several trace elements in an environment, antagonistic or synergistic effects can indeed occur (Kabata-Pendias and Pendias, 2001). Moreover, positive inter-elemental relationships have been observed: Cr-V; Cr-Ni; Cr-U are among the strongest combinations. Ni, U and V could be bioaccumulated with Cr, an essential element, in male gonads. A high level of some non-essential trace elements can reduce sperm fertility or increase the frequency of embryo malformations that can lead to death (Pagano *et al.,* 1986; Soualili *et al.,* 2008).

The population in Corsica increases considerably during summer with intense maritime and recreational activities (INSEE, 2018). It's therefore surprising that the contamination levels are lowest this season. Furthermore, studies indicate that transfers inside the echinoid occur on a relatively short time scale (typically of the order of a week) (*e.g.* Miramand *et al.*, 1982; Warnau *et al.*, 1996); These low concentrations of trace element in summer cannot be due to delays in gonad contamination. An increase in the gonad weight of *P. lividus* is correlated with a decrease in trace element concentrations, and inversely. There are two main cell populations in the germinal epithelium of the sea urchin gonad: germinal cells and somatic

cells called nutritive phagocytes (Holland and Holland, 1969; Byrne, 1990; Walker *et al.,* 2005). Assuming that the trace elements accumulate mainly in the somatic cells and not in the germinal cells (Sellem and Guillou, 2007), a dilution of the trace elements is observed during gametogenesis and a concentration during spawning (Guendouzi *et al.*, 2017). The spawning period varies by site and is influenced by environmental factors such as temperature, depth, photoperiod, quality and abundance of food (Fenaux, 1968; Byrne, 1990; Lozano *et al.,* 1995; Guettaf, 1997). The higher gonad weight is probably due to gametogenesis and its decrease to potential spawning. Trace element concentration tend to decrease in spring and summer when gonadosomatic indices are highest and increase in autumn and winter when spawning has occurred.

The size of sea urchin gonads is not necessarily related to the progress of gametogenesis alone. They also grow because somatic cells, the nutritive phagocytes, store extensive nutrient reserves before gametogenesis begins (Böttger *et al.*, 2004). Before the formation of gametes, nutritive phagocytes could store reserves and accumulate trace elements, which explains the significant positive correlation between the concentration of Zn and the gonadosomatic index. About 80% of the total proteins in the gonads, at the stage of pre-gametogenesis and the renewal of nutrient phagocytes after spawning, are in both genders MYP, the protein involved in the transport of Zn in the gonads (Unuma *et al.*, 2003; Unuma *et al.,* 2007). It is also worth noting that the trace element concentration is higher during the autumn, the period characterized by the refilling of nutrient reserves in the nutritive phagocytes (Walker *et al.,* 2013). This supports the hypothesis that TEs are accumulated in nutritive phagocytes.

The temporal variations in trace elements concentration are related to the sea urchin's physiological variations. Thus, to monitor trace element contamination in the sea urchin gonads, it would be more appropriate to avoid the spawning period. It’s also important to take into account the sex of individuals because some trace elements are naturally more abundant in males or females. The role of trace elements in sea urchins needs to be studied further in order to better interpret their dynamics and their potential consequences for the species.

In addition to physiological factors, other parameters such as food may be involved in these temporal variations. Nutritive phagocytes develop when individuals are well nourished during

the pre-gametogenesis and nutrient phagocyte replacement phase (Walker and Lesser, 1998). According to Schlacher-Hoenlinger and Schlacher. (1998), several macrophyte species have levels of some trace elements which have a marked seasonality with lower values in summer and higher in winter. Consumption of food containing higher trace elements levels in winter may amplify the bioaccumulation process. Moreover, according to Nédélec (1982), *P. lividus* shows an alternation of feeding and fasting phases; the period of fasting or low consumption when the gonads are highly developed (Leighton, 1968). Therefore, the low trace element contamination of macrophytes, coupled with the reduced consumption of sea urchins during the summer season, may explain the low concentrations recorded in summer in this study.

Biotic factors are not sufficient to explain the trace element temporal dynamics in sea urchin gonads. In the Bonifacio area, for example, gonadosomatic index variations are not related to the trace element content recorded. Bioavailability is determined by physiological and biological characteristics but also by external environmental conditions (Chapman, 2008). These conditions, such as temperature, pH, oxygen content and salinity, vary with the seasons. They modify trace element availibily and contribute to the temporal variability of trace element concentrations in the gonads.

The weight and size of individuals influence the trace element concentrations measured in the gonads of *P. lividus.* The assumption being that sea urchins feed more when they are large and thus bioaccumulate more contaminants. The use of TEPI allows a reliable comparison of TE contamination both locally and internationally (Wilkes *et al.*, 2017; Ternengo *et al.*, 2018). The seasons affect the TE concentrations in the sea urchin gonads, so it is not surprising to note that the contamination levels of the sites observed in Ternengo *et al.* (2018) in winter are different from the results of the annual TEPI in the present study.

As indicated by Ternengo *et al.* (2018), Saint-Florent 2 has a High Contamination Level probably due to the proximity of the asbestos mine and the influence of soil leaching (Andral *et al.*, 2004; Galgani *et al.*, 2006; Kantin and Pergent-Martini, 2007). High content of Ag has been measured each season and is responsible for the High Level Contamination observed at Saint-Florent 1. In Corsica, the Ag content is very low and only reflects the background level of agriculture (Luy *et al.*, 2012) but although this site is now protected, the old mining

concessions rich in Ag close to the site could explain this high concentration (Gauthier, 2011). Calvi 2, identified as having a High Contamination Level in Ternengo *et al.* (2018) and localised near the water treatment plant of the city, presents the lowest level of contamination in Corsica. This is probably due to a bias in winter sampling when some sites have sea urchins at an advanced stage of the nutrient reserve process. Thus, even if sea urchins are harvested in winter to avoid the period of gametogenesis, the results should be interpreted carefully, taking into account the physiological state. It is preferable to combine the trace element analysis and histology in order to know the physiological stage of the sea urchins and better assess the contamination. It is also necessary to consider these results carefully because despite the analysis of a large number of trace elements, many other contaminants are not taken into account.

To verify the accuracy of these results, it is of interest to compare them with other bioindicators such as *Mytilus galloprovencialis* (Lamarck, 1819) or *Posidonia oceanica*. The trace elements measured at high concentrations at the different sites of this study were also observed in these bioindicators, in particular for Saint-Florent 2 (*e.g.* Kantin and Pergent-Martini, 2007; Lafabrie *et al.*, 2007; Lafabrie *et al.*, 2008; Richir *et al.*, 2015). High concentrations of Ag were observed in *Posidonia oceanica* at Ile Rousse in Richir *et al.* (2015), certainly originating in the same type of source as for the contamination at Saint-Florent 1. In view of these results, the sea urchin *P. lividus* proves to be a good bioindicator and could complement the use of other bioindicators. An ecotoxicological study of this sea urchin would provide information on the contamination at sea and on the influence of the trophic chain and substrates, while the mussel would determine the contamination in the water column, and *Posidonia oceanica* the contamination on substrate and in the first link of the trophic chain (Richir and Gobert, 2014). In addition, depending on the sampling period, one or other of these bioindicators would be more suitable. The spawning period would be avoided for the sea urchin and the mussel while it would be more interesting to study *Posidonia oceanica* in the spring (Kantin *et al.*, 2015).

5. Conclusion

This study highlights, once again, the need to consider biotic factors (gender, reproductive activity) and abiotic factors (physical and chemical characteristics of seawater or food) in the use of sea urchins as bioindicators. Differences in concentration were observed, according to sex, for 11 trace elements. This would probably be due to gametogenesis and to antagonistic or synergistic effects between trace elements. It is necessary to extend our knowledge to assess the effects of these contaminants on the sea urchin populations. There are temporal variations marked by higher trace element concentrations in autumn and winter and, conversely, lower concentrations during the summer season. A dilution effect is observed on the trace element content in the gonads during gametogenesis. The use of gonads should be avoided during the spawning period in order to avoid biased comparisons. A difference in concentration of trace elements is observed between the different sites. Due to the influence of the soil and the former asbestos mine at Canari, the Saint-Florent area is the most contaminated. On the basis of these results, *P. lividus* appears as an interesting tool for achieving a better understanding of anthropic pressures. Associated with other bioindicators such as *Mytilus galloprovencialis* or *Posidonia oceanica*, the study of its ecotoxicological properties would enable managers to act at source and reduce degradation or improve the ecological quality of water bodies. In addition, it seems that no previous study of the sea urchin, taking all these factors into account, has analyzed so many trace elements in a Mediterranean region. These results are therefore essential and can serve as a reference state for the Medirerranean Sea.

Article 3

Trace element contamination and effects on *Paracentrotus lividus* using several approaches: pollution indices, accumulation factors and biochemical tools

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Contexte et résumé de l'article 3 « Évaluation de la contamination et des effets des éléments traces chez *Paracentrotus lividus* à l'aide de plusieurs approches : indices de pollution, facteurs d'accumulation et outils biochimiques »

Les travaux menés dans l'article précédent ont permit de mettre en évidence que le secteur localisé à proximité l'ancienne mine d'amiante à Canari (Haute-Corse) présentait des niveaux de contamination les plus élevée parmi les stations étudiées. Les éléments traces mesurés en fortes quantités s'avèrent être caractéristiques du site. Ces derniers sont dus aux rejets de l'ancienne mine et au socle géologique riche en cobalt, chrome, fer et nickel. Afin d'évaluer l'étendue de cette contamination, la concentration de 24 éléments traces a été mesurée dans les gonades et tubes digestifs de l'oursin *Paracentrotus lividus*, les macroalgues, la colonne d'eau de mer et les sédiments marins au sein de 12 stations répartis à proximité de l'ancienne mine d'amiante et dans un site de référence. Les différents facteurs d'accumulation (BAF, BMF et BSAF) indiquent que la bioaccumulation se déroule comme suit : macrophytes > tubes digestifs > gonades. Le calcul des « Trace Element Pollution Index » (TEPI) ont permis de mettre en évidence les gradients de contaminations qui sont principalement dus aux courants marins dominants permettant la migration des rebuts miniers le long du littoral vers le sud. Cette hypothèse a été confirmée par le « Trace Element Spatial Variation Index » (TESVI) qui a identifié les éléments traces caractéristiques au sud de la mine. Des teneurs élevées en peroxyde d'hydrogène (H_2O_2), associées à une activité enzymatique élevée de la catalase et de la glutathion-S-transférase, ont également été identifiées sur ces sites et sur le site de référence. Ces résultats au sud de l'ancienne mine peuvent être dus à la contamination plus élevée en éléments traces. Un stress induit par des facteurs biotiques ou la présence d'une autre source de contamination que les éléments traces pourrait être à l'origine des résultats obtenus sur le site de référence (*e.g.* la contamination microbiologique, les événements hydrodynamiques). Toutefois, aucune différence significative entre les sites n'a été mise en évidence en ce qui concerne la teneur en malondialdéhyde (MDA) et l'activité spécifique de la superoxyde dismutase (SOD) et de la glutathionne peroxydase (GPX). Ces données suggèrent que le stress oxydatif induit par la contamination de l'ancienne mine d'amiante n'affecte pas la santé de *Paracentrotus lividus*. Ce travail a fourni un ensemble de données utiles permettant une meilleure utilisation des oursins et de plusieurs outils pour évaluer la contamination par les éléments traces dans les écosystèmes côtiers.

1. Introduction

Coastal ecosystems are at the interface between the marine and terrestrial domains and provide many goods and ecosystem services of high-value (Costanza *et al.*, 2017). Coastal marine waters are particularly vulnerable to a wide range of disturbances such as climate change effects and the release of great variety of contaminants (Livingston *et al.*, 1994; Mostofa *et al.*, 2013). These habitats are subject to an increase in anthropogenic activities that can lead to high levels of contamination (Islam & Tanaka, 2004). The presence of these contaminants cause devastating effects on organisms and their ecosystems, making marine pollution a global concern (Abdel-Shafy & Mansour, 2016; Torres *et al.*, 2016).

Among the major pollutants, trace elements are a real ecological issues because of their toxicity, their persistence and their ability to accumulate in marine organisms and even biomagnified through the trophic chain (Rainbow & Luoma, 2011; Bonanno & Di Martino, 2017). The inputs of trace elements in marine environment derived from natural (geological phenomena): geogenic pollution and/or anthropogenic sources (*e.g.* mining, agriculture, petrochemical industry, aquaculture, sewage waste water) resulting in increased concentration levels (D'Adamo *et al.*, 2008; Barhoumi *et al.*, 2014). The trace elements can be classified as essential or non-essential (Jiang *et al.*, 2014). Essential elements have a biological function in organisms such as copper, iron and zinc and non-essential trace elements are not involved in any metabolic mechanism such as cadmium, mercury and lead (Amiard, 2011). Above a threshold, all trace elements present a potential threat for organisms (Nordberg *et al.*, 2007). As a result of the threats caused by trace elements, a continuous monitoring of their presence and concentration must be undertaken (Richir & Gobert, 2014).

Mediterranean Sea, which represent 0.82% of the global ocean surface, is one of the main hotspots of marine biodiversity in the world with 4–18% of identified marine species (Coll *et al.*, 2010). Nevertheless, the coastal areas surrounding this semi-enclosed sea are subjected to the increasing synergistic effects of global changes and intense anthropogenic activities leading to significant modification in the structure and functioning of trophic webs and in the degradation of coastal and marine ecosystems (Coll *et al.*, 2012; Ramírez *et al.*, 2018; Stock *et al.*, 2018a). Located in the northwestern Mediterranean Sea, Corsica Island has long been

considered as a pristine region with low anthropogenic disturbances resulting in low levels of contamination (Gobert *et al.*, 2017). However, recent studies have revealed major trace elements contamination near an old asbestos mine in northern Corsica (Cary *et al.*, 2013; Ternengo *et al.*, 2018; El Idrissi *et al.*, 2020). These high levels are originating from untreated material directly discharged into the sea during the active period of the asbestos mine between 1950 and 1965 (BRGM, 2017; Cary *et al.*, 2013). The continuous leaching of these mining residues, still present on the sides of the mine, contributes to the dispersion of nickel, chromium and cobalt along the coastline (Galgani *et al.*, 2006; Kantin & Pergent-Martini, 2007). Besides threats associated with the old asbestos mine, this region is characterized by reduced anthropogenic pressures (*i.e.* industrial, agricultural and urban activities; ASR, 2011). Because of these specificities, this sector represents a privileged study area in order to assess the trace elements contamination of a specific source within the coastal ecosystems.

Bioaccumulation of trace elements by marine organisms can occur through various pathways such as absorption and/or adsorption directly from sediments and interstitial seawater but also through ingestion, which will potentially affect other species along the food chain (Adamo *et al.* 2005; Chen *et al.* 2007; Türkmen *et al.*, 2008). In order to assess bioaccumulation and biomagnification to clarify the toxicity and fate of trace elements in organisms, it is important to determine the concentrations in the different biological and environmental matrices (water column, sediment, plants, animals; Jha, 2004). Echinoderms, and in particular sea urchins, are very appropriate organisms for use in bioindication studies (Parra-Luna *et al.*, 2020). Along the Mediterranean coast, *Paracentrotus lividus* (Lamarck, 1816) is frequently considered as a good bioindicator of local pollution because of its wide distribution, abundance, benthic behavior, sedentary habits, rapid response and its recognized sensitivity to a variety of pollutants (Sugni *et al.*, 2007; Amri *et al.*, 2017; Rouhane-Hacene *et al.*, 2017). Described as the organs that accumulate the most trace elements, its gonads and gut are of interest in studies assessing contamination levels in coastal marine ecosystems (Augier *et al.*, 1989; Warnau *et al.*, 1998; Geraci *et al.*, 2004). *P. lividus* is also a species of economic importance due to its valued consumption in several countries (Fernández-Boán *et al.*, 2013; Powell *et al.*, 2014; Sun & Chiang, 2015), and ecological significance, controlling the dynamics of seaweeds and seagrass through its grazing activity (Lawrence & Sammarco, 1982; Boudouresque & Verlaque, 2013). The use of biomarkers as bioindicators is an important approach in aquatic biomonitoring to

assess the effects and relationships between exposure to environmental pollutants and increased effects on individuals and populations (Bouzahouane *et al.*, 2018). The effects of pollutants on the marine ecosystem can be expressed in terms of biochemical endpoints (Kamel *et al.*, 2014). The exposure of aquatic organisms to trace elements can lead to the production of reactive oxygen species (ROS; Nieto *et al.*, 2010), causing an imbalance between the production of ROS and endogenous antioxidant activity (Beyersmann & Hartwig, 2008). Oxidative stress is involved in DNA damage, protein oxidation and lipid peroxidation (Winston & Digiulio, 1991). In this context, it is relevant to evaluate the activities of antioxidant enzymes acting against oxidative stress such as catalase (CAT), glutathione peroxidase (GPX), glutathione-s-transferase (GST) and superoxide dismutase (SOD). The content of malondialdehyde (MDA), by-product of lipid peroxidation, and the level of hydrogen peroxide (H2O2) in the tissues of organisms is also a good indicator of oxidative stress intensity. Therefore, oxidative stress biomarkers are widely used in marine ecotoxicology (Kamel *et al.* 2014; Benedetti *et al.*, 2015; Ghribi *et al.*, 2020).

The aims of this study are to (i) evaluate the reliability of different matrices (sea urchin, macroalgae, water column and sediment) to characterize 24 trace elements contamination levels; (ii) test the effectiveness of several indices used in ecotoxicological studies in order to confirm their use in the assessment of contamination; (iii) determine bioaccumulation, biomagnification and biota-sediment accumulation factor of trace elements and (iv) estimate the effects of trace elements contamination on the oxidative stress of *P. lividus*.

2. Material and methods

2.1. Study area

The study area is located on the northwest coast of Corsica (NW Mediterranean Sea), near the old asbestos mine of Canari (42°49'15''N, 9°19'41'' E; Fig. 1A; Fig. 1B). The old asbestos mine stretches along 1 km of rocky coastline (Fig. 1C). After the discovery of the asbestos deposit in 1898, the mine was industrially exploited from 1948 to 1965 (BRGM, 1997). During this active period of exploitation, up to 28,000 tons of asbestos were produced

yearly and approximately 4.5 million m^3 of solid mine wastes were directly discharged into the Mediterranean Sea (Méria, 2004, BRGM, 1997). Though the closure of the mine for over 50 years, the leaching of mining residues, still present along the coastline of the mine, contributes to the continuous dispersion of nickel, chromium and cobalt into the marine environment (Galgani *et al.,* 2006; Kantin & Pergent-Martini, 2007). Currently, the low levels of anthropogenic activity in this area and the local sources of contamination associated to the old mine provide a unique mesocosm to study the contamination in the marine environment (ASR, 2011).

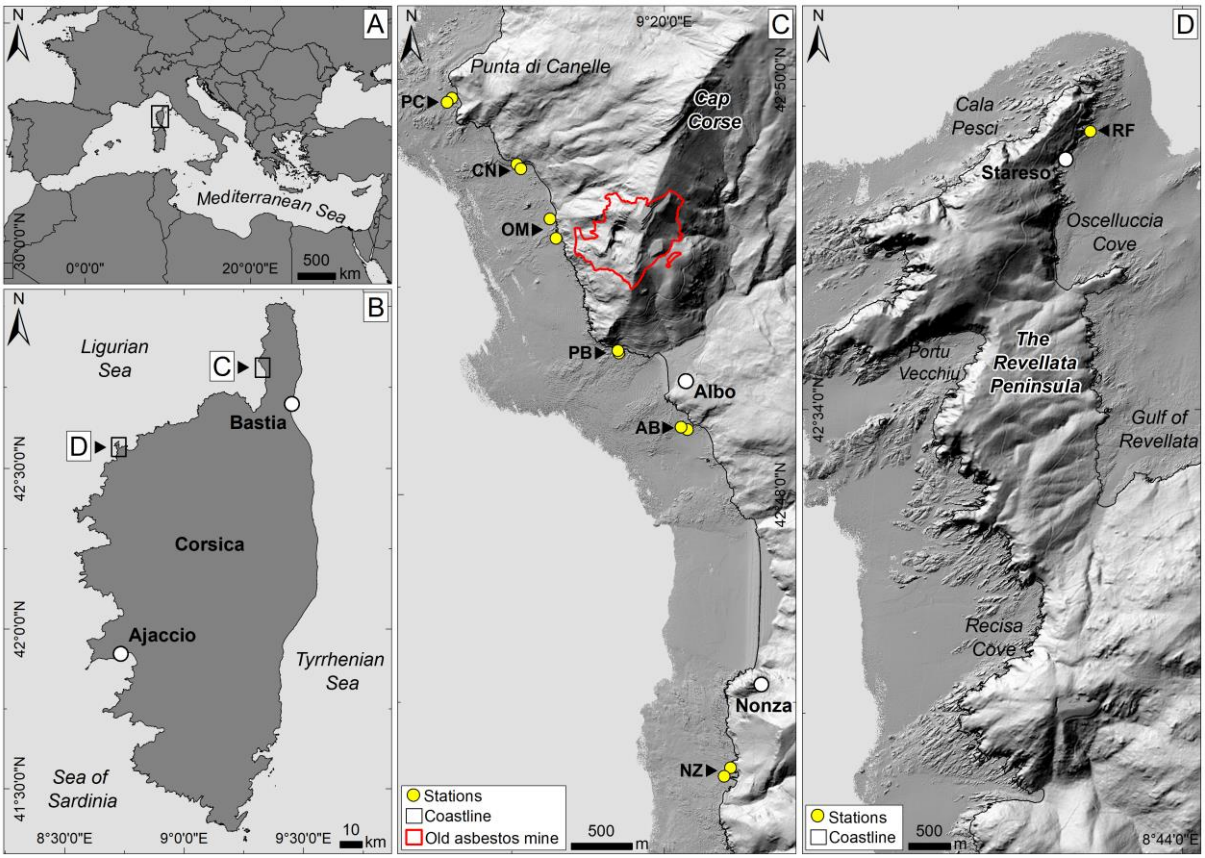

Figure 1. Location of the study area in Corsica Island (A, B) and the sampling sites (C, D). PC: Punta di Canelle; CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site

Six sampling sites located along the coast were selected (Fig. 1C): one in front of the old asbestos mine (Old Mine: OM), two to the north (Punta di Canelle: PC; Canelle: CN) and three to the south (Punta Bianca: PB; Albo: AB; Nonza: NZ). Samples were also collected in a reference site (RS), recognized to have a low level of trace elements contamination (Fig. 1D; Gobert & Richir, 2019; El Idrissi *et al.*, 2020). The selection of these sites was achieved to assess the differences in trace elements concentration in order to establish a potential gradient of

contamination and also to better understand the effects of these different concentrations on the organisms.

2.2. Collection and processing of biological samples

Individuals (n = 28) of *P. lividus* sea urchin and macroalgae species observed in each sampling site were collected at two distinct depths for each site (3 m and 6 m depth) in winter (Fig. 1C; Fig. 1D). In total, 364 sea urchins of commercial size (~50 mm) were collected and directly transported in a cooler with oxygenated seawater to the laboratory. Among the macroalgae collected in each sites, the three most dominant taxa were retained and presented in this study *(Ericaria amentacea* (C.Agardh) Molinari & Guiry, 2020, *Dictyota dichotoma* (Hudson) J.V.Lamouroux, 1809 and *Ellisolandia elongata* (J.Ellis & Solander) K.R.Hind & G.W.Saunders, 2013).

After sampling, macroalgae were rinsed with ultrapure MilliQ™ water and placed at -20°C until chemical analysis. The specimens of *P. lividus* were measured, weighed and dissected. The sex ratio has been respected to avoid bias. Gonads and guts collected on sea urchins (n = 16) from each station were immediately weighed and stored at –20 °C. Frozen samples were lyophilized (CHRIST LCG Lyochamber Guard 121550 PMMA/Alpha 1-4 LD plus) and ground with an agate mortar. Approximately 0.2 g of each dried material was mineralized in Teflon digestion vessels, in a closed microwave digestion labstation (Ethos D, Milestone Inc. Sorisole, Italy), using nitric acid (HNO_3, 60%) and hydrogen peroxide (H_2O_2, 30%) as reagents (Suprapur grade, Merck, Darmstadt, Germany).

2.3. Collection and processing of sediment samples

Sediment samples were collected at each station using a 0.1 m^2 Van Veen grab from the bottom (top 20 cm). After collection, the sediments were stored at –20 °C until chemical analysis. Frozen sediments were lyophilized (BenchTop 3L, VirTis Company Inc.) and were eluted for 4 h at room temperature with 30 mL of HCl 1N (Suprapure grade, Merck, Darmstadt, Germany), according to Townsend *et al.* (2007). A 4-h extraction time in HCl 1N ensures the removal of the available precipitated trace elements while not favouring the extraction of

natural geogenic metals (Snape *et al.,* 2004). Eluates were then diluted to an appropriate volume of 50 mL, centrifuged 10 min at 2000 rotations per minute and separated from their remaining culot prior to being analyzed.

2.4. Trace element concentrations

2.4.1. Bioavailable concentrations in the seawater column

The concentration of trace elements in the seawater column of each sites was measured using diffusive gradients in thin films (DGTs) devices (DGT Research Ltd., UK). DGT units, fixed with a nylon fishing line to a plastic stage buried in sediments, floated freely in the seawater column for one week. After recovery, DGTs were rinsed with ultrapure MilliQ™ water and stored at 4 °C until analysis. As trace metal diffusion coefficients through the diffusive layer depend on water temperature, average temperatures were recorded using HOBO TidbiT® v2 loggers (accuracy: ± 0.21 °C).

2.4.2. Trace element analysis

The concentration of 24 trace elements concentrations (Ag, Al, As, Ba, Be, Bi, Cd, Co, Cr, Cu, Fe, Hg, Li, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, U, V, Zn) were determined by Inductively Coupled Plasma Mass Spectrometry using Dynamic Reaction Cell technology (ICP-MS ELAN DRC II, Perkin Elmer), according to the method described by Richir & Gobert (2014). In order to check the purity of the chemicals used, analytical blanks were analyzed similarly to the samples and were performed every 40 samples. Analytical accuracy was checked by analyzing Certified Reference Materials (CRM): DORM-4 (fish protein), NIST1566b (oyster), NIST2976 (mussel tissue), BCR-60 (*Lagarosiphon major*), BCR-661 (*Platyhypnidium riparioides*) and GBW07603 (bush twigs and leaves). For each TE, detection limit (LD) and quantification limit (LQ) were calculated, depending on their specific blank distribution (Grinzaid *et al.* 1977; Currie, 1999). The total Hg (THg) content of biological samples was determined using atomic absorption spectrometry at 254 nm, in a Direct Mercury Analyser (DMA 80 153 Milestone, Minnesota, USA). Quality assessment was operated using replicates, standards (THg 100 ng g^{-1}), blanks (HCl 1%) and CRM at the beginning and the end of each series. The results

are expressed in milligrams of element per kilogram of dry weight (mg kg^{-1} DW) for tissue and sediment, and in milligrams of elements per liter (mg L^{-1}). TEs with values below the detection limit were removed from the database. For the others, concentrations below the LD were replaced with a value of LD/2, as reported by Skrbić *et al.* (2010).

2.5. Histological study

The gonads of six sea urchins from different stations were fixed in formaldehyde in order to perform a histological study and determine the reproductive stage. This allow to determine if trace elements levels are influenced by reproductive stage in this study. Gonadal tissues were dehydrated using ethanol (from 70 to 100%) then placed in Neo-Clear™, xylene substitute, before being embedded in paraffin. After cooling the blocks, two duplicate sections (thickness: 5 µm) were taken by using a microtome, mounted on slides, and air-dried for the deparaffinization of the tissue in preparation to the rehydration. After that, the sections were stained with Masson's Trichrome and observed under a light microscope to determine the stage of maturity based on the stages described by Byrne (1990): stage I, recovery; stage II, growing; stage III, premature; stage IV, mature; stage V, partly spawned; stage VI, spent.

2.6. Biochemical analyses

For the oxidative stress study, the gonads of six sea urchins per station were fixed in liquid nitrogen before storage at –80 °C. Samples were homogenized using a Potter-Elvehjem homogenizer in chilled phosphate buffer (100 mM, pH 7.4; 25 mg w/w per mL of buffer) containing 20% glycerol and 0.2 mM phenylmethylsulfonyl fluoride as a serine protease inhibitor. The homogenates were centrifuged at 15,000 × g for 30 min at 4 °C and the supernatant was used for biochemical assays. Protein concentration was measured as described in Bradford (1976) and was used to normalize the final unit for biomarker responses. Biomarkers including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione-S-transferase (GST) and determination of malondialdéhyde (MDA) content were assessed as described by Greani *et al.* (2017). The hydrogen peroxide (H_2O_2) concentration was determined using a PeroxiDetect Kit (Sigma, Aldrich, St. Louis, MO, United States) as described by Lourkisti *et al.* (2020).

