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Auteur : Le Vern, Lise
Promoteur(s) : Gobert, Sylvie; 16466
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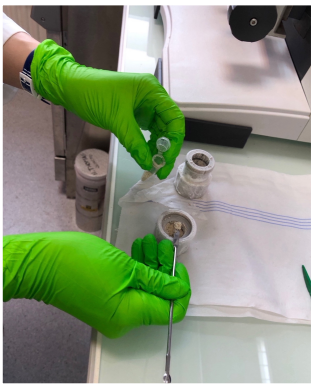
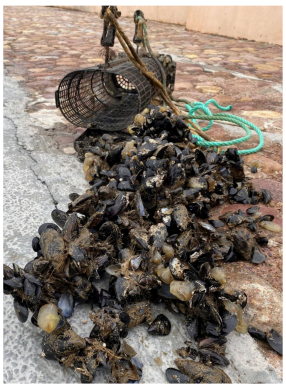
Biomonitoring of two marine mollusks, the Mediterranean mussel *Mytilus galloprovincialis* and the limpet *Patella sp.* in three North Corsican ports and inter-annual comparisons



Lise **LE VERN**

Hosting structure: UMRi 7266 LIENSs
Internship supervisor: Marion **PILLET**, Hélène **THOMAS**
University tutor: Sylvie **GOBERT**

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RÉSUMÉ

Plus de 80% de la pollution présente dans les océans provient des activités terrestres et humaines qui y sont menées. Les eaux côtières, contenant une grande partie de la biodiversité marine, sont directement impactées par ces pressions anthropiques. Plus particulièrement, les zones portuaires, qui constituent le lieu de vie de nombreuses espèces marines, sont soumises à des risques supplémentaires de pollution liées aux activités portuaires (trafic maritime, pollution chronique par les hydrocarbures ou les peintures antifouling, etc.). La surveillance de la contamination dans ces zones portuaires est un enjeu important, notamment en Corse où la population côtière peut doubler en saison estivale en raison du tourisme, augmentant considérablement les risques de pollution. Le projet QUAMPO, mis en place dans le cadre du maintien du bon état écologique proposé par la Directive Cadre Stratégie pour le Milieu Marin (DCSMM), vise à évaluer la qualité des eaux des ports de la Haute-Corse. Pour ce faire, l'utilisation d'une approche multi biomarqueurs a été réalisée sur deux mollusques marins différents : la moule méditerranéenne *Mytilus galloprovincialis* et la patelle *Patella sp*. Dans le cadre du projet QUAMPO, le présent mémoire vise à fournir les dernières données de janvier 2022. Des organismes ont été prélevés dans trois zones portuaires (Calvi, Île Rousse et St-Florent) et une sélection de biomarqueurs d'exposition (éléments traces, polluants organiques) et d'effet (stress oxydatif, métabolisme énergétique, immunité, neurotoxicité, stock énergétique) ont été analysés pour (1) évaluer l'effet induit de la pollution chronique dans ces ports, (2) confirmer ou non le potentiel de la patelle comme bioindicateur de la qualité des eaux, (3) et utiliser un indice intégratif : « Integrative Biomarker Response » (IBR) pour communiquer plus simplement les résultats aux gestionnaires des ports. En conséquence, certains biomarqueurs de la patelle répondent bien aux différents polluants présents dans les ports. Même si des traces de pollution ont pu être observées dans tous les ports, de nature et concentration différente, les ports de la Haute-Corse présentent globalement des eaux de bonne qualité. Enfin, l'indice IBR semble être un outil utile pour résumer toutes les réponses des biomarqueurs.

ABSTRACT

More than 80% of the pollution present in ocean comes from land and human activities carried out there. Coastal waters, containing a large part of marine biodiversity, are directly impacted by these anthropogenic pressures. More especially, port areas, which constitute living place to numerous marine species, are subject to additional risks of pollution specific to port activities (maritime traffic, chronic pollution by hydrocarbons or antifouling paints, etc.). Monitoring contamination in these port areas is an important issue, especially in Corsica where the coastal population can double in summer season, considerably increasing the risks of pollution. The QUAMPO project, set up in the context of achieving of maintaining the good ecological status proposed by the Marine Strategy Framework Directive (MSFD), aims to assess the quality of water in the North Corsica ports. To do so, the use of a multibiomarker approach was carried out on two different marine mollusks: the Mediterranean mussel *Mytilus galloprovincialis* and the limpet *Patella sp*. As part of the QUAMPO project, the present master thesis aims at providing the last data, of January 2022. Organisms were sampled in three ports areas (Calvi, Île Rousse and St-Florent) and a battery of biomarkers of exposure (traces elements, organic pollutants) and biomarkers of effect focusing oxidative stress, energetic metabolism, immunity, neurotoxicity, and energy stock was analyzed to evaluate the induced-effect of chronic pollution in those ports, to confirm or no the potential of limpet as a reliable bioindicators, but also used an integrative index: the integrative biomarker response (IBR) to communicate results to ports managers in a simpler way. As results, some limpet biomarkers well respond to the different pollutant present in ports. Even if trace of pollution could be observed in all port, from different nature or concentration, North Corsica ports present globally good quality waters. Finally, the IBR index appears to be a useful tool to summarise all biomarkers responses.