2.7. Elemental and isotopic analyses

Gut contents of *P. lividus* (n = 12 per site) and food sources (*D. dichotoma, E. elongata and E. amentacea*) were analyzed through elemental and isotopic analyses. The sea urchins and macroalgae samples were dried at 60 °C for 48-96 h and successively grounded in a homogenous powder with agate mortal. As acidification is known to alter N stable isotope ratios (Mateo *et al.*, 2008), acidified samples (*E. elongata*) were analyzed twice: once for stable C isotopic ratios, using decarbonated material, and once for N isotopic ratios, using native material. Samples of food source (1-5 mg) and gut content (2-3 mg) were subsequently loaded into tin capsules (8 x 5 mm, Elemental Microanalysis). Stable isotope ratios measurements were performed *via* continuous flow-elemental analysis-isotope-ratio mass spectrometry (CF-EA-IRMS) at University of Liège, using a Vario Micro Cube elemental analyser (Elementar Analysensysteme GmBH, Hanau, Germany) coupled to an Isoprime 100 mass spectrometer (Isoprime, Cheadle, UK). Isotopic ratios of C and N were expressed conventionally (Coplen 2011), using standard delta (δ) notation relative to their respective international standards, Vienna-Pee Dee Belemnite (VPDB) and atmospheric N_2. Certified reference materials (CRM) of sucrose (IAEA-C6, $\delta^{13}C$ = -10.8 ± 0.5‰) and ammonium sulphate (IAEA-N2, $\delta^{15}N$ = 20.3 ± 0.2‰) obtained from the International Atomic Energy Agency (IAEA, Vienna, Austria), were used for the measurement of isotopic ratios. These CRM, procedural blanks and internal replicates (*i.e.,* glycine and in-house seagrass reference materials) were used to assess the analytical precision. Standard deviations on multi-batch replicate measurements were 0.1‰ for $\delta^{13}C$ and 0.2‰ for $\delta^{15}N$.

The relative contribution of macroalgae to the diet of the sea urchin *P. lividus* was estimated with a Bayesian isotopic mixing model (MixSIAR, Stock and Semmens, 2016; Stock *et al.*, 2018b). The application of mixing model provided accurate information concerning the contribution of macroalgae species to the sea urchin tissues recognized the main components of the diet under different conditions (Peterson, 1999; Fry, 2006; Wing *et al.*, 2008; Cabanillas-Terán *et al.*, 2016). The model runs included the isotopic signatures of each individuals, isotopic compositions of food sources (mean ± SD) and trophic enrichment factors (TEFs; mean ± SD) corresponding to the isotopic composition difference of consumer tissues and the

potential food sources (Mascart *et al.*, 2018). Though the use of adequate TEFs is required to run mixing model, there are no specific TEF for the taxa studied here, we used a widely applicable values (*i.e.,* 0.4 ± 1.2‰ for C, 2.3 ± 1.6‰ for N) from McCutchan *et al.* (2003). Model was run using the MixSIAR 3.1.12 package in R 4.1. The results of the mixing model showing the calculated sea urchin dietary proportions were represented using mean ± SD and 95% credible intervals (CI_{95}) indicating the intervals of probability density function distributions.

2.8. Numerical procedures

In order to compare the contamination levels of the different sites, the Trace Element Pollution Index (TEPI) was calculated for each sites. The TEPI allows a reliable comparison of study sites, regardless of trace elements or the biological model used (Richir and Gobert, 2014). The index was calculated with concentrations measured in gonads, digestive tract and macroalgae to confirm its robustness. The data were standardized by mean normalization as recommended for the calculation by Richir and Gobert (2014). TEPI values were calculated using the following formula:

$$TEPI = (Cf_1 * Cf_2 * ... * Cf_n)^{1/n}$$

where Cf_n is the mean normalized concentration of the trace element at each site and n is the number of trace element examined. The higher the TEPI value, the more contaminated the site is. In the aim to classify and compare the trace elements according to their spatial variability on all the studied sites, the Trace Element Spatial Variation Index (TESVI) was determined for each element according to Richir and Gobert (2014). For each trace element, TESVI was calculated as follows:

$$TESVI = [(x_{max} / x_{min})/(\sum(x_{max} / x_i) / n)] * SD$$

where x_{max} and x_{min} are the maximum and minimum mean concentrations recorded among the n sites, x_i are the mean concentrations recorded in each of the n sites, and SD is the standard deviation of the mean ratio $\sum(x_{max} / x_i) / n$. For a given trace element, the higher the

value of the TESVI, the more its environmental levels will vary globally across the study area. Therefore, the higher the TESVI, the more representative it is of a site.

Bioaccumulation is generally referred to as a process in which the chemical concentration in an organism achieves a level that exceeds that in the environment, the diet, or both (Gobas *et al.*, 2009). The extent to which chemicals bioaccumulate is expressed by several values, including bioaccumulation factor (BAF), biomagnification factor (BMF) and biota-sediment accumulation factor (BSAF). BAF is ratio of the steady chemical concentrations in an aquatic water-respiring organism (CB, g kg^{-1} WW) and the water (CW, g L^{-1}) determined from field data in which sampled organisms are exposed to a chemical in the water and in their diet. BAF is calculated using the following formula: CB /CW. BFM is the ratio of the steady state chemical concentrations in an organism (CB, g kg^{-1} WW) and the diet of the organism (CD, g kg^{-1} WW) determined from field data. Lastly, BSAF is the ratio of the chemical concentrations in an organism (CB, g kg^{-1} DW) and the sediment (CS, g kg^{-1} DW).

Data were expressed as mean ± standard error (SE) and analyzed using XLSTAT software. The data were transformed in order to meet the conditions of application of the parametric tests and to reduce the effect of outliers skewing the data distribution. Analyses of variance (ANOVA) followed by post-hoc Tukey's honestly significant difference (HSD) tests were performed. The relationship between enzymatic activities and trace elements were measured by Pearson correlation coefficient. A significant difference was considered a p-value less than 0.05.

3. Results

3.1. Distribution of trace element content

The levels in Be and Bi levels were below the detection limit in all matrices analyzed, so they were not considered in the statistical analyses of the results. Also, Hg concentrations were also below the detection limit in the sediments and the seawater column. For clarity purpose, only 14 representative examples chosen among the 24 studied trace elements were

graphically illustrated throughout the manuscript, and discussion mainly revolves around these selected examples. The mean trace element concentrations measured in the different matrices (sea urchins, macroalgae, sediment and seawater column) are presented in Fig. 2.

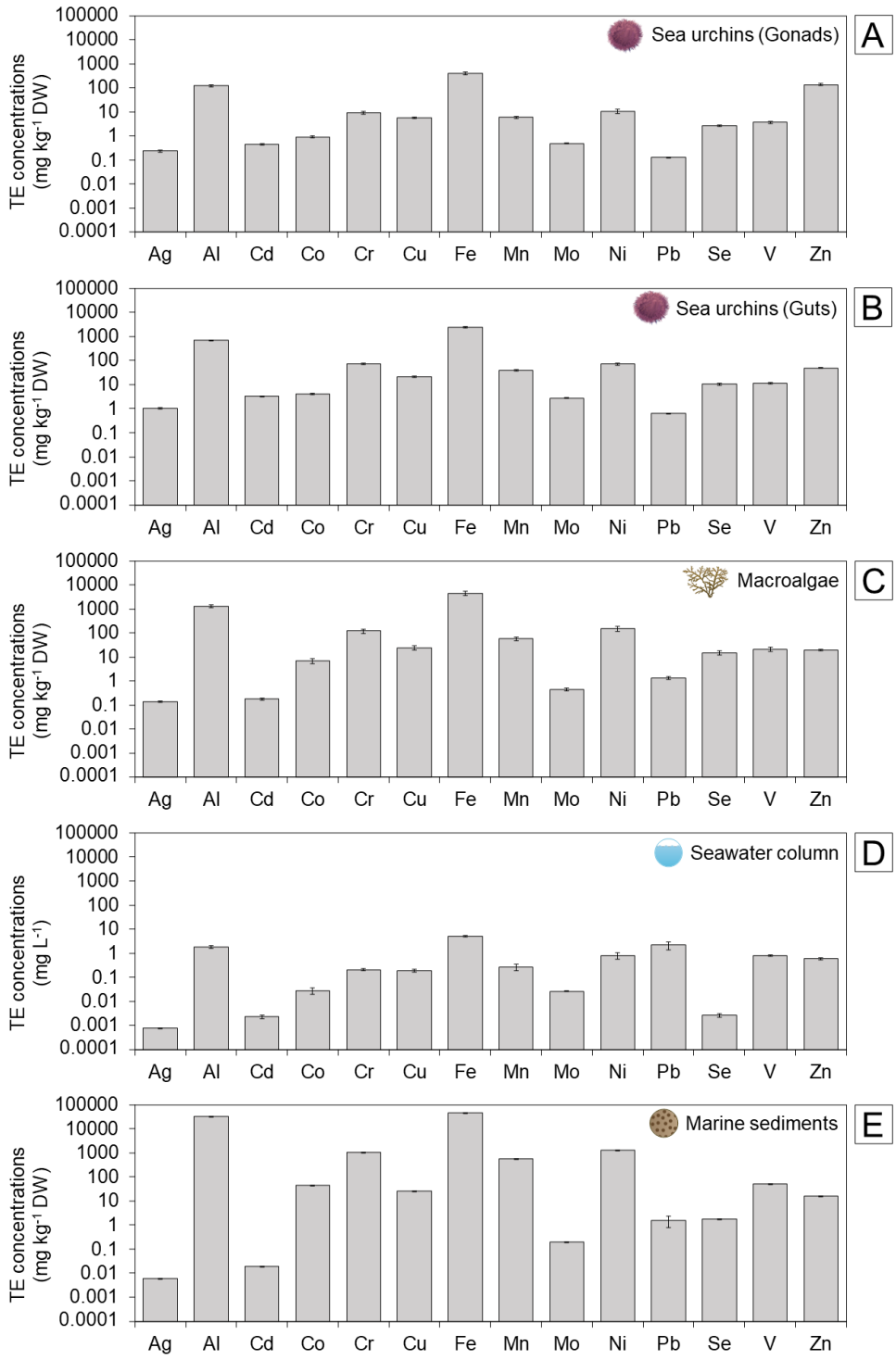

Figure 2. Distribution of trace element (TE) concentrations in sea urchins *P. lividus* (gonads: A, guts: B), macroalgae (C), seawater column (D) and marine sediments (E).

Trace element concentrations follow the sequence: Fe> Zn> Al> Ni> Cr> Mn> Cu> V> Se> Co> Mo> Cd> Ag> Pb in sea urchin gonads; Fe> Al> Cr >Ni> Zn> Mn> Cu> V> Se> Co> Cd> Mo> Ag> Pb in sea urchin guts; Fe> Al> Ni> Cr> Mn> Cu> V> Zn> Co> Pb> Mo> Cd> Ag in macroalgae; Fe> Pb> Al> Ni> V> Zn> Se> Mn> Cr> Cu> Co> Mo> Cd> Ag in seawater column and Fe > Al> Ni> Cr> Mn> V> Co> Cu> Zn> Se> Pb> Mo> Cd> Ag in sediment (Fig. 2).

3.2. Spatial variations of trace elements

There is no significant difference in trace elements concentrations between the samples collected at 3 m depth and those collected at a 6 m depth. The TEPI was calculated with concentrations in sea urchin gonads, sea urchin guts and macroalgae (Fig. 3). In gonads and guts, a gradient of contamination clearly appeared, with higher contamination to the south of the old asbestos mine, particularly in Albo (gonads: 1.367, guts: 1.172) and Nonza (gonads: 1.161, guts: 1.201; Fig. 3A; Fig. 3B). In contrast, the reference site is the least contaminated, with a TEPI of 0.456 for the gonads and 0.529 for guts (Fig. 3A; Fig. 3B).

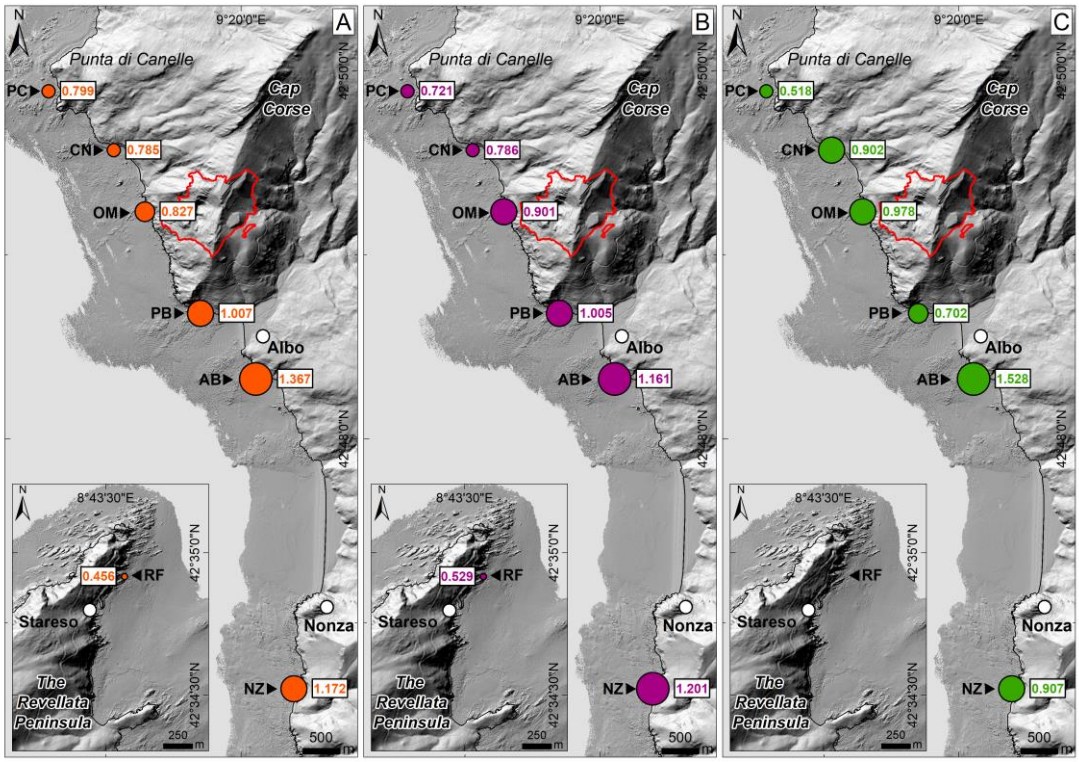

Figure 3. Trace Element Pollution Index (TEPI), determined using sea urchins (gonads: A, orange points, guts: B, purple points) and macroalgae (C, green points), of the seven sites (PC: Punta di Canelle; CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site). No macroalgae samples were collected in RF.

The TEPI calculated with macroalgae showed a more contrasting gradient with the Canelle site, located north of the mine, similarly contaminated to Nonza (0.902 and 0.907, respectively; Fig. 3C). The four trace elements with the highest TESVI in gonads, guts and macroalgae are Co, Cr, Fe and Ni (Table 1). The highest concentrations were noted at Albo for the four trace elements (Table 1). Therefore, Co concentrations are 10 to 20-fold higher at Albo than at the reference site, Cr 96 to 172-fold, Fe 2 to 9-fold and Ni approximately 38-fold.

Table 1. Trace Element Spatial Variation Index (TESVI) of 22 trace elements examined in sea urchins (gonads and guts) and macroalgae from six sites near the old asbestos mine (Corsica, NW Mediterranean Sea; PC: Punta Bianca; CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site). The higher the TESVI value, the greater the spatial variation of that element among the sampling locations. In dark grey, the higher values.

Trace elements	Sea urchins				Macroalgae	
	Gonads		Guts			
	TESVI	Site x_{max}	TESVI	Site x_{max}	TESVI	Site x_{max}
Ag	1.622	NZ	3.964	CN	0.216	PC
Al	**4.896**	**AB**	1.321	AB	0.776	AB
As	0.704	AB	0.962	OM	2.357	AB
Ba	0.710	PC	0.182	PB	n.a	n.a
Cd	1.354	PC	0.370	CN	0.639	PC
Co	**8.243**	**AB**	**31.789**	**AB**	**10.124**	**AB**
Cr	**194.926**	**AB**	**406.518**	**AB**	**13.335**	**AB**
Cu	0.589	NZ	0.750	NZ	**4.422**	**AB**
Fe	**16.854**	**AB**	**15.238**	**AB**	**10.913**	**AB**
Hg	1.703	AB	0.576	RF	n.a	n.a
Li	0.160	AB	0.247	PB	n.a	n.a
Mn	3.078	AB	**6.650**	**AB**	3.897	AB
Mo	0.454	PC	0.652	RF	1.871	AB
Ni	**56.107**	**AB**	**50.454**	**AB**	**17.229**	**AB**
Pb	0.296	RF	0.488	RF	0.422	CN
Sb	0.794	CN	0.304	RF	0.185	AB
Se	0.790	NZ	3.457	NZ	3.451	AB
Sn	1.002	AB	2.190	RF	2.893	AB
Sr	0.960	AB	0.520	PB	0.237	PC
U	3.293	CN	0.120	PB	n.a	n.a
V	3.735	AB	1.310	AB	2.000	OM
Zn	1.429	PC	0.287	PC	0.204	CN

3.3. Bioaccumulation, biomagnification and biota-sediment accumulation factors

The BAF and BSAF were calculated with the concentrations in the gonads and guts of the sea urchin and with concentrations measured in macroalgae (Table 2). Most trace elements were most bioaccumulated in macroalgae then the guts and finally the gonads (Al, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, V; Table 2). Only Zn had a higher accumulation in the gonads in comparison to the guts and macroalgae (Table 2).

Table 2. Bioaccumulation (BAF), biomagnification (BMF) and biota-sediment accumulation (BSAF) factor of 14 trace elements. TE: Trace elements; SW: Seawater.

TE	BAF			BMF	BSAF		
	gonads/ SW	guts/ SW	macroalgae/ SW	guts/ macroalgae	gonads/ sediment	guts/ sediment	macroalgae/ sediment
Ag	50.3526	163.6319	29.8274	5.5936	38.7261	167.7989	22.9402
Al	11.2258	46.8450	117.4314	0.1401	0.0038	0.0210	0.0395
Cd	33.4045	181.6255	13.1774	6.9715	23.6158	171.2038	9.3159
Co	5.5608	18.1816	41.7896	0.1351	0.0205	0.0894	0.1541
Cr	7.5379	45.2430	98.6556	0.1514	0.0086	0.0691	0.1131
Cu	5.0336	14.3122	21.8451	0.2092	0.2221	0.8419	0.9638
Fe	13.1917	59.3457	148.6595	0.1393	0.0086	0.0516	0.0970
Mn	3.7815	18.2950	36.8864	0.1556	0.0109	0.0702	0.1061
Mo	3.1471	13.0912	2.7865	3.2869	2.5737	14.2747	2.2788
Ni	2.2670	11.1937	32.2017	0.1214	0.0081	0.0532	0.1149
Pb	0.0097	0.0361	0.1022	0.1169	0.0794	0.3938	0.8352
Se	170.7810	502.6702	958.6628	0.1535	1.5232	5.9777	8.5503
V	0.7858	1.8492	4.5681	0.1535	0.0720	0.2259	0.4185
Zn	38.8205	10.4672	5.4270	0.8050	8.7230	3.1360	1.2195

Among the macroalgae studied, the isotopic analysis highlighted that sea urchins collected fed mainly on *E. elongata* (96.6 ± 2.3%; CI_{95}: 90.8-99.6%) and very low on *D. dichotoma* (2.5 ± 2.2%; CI_{95}: 0.1-8.4%) and *E. amentacea* (0.9 ± 0.9%; CI_{95}: 0-3.2%; Fig. 4). In order to calculate the BMF, a weighted average of macroalgae was used for accuracy. Therefore, the formula used in this study was:

$$CB/(CD_1 * 0.966 + CD_2 * 0.025 + CD_3 * 0.009)$$

where, CB is the concentration measured in guts, CD_1 the concentration in *E. elongata*, CD_2 in *D. dichotoma* and CD_3 in *E. amentacea*. The highest BMFs were obtained for Ag, Cd and Mo, elements for which the guts accumulate more than the other matrices (Table 2).

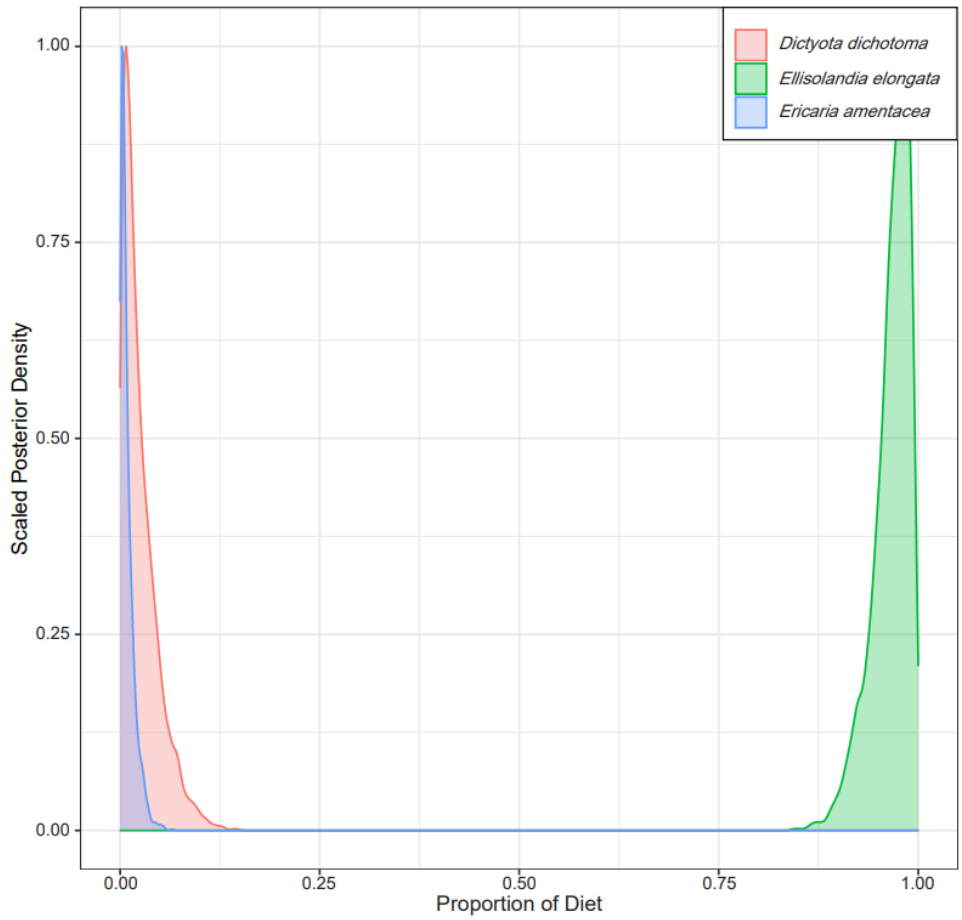

Figure 4. Relative contributions of three food sources (*Dictyota dichotoma, Ellisolandia elongata* and *Ericaria amentacea*) to the diet of *P. lividus* individuals sampled near the old asbestos mine of Canari (Corsica, NW Mediterranean).

3.4. Biotic factors of the sea urchin

The histological analysis highlighted that sea urchins were mostly at stade 4 (mature) and stade 5 (partly spawned; Fig. 5). However, variations were underlined in Punta Bianca where ~30% of the sea urchins had already spawned and in the reference site where 50% of the sea urchins reached the premature stage (Fig. 5). Significant differences were noted between the concentrations measured in the male and female gonads. Females exhibited higher concentrations of Cd, Mn, Se, and Zn while high Mo, Pb, and V levels were found in

males (p-value < 0.05). In contrast, no significant differences were found for biomarkers of stress. In the guts, the only significant difference between males and females is measured for Zn with a concentration 1.5 fold higher in females (p-value < 0.001).

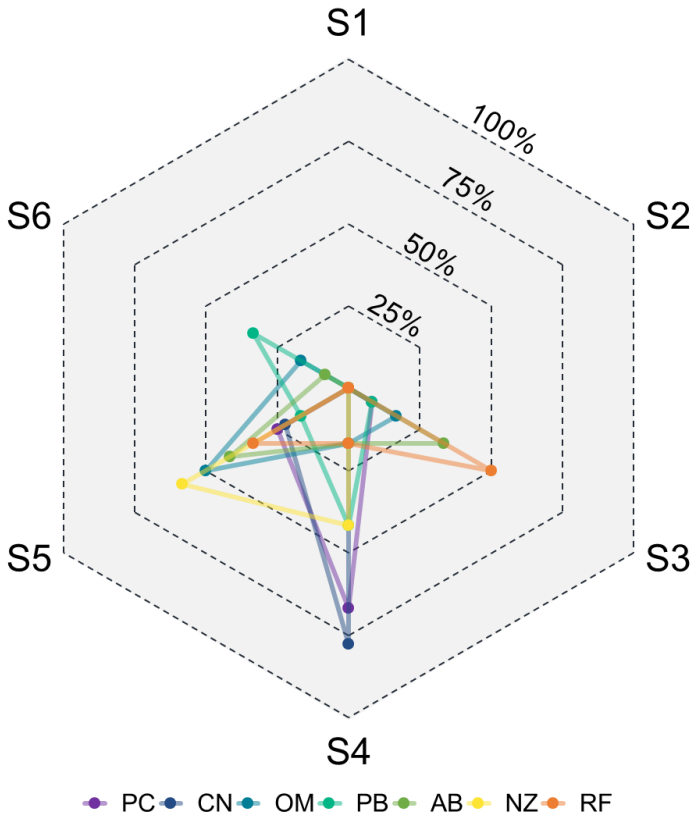

Figure 5. Maturity stage of sea urchin gonads (%) in each site (PC: Punta di Canelle; CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site).

3.5. Biochemical analyses

There was no significant difference between sites regarding MDA content and specific activities of SOD and GPX (Fig. 6). At the same time, the specific activity of CAT and the H_2O_2 levels were higher in sea urchins collected at Albo, Nonza and at the reference site (Fig. 6). Finally, the highest specific activity of GST was measured in sea urchins at Albo and three sites (Reference site, Nonza and Old Mine; Fig. 6). Specific activities of CAT and GST as well as H_2O_2 levels in sea urchin gonads showed significant positive correlation (p-value < 0.001, Table 3). In addition, there were significant positive correlations for several trace elements with CAT and H_2O_2 (Table 3). Accordingly, increased concentrations of Al, Cr, Cu, Fe, Mn, Pb, and Se resulted in higher specific CAT activity and H_2O_2 levels in the gonads (p-value < 0.05).

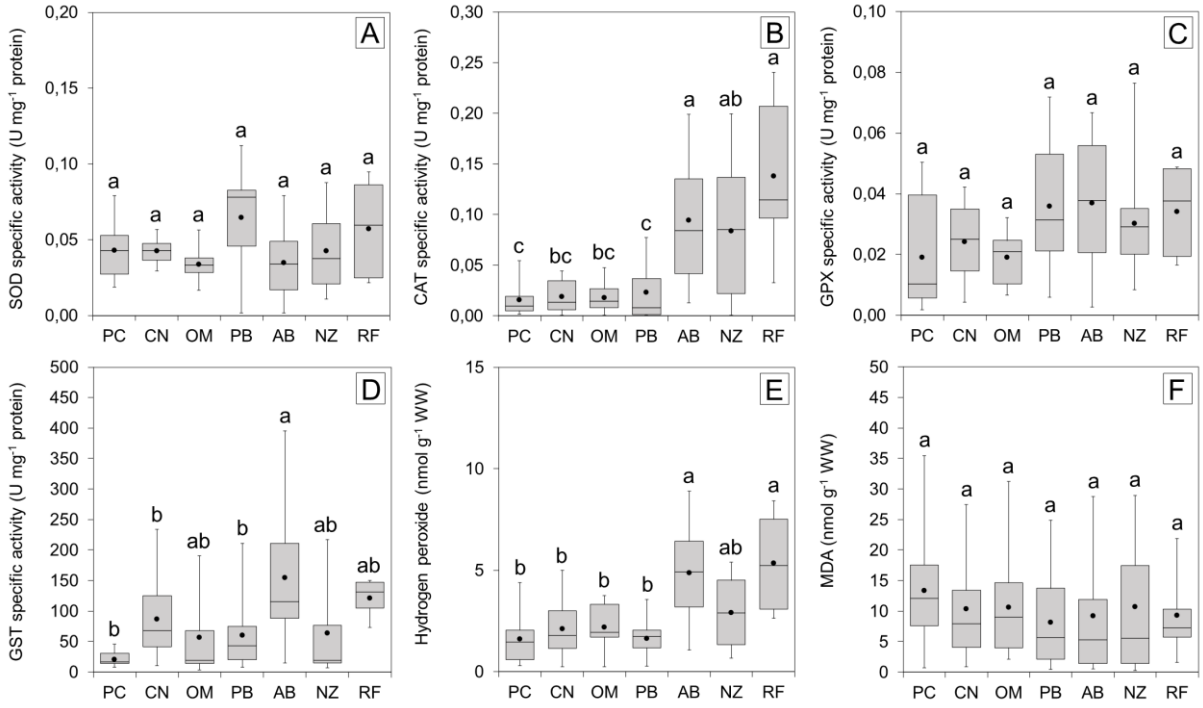

Figure 6. Changes in specific activities of (A) superoxyde dismutase (SOD), (B) catalase (CAT), (C) glutathione peroxidase (GPX), (D) glutathione-S-transferase (GST) and changes in (E) hydrogen peroxide (H_2O_2) and (F) malondialdehyde (MDA) contents in gonads of *P. lividus* collected at seven sites (PC: Punta di Canelle; CN: Canelle; OM: Old Mine; PB: Punta Bianca; AB: Albo; NZ: Nonza; RF: Reference site). Dissimilar letters denote significant differences between groups (p-value < 0.05).

Table 3. Pearson's correlation matrix between the between enzymatic activities and the concentration in trace elements measured in *P. lividus* gonads. Level of significance: *P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001. Significant correlations in bold (r value). See Fig. 6 for acronyms.

	SOD	CAT	GST	GPX	H_2O_2	MDA
SOD		**0.37*** **	**0.41*** **	**0.31*** **	0.00	0.05
CAT	**0.36*** **		**0.58*** **	**0.31*** **	**0.27*** **	0.05
GST	**0.41*** **	**0.58*** **		**0.40*** **	**0.42*** **	**0.23*** **
GPX	**0.31*** **	**0.31*** **	**0.40*** **		0.09	0.10
H_2O_2	-0.08	**0.27*** **	**0.42*** **	0.09		**0.18** **
MDA	0.05	0.05	**0.23*** **	0.10	**0.18** **	
Ag	-0.13	-0.09	0.03	-0.06	-0.07	0.04
Al	-0.10	0.13	0.07	-0.01	**0.20** **	-0.11
As	**-0.17***	-0.01	-0.02	-0.05	-0.01	-0.05
Ba	-0.09	0.09	0.09	-0.03	0.12	0.01
Cd	**-0.16***	-0.03	-0.06	-0.06	0.00	-0.07
Co	**-0.23*** **	0.03	0.00	0.00	**0.17***	**-0.14***
Cr	**-0.16***	**0.14***	0.07	0.01	**0.26*** **	-0.13
Cu	-0.10	**0.19** **	0.03	-0.05	**0.20** **	**-0.15***
Fe	**-0.15***	0.13	0.06	0.01	**0.24*** **	-0.13
Hg	**-0.16***	0.01	-0.04	0.01	**0.30*** **	-0.12
Li	0.05	**0.15***	**0.18***	-0.04	**0.22** **	-0.04
Mn	**-0.14***	**0.15***	0.07	-0.01	**0.26*** **	-0.13
Mo	-0.12	-0.01	-0.04	-0.01	-0.02	-0.06
Ni	**-0.20** **	0.03	-0.03	-0.04	0.10	-0.12
Pb	-0.13	**0.14***	0.06	-0.03	**0.14***	-0.08
Sb	-0.11	0.00	-0.02	-0.02	-0.05	-0.06
Se	-0.06	**0.20** **	0.05	-0.05	**0.21** **	-0.11
Sn	-0.07	**0.17***	0.09	0.00	0.12	-0.05
Sr	0.03	**0.16***	0.13	-0.04	0.09	-0.09
U	-0.05	-0.06	-0.03	0.00	-0.04	-0.01
V	-0.13	0.01	0.00	0.00	0.03	-0.07
Zn	-0.03	0.04	0.06	-0.06	0.03	0.06

4. Discussion

The study of environmental contamination requires a deep knowledge of the distribution of pollutants and their concentrations in biotopes and organisms (Lagadic *et al.*, 1998; Ramade, 2007). For this purpose, trace element concentrations were analyzed in sea urchins (gonads and guts), three macroalgae species, seawater column and sediment. The trace elements follow an almost similar sequence regardless of the environmental matrices studied, although there are some notable differences. Fe is the predominant trace element resulting from its essential character (Phillips & Rainbow *et al.*, 1989; Lohan & Tagliabue, 2018) or from the contamination of the ecosystem as described in the literature (Brik *et al.*, 2018). Fe is an essential trace element playing a major role for many species due to its role in various physiological (*e.g.* photosynthesis, enzymatic activity, reproduction, etc.) and it also included in geochemical processes (Sunda, 2001; Thuróczy *et al.*, 2011). However, in contrast to the literature, Fe concentrations exceeded Zn concentrations in the gonads (Strogyloudi *et al.*, 2014; Ternengo *et al.*, 2018; El Idrissi *et al.*, 2020). This is due to the remarkably high concentrations of Fe, 2 to 8-fold higher than in many studies (Warnau *et al.,* 1998; Storelli *et al.*, 2001; Soualili *et al.*, 2008; Strogyloudi *et al.*, 2014). According to Blum *et al.* (2006), the serpentinites of Cap Corse could generate significant Fe contents explaining these high contents in the studied sites. Although Fe is an essential requirement for marine organisms, an excessive concentration could be harmful to their health and must therefore be monitored (Fosmire, 1990). Zn is the second element measured in high concentration in the gonads. It is the only trace element with a higher accumulation factor in the gonads than in the guts and macroalgae. This accumulation could be due to its intervention in gametogenesis and in particular in ovogenesis explaining its high content in females compared to males (Unuma *et al.*, 2003, 2007). In contrast to the sediment, macroalgae, gonads and guts of sea urchins where Pb is one of the least concentrated, this trace element is the second most abundant in the distribution sequence of the seawater column. The latter is one of the most abundant and common non-essential trace elements in the environment (Mishra *et al.*, 2006). Its concentration in the seawater column (2.17 ± 0.83 mg kg^{-1}) is considerably higher than previously reported in the literature (Lin *et al.*, 2000; Bruland & Lohan, 2003). This is due to point contamination of the water column during the period of this study or contamination of DGTs during material handling. Seawater column analysis is reported to be less relevant in the

literature for trace element monitoring due to its high fluctuations caused by several factors such as hydrodynamic energy (Jahan & Strezov, 2019). Moreover, it is not always possible to analyze all the contaminants in this biotope, the concentrations being too low and the analytical techniques not being sensitive according to this study for Hg.

Calculation of the TEPI through measurements of trace element contents in the gonads and guts of the sea urchin allowed to determine a gradient of contamination with a higher concentration in the south of the old asbestos mine, in particular in Albo and Nonza. In the literature, it has been established that asbestos wastes were accumulated to the south of the old asbestos mine (BRGM, 1997; Cary *et al.,* 2013). These materials migrated along the coastline due to the effect of swell and prevailing marine currents (BRGM, 1997). As a result, the wastes swelled the coastline southward for 5 km from the old mine, progressively filling the beach of Albo and creating the beach of Nonza (about 1 500 m long; BRGM, 1997). A total of one million tons of excavated material has been transported to the artificial beaches during the 35 years of operation of the site (Boulmier *et al.*, 1999; Hervé *et al.*, 1997; Cary *et al.,* 2013). This observation highlights the important role that the marine environment plays in the diffusion and distribution of contaminants in the environment. Hence, it is necessary to take caution during the selection process of the sampling site for ecotoxicological studies and to consider which can contribute to the variability of contaminants at the local scale. Moreover, sites at 3 and 6 m depth are similarly impacted by diffusion, indicating that the contamination affect all the studied bathymetric range.

The Albo and Nonza beaches are characterized by black pebbles constituted by serpentine (BRGM, 1997). The serpentinites are characterized by high levels of Fe and Mg (Morrison *et al.*, 2009) which may explain the high Fe concentrations found at Albo with a high TESVI whatever the matrices studied. Co, Cr, and Ni are also present in high concentrations at Albo with significant concentration differences from the reference site. Previous works have also highlighted a high contamination of Ni, Cr and Co in marine sediments in the area adjacent (15 km of coastline) to the old asbestos mine of Canari (Andral & Tomasino, 2007; Kantin & Pergent-Martini, 2007) and an accumulation of these elements in several marine organisms (Bouchoucha & Andral, 2010; El Idrissi *et al.*, 2020). According to several authors, in addition to Fe, serpentinites are naturally enriched in other trace elements such as Cr, Ni and Co

(Morrison *et al.*, 2009; Siebecker, 2010; Tashakor *et al.*, 2011) explaining these high concentration levels in the region and particularly in the south of the old mine. These trace elements can have deleterious effects if they become available at high concentrations in the environment and therefore serpentine soils are considered a source of geogenic pollution by many researchers (Caillaud *et al.,* 2009; Tashakor *et al.,* 2011). Thus, even if mine drainage was the main cause of these contamination variations, there is a general sensitivity related to a particularly concentrated geochemical background. The assessment of ecosystem quality requires a good knowledge of the natural geochemical context in order to distinguish the trace elements naturally present in the environment from those resulting from anthropogenic activities. The TEPI of macroalgae also indicated a strong contamination in Albo but any north-south contamination gradient has been highlighted. Indeed, the macroalgae located in north part of the mine exhibited contamination levels similar to those located in south, resulting in a gradient around the mine rather than north-south. Macroalgae may be abler to accumulate with a majority of trace elements tending to be much more bioaccumulated in macroalgae as indicated by the different accumulation factors calculated in this study. It is probably more interesting to study macroalgae to assess acute contamination, as they will tend to bioaccumulate most trace elements to a greater degree than sea urchins. However, if the aim of the study is to identify the magnitude of well-integrated contamination, the sea urchin may be a better tool for assessing a contamination gradient.

It is commonly recognized that the accumulation of contaminants in marine organisms occurs through three compartments: the seawater column, food and sediments (Bouzahouane *et al.*, 2018). Accumulation can lead to high internal concentrations resulting in toxicity, even when external concentrations are low (ECHA, 2017). According to some authors, sediment often plays the role of a local sink for contaminants, which can increase the concentrations of benthic organisms that indiscriminately ingest sediment particles while feeding (Li *et al.*, 2019; Jacobs, 1998). In the present study, the BSAFs are mostly low (less than 1) indicating low bioaccumulation (Warnau *et al.*, 1998). These low values are notably related to high trace element concentrations in the sediment. Only Ag, Cd, Mo, Se and Zn have BSAFs higher than 1 with concentrations in organisms equivalent to those recorded in the literature (*e.g.* Guendouzi *et al.*, 2017; Rouane-Hacene *et al.,* 2017; Warnau *et al.*, 1998). A high BSAF does not necessarily indicate a risk and rather suggests high concentrations in the organism due to

its essentiality in physiological processes and low concentrations in sediments as with Zn for instance. Therefore, the efficiency of trace element absorption from different sources may vary according to ecological needs, organism metabolism and concentrations in different compartments (Bouzahouane *et al.*, 2018). Variations in trace elements within the same species can therefore have different origins. The reproductive stage of the sea urchin is known as a parameter influencing trace element concentrations (El Idrissi *et al.*, 2020). In the present study, the sea urchins are almost all at the same stage of reproduction except at Punta Bianca where about 30% of the sea urchins have already spawned (stage 5) and at the reference site where 50% of the gonads are premature (stage 3). This information probably indicates a low overestimation of gonadal contamination levels at Punta Bianca and reference site compared to the other investigated sites where a dilution effect occurs (El Idrissi *et al.*, 2020). In this study, BAFs indicate that the majority of trace elements are most bioaccumulated in macroalgae then the gut and later the gonads. Therefore, the use of guts might be more interesting than gonads in studies assessing local variations in trace element contamination. Indeed, this compartment of the sea urchin bioaccumulates more making higher concentrations and therefore an analytical analysis more accurate. In addition, contrary to the gonads, the majority of the trace elements does not vary according to the sex. Investigations should be conducted to assess whether reproductive stages and the source of food have an impact on the concentrations in the guts. In the present study, we estimated BMF using the guts and macroalgae that the urchin could potentially feed on. Indeed, although in some conditions, *P. lividus* can be omnivorous (Wangensteen *et al.*, 2011), it remains mainly herbivorous (Agnetta *et al.*, 2013; Boudouresque & Verlaque, 2013). Several factors such as seasonality (Verlaque, 1987), abiotic parameters or resources available within the biotope (Paine & Vadas, 1969; Frantzis *et al.,* 1988) can influence its choice. Therefore, for this work, three species of macroalgae were collected because of the presence of *P. lividus* in their beds and their high abundance in the sites. Isotopic analyses identified *E. elongata* as the main food source compared to *D. dichotoma* and *E. amentacea*. Frantzis & Grémare (1992) has already demonstrated a preference of *P. lividus* for this macroalgae which is coherent with our results. The most biomagnified trace elements (Ag, Cd and Mo) are those measured at low concentrations and are among the last elements in the distribution regardless of the matrices studied. Luy *et al.* (2011) have also studied these elements and, according to them, the levels of Ag and Mo measured in seagrass reflect the background level of agriculture in this area.

Several studies suggest that exposure to trace element contamination can induce a cascade of events such as ROS production (Farombi *et al.*, 2007; Nieto *et al.*, 2010). Thus, antioxidant defense mechanisms are crucial to maintain the redox balance between pro-oxidants and antioxidants in aerobic organisms (Limon-Pacheco & Gonsebatt, 2009; Chan & Wang, 2019). In order to estimate the effects of trace element contamination on the oxidative stress of *P. lividus*, analyses were performed on the gonads of sea urchins collected at different locations of the contamination gradient (north to south). The highest specific activity of CAT, GST and H_2O_2 was reported in the south of the old asbestos mine where the contamination is the highest. A positive correlation between the specific activity of CAT, H_2O_2 and some trace elements is noted confirming an effect of contaminants on the oxidative stress of sea urchin and on the reliability of biomarkers used in this study. In the mitochondrial respiratory chain, H_2O_2 can react directly with metal ions such as Fe or Cu, by the Fenton reaction, and form the hydroxyl radical which is a powerful oxidant (Regoli & Giuliani, 2014; Mejdoub *et al.*, 2017). Consequently, the elimination of H_2O_2 is a key strategy of organisms against oxidative stress (Regoli *et al.*, 2002a; Mejdoub *et al.*, 2017). According to Giarratano *et al.* (2013), the simultaneous induction of GST and CAT activities suggests a similar pattern for hydrogen peroxide removal. Therefore, the increase in these enzymes suggests an activation of detoxification processes, probably reflecting high stress (Louiz *et al.*, 2016). A large number of other studies have shown higher activity of CAT and GST in organisms collected from contaminated sites (Bougherira *et al.*, 2015; Keblouti *et al.,* 2015; Bouzenda *et al.,* 2017). Regoli *et al.* (2002b) consider CAT a sensitive and important biomarker of oxidative stress superior to SOD, explaining the invariance of specific SOD activity in this study. These high specific activities of CAT and GST as well as this high H_2O_2 content were also measured in the reference site which however has a low concentration of trace elements. The main difficulty in using biomarkers in the natural environment is the interference with other environmental factors (Lagadic *et al.*, 1998). In contrast to experiments under controlled conditions, different factors can lead to responses of biochemical parameters in the natural environment (Lagadic *et al.*, 1998; Ramade, 2007). As a result, many factors such as meteorological conditions, interactions with other contaminants than those studied, or interspecific relationships can complexify the interpretation of oxidative stress responses (Lagadic *et al.*, 1998). In this case, the same habitat in the reference site was chosen as for the sites near the old asbestos mine in order to minimize variation, then it appears that other factors allowed these high values.

Further study would be relevant to determine the source of this significant oxidative stress response. Finally, despite the higher levels of H_2O_2 in sea urchin gonads collected at the south of the mine and at the reference site, the level of MDA, a by-product of lipid peroxidation, did not significantly vary, suggesting that the antioxidant enzyme system of *P. lividus* prevented oxidative damage from occurring (Amri *et al.*, 2017; Ding *et al.*, 2018).

5. Conclusion

This study assessed the reliability of sea urchins and macroalgae in the evaluation of contaminations in coastal ecosystems. The sea urchin guts appear to be a good bioindicator tool for assessing trace element levels. In this way, it would be interesting to perform complementary studies in order to understand the influence of several parameters such as the season and the food sources of the sea urchin. TEPI allowed to identify the different levels of contamination by highlighting the gradients of contamination in the old asbestos mine using sea urchins and macroalgae. TESVI enabled to determine with efficiency the trace elements characteristics of the Albo site associated with the discharges of the old mine dragged by the sea current and the geology of the region. The contamination generated by the old asbestos mine causes oxidative stress very well regulated by the antioxidant mechanisms of the sea urchin. This study also highlighted the necessity of cautions when interpreting biomarkers of stress under environmental conditions due to the different factors involved. Finally, this research shows that the effects of the old asbestos mine are still present and in order to prevent them, it is essential to stabilize the surrounding environment and to maintain monitoring of the marine environment.

CHAPITRE 3

EFFETS DES CONTAMINANTS SUR LES STADES EMBRYO-LARVAIRES DE L'OURSIN *PARACENTROTUS LIVIDUS*

Plan et objectif du chapitre

Le chapitre 3 s'appuie sur l'utilisation de l'oursin pour évaluer les effets des contaminations en éléments traces dans le milieu marin. Il est composé de deux articles scientifiques.

- **L'article 4** étudie les les effets d'une exposition chronique à un mélange d'éléments traces sur le développement embryolarvaire de *Paracentrotus lividus ;*

- **L'article 5** évalue la capacité des stades larvaires à répondre au stress induit par des contaminations en éléments traces.

Article 4

Effects of trace elements contaminations on the larval development of *Paracentrotus lividus* using an innovative experimental approach

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Contexte et résumé de l'article 4 « Effets des contaminations en éléments traces sur le développement larvaire de *Paracentrotus lividus* à travers une approche expérimentale innovante »

L'oursin est reconnu pour son rôle de bioindicateur et peut être utilisé afin d'évaluer l'impact potentiel des contaminants présents dans l'écosystème. Les bioessais utilisant ses larves sont aujourd'hui parmi les mieux standardisés en écotoxicologie. L'objectif de cet article est d'évaluer les effets des contaminations en éléments traces sur le développement larvaire de *Paracentrotus lividus*. Plusieurs expérimentations ont été réalisées sur des élevages larvaires afin de déterminer les conséquences de différentes contaminations chroniques par des mélanges de (i) quinze éléments traces (argent, arsenic, aluminium, cadmium, cobalt, chrome cuivre, fer, mercure, manganèse, molybdène, nickel, fer, vanadium et zinc) à des concentrations similaires à celles mesurées dans les mers et océans et (ii) sept éléments traces à des concentrations mesurées en face de l'ancienne mine d'amiante de Canari. Les sept éléments traces étudiés ont été choisis dans trois buts distincts : (i) le cobalt, le chrome et le fer ont été mesurés à des concentrations élevées au niveau de l'ancienne mine d'amiante, (ii) le fer, le cuivre et le zinc sont des éléments traces essentiels habituellement présents à des niveaux élevés dans les gonades des oursins et (iii) le mercure fait l'objet de nombreuses discussions depuis plusieurs années au sein de la communauté scientifique en raison de son impact potentiel. Afin d'estimer les effets d'une augmentation des pollutions marines, des concentrations plus importantes ont également été testées. Ces bioessais ont été effectués *via* des géniteurs prélevés sur un site référence (Calvi) et un site contaminé par de fortes teneurs en éléments traces (Albo). Les effets des éléments traces ont été étudiés sur l'ensemble du développement larvaire. Un retard de développement ainsi qu'une hausse de la taille des bras sont observés lorsque le nombre et la concentration en éléments traces augmentent. Le développement des larves contaminées par le mélange de sept éléments traces à des concentrations moyennes a été plus rapide, probablement en raison d'un phénomène d'hormèse. Ce travail a également mis en évidence l'importance de l'origine des géniteurs dans les études écotoxicologiques. À notre connaissance, il s'agit de la première étude à examiner les effets d'une combinaison aussi large d'éléments traces pour une contamination chronique sur le stade larvaire complet de l'oursin.

1. Introduction

Since the beginning of the Industrial Revolution, anthropic pressures have increased due to strong population and economic growth. These pressures have led to the release of toxic substances into coastal and marine ecosystems (Bonanno & Di Martino, 2017). Trace elements are among the most common contaminants in the marine ecosystem leading to considerable long term ecological damage to aquatic organisms and the alteration of the dynamics of a system (Sandilyan & Kathiresan, 2014). The dispersion of these contaminants in the marine environment results mainly from anthropic sources but is also due to various geological phenomena (Baize, 2009). Trace elements can be classified as (i) “essential”, allowing the growth and normal development of an organism within a well-defined interval, and (ii) “non-essential”, having, in the present state of knowledge, no beneficial role in biological functions (Amiard, 2011). Essentiality is a characteristic which changes according to knowledge and whose sensitivity differs according to the authors. Beyond a critical threshold, any trace element, whether essential or not, causes disturbances at the molecular and cellular levels, thus impacting individual organisms, entire populations, and ultimately potentially ecosystems. (Amiard, 2011). Although marine organisms may have capacities of resistance and resilience to such abiotic stresses (Loizeau & Tusseau-Vuillemin, 2014), chronic contamination of the marine environment, particularly with trace elements, is a major issue for wildlife conservation (Amri *et al.*, 2017).

The study of these contaminants is particularly important in the Mediterranean Sea because of its geomorphological and hydrodynamic characteristics (Bethoux *et al.*, 1999). Within the Western Mediterranean basin, Corsica Island is often considered as a reference region on account of its high water quality and the low anthropic pressures (Richir *et al.*, 2013). In spite of this, high levels of trace elements were observed in the gonads of sea urchins near an old asbestos mine at Canari (Corsica, France; El Idrissi *et al.*, 2020; Ternengo *et al.*, 2018). The high levels found in this area originate from the untreated excavated material discharged into the sea, representing about 11 million tons of sediment (Kantin & Pergent-Martini, 2007). The continuous leaching of mining residues, still present on the sides of the mine, contributes to the dispersion of nickel, chromium and cobalt along the coastline (Kantin & Pergent-Martini, 2007).

By its ecological features, high tolerance of contaminants and ability to bioaccumulate trace elements, *Paracentrotus lividus* (Lamarck, 1816) is recognized as a bioindicator (El Idrissi *et al.*, 2020; Warnau *et al.*, 1996). The embryo-larval and adult life stages of the sea urchin are the most studied and used in testing (Bielmyer *et al.*, 2005). Adult sea urchins are extensively studied to assess the levels of trace elements contamination in coastal ecosystems (*e.g.*, El Idrissi *et al.*, 2020; Ternengo *et al.*, 2018). Although this allows determination of the degree and nature of pollution, no evidence regarding the biological consequences can be provided (Chapman *et al.*, 1987). Bioassays enable the detection of these effects by measuring the biological responses of marine organisms (Fernandez & Beiras, 2001). Embryo-larval development of several species of sea urchins has been widely studied since the 1950s in order to monitor contaminants in the marine environment (*e.g.*, Okubo & Okubo, 1962; Tabata, 1956). Currently, bioassays using sea urchin larvae are among the most standardized in ecotoxicology (Pétinay *et al.*, 2009).

Many studies have been carried out to test the influence of trace elements, in bioassays using sea urchin larvae. However, most studies tested one or cocktails of only few contaminants, most of time at high concentrations relative to the environment (*e.g.,* Bielmyer *et al.*, 2005; Chiarelli *et al.*, 2014). The single metal study allows to establish a metal-related effect but does not make it possible to understand the effects due to the mixtures of several trace elements. Since marine environments normally contain more than one trace element (Fernandez & Beiras, 2001), it's essential to carry out work on the effects of interactions of trace elements. In addition, studies have focused on the endotrophic stages (embryonic and first larval stage) and the effects of trace elements on the entire larval development has never been explored. The acquisition of knowledge through studies on *P. lividus* is crucial in the context of degradation of the quality of coastal waters owing to its scientific, ecological and economic interest (Carballeira *et al.*, 2012).

The aim of this study is to estimate the effects of chronic exposure to a mixture of trace elements on the development of *P. lividus* – from the fertilized egg to the end of larval development – for (i) the world ocean seawater average concentration (mixture of fifteen trace elements), and (ii) contamination observed near the old asbestos mine of Canari (Corsica, France), resulting from mining activity (mixture of seven trace elements). This study

will also enable us to understand the effects of an increase in marine pollution on the larvae of *P. lividus* and to determine whether contamination of spawners has an influence on larval development in contaminated areas by using spawners collected from Calvi (reference site, Corsica), and Albo (mining area, Corsica). This will allow an assessment of whether there is some adaptation of the spawners that could be transmitted to the larval population and how this might have important implications in ecotoxicological studies.

2. Material and methods

2.1. Biological collection

Adult sea urchins were collected in a spawning period in Corsica (Mediterranean Sea) at two distinctive sites: (i) Albo (42°46.313 N, 9°20.150 E), contaminated due to its proximity to the old asbestos mine of Canari, and (ii) Calvi (42°34.916 N, 8°43.589 E), a reference site, with very low levels of trace elements contamination (El Idrissi *et al.*, 2020; Gobert & Richir, 2019**;** Ternengo *et al.*, 2018). Individuals sampled were immediately transported in an insulated box to the laboratory and gametes were obtained by dissecting mature organisms. In order to optimize the genetic mixing, thirty males and thirty females for each site were used to consider the maximum of expected variability, that could be genetically driven, in a natural larval population. The spawning was carried out in reference seawater (control) in order to achieve *in vitro* fertilization (Buttino *et al.*, 2016). One hour after fertilization, the embryos were observed to verify that the fertility rate is above 90% (Buttino *et al.*, 2016; Pétinay *et al.*, 2009).

2.2. Larval development bioassays

Three experiments were carried out using larvae produced by sea urchins from Albo or Calvi. A mixture of trace elements was produced in deionized water using analytical grade solutions (1 g L^{-1}; Certipur®, Merck, Germany; Appendix A) and was diluted in 10 mL of seawater. This mixture was poured into the rearing tank containing reference sea water. The concentration and the number of trace elements varied according to the experiment and treatment (Table 1; Table 2). Three replicates were performed per treatment and a control

was carried out for each experiment. Temperature was adjusted at the beginning of the experiments (20 °C) and was continuously monitored using HOBO TidbiT® v2 loggers (accuracy: ± 0.21 °C). The sea water was renewed daily and food, composed of a mixture of phytoplankton, was provided *ad libitum*. This work was carried out in accordance with French regulations for animal experiments.

Table 1. Trace elements concentrations (µg L^{-1}; world ocean seawater average concentration; more details in Appendix B), tested from larvae produced by sea urchins sampled at Calvi (experiment 1).

	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	V	Zn
Treatment 1: Average concentration	0.1	0.7	1.6	0.1	0.1	0.2	0.3	1.1	0.4	0.3	11.1	0.4	0.2	1.8	1.3
Treatment 2: 5– fold concentration	0.5	3.5	8	0.5	0.5	1	1.5	5.5	2	3	55.5	2	1	9	6.5
Treatment 3: 10– fold concentration	1	7	16	1	1	2	3	11	4	6	111	4	2	18	13
Treatment 4: 50– fold concentration	5	35	80	5	5	10	15	55	20	30	555	20	10	90	65

The first experiment was realized to assess the impact of the contamination measured in the world ocean seawater. The fertilized eggs from Calvi were placed in fifteen rearing tanks (120 000 eggs per tank): three control and twelve contaminated. Four treatments at different concentrations were tested: world ocean seawater average concentration (treatment 1) and the same concentration multiplied by 5 (treatment 2), 10 (treatment 3) and 50 (treatment 4; Table 1). The average concentrations of world ocean seawater were calculated by compiling data from the literature (Richir *et al.*, 2013; Appendix B). In total, fifteen trace elements were studied: silver (Ag), arsenic (As), aluminum (Al), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), mercury (Hg), manganese (Mn), molybdenum (Mo), nickel (Ni), lead (Pb), vanadium (V) and zinc (Zn).

The second and third experiments were carried out to assess the impact of the contamination measured in the seawater column in front of the old asbestos mine (Canari, Corsica, France).

Seven trace elements were studied for three distinct purposes: (i) Co, Cr and Ni, measured in high concentrations; (ii) Fe, Cu and Zn, essential trace elements usually found in high levels in the gonads of sea urchins, and (iii) Hg, which has been the subject of frequent discussions for several years within the scientific community in view of its potential impact (Buttino *et al.*, 2016; El Idrissi *et al.*, 2020; Ternengo *et al.*, 2018). Three treatments at different concentrations were tested: the average of concentrations measured in the seawater column in front of the old asbestos mine (treatment 1), and this same concentration multiplied by 5 (treatment 2) and 10 (treatment 3; Table 2). These treatments were applied to larvae produced by sea urchins from Calvi (experiment 2) and Albo (experiment 3) to verify whether the contamination status of spawners has an influence on larval development. The fertilized eggs from each site were placed in twelve distinct rearing tanks: three control and nine contaminated.

Table 2. Trace elements concentrations (µg L^{-1}; concentration measured near an old asbestos mine at Canari, Corsica, France; more details in Appendix B) tested from larvae produced by sea urchins sampled at Calvi (experiment 2) and Albo (experiment 3).

	Co	Cr	Cu	Fe	Hg	Ni	Zn
Treatment 1: Average concentration	0.02	0.15	0.30	1.10	0.40	1.38	1.30
Treatment 2: 5-fold concentration	0.1	0.75	1.5	5.5	2	6.9	6.5
Treatment 3: 10– fold concentration	2	1.5	3	11	4	13.8	13

2.3. Monitoring of larval development

Larval development was monitored daily from the 4th day after fertilization. Each individual was photographed under a stereomicroscope (40-fold magnification) and measured using ImageJ software to determine total length, body width and length and arm length (Fig. 1). In total, more than 10 000 larvae were measured in this study in order to have a robust dataset. 100-fold magnification allowed determination of larval malformation according to the criteria of Carbaillera *et al.* (2012). To assess developmental delay, the larval stage was determined. The larval stages considered are the following: the 4-arm stage, the 6-arm stage and the 8-arm stage (Fig. 2); the shift to the next stage was determined from the appearance of the two additional arms.