TABLE OF CONTENTS

TABLE OF ILLUSTRATIONS

1. List of figures

2. List of tables

3. List of appendixes

ABBREVIATION

ACHE: Acetylcholinesterase

ADP: Adenosine 5'-diphosphate

Ag: Silver

Al: Aluminum

As: Arsenic

Ba: Barium

CAT: Catalase

Cd: Cadmium

cDNB: 1-chloro-2,4-dinitrobenzene

Co: Cobalt

Cr: Chromium

Cu: Copper

dGDP: Deoxyguanosine-5'-diphosphate

DO: dissolved oxygen

DTNB: 5,5′-Dithiobis(2-nitrobenzoic acid)

EDTA: Ethylenediaminetetraacetic acid

EPA: Environmental protection agency

Fe: Iron

Fz: Fairing zone

GOD-POD: Glucose oxidase peroxidase

GPX: Glutathione peroxidase

GR: Glutathione reductase

Gs: Gas station

GS-DNB: Glutathionyl-dinitrobenzene

GSH: Glutathione

GSSH: Glutathione-oxydo-reductase

GST: Glutathione-S-transferase

IBR: Integrated Biomarker Response

ICP: Inductively coupled plasma

ICP-MS: ICP mass spectrometry

ICP-OES: ICP optical emission spectrometry

LAC: Laccase type phenoloxidase

LDH: Lactate dehydrogenase

NADH: β-Nicotinamide adenine dinucleotide

NADPH: β-Nicotinamide adenine dinucleotide phosphate

MDH: Malate dehydrogenase

Mn: Manganese

Mo: Molybdenum

MSFD: European Marine Strategy Framework Directive

Ni: Nickel

NIST: National Institute of Standards and Technology

PAHs: Polycyclic aromatic hydrocarbons

Pb: Lead

PBS: Phosphate-buffered saline

PCBs: Polychlorobiphenyl

PEP: Phosphoenolpyruvate

PEPCK: Phosphoenolpyruvate carboxykinase

PPD: *p*-phenylenediamine

PK: Pyruvate kinase

ROS: Reactive oxygen species

RS: Reacting solution

Sb: Antimony

SBSEGC-MS/MS: Stir bar sportive extraction-thermal desorption-gas chromatography-tandem mass spectrometry

Se: Selenium

Sn: Tin

SOD: Superoxide dismutase

tBHP-2: Tert-butyl hydroperoxide solution

TE: Trace elements

V: Vanadium

WFD: Water Framework Directive

Zn: Zinc

INTRODUCTION

1. Marine chemical pollution

1.1. Context

Since the 19th century industrial revolution, and especially during the last fifty years, worldwide marine ecosystems have been increasingly threatened by man-made chemical pollution[1] from urban, industrial, and agricultural sources (Bonacci *et al.,*2007; Holon *et al.,*2018). About 80% of this marine pollution comes from terrestrial human activity (Gobert & Richir, 2019; Parra-Luna *et al.,*2020; United Nations, 2020), through aquatic effluents, atmospheric emissions or accidental spillages (Chase *et al.,*2001).

Oceans, covering about 71% of Earth surface and representing about 98% of water present on the planet, constitute the final receptacle of anthropogenic waste (Beiras, 2018; Gobert & Richir, 2019). Chemical contaminants are toxic, persistent in the environment, can cause damage, as endocrine disruption, on living organisms, and for some of them, can bioaccumulate and biomagnify through trophic chains (Parra-Luna *et al.,*2020). Oceanics currents participate in the distribution of chemicals in marine ecosystems all over the world, sometimes very far from their initial emission site (Muir *et al.,*1992; Debier *et al.,*2003). Coastal waters are especially of concern. Concentrating near 90% of marine biodiversity, they received the largest exposures to chronic chemical pollution because of their proximity to sources (e.g. fishing, shipping at sea, shipyards, tourism, wastewater treatment plant, accidental spills, agricultures and industries). It seems important to remember that 8 of 10 world's bigger cities are located in littoral area, concentrating about 44% of human population (Yin *et al.,*2000; Chase *et al.,*2001; Islam & Tanaka, 2004; Fasulo *et al.,*2012; Solaun *et al.,*2013; Beiras, 2018).