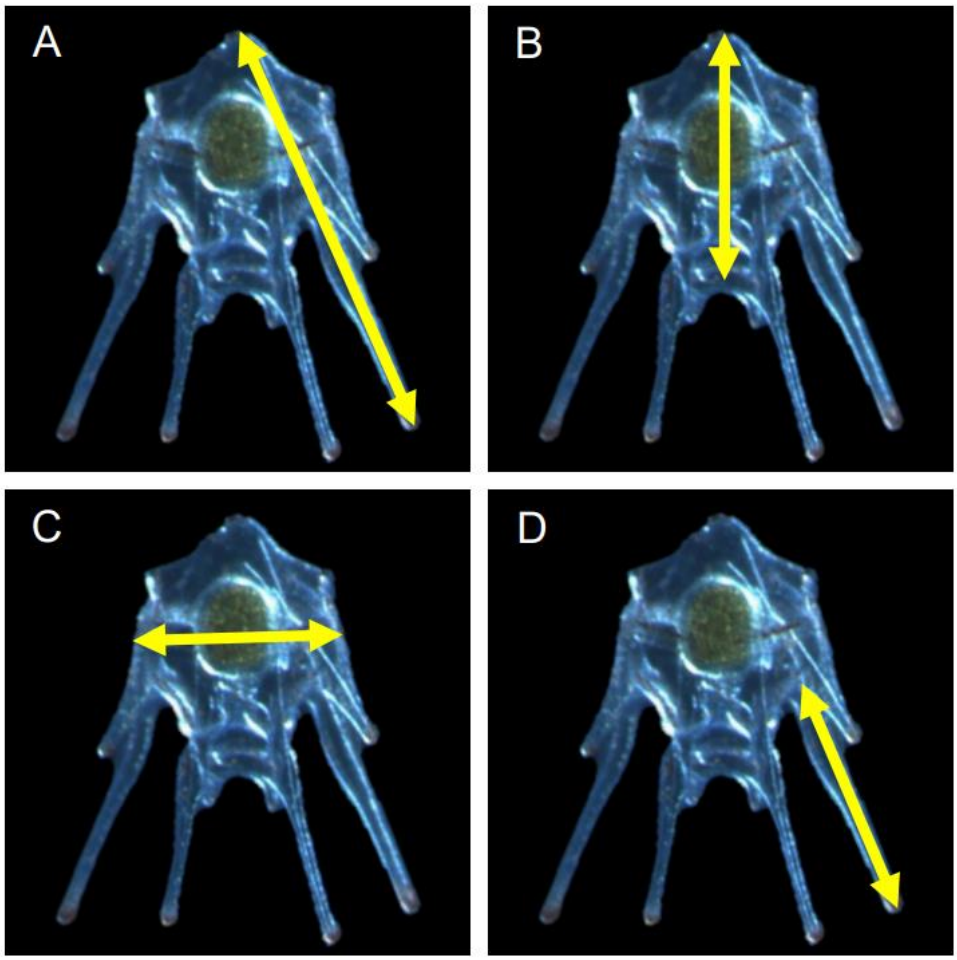

Figure 1. Measurements of total length (A), body length (B), body width (C) and arm length (D) performed on *P. lividus* larvae photographed under a stereomicroscope (40-fold magnification).

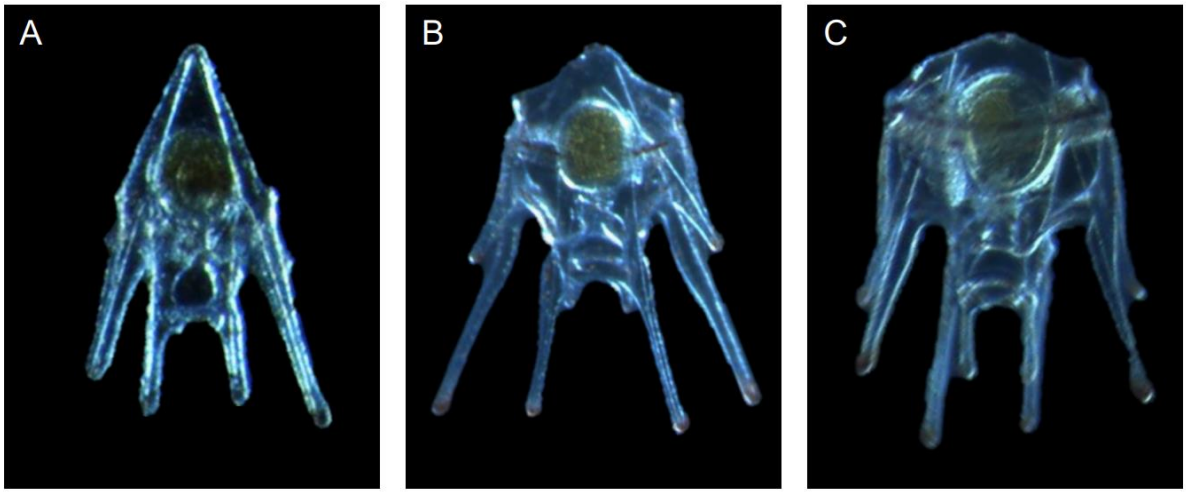

Figure 2. Larval stages studied in this study with 4-arm (A), 6-arm (B) and 8-arm (C) performed on *P. lividus* larvae photographed under a stereomicroscope (40-fold magnification).

2.4. Data presentation and statistical analyses

Larval growth was illustrated using a logarithmic trendline; this is the curved line with the best coefficient of determination (close to 1) and the most suitable for this dataset. Data were transformed when necessary, to satisfy the conditions of application of the parametric tests (data normality and homogeneity of variances). Growth data (*i.e.,* total length, body length, width and arm length) were analyzed using several repeated measures of analysis of variance (ANOVA) to evaluate differences between treatments and experiments. The significance level adopted was 95 % ($\alpha < 0.05$). Analyses and graphical representations were performed using XLSTAT® software.

3. Results

3.1 Effects of world ocean seawater average concentration

The aim of the first experiment was to determine the effects of chronic exposure to a mixture of trace elements on the development of *P. lividus* for concentrations measured in seas and oceans and with concentrations from 5- to 50-fold higher. No malformation was observed under the stereomicroscope but larvae exhibited a significantly greater total length (from 11th day) in contaminated rearing tanks compared to the control (p-value < 0.001; Fig. 3A). A differential influence among metals concentration could be observed as the highest concentration generally resulted in lower total length at early development times, and to higher total length at the end of development. In contrast, the general trend of body length and width variation are similar to the control, although control larvae had a greater body length and width than contaminated larvae (Fig. 3B; Fig. 3C). Body length tended to be significantly shorter in the most contaminated rearing tanks (p-value < 0.001; Fig. 3C). The arms were significantly longer from the 10th day post fertilization in the larvae in the contaminated rearing tanks compared to the control (p-value < 0.001; Fig. 3D). In the control, no significant increase of the arm length was observed in contrast to those in contaminated tanks (Fig. 3D). There was a timeframe during which the length variabilities are high (*e.g.* between 12th day to 18th). This was probably due to greater stage variability during this period (Fig. 4) but also to variability of samples.

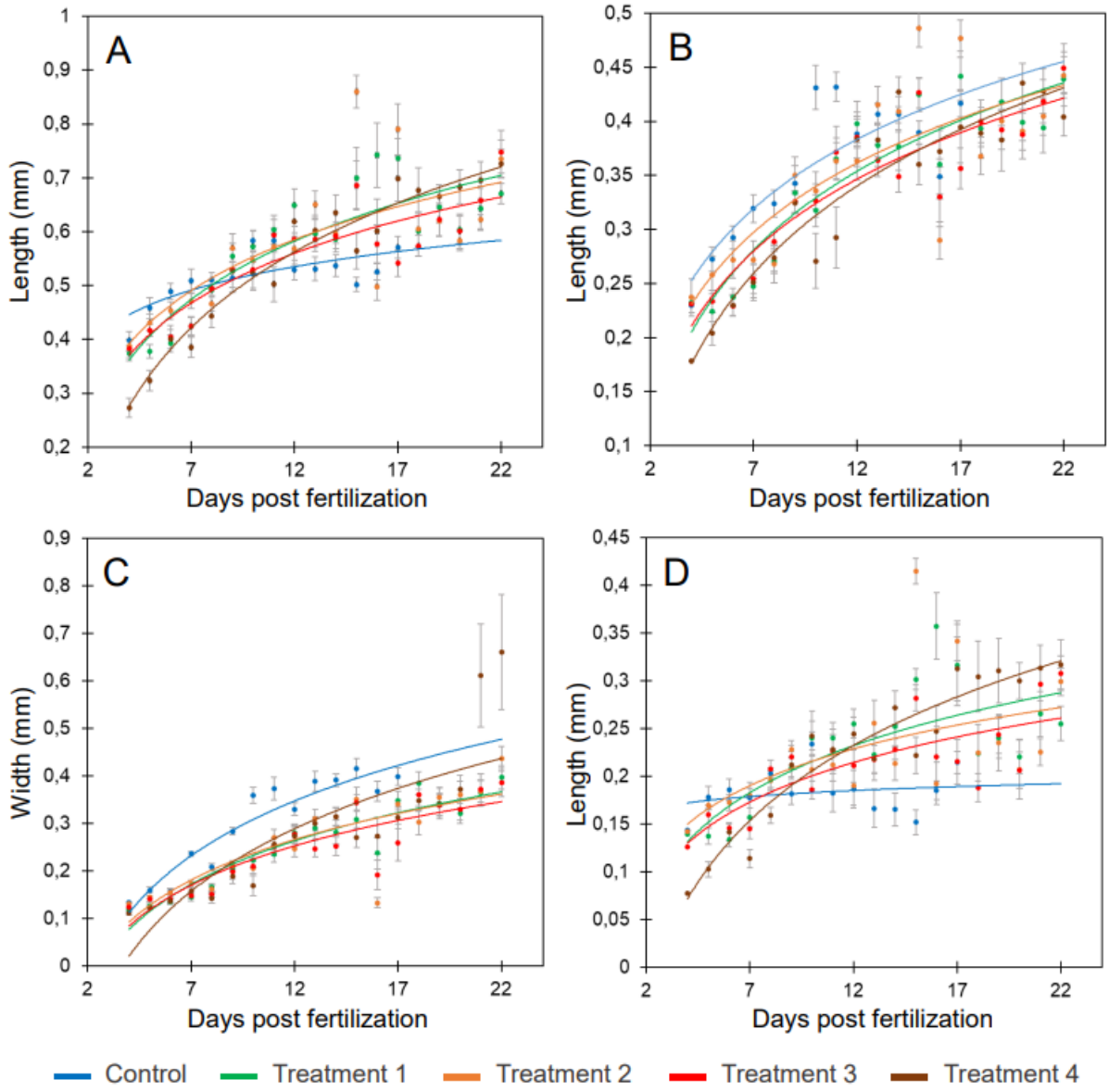

Figure 3. Effects of chronic exposure to a mixture of trace elements on development of *P. lividus* larvae produced by sea urchins from Calvi (A: total length; B: body length; C: body width and D: arm length) for world ocean seawater average concentration and 5- to 50-fold higher concentrations (experiment 1).

The larvae were all in the 4-arm stage at 4 days post-fertilization (Fig. 4). The larvae reached the 6-arm stage from the 9th day post-fertilization and 8-arm stage from the 11th day post-fertilization in the control tanks (Fig. 4A). Under metallic exposure, only a fraction of the larvae could complete their development until the 8-arm stage, and that delays were observed for both 4- to 6-arms and 6- to 8-arms transitions (Fig. 4). Whatever the metallic concentration, a similar delay was noted to observe the 6-arms larvae (2 to 3 days). By contrast, the delays observed for the appearance of 8-arms stage is a function of the metallic concentrations,

varying from 6 days to 10 days (Fig. 4). Moreover, the metallic concentration has an influence on the proportion of larvae that could effectively develop. The higher the concentration, the lower the proportion of larvae at both 6- and 8-arms stages (Fig. 4).

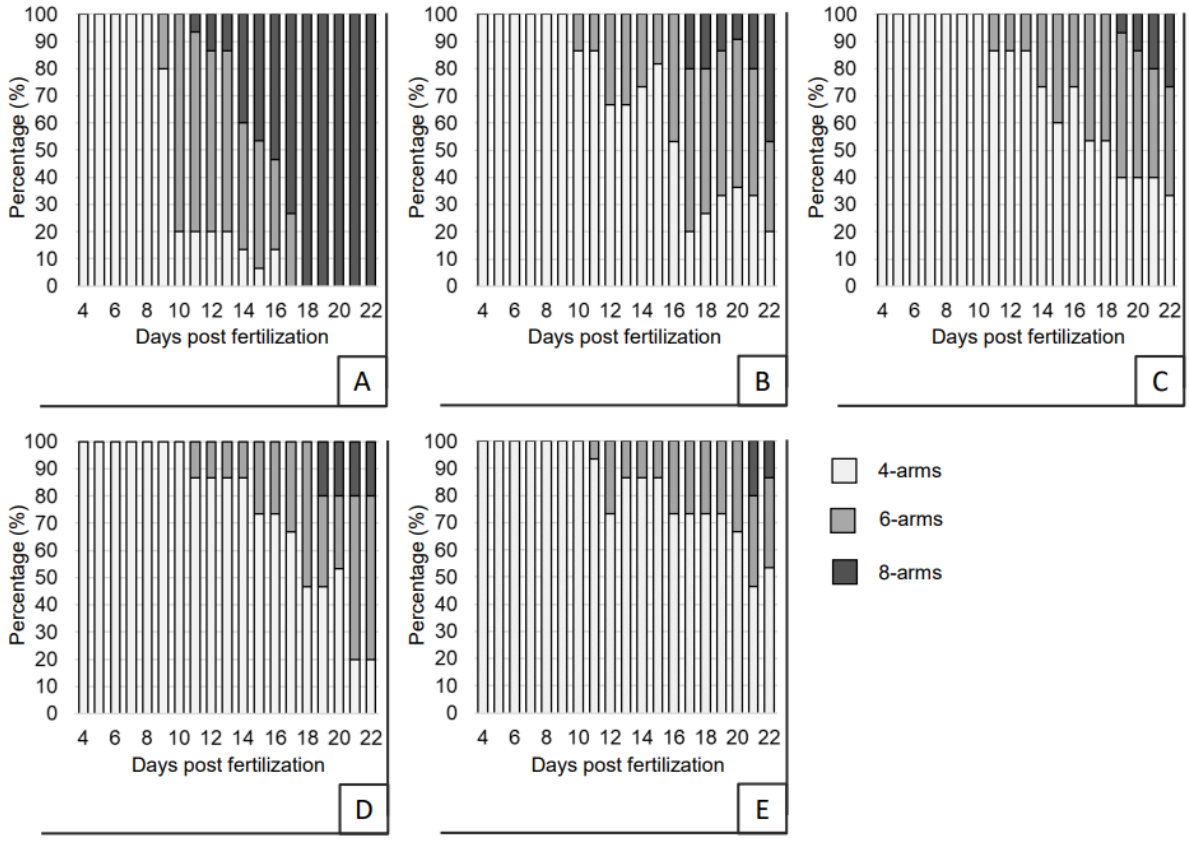

Figure 4. Effects of chronic exposure to a mixture of trace elements on stage development of *P. lividus* larvae produced by sea urchins from Calvi (experiment 1; A: control; B: treatment 1; C: treatment 2; D: treatment 3 and E: treatment 4).

3.2. Effects of contamination resulting from mining activity

The aim of the second and third experiments, with respectively spawners from reference and contaminated sites, was to determine the effects of asbestos mine contamination on the development of *P. lividus* larvae. No malformation was observed under the stereomicroscope using a 100-fold magnification. The same trends were observed for the measurements of the larvae according to the different treatments (Fig. 5; Fig. 6). Control larvae exhibited significantly shorter total and arm lengths compared to contaminated larvae (p-value < 0.001; Fig. 5A, 5D, 6A, 6D). Body width tended to be significantly greater in larvae contaminated with treatment 1 (average contamination) and significantly lower in larvae

contaminated with the most contaminated treatment (p-value < 0.001, Fig. 5C; Fig. 6C). The body length differed between experiments 2 and 3 (Fig. 5B; Fig. 6B). In experiment 2, larvae tended to have significantly higher body length in treatment 1 while in experiment 3, all treatments resulted in significantly higher body length than control (p-value < 0.001). Overall, larvae contaminated with average contamination (treatment 1) tended to be significantly wider than the others.

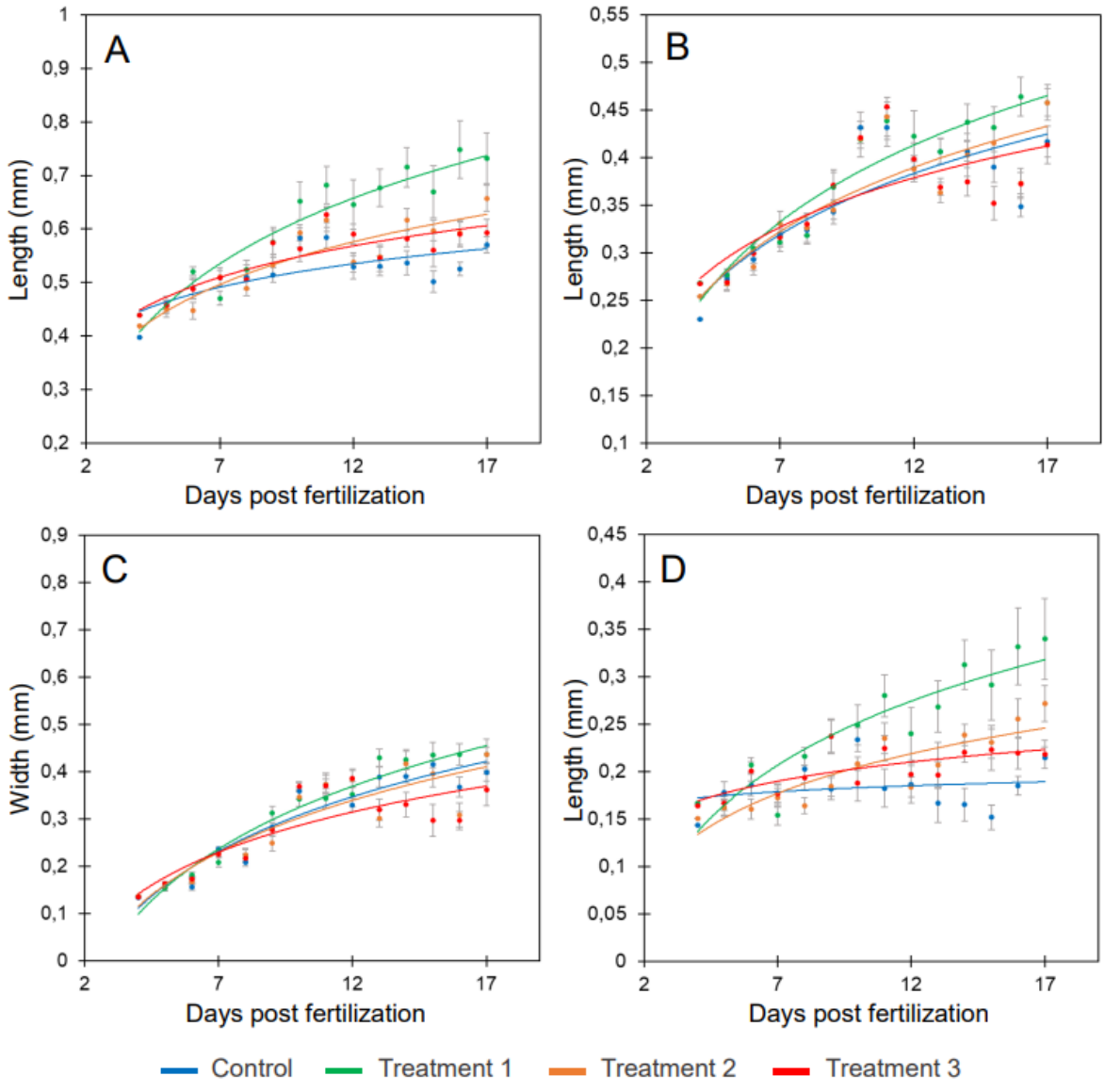

Figure 5. Effects of chronic exposure to a mixture of trace elements on development of larvae of *P. lividus* (A: total length; B: body length; C: body width and D: arm length) produced by sea urchins from Calvi (reference site) for concentrations measured in an old asbestos mine at Canari (Corsica. France) and 5- to 50-fold higher concentrations (experiment 2).

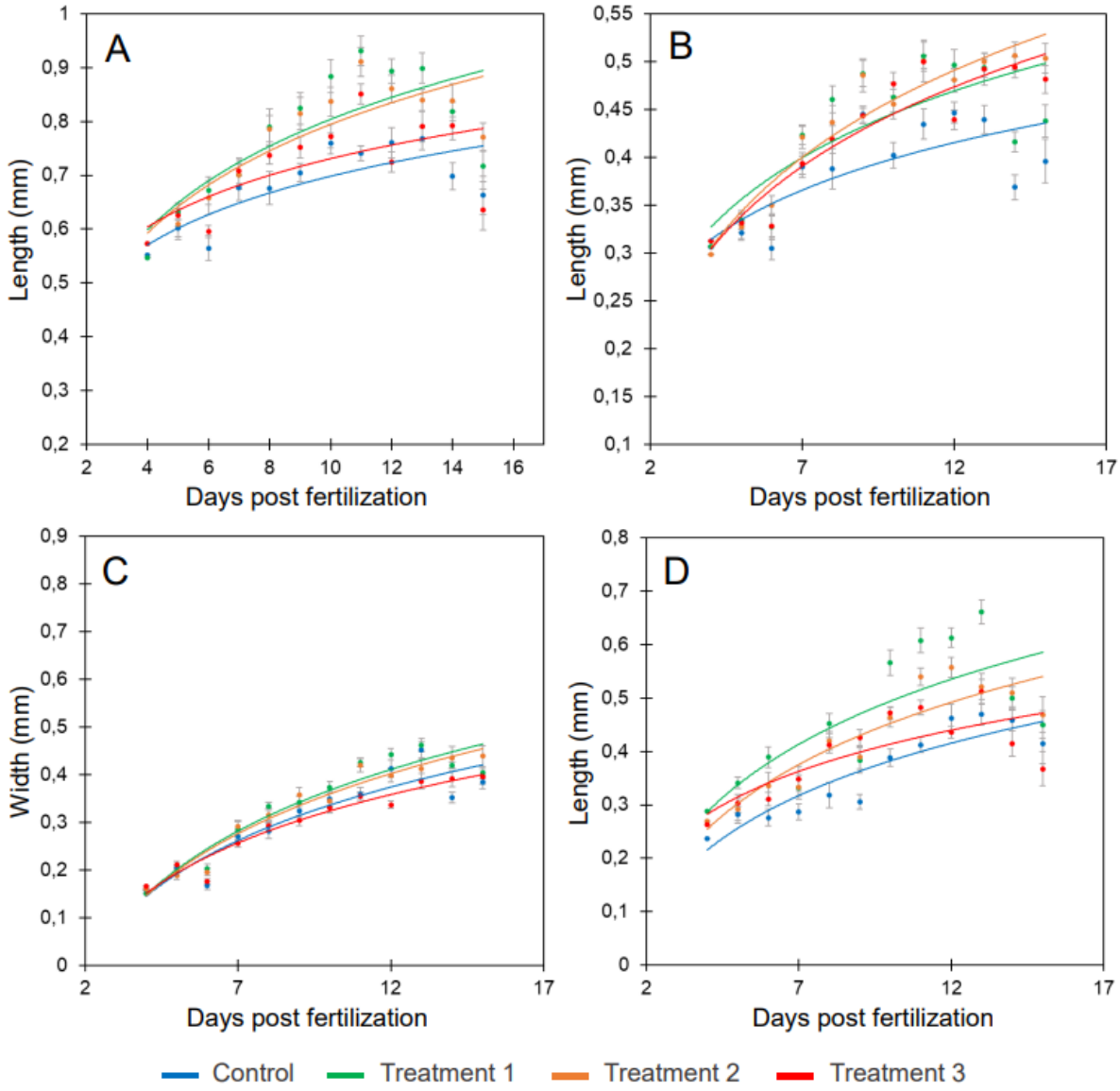

Figure 6. Effects of chronic exposure to a mixture of trace elements on development of larvae of *P. lividus* (A: total length; B: body length; C: body width and D: arm length) produced by sea urchins from Albo (contaminated site) for concentrations measured in an old asbestos mine at Canari (Corsica. France) and 5- to 50-fold higher concentrations (experiment 3).

Larvae obtained from sea urchins from Albo (experiment 3) tended to be significantly larger compared to larvae from sea urchins collected at Calvi (experiment 2). There were also some differences between the two experiments regarding the stage of development (Fig. 7; Fig. 8). All larvae had four arms at 4 days post-fertilization. In experiment 3, the 6-arm stage appeared earlier (7 days post-fertilization; Fig. 8B, 8C) in treatments 1 and 2 (average and 5-fold concentration, respectively).

The larvae acquired the 8-arm stage from the 11th or 12th days post-fertilization in controls and treatments 1 and 2 for both experiments. In treatment 3, the 8-arm stage appeared from the 13th or 14th day post-fertilization (Fig. 7D; Fig. 8D). Despite the similarities between the two experiments, the number of larvae at the 8-arm stage was higher in experiment 3 (over than 70% at the 15th day post-fertilization; Fig. 8), notably in treatment 1 where all the larvae had eight arms from the 13th day post-fertilization (Fig. 8B).

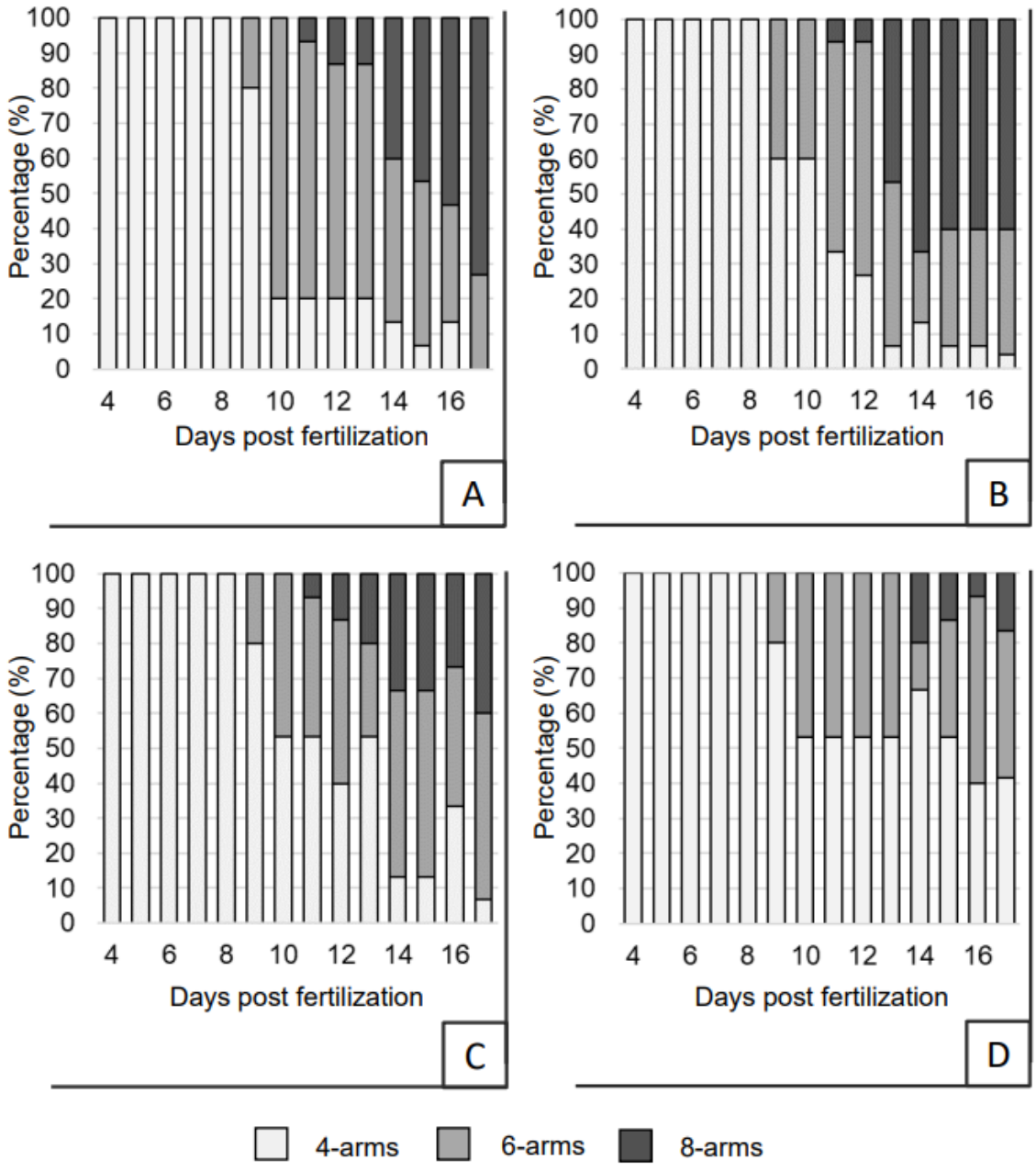

Figure 7. Effects of chronic exposure to a mixture of trace elements on stage development of *P. lividus* larvae produced by sea urchins from Calvi (experiment 2; A: control; B: treatment 1; C: treatment 2 and D: treatment 3).

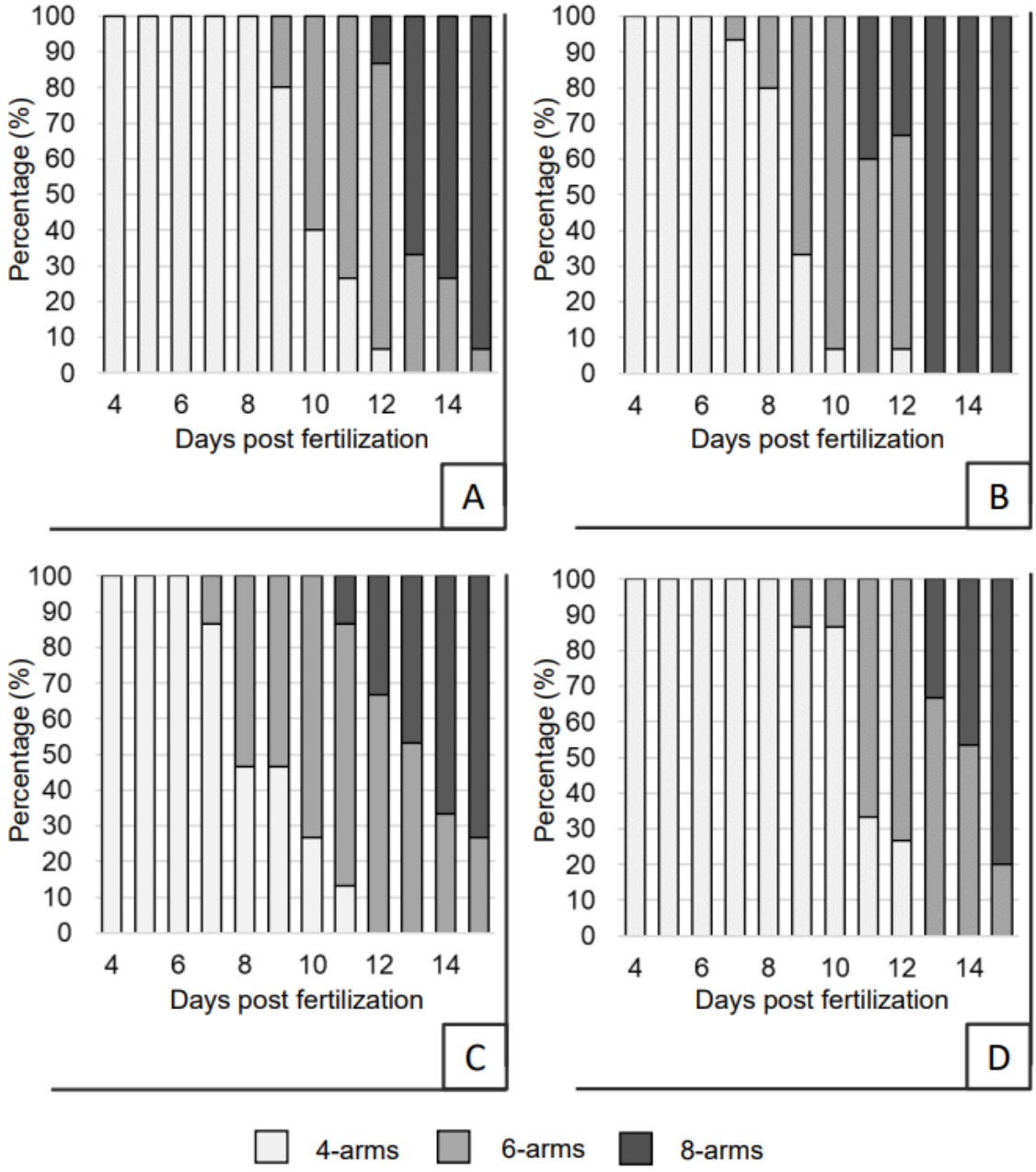

Figure 8. Effects of chronic exposure to a mixture of trace elements on stage development of *P. lividus* larvae produced by sea urchins from Albo (experiment 3; A: control; B: treatment 1; C: treatment 2 and D: treatment 3).

3.3. Comparison of the effects of different contaminations

Experiments 1 and 2 were carried out with larvae from adult sea urchins from Calvi (reference site). The first experiment was performed in order to study the effects of world ocean seawater average concentration while the second experiment was carried out to understand the effects of contaminations resulting from mining on the larval development of *P. lividus*.

Three treatments at different concentrations were considered: the average concentrations (treatment 1), and this same concentration multiplied by 5 (treatment 2) and 10 (treatment 3; Table 1; Table 2). The arm and total length of the larvae tended to be significantly greater in experiment 1 (p-value < 0.001; Figs. 3A, 3D, 5A, 5D). Conversely, body length and width tended to be greater in experiment 2 (Figs. 3B, 3C, 5B, 5C). The 6-arm stage appears in all treatments between the 9th and 11th day post-fertilization of both experiments (Fig. 4; Fig. 7). However, the acquisition of 8-arms stage appeared later for experiment 1, depending on the trace elements concentration (17th-21st day; Fig. 4).

4. Discussion

The chronic toxicity of trace elements on the larval cycle of *P. lividus* remains largely unknown. To our knowledge, no study has focused on the last larval stages of sea urchins (6- and 8-arms). The toxic properties of some trace elements have been demonstrated for several years (*e.g.* Dermeche *et al.*, 2012; Radenac *et al.*, 2001). However, their effects on larval development of sea urchin have often been studied in the context of acute exposure to one or a combination of two trace elements and at levels higher than those recorded in the environment (*e.g.* Bielmyer *et al.*, 2005; Dermeche *et al.*, 2012). This methodology is important to determine the median effective concentrations (EC_{50}), *i.e.* the metal concentrations reducing embryogenesis success to 50% of the control values, and the lowest observed effect concentrations (LOEC), *i.e.* the lowest concentrations significantly inhibiting growth (Fernandez & Beiras, 2001). In order to determine the effects of trace elements on marine organisms, it is necessary to consider both low concentrations and a mixture of several contaminants. This approach is difficult due to the large number of possible combinations and interactions in the mixture.

The toxicity of a chemical can be increased (synergy), reduced (antagonism) or unaffected (no interaction) by the presence of another toxicant (Amiard, 2011; Fernandez & Beiras, 2001). According to Fernandez & Beiras (2001), from the environmental standpoint, the main purpose is to predict the toxicity of mixtures of pollutants, rather than to classify the combinations of toxicants by their types of interaction. Therefore, the main aim of this study was to evaluate the impact of trace element mixtures measured in the environment. Mixtures

of seven and fifteen classic and emerging trace elements were tested to better understand the impact on sea urchins considering contamination levels found in the environment.

Larvae contaminated with the mixture of fifteen trace elements have a higher total length associated with the significantly longer arms likely resulting from morpho-anatomical abnormalities. Numerous larval malformations have already been observed in the literature, skeletal anomalies being the main consequences (*e.g.* Kobayashi & Okamura, 2004; Roccheri *et al.*, 2004). The sensitivity of skeletogenesis related to specific contaminants has been widely demonstrated (*e.g.* Moureaux *et al.*, 2011; Radenac *et al.*, 2001). Malformations and skeletal developmental delays in pluteus are mainly caused by Zn, Cd and Co (*e.g.* Kobayashi & Okamura, 2004; Mannaerts, 2007). Longer arms have likely been caused by skeletal elongation of the larvae even if no evidence of malformations was detected during stereomicroscopic observations, as described at Carbaillera *et al.* (2012). Nickel may also play a role because its presence is frequently associated with decreased Ca^{2+} uptake (Blewett *et al.*, 2016) which is essential for the calcification of the internal skeleton (Tellis *et al.*, 2013). Several authors have reported a decreased skeletal formation at higher Ni concentrations (Hardin *et al.*, 1992; Kobayashi & Okamura, 2004). Larvae with significantly reduced body length and width observed in the contaminated rearing tanks seem to confirm this hypothesis. Recently, skeletal integrity has been reported as a more sensitive criterion compared to description of malformations or EC_{50} determination, ranking abnormalities according to the severity of skeletal alteration (Carballeira *et al.*, 2012). However, Buttino *et al.* (2016) suggest that morphological abnormalities are not always determined by incorrect skeletal structures. In this experiment, the differences reported are not necessarily due to skeletal structures, but may also lead to decreased mobility making feeding, predator avoidance and settlement more difficult (O'Donnell *et al.*, 2010). Besides the measurement data, no signs of malformation were visible under stereomicroscopy. Thus, despite a broad mixture of trace elements, the reduced concentrations may be insufficient to cause malformations, confirming that larval measurements are essential to clarify the effects of low contaminant levels on larval development. Fernandez & Beiras (2001) indicate that larval length reflects a more gradual response to trace elements concentration allowing a better sensitivity of the bioassay with low levels of contamination.

Higher number of trace elements could induce more synergistic effects causing the arm elongation observed in experiment 1. Indeed, arm and total length tended to be greater when larvae were contaminated with fifteen trace elements (experiment 1) compared to larvae contamined with seven trace elements (experiment 1). Synergistic effects are known for mixtures of trace elements such as Cu-Zn (MacInnes, 1981), Hg-Pb (Fernandez & Beiras, 2001), Cu-Ag (Coglianese & Martin, 1981), Mn-Mo (Morgan *et al.*, 1986). However, since the notion of synergy or antagonism depends on the organism studied, it will be necessary to be vigilant. For example, the Zn-Cd mixture has been reported as synergistic for white shrimp, *Litopenaeus setiferus* (Linnaeus, 1767; Vanegas *et al.*, 1997), and antagonistic for bivalve embryos (Pavicic, 1980). Furthermore, the toxicity of trace elements depends on abiotic (*e.g.* pH, salinity) or biotic (*e.g.* organic ligands) factors (Fernandez & Beiras, 2001). The high number of trace elements probably also increases competitive interactions with essential ions due to the chemical similarity (Nogueira *et al.*, 2020). This could explain a body length and width tending to be smaller in larvae contaminated with the mixture of 15 trace elements. Overall, the results obtained in experiment 1 on the development of the larval stages showed few differences for the 4- and 6-arm stages. However, there was a significant delay in the development of 8-arms stage in contaminated larvae. This delay is even greater as the number and concentration of trace elements increases. This may be related to morpho-anatomical abnormalities causing elongation of the arms. Therefore, it is likely that mixing trace elements induces slowing or inhibition of larval development as concentrations increase. Several authors highlighted growth inhibition linked to the concentration of trace elements (Chiarelli *et al.*, 2014; Filosto *et al.*, 2008; Roccheri *et al.*, 2004). According to MacInnes (1981), the degree of synergy is greater as the concentrations in mixtures increase. This confirms the hypothesis of an inhibition of larval development when number and concentrations of trace elements increase. Furthermore, exposure time appears to be a factor in the occurrence of abnormalities and developmental delays (Filosto *et al.*, 2008) confirming the results observed in experiment 1 where the larvae were contaminated for 22 days. According to many studies, first developmental stages of marine invertebrates were recommended for performing bioassays due to their sensitivity to contaminants (*e.g.*, Paredes & Bellas, 2015; Pétinay *et al.*, 2009). This high sensitivity may be due to the important processes and vital events that occur during the early hours of development (Paredes & Bellas 2015). In this study, the stage at which the differences between control and contaminated larvae tended to be greatest was

near the end of the larval cycle. Therefore, it appears of interest to study the entire larval cycle when the aim is to understand the real effects of contamination on the organisms.

In experiments 2 and 3, dealing with contamination near the old asbestos mine, larvae contaminated at an average concentration (treatment 1) tended to be significantly larger in size and to develop more quickly. This may be due to the many essential trace elements present in the contaminant mixture, which are of nutritional interest and play a role in the enzyme systems (Chiarelli & Roccheri, 2014). A phenomenon of hormesis has already been described in the literature (*e.g.,* Nogueira *et al.*, 2020; Pétinay *et al.*, 2009), which is a biphasic dose-response relationship characterized by improvement of the biological aptitude at low doses and inhibitory or toxic effects at high doses (Mattson, 2008). Pétinay *et al.* (2009) observed a relative increase in the size of larvae with a concentration of 10 µg L^{-1} of Cu which they describe as the result of this phenomenon. Thus, the biological response to organic or inorganic stress does not necessarily lead to a negative effect on short-term growth. Therefore, each effect, positive or negative, must be interpreted with some caution.

Larvae from contaminated sea urchins tend to be wider than larvae from reference sea urchins in contaminated rearing tanks. Larvae contaminated at the average concentration tend also to develop faster when the spawners are from Albo. These results are surprising because the most common assumption would have been that larvae respond less well to contamination when they are from contaminated sea urchins. Indeed, according to several authors, the gametes of lower quality are more likely to be sensitive to a toxic agent (*e.g.* Bougis & Corre, 1979; Pétinay *et al.*, 2009). Bougis & Corre (1979) suggest that the effect of Cu is variable depending on the quality of the spawners. The effects of trace elements could be variable depending on the biochemical composition of the eggs, the genetic quality of the spawners or the water temperature (Bougis & Corre, 1979). This would also explain the different results obtained between the various authors (Pétinay *et al.*, 2009). Here, the temperature in rearing tanks was controlled to remain at 20 °C. Then, the first hypothesis would assume that the sea urchins from Calvi were subject to other sources of pressure; therefore, the spawners would have gametes of less good quality. However, the site has been described as a reference site in the literature (El Idrissi *et al.*, 2020; Richir *et al.*, 2013). The second hypothesis would be that the hormesis phenomenon is greater in larvae from contaminated spawners. These larvae

would be more sensitive to toxic agents and the stress induced by contaminants allowing a higher growth rate. A last hypothesis is that prior exposure of sea urchins from Albo to contaminants may make their gametes more resistant (Crean & Immler, 2021; Wells *et al.*, 1998). It would be interesting to investigate this hypothesis by performing biochemical analyses in order to understand if there is an oxidative stress or a higher expression of certain genes in larvae related to spawner contamination. These hypotheses demonstrate the importance of considering spawner quality in ecotoxicological studies. Pétinay *et al.* (2009) propose to use standardization of spawner conditioning to achieve the same quality and avoid bias. Cryopreservation of gametes or fertilized eggs may simplify the technique and make it available to all laboratories (Paredes & Bellas, 2015). However, to assess the impact of contaminants at site-scale, it would be more appropriate to perform the tests onorganisms from the same study site in order to have a more reliable response and better visibility regarding the consequences in the environment.

5. Conclusion

This study has contributed to better understanding of the effects of chronic exposure to trace element contamination on the entire larval stage of *P. lividus*. No visible malformations were observed at the concentrations studied. However, the increase in the number and concentration of trace elements caused an elongation of the arms and a delay in the larval development which increased with time. Therefore, this work highlights the need to consider the entire larval cycle of the sea urchin. In contrast, the larvae contaminated by 7 trace elements (Co, Cu, Cr, Fe, Hg, Ni and Zn) developed more quickly. This may be due to the several essential trace elements present in the mixture but also to a phenomenon of hormesis already observed in the literature for certain trace elements. The origin of the spawners also plays a role in the results observed in this study. It is therefore necessary to include this factor in the ecotoxicology bioassays.

Article 5

Stress response to trace elements mixture of different embryo-larval stages of *Paracentrotus lividus*

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Contexte et résumé de l'article 5 « Réponse au stress des stades embryo-larvaires de *Paracentrotus lividus* à différents mélanges d'éléments traces »

Au niveau de l'ancienne mine d'amiante de Canari, de fortes teneurs en éléments traces ont été mesurées (Article 2) et leurs effets sur le développement larvaire et sur la morphologie des larves de *Paracentrotus lividus* ont été étudiés (Article 4). Cette partie du chapitre vise à déterminer les réponses que présentent les larves face aux contaminations en utilisant des biomarqueurs biochimiques et biométriques. Ainsi, en parallèle des mesures de larves, une analyse des activités spécifiques de la catalase (CAT), superoxyde dismutase (SOD), glutathione peroxidase (GPX) et glutathione-S-transferase (GST) a été effectuée. Dans ce but, différents stades embryolarvaires (4 bras, 6 bras et 8 bras) ont été contaminés par sept éléments traces (cobalt, chrome, cuivre, fer, mercure, nickel et zinc) pendant 48 h à des concentrations mesurées dans la zone de l'ancienne mine d'amiante à des facteurs de 5 et 10. Plusieurs paramètres ont été pris en compte dans cette étude comme la pré-exposition des larves pendant le stade embryonnaire, l'origine des géniteurs et les niveaux de contamination. Les résultats suggèrent que les concentrations mesurées au niveau de l'ancienne mine d'amiante ne sont pas suffisamment élevées pour induire un stress oxydatif majeur, même à des facteurs de 5 et 10. Des différences biométriques ont néanmoins été observées avec des larves plus longues lors des contaminations. Ces différences font de cette méthode une approche plus sensible pour évaluer les effets d'un mélange d'éléments traces à des concentrations environnementales. Les mesures des activités spécifiques des enzymes antioxydantes à chaque stade ont suggéré une forte capacité des larves à répondre au stress oxydatif. Cette activité normale de l'organisme doit être prise en compte dans les futurs travaux de recherche afin d'interpréter avec prudence les réponses des biomarqueurs de stress. Cette étude a également mis en évidence l'importance de la provenance des géniteurs dans les études écotoxicologiques avec des différences significatives dans les analyses biométriques et biochimiques. Ces données sont essentielles pour mieux comprendre les réponses au stress des larves d'oursins et fournir des informations de base pour les recherches ultérieures d'évaluation environnementale.

1. Introduction

The intensification of anthropogenic practices such as agricultural, urban and industrial discharges can lead to high levels of pollution in coastal habitats and affect marine ecosystems (Islam & Tanaka, 2004; Mostofa *et al.*, 2013). These increasing discharges of pollutants of different kinds cause pressure on the marine ecosystem, disrupting its natural balance (Halpern *et al.,* 2007; Benedetti *et al.,* 2015). Due to their continuous inputs, persistence, toxicity, numerous discharge routes and ability to accumulate in organisms, trace elements are a major issue in the marine environment (Spencer *et al.*, 2006; Ali *et al.*, 2013).

Within the Mediterranean Sea, Corsica Island has long been considered as a site subject to low anthropic pressure, in contrast to other Mediterranean coasts (Gobert *et al.*, 2017). Nevertheless, concerning levels of trace elements were measured in the north-west of Corsica, near an old asbestos mine (Cary *et al.*, 2013; Ternengo *et al.*, 2018; El Idrissi *et al.*, 2020). Studies have shown high concentrations of chromium (Cr), cobalt (Co), and nickel (Ni) in the area adjacent to the old mine due to the release of 4.5 x10^6 m^3 of solid waste rock into the sea during the active mining period between 1950 and 1965 (BRGM, 1997; Andral *et al.*, 2004; Cary *et al.*, 2013). An accumulation of these trace elements has been recorded in several organisms implanted in this area (*e.g.* mussels, sea urchin, Mediterranean tapeweed (seagrass) *Posidonia oceanica*; Lafabrie *et al.*, 2007; Kantin *et al.*, 2015; El Idrissi *et al.*, 2020). A variety of methods are used to assess the levels of contamination in marine organisms and ecosystems. Quantification of chemicals by analytical tools can measure the extent and significance of environmental contamination (Guendouzi *et al.*, 2017; Rouane-Hacene *et al.*, 2017). However, analytical techniques are not adequate to assess the effects of the presence of contaminants on organisms or on the health of the ecosystem (Lagadic *et al.*, 1998; Ramade, 2007). Indeed, the effects of trace elements on organisms vary considerably depending on their forms (Xian, 1989) and the synergistic or antagonistic actions that may occur in the environment (Fernandez & Beiras, 2001; Nogueira *et al.*, 2021), but also on the organism considered, its stage of development or its physiological state (Paredes & Bellas, 2015).

In recent years, bioassays have been used more frequently to better predict the effects of contaminants on organisms exposed to pollutants (*e.g.* Ruocco *et al.*, 2020; Nogueira *et al.*, 2021; Rendell-Bhatti *et al.*, 2021). It has been widely demonstrated in controlled experiments that biomarkers can be used to assess the effects of xenobiotics on the organism's vital structures and functions (*e.g.* Roccheri *et al* 2004; Richardson *et al.*, 2021). Marine organisms are very sensitive to many environmental stresses, and therefore the analysis of their response to different stressors is crucial for our understanding of defense mechanisms (Roccheri *et al.*, 2004). Numerous studies suggest that exposure to various trace elements can cause the production of reactive oxygen species (ROS) resulting in irreversible damage in marine organisms (Nieto *et al.*, 2010). One of the predominant mechanisms for the toxicity of trace elements is interference with cellular redox regulation and induction of oxidative stress (Beyersmann & Hartwig, 2008). It is therefore of interest to consider the activities of antioxidant enzymes such as catalase (CAT), glutathione peroxidase (GPX), glutathione-s-transferase (GST), superoxyde dismutase (SOD), acting against oxidative stress.

The choice of bioindicators for bioassays must take into account many factors such as sensitivity and reliability but also distribution and environmental relevance (Chapman, 2002a). Besides its key role in structuring benthic communities through grazing activity, the sea urchin is one of the most suitable test organisms for acute bioassays of marine pollution (Boudouresque & Verlaque, 2013). Indeed, sea urchin larvae have many advantages: widespread geographical distribution, rapid development, ease of handling and rearing under laboratory conditions (Geraci *et al.*, 2004; Salvo *et al.*, 2014). Consequently, bioassays on embryo-larval stages are frequently used in the determination of the availability of chemicals and assessment of their toxicity (*e.g.* Migliaccio *et al.*, 2014; Ruocco *et al.*, 2020; Nogueira *et al.*, 2021). The sensitivity of the sea urchin larvae and morphological abnormalities due to pollutants exposure have been reported under controlled laboratory conditions or in natural marine-polluted ecosystems (Saco-Álvarez *et al.*, 2010; Beiras *et al.*, 2012; Gharred *et al.*, 2021). While larvae have been used to assess trace elements contamination, no study has examined induced oxidative stress over the entire larval cycle. It is therefore essential to improve the understanding of biomarker responses to environmentally relevant concentrations for all larval stages.

The overall aim of this study is to assess the capacity of larval stages to respond to stress induced by trace elements concentrations of concern (levels measured near the old asbestos mine at Canari (Corsica, France)). This study has also assessed the responses according to the stage of development, the origin of the spawners and also the pre-exposure of the fertilized eggs to contamination. These data, combined with the monitoring of larval development, will allow a better understanding of the effects of contaminants on aquatic organisms and also a better use of sea urchin larvae as bioindicators.

2. Material and methods

Adult sea urchins, *Paracentrotus lividus* (Lamarck, 1816), were collected in spawning period, in March by scuba diving in Corsica (France, Mediterranean Sea) at two specific stations: (i) Albo (42°46.313 N, 9°20.150 E), a contaminated site due to its proximity to the old asbestos mine at Canari, and (ii) Calvi (42°34.916 N, 8°43.589 E), a reference site characterized by very low levels of trace elements contamination (Fig. 1; Ternengo *et al.*, 2018; Gobert & Richir, 2019; El Idrissi *et al.*, 2020). Directly after the field sampling, individuals were transported in an insulated box to the laboratory. For each site, 30 males and 30 females were spawned. Concentrated sperm was collected dry, mixed and kept on ice until use. In order to optimize the genetic mixing, equal amounts of eggs from each female were mixed and suspended in natural filtered seawater. A concentrated sperm was added and after 45 min, fertilization success was checked (fertility rate above 90%; Pétinay *et al.*, 2009; Buttino *et al.*, 2016). The number of fertilized eggs was assessed and distributed in the rearing tanks. Temperature was adjusted at the beginning of the experiments (20 °C) and was continuously monitored using HOBO TidbiT® v2 loggers (accuracy: ± 0.21 °C). All animal procedures were in compliance with the guidelines of the European Union (Directive 609/86/CEE).

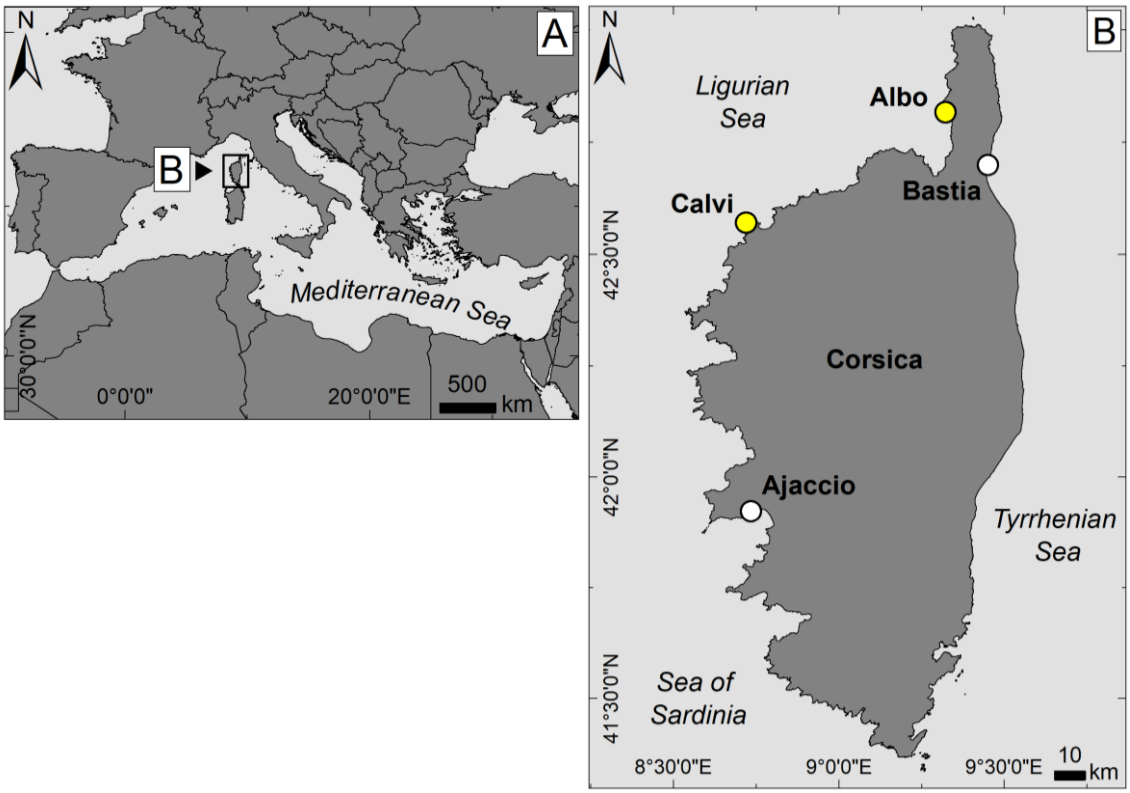

Figure 1. Location of the sampling stations (yellow dots; reference site: Calvi; contamined site: Albo - old asbestos mine) in Corsica Island (NW Mediterranean).

Bioassays were performed to assess the impact of the contamination measured in the seawater column in front of the old asbestos mine at Canari (Fig. 2). The effects on the embryo-larval stages were reproduced by using synthetic polluted seawater characterized by a mixture of seven trace elements selected for distinct purposes: (i) cobalt (Co), chromium (Cr) and nickel (Ni) are measured at high concentrations in the study area (Ternengo *et al.,* 2018); (ii) iron (Fe), copper (Cu) and zinc (Zn) are essential trace elements usually recovered at high levels in sea urchin gonads (El Idrissi *et al.*, 2020), and (iii) mercury (Hg) has been frequently discussed for several years in the scientific community in view of its potential impact (*e.g.* Streets *et al.*, 2019; Zheng *et al.,* 2019). Two treatments were tested using the average concentrations measured in the seawater column in front of the old asbestos mine multiplied by 5 (treatment 1) and multiplied by 10 (treatment 2; Table 1). Trace element stock solutions diluted in deionized water were prepared using the following analytical grade solutions (1 g L^{-1} Certipur®, Merck, Germany): $Co(NO_3)_2$, $Cr(NO_3)_3$, $Cu(NO_3)_2$, $Fe(NO_3)_3$, $Hg(NO_3)_2$, $Ni(NO_3)_2$, $Zn(NO_3)_2$. Synthetic polluted seawater was prepared by adding trace elements into control seawater to reach the desired concentrations.

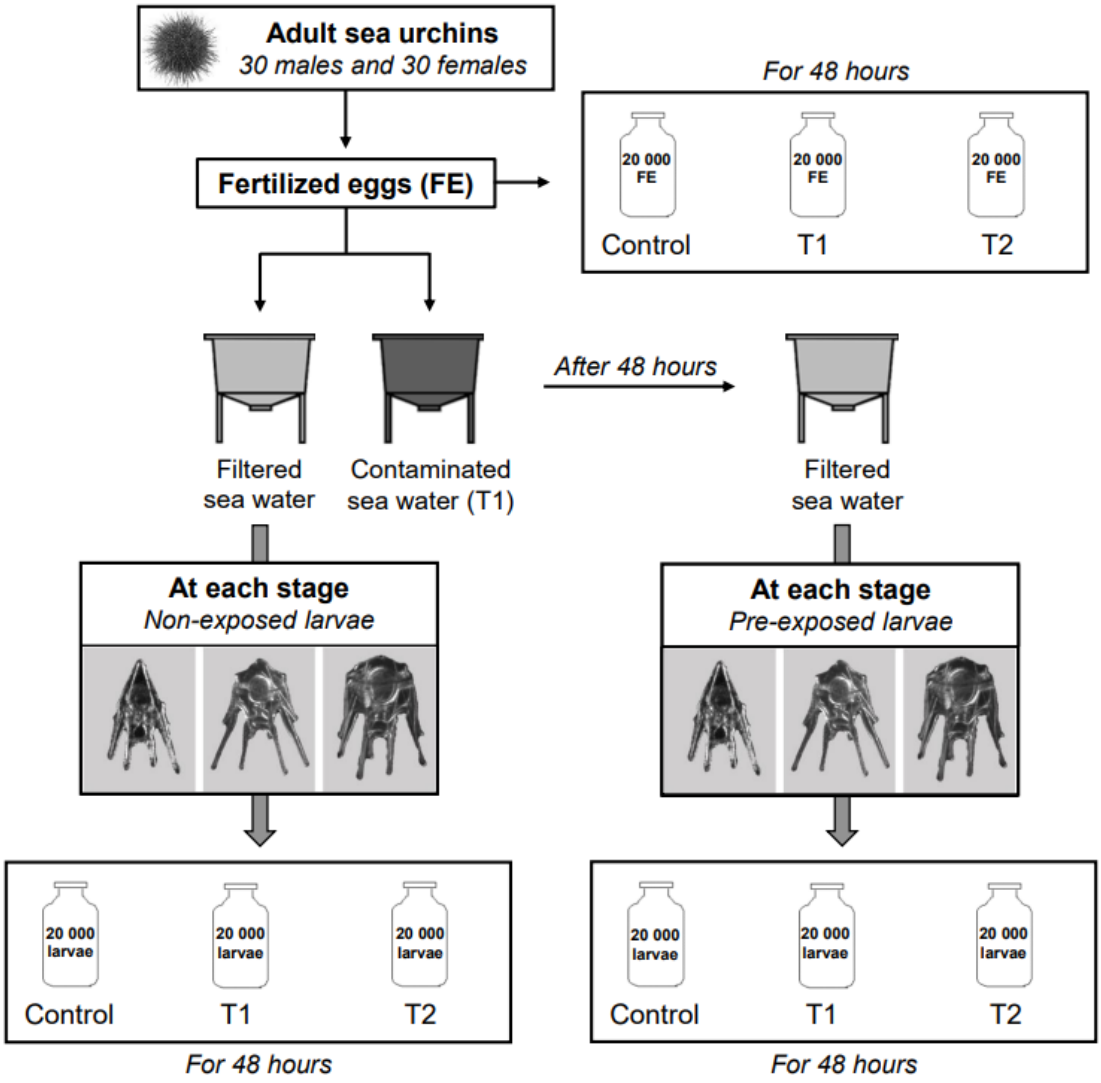

Figure 2. Schematic overview of the experimentation performed with sea urchins from each site. The experiments with larvae from Albo and Calvi spawners were conducted simultaneously. All embryolarval stages studied (fertilized eggs: FE; 4-arms; 6-arms and 8-arms) were exposed for 48 h to a mixture of trace elements at a concentration measured in the area adjacent toan old asbestos mine at factors of 5 (Treatment 1: T1) and 10 (Treatment 2: T2).

Two experiments were carried out using larvae produced by sea urchins from Albo and Calvi and were conducted simultaneously so as to have the same experimental conditions. Fertilized eggs from each site were placed in rearing tanks filled with filtered seawater and in contaminated water (treatment 1; Fig. 2). After 48 hours, the pre-exposed larvae were transferred to rearing tanks with filtered seawater. The seawater was renewed daily and food, composed of a mixture of phytoplankton, was provided *ad libitum*.

Table 1. Average concentration recorded near an old asbestos mine at Canari, Corsica, France (µg L-1; El Idrissi *et al.*, 2022) and trace elements concentration tested from larvae produced by sea urchins sampled at Calvi and Albo.

	Co	Cr	Cu	Fe	Hg	Ni	Zn
Average concentration	0.02	0.15	0.30	1.10	0.40	1.38	1.30
Treatment 1: 5-fold concentration	0.1	0.75	1.5	5.5	2	6.9	6.5
Treatment 2: 10-fold concentration	2	1.5	3	11	4	13.8	13

In total, four rearing tanks of 80 L were used (two rearing tanks for the larvae from the spawners of each site). For each population, one of the two rearing tanks received pre-exposed larvae and the other, non-exposed larvae (Fig. 2). About 20 000 larvae from each rearing tank were collected at different stages (fertilized eggs; 4-arms; 6-arms; 8-arms) and placed in contaminated water at different concentrations (treatments 1 and 2; Fig. 2). After 48 hours of exposure to contaminants, larvae were frozen in liquid nitrogen and immediately stored at −80 °C for biochemical analyses. Duplicates were performed for each treatment and a control was carried out for each experiment.

Samples were homogenized using a Potter-Elvehjem homogenizer in chilled phosphate buffer (100 mM, pH 7.4; 25 mg w/w per mL of buffer) containing 20% glycerol and 0.2 mM phenylmethylsulfonyl fluoride as a serine protease inhibitor. The homogenates were centrifuged at 15,000 × g for 30 min at 4 °C and the supernatant was used for biochemical assays. Protein concentration was measured as described in Bradford (1976) and was used to normalize the final unit for biomarker responses. Biomarkers including catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPX) and glutathione-S-transferase (GST) were assessed in whole body tissues of *P. lividus* larvae as described by Greani *et al.* (2017). All enzyme activities were expressed in units mg^{-1} protein (specific enzymatic activities). Antioxidant enzyme activity was determined using a Specord 205 spectrophotometer (Analytic Jena, Wembley, UK).

In order to have a better understanding of the effects of contaminants, at each stage studied, sixty larvae from each treatment were photographed under a stereomicroscope. The larvae were then measured using ImageJ software to determine the total length, body width and length and arm length.

All statistical analyses and graphical representations were conducted using XLSTAT® software. The results were expressed as mean ± standard error (SE). Data were transformed when conditions of normal distribution and homogeneity of variance were not met and were analyzed using analysis of variance (ANOVA) followed by post-hoc Tukey's honestly significant difference (HSD) tests. The relationship between enzymatic activities was measured by Pearson correlation coefficient. Differences were considered statistically significant at $p<0.05$.

3. Results and discussion

Recent studies suggest that exposure to different contaminants, including trace elements, may cause a cascade of physiological events such as the production of ROS (Roccheri *et al.*, 2004; Benedetti *et al.*, 2015; Chan & Wang, 2019). When ROS production exceeds the cellular antioxidant defences, it results in oxidative stress, involved in DNA damage, protein oxidation, lipid peroxidation impacting the survival of marine organisms (Winston & Di Giulio, 1991; Nieto *et al.*, 2010; Halliwell & Gutteridge, 2015; Chan & Wang, 2019). In order to offset molecular and cellular damages, aerobic organisms have developed mechanisms including an assortment of antioxidant enzymes (Valavanidis *et al.*, 2006, Vlahogianni & Valavanidis, 2007; Ighodaro & Akinloye, 2018). Some studies reported biomarker responses of oxidative stress in organisms to trace elements contamination (Farombi *et al.*, 2007; Sappal *et al.*, 2009; Liu & Wang, 2016; Chan & Wang, 2019). Interference with cellular redox regulation is a major mechanism for the toxicity of trace elements (Beyersmann & Hartwig, 2008; Benedetti *et al.*, 2015). For example, Cu and Fe are widely known to actively mediate ROS production through Fenton and Haber-Weiss reactions and could alter cellular redox balance (Chan & Wang, 2018). However, the results of this study show that there is no significant difference in the specific activity of antioxidant enzymes depending on the concentrations used (Fig. 3A). Thus, these concentrations are probably not high enough to create oxidative stress in *P. lividus* larvae, although they are 5 to 10 times higher than those at the contaminated sites (Table 1; Ternengo *et al.*, 2018; El Idrissi *et al.*, 2022). A recent study has shown that the number of trace elements also plays an important role in the effects on sea urchin larvae (El Idrissi *et al.*, 2022). A mixture with higher diversity of trace elements would probably have had a greater impact on the mechanism against oxidative stress in sea urchin larvae.

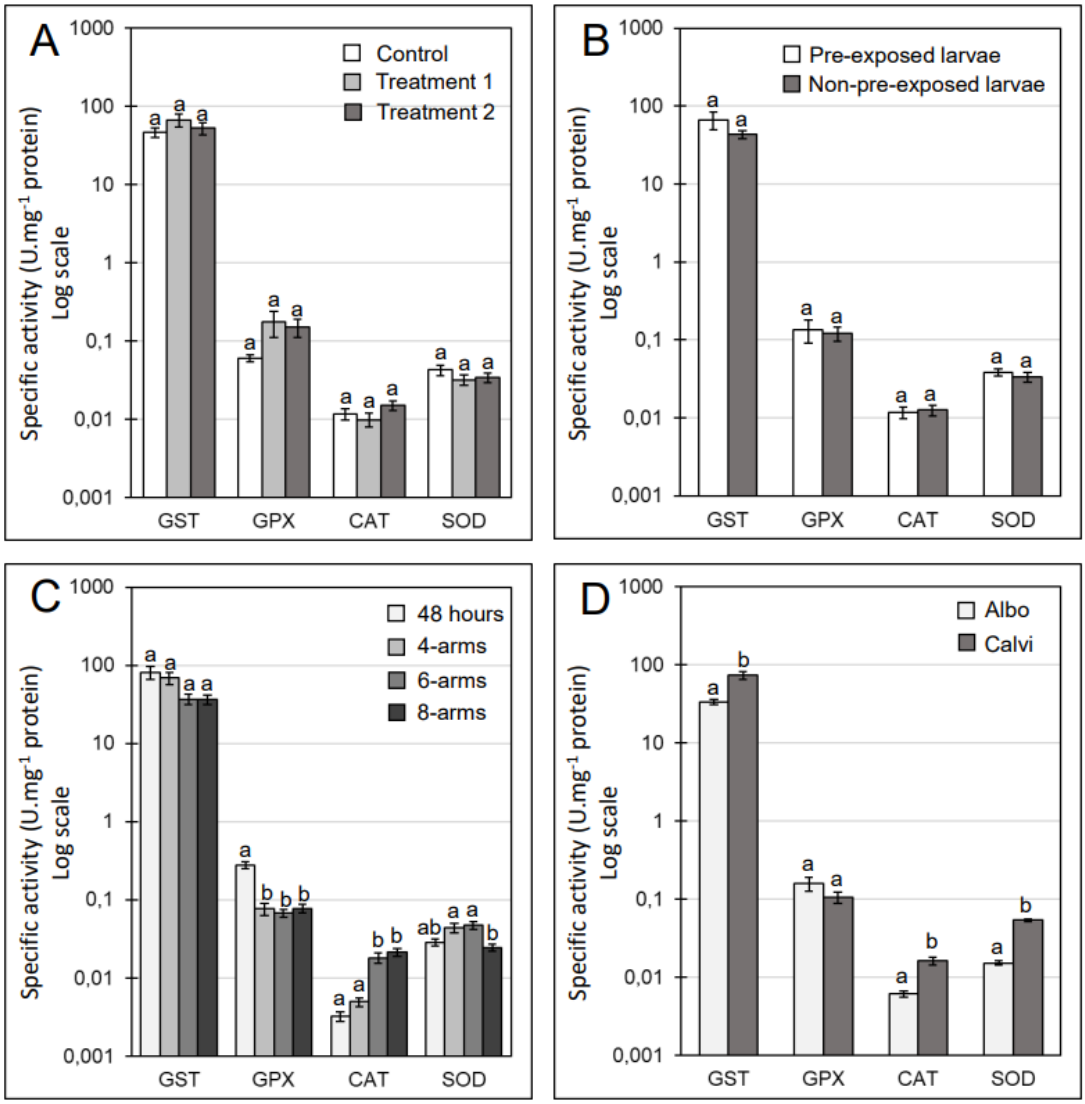

Figure 3. Changes in specific activities of glutathione-S-transferase (GST), glutathione peroxidase (GPX), catalase (CAT) and superoxyde dismutase (SOD) in *P. lividus* larvae according to (A) contaminant concentrations, (B) pre-exposure of the fertilized eggs, (C) developmental stage and (D) the origin of the spawners. Dissimilar letters denote significant differences between groups (p-value < 0.05).

Despite the absence of significant differences in enzymatic response to the various treatments, biometrics differences have been reported (Fig. 4). This method of measuring larvae is therefore probably more suitable and sensitive when concentrations are low. Larvae are significantly lengthier when they are contaminated (Fig. 4). This could be justified by the fact that the mixture used in this study is composed for the most part by essential trace elements, fundamental for most physiological processes of living organisms (Janssens *et al.*, 2009; Yamaguchi *et al.*, 2009; Ghribi *et al.*, 2020). A phenomenon of hormesis characterized by an improvement of the biological aptitude at low dose had been assumed in the study of

El Idrissi *et al.* (2022). This is a response, generally favorable, to low-dose exposures to contaminants or stress-generating phenomena (Mattson, 2008). Thus, the biological response to organic or inorganic stress would not necessarily lead to a negative effect on short-term growth (Chapman, 2002b; Pétinay *et al.*, 2009). In this study, this hypothesis can be refuted because there is no difference in specific stress biomarker activity when the larvae were contaminated or not (Fig. 3A).

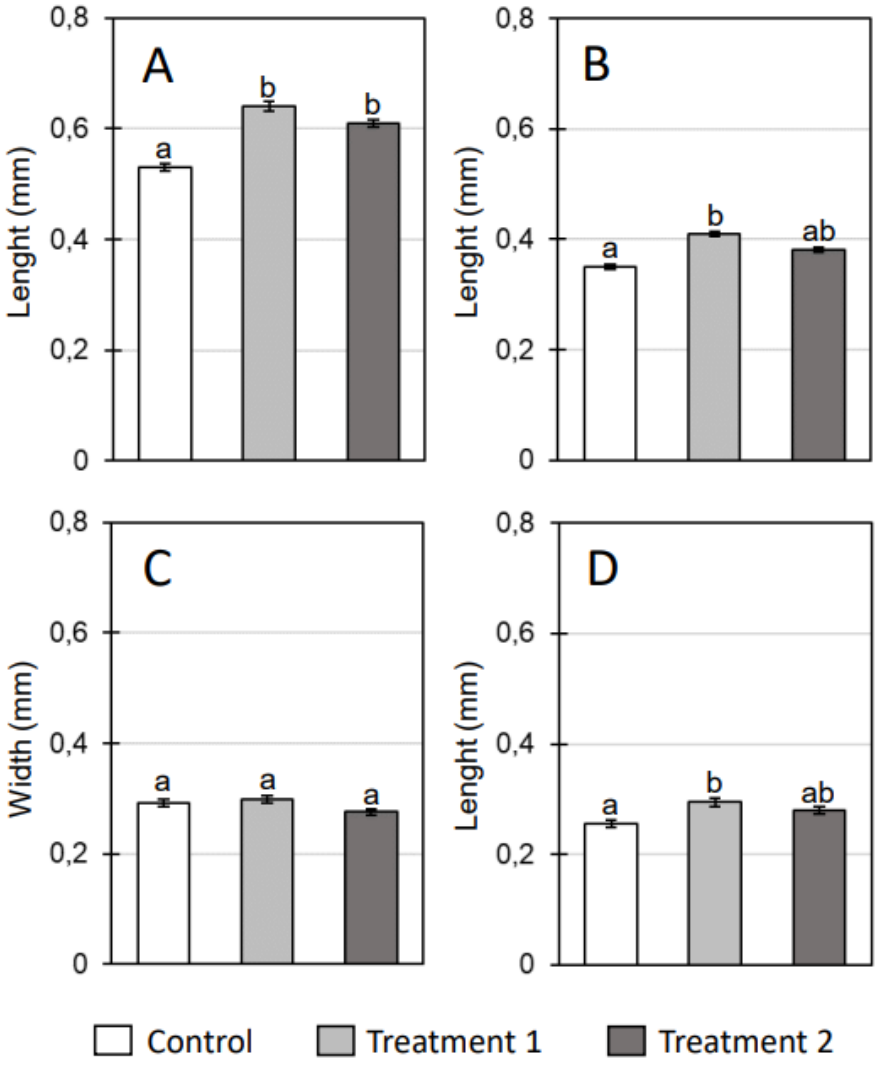

Figure 4. Effects of exposure to different concentrations of a mixture of trace elements on development of *P. lividus* larvae (A: total length; B: body length; C: body width and D: arm length). Dissimilar letters denote significant differences between groups (p-value < 0.05).

Several authors reported the sensitivity of the morphology of sea urchin larvae, especially with abnormalities, during different exposures to pollutants (Kobayashi & Okamura, 2004; Beiras

et al., 2012; Migliaccio *et al.*, 2014; Gharred *et al.*, 2016). However, in this work, there was no malformation as described by Carballeira *et al.* (2012). The concentrations used in this research are probably not sufficient to induce a stress biomarker response and therefore malformation of the larvae. Transcriptional responses might be more sensitive but these do not necessarily correspond to contaminant effects and are more useful as biomarkers of exposure (Giuliani *et al.*, 2014; Benedetti *et al.*, 2015).

A first stressor may precondition the species to be less sensitive to a second stressor (Crain *et al.*, 2008). However, it appears that pre-exposure had no effect on sea urchin larvae in this investigation (Fig. 3B). Indeed, there is no variation of the specific activity of the four studied enzymes involved in oxidative stress when the larvae are in contact or not with contaminants at the embryonic stage (Fig. 3B). This seems to confirm the hypothesis that the contaminations used in these experiments are insufficient to induce an increase in oxidative stress.

The larvae were contaminated for only 48 h at each stage to determine whether there were different responses depending on the stage (Fig. 3C). This short period prevents organisms from acquiring resistance to contamination through physiological acclimatization (Benedetti *et al.*, 2015). The embryonic development of sea urchin is characterized by crucial successive stages from fertilization to organogenesis (Byrne *et al.*, 2009; Gharred *et al.*, 2021). This early stage is therefore likely to be more sensitive than later stages and could explain a significantly higher GPX specific activity (Fig. 3C). SOD has a higher specific activity in stage 4 and 6-arms (Fig. 3C). The latter is a metalloenzyme using trace elements such as Cu and Fe as cofactor (McCord & Fridovich, 1969; Li *et al.*, 2009; Zhang *et al.*, 2010). Santon *et al.* (2003) and Zhang *et al.* (2010) demonstrated that Zn contamination at an environmentally relevant concentration can support SOD activity and thus play a protective role in delaying oxidative processes. This enzyme detoxifies the superoxide radical and thus provides protection against damage induced by free radicals (Zhang *et al.*, 2010). This mechanism is one of the first lines of defence against oxygen toxicity (Winston & Di Giulio, 1991). Stages 4 and 6-arms probably have a better ability to respond to early signs of oxidative stress by catalyzing the conversion of the superoxide radical to hydrogen peroxide. The latter is also an important ROS (Rojkind *et al.*, 2002) and leads to the formation of the hydroxyl radical in the presence of transition metals *via* the Fenton and Haber-Weiss reactions (Haber & Weiss, 1932; Winterbourn, 1995).

It is important to underline that the hydroxyl radical is the most powerful oxidant of ROS with a high rate constant (Di Giulio *et al.*, 1989; Gligorovski *et al.*, 2015). It is therefore essential to eliminate hydrogen peroxide by metabolizing it into water and oxygen using certain enzymes such as CAT in order to avoid potential damage (Roméo *et al.*, 2000; Ko *et al.*, 2000). The results exhibited an increasingly higher CAT activity during larval development with 0.0032 ± 0.0004 $U.mg^{-1}$ proteins at the embryonic stage and 0.2139 ± 0.0065 $U.mg^{-1}$ proteins at the 8-arm stage (Fig. 3C). In this way, hydrogen peroxide potentially metabolized by SOD during stages 4 and 6-arms can be eliminated by CAT activity in order to limit or eliminate the potential impacts due to ROS. This suggests the ability of the larvae to respond to oxidative stress. Moreover, the specific activity of CAT is positively correlated with the specific activity of SOD (0.228; p-value 0.002). It is important to take this information into account in future works studying the responses of sea urchin larvae to different stresses. Indeed, ROS are produced during normal aerobic metabolism, and organisms use a number of enzymes to trap them, even in the absence of stress (Del Río *et al.*, 2006; Mittler *et al.*, 2004; Navrot *et al.*, 2007). Moreover, ROS not only indicates the presence of deleterious oxidative stress but is also involved in the regulation of metabolic processes (Levine, 2002; Migdal & Serres, 2011). Consequently, it is necessary to interpret the responses of the stress biomarkers with prudence, taking into account the normal activity of the organism studied. It would be interesting to reproduce the experiment with higher concentrations in order to verify the hypotheses expressed in this study regarding the ability of the larvae to resist stress.

Several authors have indicated the importance of considering spawners quality in studies of the effects of contaminants on larvae (*e.g.* Bougis & Corre, 1979; Pétinay *et al.*, 2009). El Idrissi *et al.* (2022) demonstrated significant differences in development and size between the larvae obtained by the spawners from Albo and those produced by spawners from Calvi. These size differences are confirmed in this work with significantly larger larvae when they are obtained by sea urchins from Albo (Fig. 5). Durkina & Evtushenko (1991) observed an acceleration in development in the descendants of individuals exposed to Cu. It is likely that larvae from Albo spawners develop more rapidly and are lengthier due to the contamination of the spawners (Ternengo *et al.,* 2018; El Idrissi *et al.,* 2020).

The specific activity of GST, CAT and SOD is significantly higher in larvae produced by sea urchins from Calvi (Fig. 3D). El Idrissi *et al.* (2022) has mentioned that Calvi is exposed to other sources of pressure than trace elements inducing spawners with lower quality gametes, explaining the slower development and smaller larvae. The quality of gametes is known to be greatly influenced by different environmental factors such as temperature, quality of the seawater (*e.g.* chemical quality, bacteriological quality) and also spawners feeding condition (*e.g.* Zhao *et al.*, 2015; Gallo *et al.*, 2020). This potential pressure, unknown at this time, may lead to larvae more sensitive to stressors with higher specific antioxidant enzyme activity (Fig. 3D). According to Benedetti *et al.* (2015), the difference in the ability to resist the environmental conditions would indicate the occurrence of adaptive mechanisms in chronically exposed organisms. The possibility of a genetic adaptation has already been demonstrated in long-term exposure (Uthicke *et al.*, 2019; Ruocco *et al.*, 2020) and it is now widely accepted that offspring inherit epigenetic information from their parents (Benedetti *et al.*, 2015; Crean & Immler, 2021). This may provide some protection (Amiard-Triquet *et al.*, 2011; Munday *et al.*, 2013; Foo & Byrne, 2016) but the effects are not necessarily adaptive and epigenetic inheritance may also increase negative consequences of environmental change if parents transmit stress to future generations (Bonduriansky & Crean, 2018; Crean & Immler, 2021). In accordance with Lawrence (1990), biological stress would result in a decrease in production. This suggests that energy will be used in priority to metabolize enzymes against oxidative stress and less for growth. Therefore, sea urchins are characterized by high levels of phenotypic plasticity (Zhadan *et al.*, 2017; Ruocco *et al.*, 2020) and their morphological and physiological characteristics are subject to changes during the adaptation to selected environmental conditions (Ruocco *et al.*, 2020).

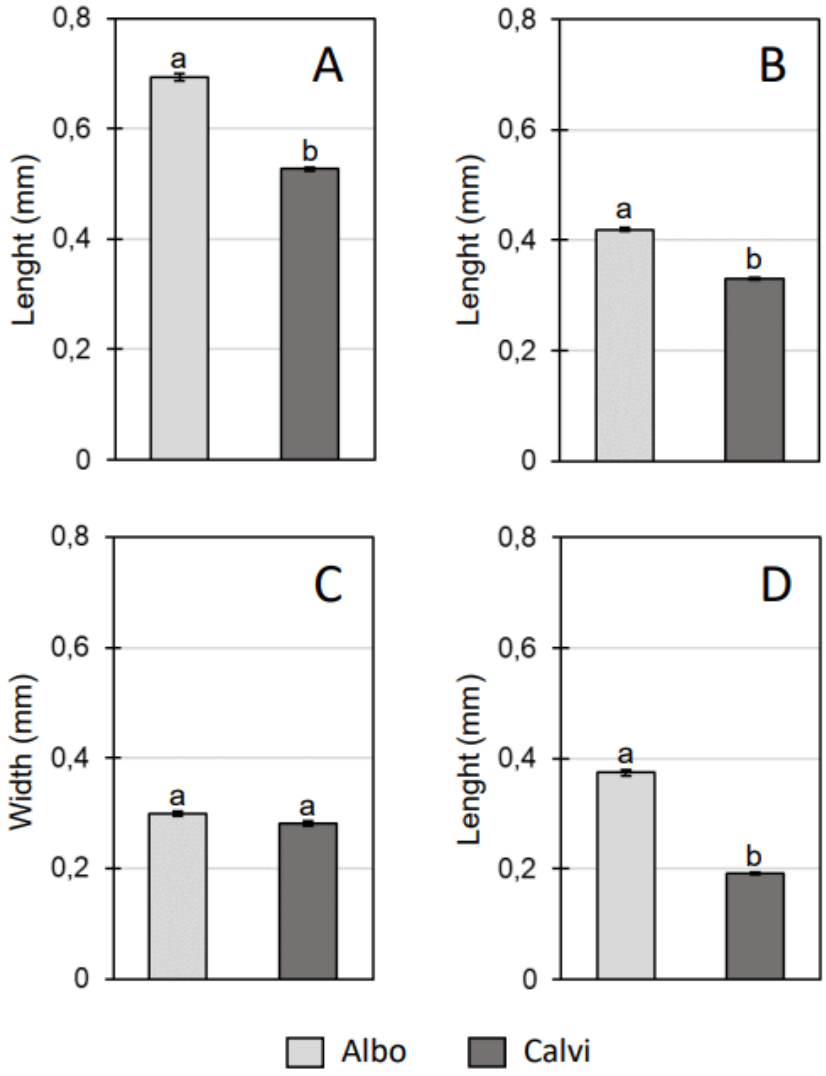

Figure 5. Morphological measurements (A: total length; B: body length; C: body width and D: arm length) of larvae from spawners harvested at Albo and at Calvi. Dissimilar letters denote significant differences between groups (p-value < 0.05).

The lower specific activity in larvae produced by spawners from Albo may also be attributed to an inhibition caused by contaminants. While some defences can be induced, others might be inhibited, or induced as the first phase of a response and then depleted at longer periods (Benedetti *et al.*, 2015). These larvae are susceptible to being more impacted by oxidative stress, because less protected, while those produced by the spawners from Calvi have a better defence with a higher specific activity. In order to confirm or refute these hypotheses, it would be interesting to reproduce this experiment with higher concentrations of trace elements, to explore the level of expression of key genes in the adaptation of the stress response and also to undertake an extensive study in the Calvi area.

4. Conclusion

Sea urchins are key species in the structuring of marine ecosystems (Boudouresque & Verlaque, 2013). Their larval stage constitutes a critical period in their life cycle because the decrease in their survival rate can reduce the long-term viability of adult populations (Martin *et al.*, 2011; Richardson *et al.*, 2021). Consequently, it is necessary to improve our understanding of the effects of stress responses to different biotic and abiotic parameters. To our knowledge, this is the first time that a study has assessed stress responses over the entire larval cycle. Hence, this study provides baseline information for future investigations assessing the effects of environmental stress on sea urchin larvae.

CHAPITRE 4

SYNTHESE GENERALE ET PERSPECTIVES

De nombreux polluants d'origine et de nature différentes peuvent pénétrer dans les écosystèmes marins en raison d'activités anthropiques (Amar, 2010 ; Häder *et al.*, 2020). Leur présence a des conséquences considérables sur les organismes marins et leurs écosystèmes, faisant de la pollution marine une préoccupation majeure (Abdel-Shafy & Mansour, 2016 ; Torres *et al.,* 2016). Parmi les principaux polluants, les éléments traces constituent un véritable enjeu écologique en raison de leur toxicité, de leur persistance et de leur capacité à s'accumuler dans les organismes marins, voire à se bioamplifier à travers la chaîne trophique (Rainbow & Luoma, 2011 ; Bonanno & Di Martino, 2017). Reconnu comme un bon bioindicateur par plusieurs chercheurs, l'oursin violet, *Paracentrotus lividus*, est un modèle pertinent pour l'étude des contaminations en éléments traces et a souvent été utilisé comme un outil pour évaluer la contamination des écosystèmes côtiers (*e.g.* Gharred *et al.*, 2015 ; Guendouzi *et al.*, 2017 ; Soualili *et al.*, 2008 ; Warnau *et al.,* 1998).

Quels sont les niveaux de contamination à l'échelle de la Méditerranée ?

L'étude de la contamination de l'environnement nécessite une connaissance approfondie de la distribution des polluants et de leurs concentrations dans les biotopes et les organismes (Lagadic *et al.*, 1998 ; Ramade, 2007). Dans cet objectif, les niveaux de contamination en éléments traces ont été évalués dans le deuxième chapitre. Les indices de pollution en éléments traces de plusieurs régions de la Méditerranée ont été calculés dans l'article 1 à partir des données disponibles dans la littérature (Ternengo *et al.,* 2018). Cela a permis de mettre en évidence **une contamination hétérogène au sein de la Méditerranée avec de hauts niveaux de contamination en Algérie et en Grèce**. Les niveaux de contamination en Algérie sont principalement dus au trafic maritime ainsi qu'aux rejets domestiques non traités (Soualili *et al.,* 2008 ; Rouane-Hacene *et al.,* 2017), tandis que celles en Grèce sont essentiellement dues aux activités industrielles et urbaines associées à un fond géochimique (Portocali *et al.,* 1996 ; Strogyloudi *et al.,* 2014). Deux sites en Corse ont également été répertoriés comme ayant de forts niveaux de contamination. Ces deniers sont situés à proximité d'Ajaccio (Corse du Sud), site proche d'une station d'épuration et supposé impacté, et Saint-Florent 2 (Haute-Corse), site proche d'une ancienne mine d'amiante (Tableau 1).

Tableau 1. Caractérisation des sites de prélèvements sur les 8 sites étudiés en Corse dans l'article 1 et 2.

Site de prélèvement	Source de contamination
Ajaccio 1	Site référence
Ajaccio 2	Station d'épuration
Bonifacio 1	Site référence
Bonifacio 2	Port
Calvi 1	Site référence
Calvi 2	Station d'épuration
Saint-Florent 1	Site référence
Saint- Florent 2	Ancienne mine d'amiante

La figure 1 permet de comparer nos résultats mesurés en Corse avec ceux obtenus dans d'autres zones géographiques de la Méditerranée et de l'Atlantique. **Dans l'ensemble, les teneurs en éléments traces en Corse semblent être similaires, voir inférieures, à celles des autres régions.** Ainsi, malgré quelques niveaux de contamination élevés, les concentrations restent peu importantes en raison des faibles pressions anthropiques dans la région (*e.g.* activité industrielle réduite, peu de zones fortement urbanisées ; Gobert *et al.,* 2017).

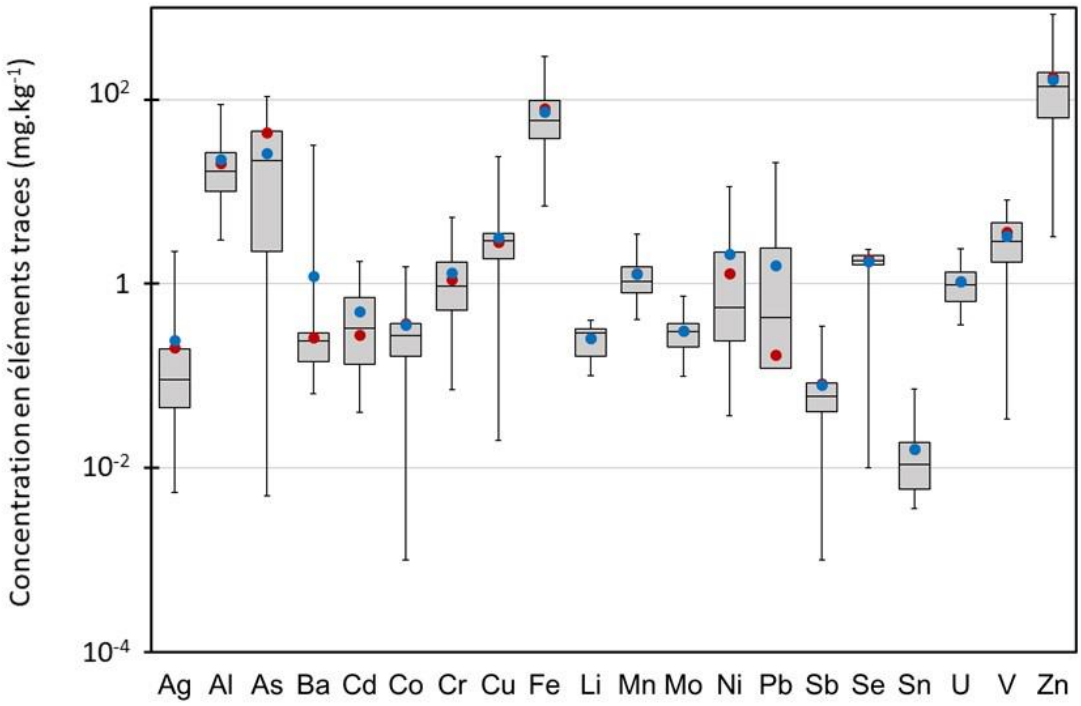

Figure 1. Concentrations en éléments traces dans les gonades d'oursin de *P. lividus* en Corse (points rouges) et dans les autres secteurs géographiques de la Méditerranée et de l'Atlantique (points bleus).

Cependant, **aucune étude sur *P. lividus* n'a analysé un nombre aussi important d'éléments traces dans la région méditerranéenne**, ce qui rend difficile les comparaisons avec les précédentes recherches (Annexe C). Ces résultats sont donc essentiels car ils pourront servir de référence dans le suivi des niveaux des contaminations.

Quelles sont les contaminations à l'échelle de la Corse ?

Les teneurs en éléments traces à l'échelle de la Corse ont été mesurées dans les gonades d'oursins dans le chapitre 2 et leurs variations spatiales ainsi que leurs sources de contamination ont été déterminées. Dans l'ensemble, **les niveaux élevés de contamination mesurés en Corse sont dus à des pressions localisées ou aux caractéristiques particulières du site étudié**. Ainsi, sur le site d'Ajaccio 2, le rejet de matière organique par la station d'épuration pourrait être la cause d'une bioaccumulation plus importante de certains éléments (Ozdemir *et al.*, 1998). En effet, les eaux usées urbaines et industrielles sont responsables de l'augmentation des apports de matière organique et ces effluents chargés de particules organiques constituent un support idéal pour les éléments métalliques (Egemen *et al.*, 1998). À Ajaccio 1, site « référence » relativement éloigné de tous types d'activités anthropiques, présente quant à lui un socle de gabbro-diorites indifférenciées dont les minéraux sont riches en Al (BRGM, 2018) expliquant la teneur élevée de cet élément sur ce site (Tableau 2). Ainsi, la distribution spatiale de cet élément résulterait d'une hétérogénéité naturelle plutôt que d'activités anthropiques (Luy *et al.*, 2012). De par la large utilisation de Sn dans les peintures antifouling comme le tributylétain sur les coques des bateaux (Luy *et al.,*2012), les teneurs plus élevées mesurées dans la région de Bonifacio sont probablement dues aux activités maritimes (Tableau 2).

Contre toute attente, des sites supposés impactés tels que Calvi 2 et Bonifacio 2 présentent des niveaux de contamination globalement faibles. Inversement, le site de Saint-Florent 1, supposé être un point de référence et classé Natura 2000, présente un niveau de contamination élevé (article 2, El Idrissi *et al.,* 2020). Les fortes teneurs en Ag à Saint-Florent 1 semblent être responsables de ce niveau de contamination et sont certainement dues à une ancienne concession minière riche en Ag située à proximité (Tableau 2 ; Gauthier, 2011). Il est,

par conséquent, nécessaire d'être prudent lors des processus de sélection des sites d'échantillonnages dans le cadre d'études écotoxicologiques.

Tableau 2. TESVI des 20 éléments traces mesurés à chaque saison dans les gonades de *P. lividus* dans les huit sites corses (données de l'article 2, El Idrissi *et al.,* 2020).

Eléments trace	(x_{max} / x_{min})	$\sum(x_{max} / x_i) / n \pm SD$	TESVI	Station
Ag	62,95	16,10 ± 20,01	78,24	Saint-Florent 2
Al	6,31	2,74 ± 1,79	4,11	Albo 1
As	2,81	1,82 ± 0,65	1,02	Saint-Florent 1
Ba	2,93	2,00 ± 0,59	0,86	Albo 2
Cd	4,33	2,38 ± 1,37	2,50	Saint-Florent 1
Co	5,87	4,01 ± 1,58	2,31	Saint-Florent 2
Cr	7,14	4,60 ± 2,09	3,23	Saint-Florent 2
Cu	1,36	1,21 ± 0,13	0,14	Bonifacio 2
Fe	4,23	2,75 ± 0,98	1,50	Saint-Florent 2
Li	1,40	1,21 ± 0,11	0,13	Calvi 2
Mn	2,13	1,68 ± 0,36	0,46	Saint-Florent 2
Mo	1,91	1,43 ± 0,35	0,47	Calvi 1
Ni	11,61	8,16 ± 3,60	5,12	Saint-Florent 2
Pb	2,43	1,63 ± 0,55	0,82	Albo 2
Sb	2,74	1,81 ± 0,61	0,92	Bonifacio 1
Se	1,38	1,14 ± 0,13	0,15	Saint-Florent 2
Sn	6,16	3,34 ± 1,84	3,40	Bonifacio 1
U	2,73	1,93 ± 0,70	0,99	Saint-Florent 1
V	2,59	1,94 ± 0,61	0,81	Saint-Florent 1
Zn	2,02	1,51 ± 0,39	0,52	Calvi 2

Nous avons pu également constater dans l'article 2 que la **contamination du site proche de l'ancienne mine d'amiante (Saint-Florent 2) est relativement élevée quelle que soit la saison étudiée** (El Idrissi *et al.,* 2020). Les **fortes teneurs en Co, Cr, Fe et Ni** mesurées sur le site sont **dues aux rebuts et stériles miniers** directement rejetés en mer par l'usine de traitement de la mine de 1948 à 1965 (BRGM, 1997 ; Cary *et al.,* 2013). Malgré la fermeture de la mine depuis plus de 50 ans, le lessivage des résidus miniers, encore présents sur les flancs de l'ancienne mine d'amiante, et du socle géologique, constituée de serpentine naturellement riche en éléments traces, contribue toujours à la dispersion de ces derniers le long du littoral (Morrison *et al.,* 2009 ; Siebecker, 2010 ; Tashakor *et al.,* 2011). Les sols serpentins sont considérés comme une source de pollution géogénique par de nombreux chercheurs de par les effets délétères des éléments traces lorsqu'ils deviennent biodisponibles à de fortes concentrations (Caillaud *et al.,* 2009 ; Tashakor *et al.,* 2011). Cela souligne la nécessité d'avoir une bonne

connaissance du contexte géochimique naturel lors de l'évaluation de la qualité des écosystèmes afin de distinguer les éléments traces naturellement présents dans l'environnement de ceux résultant d'activités anthropiques.

Dans le cadre de l'article 3, **le recours au TEPI a permis d'identifier** les différents niveaux de contamination dans la zone adjacente de l'ancienne mine, révélant **un gradient de contamination avec des niveaux de contaminations plus élevés au sud** (El Idrissi *et al., soumis*). Cette situation est due à la dispersion des débris miniers vers le sud sous **l'effet de la houle et des courants marins dominants** et à leur accumulation progressive entraînant la création et l'engraissement des plages (BRGM, 1997 ; Cary *et al.*, 2013). Cette observation met en évidence le rôle important que joue la dynamique du milieu marin dans la diffusion et la distribution des contaminants dans le milieu. En conséquence, les éléments traces peuvent être dispersés à plusieurs kilomètres des sites d'origine rendant le phénomène difficile à contrôler (Bell *et al.*, 2001 ; Schwartz *et al.*, 2001 ; Passariello *et al.*, 2002). L'utilisation d'indices, tels que TEPI et TESVI, dans le chapitre 2 a permis une comparaison fiable de la contamination par les éléments traces entre différents sites, d'une échelle spatiale locale à internationale.

Les concentrations en éléments traces dans les gonades d'oursin violet présentent-elles des variations temporelles ?

L'article 2 a permis d'étendre nos connaissances sur la variabilité des concentrations en éléments traces dans les gonades d'oursins en mettant l'accent sur les fluctuations saisonnières (El Idrissi *et al.,* 2020). Une **importante variabilité des concentrations** a été constatée selon la saison à laquelle les prélèvements d'oursins ont été effectués. Ainsi, lorsque le TEPI est évalué à l'échelle de la Corse dans l'article 1, Ajaccio 2, Calvi 2 et Saint-Florent 2 présentent des niveaux élevés de contamination en hiver tandis que lorsque l'on considère les concentrations annuelles mesurées dans l'article 2, les deux sites de Saint-Florent sont les plus contaminés (El Idrissi *et al.,* 2018, 2020). Dans l'ensemble, **les concentrations en éléments traces sont plus élevées en automne et en hiver et, à l'inverse, plus faibles lors de la saison estivale**. Les fluctuations temporelles des concentrations en éléments traces dans les gonades

sont **étroitement liées aux variations physiologiques de l'oursin**. En supposant que les éléments traces s'accumulent principalement dans les cellules somatiques et non dans les cellules germinales (Sellem & Guillou, 2007), **une dilution se produit au cours de la gamétogenèse** (Guendouzi *et al.*, 2017). Par conséquent, il est recommandé de considérer ce paramètre lors des comparaisons des niveaux de contamination et d'éviter l'utilisation des gonades pendant la période de frai afin de limiter les biais dus à l'asynchronie des pontes. Malheureusement, peu d'études prennent en compte les variations saisonnières liées à la physiologie de l'oursin (Annexe C). Une étude histologique en parallèle de l'analyse des éléments traces est souhaitable afin de comparer et d'évaluer au mieux les contaminations en considérant le stade physiologique des oursins. Des études supplémentaires seront indispensables pour comprendre les mécanismes impliqués dans ce phénomène de dilution pour une meilleure utilisation des gonades dans l'évaluation des contaminations.

Par ailleurs, comme les concentrations en éléments traces des espèces macrophytes varient également en fonction de la saison, l'alimentation peut aussi être impliquée dans les variations temporelles mesurées chez les oursins. Enfin, les conditions environnementales telles que la température, le pH, la teneur en oxygène et la salinité font varier la biodisponibilité et entrent également en jeu dans les variations saisonnières. Il est donc nécessaire de tenir compte à la fois des facteurs biotiques (stade de reproduction) et abiotiques (caractéristiques physiques et chimiques de l'environnement) dans l'utilisation des oursins comme bioindicateur.

L'oursin violet est-il un bon bioindicateur des contaminations en éléments traces ?

En raison de leur rôle dans diverses fonctions physiologiques, **les éléments traces essentiels** tels que le Cu, le Fe, le Mn ou le Zn, **vont avoir tendance à être davantage bioaccumulés** par *P. lividus* **à l'inverse des éléments traces dit non essentiels** tels que le Pb, le Sb ou encore le Sn (*e.g.* Storelli *et al.,* 2001 ; Guendouzi *et al.,* 2017). Le Zn fait partie des éléments traces essentiels pour la gamétogenèse, ce qui explique les fortes teneurs retrouvées dans les gonades (Unuma *et al.*, 2007). L'ovogénèse nécessitant de plus grandes

quantités en Zn que la spermatogénèse (Unuma *et al.,* 2003, 2007), sa concentration est plus élevée chez les femelles. En raison des **différences naturelles de concentration de certains éléments traces entre les mâles et les femelles**, il est également important de prendre en compte le sexe des individus.

Dans l'article 3, nous avons pu observer que la bioaccumulation des éléments traces se déroule de la manière suivante : **macroalgues > tubes digestifs > gonades** (El Idrissi *et al.*, soumis). Ainsi, les **macroalgues ont une meilleure capacité à accumuler les éléments traces** que les oursins comme en témoignent les facteurs de bioaccumulation calculés dans l'étude. L'utilisation des macroalgues peut présenter un intérêt lorsqu'on souhaite savoir si un contaminant est présent sur le site étudié. Toutefois, il est nécessaire de prendre des précautions lors de l'interprétation des niveaux de contamination au risque de les surestimer. Richir (2013) recommande de sélectionner soigneusement les stations d'échantillonnage lors de l'utilisation de *Posidonia oceanica* comme bioindicateur unique afin de ne pas surévaluer l'état de contamination de la zone étudiée.

Nous avons également constaté une compartimentation bien définie des concentrations en éléments traces chez les oursins avec des **teneurs plus élevées dans le tube digestif**. Ainsi, l'utilisation du tube digestif semble être une solution plus intéressante que les gonades dans l'évaluation des variations de la contamination en éléments traces. En effet, les concentrations plus élevées dans ce compartiment demandent moins de matière à analyser. Par ailleurs, contrairement aux gonades, la plupart des éléments traces ne varient pas selon le sexe (article 3, El Idrissi *et al.,* soumis). Toutefois, des recherches doivent être menées afin de déterminer si le stade de reproduction de l'oursin et la source de nourriture ont des répercussions sur la variabilité des concentrations dans le tube digestif.

L'évaluation des teneurs en éléments traces dans les oursins a clairement confirmé le rôle de *P. lividus* comme bioindicateur présentant différents niveaux de contamination y compris lorsque la zone d'étude est restreinte, comme décrit dans l'article 3 (El Idrissi *et al.*, soumis). De plus, suite aux mesures de plus de 20 éléments traces dans le chapitre 2, l'utilisation de l'oursin en tant que bioindicateur, déjà reconnu dans le suivi des contaminations côtières d'un

grand nombre d'éléments classiquement étudiés, peut être étendue aux éléments préoccupants émergents.

Une fois qu'un organisme est considéré comme un outil potentiel pour la surveillance de la pollution côtière, il est intéressant d'étudier expérimentalement comment ce bioindicateur répondra aux changements environnementaux. Cependant, les informations actuelles sur les oursins sont principalement limitées aux niveaux de contamination dans l'environnement, et les études portant sur les cinétiques de contamination et de dépuration sont rares (*e.g.* Strogyloudi *et al.*, 2014 ; Guendouzi *et al.*, 2017). Dans cet objectif, une expérimentation de mise en cage d'oursins dans des sites contaminés et références permettrait d'étudier les processus d'accumulation et leurs effets sur les populations d'oursins, mais aussi de mieux comprendre les mécanismes de dépuration et la durée de celle-ci. Cette étude a été réalisée en parallèle des travaux de cette thèse et les résultats feront l'objet d'un article scientifique à venir.

Outre sa capacité à bioaccumuler, l'utilité de l'oursin dans l'évaluation des effets des contaminations en éléments traces à des concentrations environnementales a également été démontré, notamment grâce à l'utilisation de mesures biométriques. On peut conclure que chaque bioindicateur a ses avantages et ses inconvénients et qu'il est judicieux de faire un choix en fonction de l'étude à réaliser. Cependant, lorsque cela est possible, il est avantageux d'utiliser plusieurs bioindicateurs tels que les oursins, les moules et les macrophytes afin de comparer les niveaux de contamination et d'avoir une meilleure vue d'ensemble.

Quels sont les effets des mélanges en éléments traces sur les individus adultes de *P. lividus* ?

Des niveaux de contamination plus élevées dans certaines régions de la Corse ont été mesurés dans le chapitre 2, notamment à proximité de l'ancienne mine d'amiante de Canari, en haute Corse. Les faibles niveaux d'activité anthropique dans cette zone et les sources locales de contamination associées à l'ancienne mine offrent un mésocosme unique pour étudier la contamination dans le milieu marin (ASR, 2011).

Plusieurs études suggèrent que l'exposition à la contamination par des éléments traces peut induire une cascade d'événements tels que la production d'espèces réactives de l'oxygène (Farombi *et coll*., 2007 ; Nieto *et al.,* 2010). Des teneurs élevées en peroxyde d'hydrogène, associées à des activités enzymatiques importantes de la CAT, de la GPX et de la GST ont été rapportées dans la partie sud de l'ancienne mine d'amiante où les concentrations en éléments traces sont plus élevées (article 3, El Idrissi *et al.*, soumis). **L'augmentation de l'activité spécifique de ces enzymes suggère une activation des processus de détoxification, reflétant probablement un stress élevé dû aux contaminations en éléments traces** (Louiz & coll, 2016). Toutefois, **les faibles teneurs de MDA semblent suggérer que le stress oxydatif induit** par la contamination **n'affecte pas la santé des individus** adultes de *P. lividus*. Il conviendrait de mener une étude complémentaire afin d'évaluer si ces contaminations ont un effet sur d'autres paramètres tels que la reproduction des oursins.

Ce travail montre que les effets de l'ancienne mine d'amiante sont toujours présents. Il est donc indispensable de stabiliser le milieu et de maintenir une surveillance de l'environnement marin. Depuis plus de vingt ans, des travaux d'urgence ont été conduits sur ce site afin d'empêcher des éboulements et des glissements de terrain provoqués par les intempéries (ADEME, 2014). Ces derniers se sont toutefois heurtés à des contraintes inhabituelles telles que la recherche d'entreprises agréées, des règles de protection drastiques, la réduction du temps de travail afin d'éviter un contact continu avec l'amiante ou encore un arrosage régulier du site pour empêcher les poussières de s'envoler (BRGM, 1999). Au total, l'État a financé quelque 22 millions d'euros de travaux de mise en sécurité. Actuellement, un vaste projet de réhabilitation de l'ancienne mine d'amiante est en cours. Une étude des effets de ces travaux sur les écosystèmes marins environnants serait pertinente à moyen terme, dus au risque accru de contamination lié au chantier, et à long terme afin de suivre les effets bénéfiques potentiels.

Quels sont les effets des mélanges en éléments traces sur les stades embryolarvaires *P. lividus* ?

Les effets des contaminations chroniques sur le cycle larvaire de *P. lividus* reste largement méconnue et, à notre connaissance, aucune étude n'a porté sur la totalité du cycle larvaire ou sur les effets d'un mélange de plus de trois éléments traces. D'après les expérimentations du chapitre 3, il apparaît que **l'augmentation du nombre et de la concentration des éléments traces provoque un allongement des bras et un retard du développement larvaire qui s'accentue avec le temps** (article 4, El Idrissi *et al.,* 2022a). Les larves utilisent les bandes ciliaires le long de leurs bras pour se nourrir, ainsi la capture de nourriture est directement liée à la longueur du bras (Strathmann *et al.*, 1992 ; Strathmann & Grunbaum, 2006). Ainsi, les larves de nombreux oursins développent des bras plus longs dans des conditions de faible alimentation afin de faciliter la capture de nourriture rare et des bras plus courts dans des conditions d'abondance de nourriture pour allouer plus de ressources au développement et réduire le temps de métamorphose (Pedrotti & Fenaux, 1992 ; Strathmann *et al.*, 1992 ; Soars *et al.*, 2009. Kalachev *et al.*, 2018). Lors des expérimentations réalisées dans le cadre de cette thèse, toutes les larves ont été nourries *ad libitum*. Aussi, bien qu'aucun signe de malformation ne soit visible au stéréomicroscope selon le critère de Carballeira *et al.* (2012), le mélange de contaminant pourrait provoquer des effets sur les cils comme une diminution de ces derniers. La taille des bras des larves permettrait ainsi de compenser cette altération. Une étude au microscope électronique serait nécessaire afin de vérifier cette hypothèse.

Il a également été mis en évidence des **larves plus grandes lorsqu'elles sont en contact de nombreux éléments traces essentiels à des contaminations environnementales** (article 4, El Idrissi *et al.*, 2022a). Ces derniers présentent, en effet, un intérêt nutritionnel et jouent un rôle dans les systèmes enzymatiques (Chiarelli & Roccheri, 2014). **Le mélange ainsi que les concentrations étudiées ne semblent pas avoir d'effet sur le stress oxydatif chez les larves de *P. lividus*** (article 5, El Idrissi *et al.,* 2022b). De plus, l'activité spécifique des enzymes mesurée dans les différents stades embryo-larvaires suggère **la capacité des larves à répondre au stress oxydatif** (article 5, El Idrissi *et al.,* 2022b). À notre connaissance, c'est la

première fois qu'une étude évalue les réponses au stress sur l'ensemble du cycle larvaire. Par conséquent, cette étude fournit des informations de base pour de futures études évaluant les effets du stress environnemental sur les larves d'oursins.

Au vu des résultats, les mesures biométriques semblent plus adaptées et sensibles dans l'évaluation des effets lorsque les concentrations sont faibles. Il est nécessaire de considérer ces résultats avec prudence, car malgré l'analyse d'un grand nombre d'éléments traces, de nombreux autres contaminants ne sont pas pris en compte. De plus, plusieurs études ont souligné que l'exposition aux produits chimiques entraîne une augmentation de la sensibilité au changement climatique des organismes marins. Par conséquent, dans le cadre des scénarios actuels de réchauffement, les effets induits par les éléments traces peuvent être importants avec des impacts potentiels sur les stocks de population, qui peuvent entraîner une perte de biodiversité et des impacts socioéconomiques. Il est donc essentiel de comprendre comment les facteurs climatiques et anthropiques influencent les écosystèmes côtiers afin de garantir leur préservation et leur durabilité.

La contamination des géniteurs a-t-elle une influence sur le développement larvaire dans les zones contaminées ?

Plusieurs auteurs ont signalé l'importance de tenir compte de la qualité des géniteurs dans les études écotoxicologiques (*e.g.* Bougis & Corré, 1979 ; Pétinay *et al.*, 2009). Dans le chapitre 3, nous avons mis en évidence **des différences significatives de développement et de taille entre les larves obtenues par les géniteurs d'Albo et celles produites par les géniteurs de Calvi**. **Une activité spécifique plus élevée de la CAT, de la GST et de la SOD chez les larves issues d'oursin de Calvi** a également été constaté. Parmi les différentes hypothèses émises, la qualité probablement inférieure des gamètes de Calvi pourrait expliquer un développement plus lent et les larves plus petites. Le site de prélèvement ayant de faibles niveaux de contamination (chapitre 2), la supposition la plus vraisemblable semble être que Calvi soit exposé à d'autres sources de pression que les éléments traces. Les résultats obtenus dans le cadre de l'article 3 semblent confirmer cette hypothèse (El Idrissi *et al.*, soumis). En effet, **les activités spécifiques élevées des enzymes de stress ainsi que la forte teneur en**

H_2O_2 mesurées chez les oursins adultes des sites contaminés ont également été observées dans le site de référence de Calvi. Il serait donc pertinent d'entreprendre une étude approfondie dans cette zone d'étude afin de définir les sources de stress possibles.
Ces travaux témoignent de la vigilance dont il faut faire preuve dans la sélection des sites dits de référence et démontrent l'importance des géniteurs dans les études écotoxicologiques. Ainsi, pour évaluer l'impact des contaminants à l'échelle locale, il est plus pertinent de réaliser des bioessais sur des organismes provenant du site d'étude afin d'avoir une réponse fiable et une meilleure visibilité des effets de ces contaminants.

En conclusion, de par l'étude des variations spatio-temporelles des niveaux de contaminations, des analyses biochimiques et des expérimentations sur les stades larvaires, le présent travail de thèse a fourni un ensemble de données utiles pour une meilleure utilisation des oursins en tant que bioindicateur.

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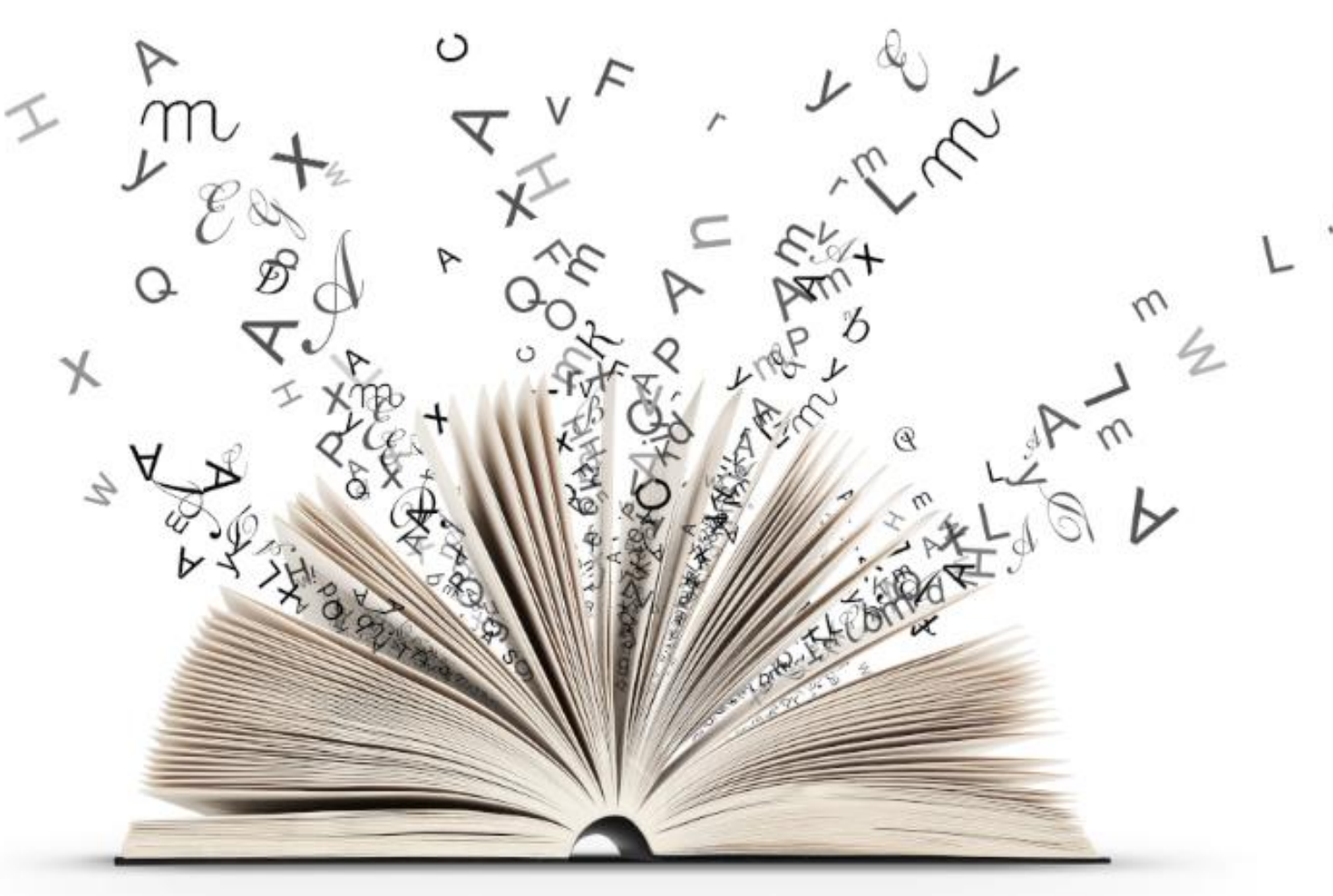

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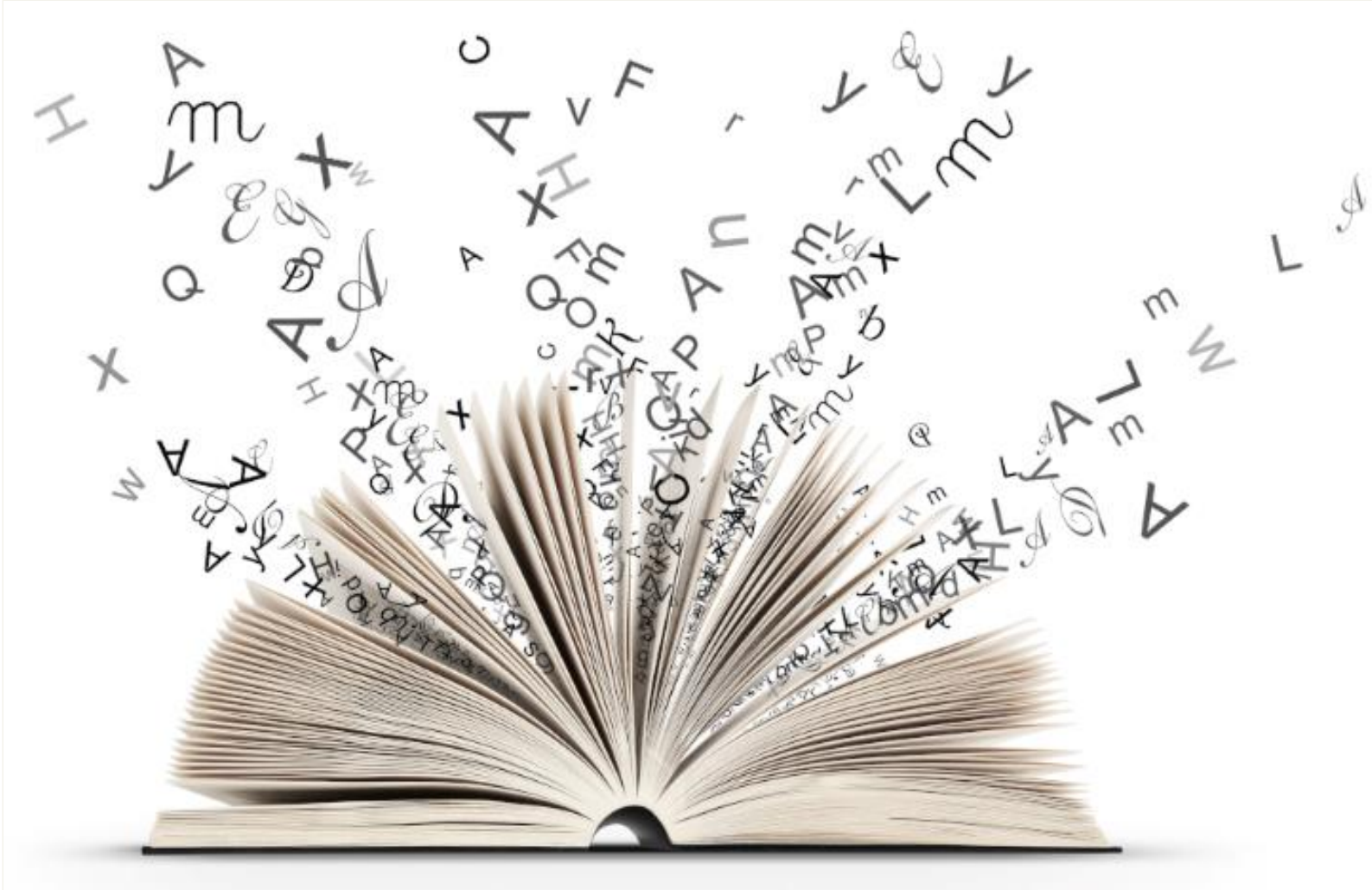
Annexes



Appendices

Annexe A - Appendix A. Information about trace elements used during contamination.

Ag	$AgNO_3$
Al	$Al(NO_3)_3$
As	H_3AsO_4
Cd	$Cd(NO_3)_2$
Co	$Co(NO_3)_2$
Cr	$Cr(NO_3)_3$
Cu	$Cu(NO_3)_2$
Fe	$Fe(NO_3)_3$
Hg	$Hg(NO_3)_2$
Mn	$Mn(NO_3)_2$
Mo	$(NH_4)_6Mo_7O_{24}$
Ni	$Ni(NO_3)_2$
Pb	$Pb(NO_3)_2$
V	NH_4VO_3
Zn	$Zn(NO_3)_2$

Annexe B - Appendix B. Non-exhaustive compilation of surface seawater trace element concentrations (µg L^{-1};) available in the literature. (**a**) World mean seawater composition. (**b**) Trace element concentrations in locations sampled inside the Mediterranean (modified from Richir *et al.*, 2013). Mean concentrations are calculated without values considered as high (in bold). *The associated references are listed below the table.

(a)

Geographic site	Ag	Al	As	Cd	Co	Cr	Cu	Fe	Hg	Mn	Mo	Ni	Pb	V	Zn	References*
Northern French coastal waters (France)														2.02		Abbasse *et al.*, 2002
Tokyo Bay (Japan)		**6.5**			0.14	0.07	0.31	**14.4**		**36.1**		1.5	**1.7**		2	Akagi *et al.*, 1985
Hiroshima Bay (Japan)		**9.6**			0.14	0.09	0.19	1.27		1.06		0.18			0.8	
Sagami Bay (Japan)		0.95				0.07	0.067	0.66		1.05					0.12	
Seawater mean composition			1.7													Andreae, 1979; Andreae & Andreae, 1989; Li, 1991
Seawater mean composition	0.0023	0.06	1.78	0.0698	0.0012	0.215	0.197	0.029		0.02	10.4	0.486	0.002	1.74	0.338	Bruland & Lohan, 2003
N. Pacific Surface Water															0.015	Bruland *et al.*, 1994
N. Pacific Deep Water															0.523	
North Sea, Offshore									0.34							Coquery & Cossa, 1995
North Sea, Nearshore									0.72							
Lapdev Sea, N. Russia: min									0.8							
Lapdev Sea, N. Russia: max									**2.7**							
Kara Sea, N. Russia: min									0.14							
Kara Sea, N. Russia: min									**3.4**							
Northeast Pacific Ocean											10.3					Collier, 1985
Chao Phraya estuary (Gulf of Thailand)											11.9					Dalai *et al.*, 2005
German Bight offshore (North Sea)										0.66	9.9					Dellwig *et al.*, 2007
NE Atlantic All Depths: min															0.011	Ellwood *et al.*, 2000
NE Atlantic All Depths: max															0.13	
Seawater mean composition	0.0399	0.51	1.5	0.01	0.0501	0.302	0.102	2.01		0.2	9.6	0.2	0.003	2.5	0.098	Fischer & Wartel, 1992
Dogger Bank, North Sea: min				0.015					0.19							Fileman *et al.*, 1991
Dogger Bank, North Sea: max				0.025					0.42							
Pacific Ocean				0.067												Frew & Hunter, 1995

Nort Atlantic Ocean: min									0.1							Guentzel *et al.*, 1996
Nort Atlantic Ocean: max									0.5							
N. Atlantic Surface Water: min													0.002			Helmers, 1996
N. Atlantic Surface Water: max													0.029			
S. Atlantic Surface Water													0.003			
North Sea: min				0.006			0.089									Hydes & Kremling, 1993
North Sea: max				0.025			0.43									
Irish Sea: min				0.013			0.22									Laslett, 1995
Irish Sea: max				0.081			0.43									
North Sea: min				0.01			0.2								0.11	
North Sea: max				0.051			0.83								2.2	
British coastal waters: min				0.004											0.22	Law *et al.*, 1994
British coastal waters: max				0.081											2.1	
NE Atlantic Surface Water: min							0.051									Le Gall *et al.*, 1999
NE Atlantic Surface Water: max							0.095									
Seawater mean composition	0.0026	0.31	1.76	0.0818	0.0012	0.261	0.217	0.259	**0.00042**	0.07	10.4	0.549	0.003	2.23	0.331	Li, 1991
East China Sea: min													0.041			Lin *et al.*, 2000
East China Sea: max													0.517			
Antarctic: min															0.085	Löscher, 1999
Antarctic: max															0.71	
North Atlantic Surface Water				0.31												Mason *et al.*, 1995
British coastal waters: min				0.002												McManus & Prandle, 1996
British coastal waters: max				**1.38**												
North USA Atlantic inshore waters		0.22														Measures *et al.*, 1984
Gulf Stream (North Carolina, USA)		0.42														
Sargasso Sea		1.12														
Carribean Sea		1.12														
Panama Bassin		**2.1**														
Atlantic Ocean			1.2											1.66		Middelburg *et al.*, 1988
Huelva Estuary (Spain)			**13**	**2.8**			**76**		0.1	**141**		**5.9**			**309**	Morillo & Usero, 2008

Northeast Atlantic Ocean											10.7			1.19		Morris, 1975
NE Atlantic Surface Water: min							0.057									Muller *et al.*, 1994
NE Atlantic Surface Water: max							0.35									
Open-ocean surface waters: min						0.1										Neff, 2002
Open-ocean surface waters: max						0.55										
Scotia/ Weddell Sea, Antarctica: min				0.019			0.094								0.09	Nolting & de Baar, 1994
Scotia/ Weddell Sea, Antarctica: max				0.107			0.41								0.91	
S. China Sea: min				0.003												Pai & Chen, 1994
S. China Sea: max				0.118												
Philippine Sea: min				0.003												
Philippine Sea: max				0.119												
Eastern Atlantic Ocean: min				0.001											0.006	Pohl *et al.*, 1993
Eastern Atlantic Ocean: max				0.018											0.118	
Greenland Sea: min				0.007									0.004		0.033	
Greenland Sea: max				0.028									0.104		0.36	
Pacific Ocean	0.0002	0.11	1.55	**0.0003**	0.007	0.277	0.035	0.042		0.14	10.4	0.151	0.014		0.007	Quinby-Hunt & Wilde, 1986-87
Atlantic Ocean		0.81		**0.0002**			0.072	0.098	0.25	0.14	10.4	0.13	0.551	1.24	0.003	
Indian Ocean: min				0.083												Saager *et al.*, 1993
Indian Ocean: max				0.113												
Shibukawa Sea (Okayama, Japan)											9.4					Sabarudin *et al.*, 2007
North East Atlantic Ocean							0.254					0.478		2.27		Santos-Echeandia *et al.*, 2008
Nagoya port (Japan)		**5.6**	0.71		0.33	0.25	1	4		**54**		**11**	0.088		5.7	Sawatari *et al.*, 1995
Ross Sea, Antarctica: min													0.005			Scarponi *et al.*, 1997
Ross Sea, Antarctica: max													0.027			
Louisiane Shelf (USA)														0.99		Shiller & Mao, 1999
S. North Sea Surface Water: min													0.008			Statham *et al.*, 1999
S. North Sea Surface Water: max													0.2			
S. North Sea Bottom Water: min													0.017			
S. North Sea Bottom Water: max													0.087			
Northwestern Gluf of Mexico: min				0.001												Trefry *et al.*, 1995

	Northwestern Gluf of Mexico: max				0.02													
	Seawater mean composition	0.28	1	2.6	0.11	0.39	0.2	0.9	3.4		0.4	10	**6.6**	0.03	1.9	5		Turekian, 1968
	Tianjin (China)	0.0324			0.1275	0.0271		**2.758**			0.01			0.059				Wen *et al.*, 1999
	Dalian (China)	0.0189			0.0472	0.0777		1.152			0.01			0.057				
	Qingdao (China)	0.0543			0.0345	0.081		0.276			0.05			0.081				
	Qinghuangdao (China)	0.0203			0.07	0.0522		0.132			0.07			0.077				
	South Atlantic Ocean: min				0.028													Yeats *et al.*, 1995
	South Atlantic Ocean: max				0.084													
	North Atlantic				0.0031			0.073	0.056				0.17	0.019		0.141		Yoon *et al.*, 1999
	20 sites in Bohai Bay (China): min				0.107											**15.2**		Zhang *et al.*, 2010
	20 sites in Bohai Bay (China): max				0.182											**24.3**		
(b)	Favigna Island (Siciliy, Italy)				0.12		0.09	0.63						0.57		3.1		Campanella *et al.*, 2001
	Northwestern Mediterranean Sea		1.65															Chou & Wollast, 1997
	Tyrhennian Sea: min															0.54		Ciceri *et al.*, 1992
	Tyrhennian Sea: max															6.7		
	Ustica Island (Italy)				0.2		0.21	1.21						0.97		**13.35**		Conti *et al.*, 2007
	Linosa Island (Italy)				0.33		0.16	**2.34**						0.97		**13**		Conti *et al.*, 2010
	Mediterranean Sea: min									0.16								Cossa *et al.*, 1997
	Mediterranean Sea: max									1.28								
	Mersin Bay (Turkey)				0.37			**1.94**	4.5				**4.8**	1.2		2.8		Elçi *et al.*, 1997
	Inner Saronikog Gulf (Greece)	0.3																Grimanis *et al.*, 1994
	Open Saronikos Gulf (Greece)	0.27																
	Aegean Sea	0.18																
	Calvi (Corsica, France)	0.01			0.008	0.006	0.048	0.094					0.37	0.047				Kantin *et al.*, 2015
	Canari (Corsica, France)				0.029		0.133	0.172					1.330	0.0595				
	Canari (Corsica, France)				0.016	0.017	0.152						1.38	0.048				Lafabrie *et al.*, 2007
	Livorno (Toscana, Italy)				0.006	0.008	0.616						0.197	0.038				
	Porto-Tores (Sardinia, Italy)				0.009	0.016	0.282						0.378	0.075				
	Algeciras Bay (Spain)			1.7	0.02			0.5		0.1	**2.1**		0.6			9.8		Morillo & Usero, 2008
	Mediterranean Sea: min				**0.0002**			0.095										Saager *et al.*, 1993

Mediterranean Sea: max				**11**			0.2									
4 sites in Sardinia (Italy): min.				0.001			0.005					0.033	0.004			Schintu *et al.*, 2008
4 sites in Sardinia (Italy): max.				0.035			0.08					0.12	0.147			
Gulf of Elefsis (Greece)															18.3	Scoullos, 1981
Tyro Basin (Cretan Sea)			1.52								15.5			1.78		Van der Weijden *et al.*, 1990
Bannock Basin (Cretan Sea)			1.5								14.8			1.7		
Western Strait 2004 (Aegean Sea, Greece)				0.022	0.019	0.063	0.194			0.42		0.327	0.49			Voutsinou-Taliadouri *et al.*, 1997
Cretan Sea 2004 (Aegean Sea, Greece)				0.0182	0.0122	0.059	0.171			0.31		0.289	0.416			
Eastern Strait 2004 (Aegean Sea, Turkey)				0.0232	0.0162	0.045	0.306			0.43		0.268	0.408			
Gulf of Cadiz				0.0119			0.129	0.098				0.199	0.028		0.666	Yoon *et al.*, 1999
Alboran Sea				0.0037			0.084	0.144				0.178	0.015		0.188	
Western Mediterranean Bassin				0.0096			0.104	0.077				0.187	0.026		0.171	
Sicilian Strait				0.008			0.101	0.088				0.188	0.025		0.159	
Number of data	12	12	11	57	18	20	41	15	13	16	13	22	41	12	36	**Number of data**
World ocean seawater average concentration	0.1	0.7	1.6	0.1	0.1	0.2	0.3	1.1	0.4	0.3	11.1	0.4	0.2	1.8	1.3	**World ocean seawater average concentration**
Canari (Corsica, France) seawater average concentration)				0.02	0.02	0.15						1.38				**Canari (Corsica, France) seawater average concentration)**
	Ag	**Al**	**As**	**Cd**	**Co**	**Cr**	**Cu**	**Fe**	**Hg**	**Mn**	**Mo**	**Ni**	**Pb**	**V**	**Zn**	

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Annexe C - Appendix C. Concentrations en éléments traces (mg kg^{-1};) mesurées dans les gonades de l'oursin *Paracentrotus lividus* au sein de la région méditerranéenne. Pour les saisons, Pr: Printemps, Et: Eté, Au: Automne, Hi : Hiver.

Pays	Site	Sexe	Saisons	Ag	Al	As	Ba	Cd	Co	Cr	Cu	Fe	Hg	Li	Mn	Mo	Ni	Pb	Sb	Se	Sn	Sr	U	V	Zn	Références
Algérie	Sidi Lakhdar	F	Au					0.0057			0.0717	1.890						0.193							0.136	Boukhelf *et al.*, 2019
		M						0.005			0.068	1.837						0.198							0.345	
	Salamandre	F						0.006			0.0763	1.837						0.194							0.165	
		M						0.007			0.065	1.801						0.194							0.398	
	Sidi Lakhdar	F	Hi					0.0045			0.06	2.005						0.205							0.441	
		M						0.0043			0.154	2.37						0.205							0.215	
	Salamandre	F						0.0045			0.054	1.947						0.053							0.69	
		M						0.005			0.061	2.007						0.198							0.178	
	Sidi Lakhdar	F	Pr					0.005			0.078	2.114						0.193							5.665	
		M						0.005			0.064	1.98						0.194							0.581	
	Salamandre	F						0.006			0.057	2.018						0.193							4.231	
		M						0.007			0.058	1.87						0.193							2.225	
	Sidi Lakhdar	F	Et					0.005			0.066	1.917						0.192							2.398	
		M						0.005			0.061	2.167						0.198							0.725	
	Salamandre	F						0.006			0.09	1.893						0.198							0.565	
		M						0.005			0.224	1.877						0.192							0.138	

Sidi Mejdoub	n.a.	Au		1.670	3.560		2.850	3.230	Guendouzi *et al.*, 2017
Abdelmalek Ramadan				1.550	3.430		4.510	120.330	
Bateau Cassé				1.000	3.450		5.130	155.720	
Sidi Mejdoub		Hi		1.430	6.280		2.680	214.210	
Abdelmalek Ramadan				1.430	3.570		2.410	167.100	
Bateau Cassé				0.900	3.470		5.170	271.390	
Sidi Mejdoub		Pr		1.350	3.470		3.070	158.300	
Abdelmalek Ramadan				1.460	3.480		3.000	94.270	
Bateau Cassé				0.940	3.050		2.790	97.380	
Sidi Mejdoub		Et		1.450	3.310		4.890	139.840	
Abdelmalek Ramadan				1.750	0.500		2.700	244.580	
Bateau Cassé				1.040	3.230		5.730	424.630	
Oran Harbor		Annual		0.860	8.820		6.080	114.930	Rouhane-Hacene *et al.*, 2017
Ain Defla				0.680	3.180		4.850	66.230	
Hadjaj				0.600	2.450		3.590	56.220	
Algiers Beach	F	Pr		0.140	2.840	73.800	6.140	385.500	Soualili *et al.*, 2008
Algiers Beach	M			0.080	3.190	19.300	7.780	32.900	
Tamentfoust	F			0.120	2.490	113.000	1.500	538.200	
Tamentfoust	M			0.050	3.880	112.600	0.880	76.100	
Sidi-Fredj	F			0.140	3.420	71.100	0.680	366.900	

	Sidi-Fredj	M						0.050		4.420	92.700						0.900									52.900	
Espagne	Espagne	n.a.				1.155		0.100	0.172	0.501		11.203				0.085	0.064							0.034			Salvo *et al.*, 2015
France	La Couronne	n.a.	Hi							6.900		0.080														403.000	Augier *et al.*, 1994
	L'Auguette									5.500		1.370														795.000	
	Darse Sud									4.000		0.080														275.000	
	Darse 1									5.800		0.150														293.000	
	Anse de Carteau									3.500		0.090														283.000	
	France		n.a.	1.570	88.400	0.005	31.800		0.001	0.446	81.200		0.227	1.060	0.159	0.287	0.014	0.001	0.650		21.200		1.120	7.660		Guérin *et al.*, 2011
								0.095				0.040					0.065										Noel *et al.*, 2011
						5.033		0.315	0.375	0.411		3.082				0.113	0.011						2.101				Salvo *et al.*, 2015
	Calvi		Pr					0.150	0.900	3.470	51.000						2.250									124.000	Warnau *et al.*, 1998
	Marseille							0.190	1.270	3.510	39.000						2.680									109.000	
	Calvi		Et					0.210	0.920	1.620	26.000						1.330									67.000	
	Marseille							0.240	1.160	2.720	23.000						1.000									55.000	
	Calvi		Au					0.320	0.670	2.200	60.000						0.740									383.000	
	Marseille							0.550	0.950	2.850	34.000						1.080									156.000	
	Calvi		Hi					0.290	1.040	3.430	51.000						1.200									69.000	
	Marseille							0.830	2.160	3.800	45.000						1.880									168.000	
Corse	Ajaccio 1	Sex ratio 1:1	Pr	0.045	22.587	23.420	0.155	0.130	0.158	0.399	2.927	55.327		0.136	0.803	0.188	0.201	0.107	0.041	1.393	0.011		0.433	1.838	102.933		El Idrissi *et al.*, 2020

(France)	Ajaccio 2	(males:females)		0.109	13.633	18.987	0.196	0.101	0.137	0.356	3.315	66.473	0.250	0.896	0.155	0.362	0.117	0.030	1.367	0.012	0.502	1.999	153.133
	Bonifacio 1			0.036	16.753	31.200	0.285	0.165	0.313	0.764	2.941	53.167	0.215	0.922	0.377	0.455	0.207	0.055	1.898	0.046	1.015	3.709	114.267
	Bonifacio 2			0.020	10.087	21.760	0.254	0.126	0.276	0.731	3.969	61.787	0.317	1.388	0.325	0.525	0.170	0.044	1.916	0.029	0.786	3.314	150.733
	Calvi 1			0.124	9.013	44.707	0.130	0.376	0.267	0.917	2.402	42.500	0.218	0.733	0.434	0.507	0.120	0.045	1.797	0.007	1.417	4.493	84.467
	Calvi 2			0.092	3.360	27.533	0.119	0.198	0.120	0.379	3.486	40.133	0.312	1.122	0.226	0.246	0.091	0.053	1.615	0.004	0.450	1.547	100.800
	Saint-Florent 1			0.800	32.247	50.633	0.655	0.461	0.357	1.106	2.373	59.113	0.290	1.002	0.526	0.523	0.154	0.094	1.677	0.012	1.378	5.600	132.800
	Saint-Florent 2			0.047	25.180	38.420	0.107	0.365	0.729	2.473	2.181	103.133	0.179	1.399	0.255	3.164	0.085	0.026	1.779	0.005	0.723	2.568	63.200
	Ajaccio 1			0.016	14.687	40.860	0.144	0.116	0.163	0.447	1.913	47.193	0.112	0.593	0.201	0.209	0.118	0.041	1.560	0.005	0.630	2.201	143.533
	Ajaccio 2			0.052	5.527	18.960	0.086	0.109	0.129	0.322	1.630	44.120	0.115	0.412	0.090	0.221	0.081	0.023	1.042	0.010	0.417	1.673	121.867
	Bonifacio 1			0.109	16.907	45.467	0.178	0.328	0.379	1.127	1.877	64.947	0.151	0.679	0.366	0.588	0.152	0.051	1.750	0.016	1.123	5.783	165.333
	Bonifacio 2			0.010	6.493	32.060	0.144	0.116	0.167	0.389	2.066	29.780	0.161	0.504	0.139	0.194	0.118	0.019	1.587	0.012	0.359	1.436	113.600
	Calvi 1	Et		0.089	2.993	60.160	0.064	0.247	0.178	0.476	1.743	27.080	0.101	0.416	0.205	0.239	0.070	0.027	1.628	0.010	0.870	2.107	139.933
	Calvi 2			0.061	3.300	46.473	0.093	0.222	0.121	0.414	1.921	49.620	0.162	0.459	0.130	0.183	0.076	0.029	1.537	0.005	0.394	1.419	164.533
	Saint-Florent 1			2.260	12.667	107.787	0.126	0.713	0.504	1.750	1.961	59.427	0.142	0.546	0.536	0.787	0.166	0.069	2.091	0.008	2.027	8.179	209.667
	Saint-Florent 2			0.026	14.520	68.840	0.095	0.425	0.774	1.877	1.843	99.393	0.107	0.932	0.253	1.971	0.089	0.031	1.825	0.004	1.154	4.027	97.133
	Ajaccio 1			0.083	43.253	36.633	0.293	0.160	0.281	0.756	3.114	88.000	0.304	1.415	0.344	1.431	0.363	0.064	1.904	0.013	1.273	4.302	197.467
	Ajaccio 2			0.142	26.733	47.953	1.172	0.201	0.342	0.751	2.781	186.667	0.310	1.403	0.305	1.862	0.266	0.078	1.887	0.018	1.013	4.091	139.400
	Bonifacio 1	Au		0.063	20.180	57.713	0.190	0.331	0.373	1.059	2.896	68.580	0.309	1.332	0.402	0.616	0.226	0.084	2.315	0.025	1.230	4.754	499.067
	Bonifacio 2			0.005	23.953	26.347	0.240	0.090	0.258	0.950	2.988	108.533	0.307	0.913	0.187	1.166	0.311	0.060	1.767	0.071	0.745	3.346	138.800
	Calvi 1			0.072	11.127	71.367	0.305	0.545	0.529	1.647	2.425	59.667	0.366	1.171	0.730	2.849	0.199	0.064	2.270	0.006	2.407	6.848	152.200

	Calvi 2			0.198	9.580	52.707	0.281	0.484	0.288	1.039	2.559	113.740	0.400	0.797	0.274	1.216	0.164	0.058	1.857	0.011	1.316	3.779	381.333	
	Saint-Florent 1			0.439	18.200	66.553	0.295	0.409	0.434	1.363	2.223	66.733	0.273	0.830	0.240	1.485	0.121	0.100	2.273	0.008	1.975	7.268	191.733	
	Saint-Florent 2			0.191	30.460	66.753	0.260	0.534	1.530	4.697	2.859	246.267	0.327	2.389	0.289	6.515	0.136	0.082	2.381	0.009	2.101	6.473	175.267	
	Ajaccio 1			0.027	85.560	26.667	0.479	0.085	0.149	0.399	4.563	115.147	0.326	3.043	0.239	0.221	0.177	0.163	1.789	0.017	0.431	1.937	201.867	
	Ajaccio 2			0.126	38.687	24.067	0.432	0.134	0.254	0.589	4.091	95.193	0.325	2.509	0.321	0.470	0.508	0.159	1.766	0.020	0.679	2.823	303.867	
	Bonifacio 1			0.040	24.207	35.260	0.240	0.159	0.218	0.563	3.651	59.547	0.325	1.664	0.337	0.309	0.167	0.348	2.192	0.057	0.943	2.931	195.133	
	Bonifacio 2			0.024	28.460	20.980	0.349	0.121	0.260	0.683	4.705	52.020	0.303	2.247	0.379	0.449	0.394	0.181	2.036	0.023	0.748	2.533	133.200	Ternengo *et al.*, 2018
	Calvi 1		Hi	0.091	13.273	33.580	0.258	0.189	0.120	0.376	3.574	46.827	0.347	1.539	0.303	0.336	0.127	0.060	1.629	0.006	0.645	1.650	115.933	
	Calvi 2			0.234	10.093	45.600	0.352	0.351	0.306	0.795	3.557	38.787	0.354	1.932	0.361	0.397	0.111	0.064	2.187	0.022	1.109	3.187	345.867	
	Saint-Florent 1			0.270	13.307	59.407	0.142	0.383	0.287	0.986	3.545	37.380	0.293	1.698	0.373	0.501	0.110	0.103	1.963	0.005	1.757	4.628	193.000	
	Saint-Florent 2			0.087	49.513	56.747	0.188	0.488	1.369	5.241	3.461	296.760	0.369	3.481	0.429	11.199	0.09	0.276	2.359	0.006	1.615	5.055	192.467	
	Akoli	F									16.880					6.950							147.940	
		M	Hi/Pr								5.900					7.420							140.800	Portocali *et al.*, 1997
Grèce	Patras	F									2.520					2.580							215.110	
		M	Hi								3.500					6.700							243.580	
	Agios Thomas	F						0.040		1.700	7.500	236.000				11.300							851.000	
	Koronisia	F	Pr					0.090		2.500	5.300	249.000				10.300							215.000	Strogyloudi *et al.*, 2014
	Mytikas	F						0.370		3.600	3.800	146.000				10.700							152.000	
Italie	Syracuse	n.a.	n.a.			4.870		1.180		1.640	0.580					1.430	1.280				1.460			Salvo *et al.*, 2014

	Ragusa				4.120	1.220	0.920	0.270			1.150	1.570		0.920		
	Messina				7.680	1.410	2.700	1.840			2.280	2.480		6.680		
	Milazzo				10.370	1.520	4.170	2.950	0.003		4.310	3.450		6.470		
	Priolo				21.560	1.260	2.990	24.170	0.177		3.430	2.720		1.840		
	Gela				6.000		2.170	2.080	0.029		0.720	20.740				
	Brolo				2.730			0.810	0.026							
	Catania				1.140			0.020	0.030		0.120					
	Filicudi				7.680			1.150	0.027		0.037	0.030				
	Sardaigne				0.536	0.435	0.300	1.577	0.062		0.070	0.144		0.586		Salvo *et al.*, 2015
	Sicile				0.066			1.500	0.020			0.091				
	Citavecchia		Au/Hi		14.600	0.070	3.600				1.150	1.180				Scanu *et al.*, 2015
	Apulian coast		Pr			0.240		5.190	183.670	0.100		0.860			157.130	Storelli *et al.*, 2001
	Ischia		Pr			0.410	2.010	3.410	90.000			3.020			140.000	Warnau *et al.*, 1998
			Et			0.260	0.960	1.030	65.000			1.300			53.000	
			Au			0.930	1.750	2.500	114.000			1.150			271.000	
			Hi			0.330	1.590	3.830	119.000			2.250			112.000	
Maroc	Maroc	n.a.			0.571	0.104	0.071	1.226		9.854	0.222	0.142		0.605		Salvo *et al.*, 2015
Portugal	SW Atlantica	n.a.	Pr		2.120	0.040	0.090	0.660	16.600	0.009	0.050	0.021	0.010		45.400	Camacho *et al.*, 2018
	Viana do Castelo	Sex ratio 1:1 (males:females)	Au		1.680			0.640				0.110			40.290	Mamede *et al.*, 2022
	Matosinhos				1.880			0.490				0.130			51.270	

Lisbon			1.650		0.700			0.430		40.280		
Sines			1.190		0.890			2.590		142.090		
Viana do Castelo		Su	1.790		0.850			0.000		53.750		
Matosinhos			2.650		0.860			0.180		36.510		
Lisbon			1.240		2.070			0.240		56.800		
Sines			0.990		2.050			1.140		41.440		
Sagres			1.820		1.060			1.430		60.580		
Praia Norte	n.a.	Sp	2.2	0.01	0.05	0.7	13.000	0.008	0.38	0.01	37.000	Rocha *et al.*, 2019
Carreço			2.5	0.02	0.79	0.8	19.000	0.01	0.59	0.01	51.000	
Praia Norte		Su	3.8	0.0222	0.093	0.5	15.000	0.013	0.15	0.016	17.5	
Carreço			3.2	0.022	0.07	0.43	13.000	0.012	0.16	0.02	12.000	
Praia Norte		Au	3.5	0.021	0.1	0.6	7.000	0.010	0.14	0.008	18.8	
Carreço			3.4	0.020	0.13	0.4	105.000	0.013	0.2	0.019	12.000	
Praia Norte		Wi	2.4	0.016	0.030	0.6	11.000	0.0055	0.12	0.007	18.000	
Carreço			2.5	0.017	0.11	0.6	11.000	0.011	0.21	0.015	18.000	
Praia Norte		Sp	3.6	0.0139	0.056	0.5	8.000	0.0066	0.08	0.018	15.000	
Carreço			3.5	0.019	0.22	0.7	11.000	0.0122	0.19	0.025	28.000	