More particularly, confined areas such as enclosed ports are commonly described in literature to have poor water quality because of a low hydrodynamism, limited renewal through open seawaters, and an intense anthropogenic pressure, causing diffuse and chronic pollution (Guerra-García *et al.,*2021; Briant *et al.,*2022). Marine communities residing inside are exposed to a high and complex "cocktail" of pollutants, potentially linked to port activities (maritime traffic, boat careening, intra-port gas station, use of antifouling paint, etc), and are consequently

[1] Marine pollution is "the introduction by man, directly or indirectly, of substances or energy into the marine environment (including estuaries) resulting in such deleterious effects as harm to living resources, hazards to human health, hindrance to marine activities including fishing, impairment of quality for use of sea water, and reduction of amenities." (Art. 1.4 of UNCLOS, (GESAMP, 1969) (European Commission, 2008; Potters, 2013; Beiras, 2018; Parra-Luna *et al.,*2020).

more vulnerable to these permanent stress conditions (Chase *et al.,*2001; Bocchetti, Fattorini, *et al.,*2008; Sureda *et al.,*2011; Fasulo *et al.,*2012). In particular, intra-port gas station (Gs) areas are more prone to hydrocarbons spills in seawater, resulting in significant exposure by harmful pollutants such as polycyclic aromatic hydrocarbons (PAHs) which can affect the neighboring marine populations (Lacroix *et al.,*2015; Breitwieser *et al.,*2017; Honda & Suzuki, 2020). Another particularly risky area is the fairing zone (Fz), where antifouling paint, used as biocide for fouling organisms, are removed to be replaced. This boat maintenance process generates waste containing biocides but also hydrocarbons, microplastics, solvents, etc. (Briant *et al.,*2022; Droit, 2020). Notice that, for some pollutants, toxicity appears only at high concentrations (essentials and natural components), but others can cause important detrimental changes even at very low levels of concentrations (Chahouri *et al.,*2022).

1.2. The case of Mediterranean Sea

In Europe, the Mediterranean Sea is surrounded by 21 different countries. Characterized by relatively warm, saline and nutrient-poor waters, it is the deepest (maximum depth of 5267 m, average depth of 1460 m) and the largest (2969000 km^2) semi-enclosed sea on Earth, and its coastal waters represent 20% of total Mediterranean waters (Duarte *et al.,*1999; Danovaro, 2003a; Coll *et al.,*2010). While it only represents less than 1% of the total world ocean area, it is considered by ecologists to be a hot-spot of biodiversity (Bianchi & Morri, 2000; Coll *et al.,*2010; Bonanno & Orlando-Bonaca, 2018a; Tovar-Sánchez *et al.,*2019). However, in addition to being one of the busiest shipping routes in the world, it is the final receptacle of waters coming from very populated watersheds (Nile, Po, Ebro, etc), directly discharging wastewaters from industries, farms, or domestic life, sometimes contaminates. Those wastewaters can come from less developed countries, which don't even have access to water treatment plants (Coll *et al.,*2010; Beiras, 2018; Parra-Luna *et al.,*2020; Marengo *et al.,*2020). According to Gobert & Richir (2019), as many as 80% of sewage are directly released in coastal waters without any prior treatment. In addition, the coastline of the Mediterranean region, already highly populated all year, also constitute the first touristic destination in the world, with more than 330 million tourists in 2016, increasing exponentially the pressure on coastal ecosystems (Gobert & Richir, 2019; Tovar-Sánchez *et al.,*2019). Since coastal areas provide the major part of fishing resources, around 99% according to Parra-Luna *et al.* (2020), their contaminations by made-man chemicals compounds have therefore become a major threat for both marine ecosystems and therefore human health, worrying more and more countries (Gobert & Richir, 2019; Breitwieser *et al.,*2020; Parra-Luna *et al.,*2020).

1.3. Legislation

As these problems of water pollution increasingly concern industrialized countries in the last decades, legislation has been developed to conserve, protect, and manage fresh and marine waters (Parra-Luna *et al.,*2020). Through the Water Framework Directive (WFD; Directive 2000/60/EC) adopted in 2000 or the European Marine Strategy Framework Directive (MSFD, Directive 2008/56/EC) adopted in 2008, the European Commission incited European states to achieve or maintain a "Good Environmental Status" before 2020, meaning that contaminants concentration levels are low enough to avoid pollution (Burgeot *et al.,*1996; European Commission, 2008; Lacroix *et al.,*2015). Those two main directives permit to centralized more than 30 different regional directives such as for instance Convention of Barcelona (1976), OSPAR for North-East Atlantic ocean (1992), or HELCOM for Baltic Sea (1992). They were all created for a type of ecosystem, water mass, pollution or after an accidental pollution (Danovaro, 2003b; Besse *et al.,*2012; Rodrigo *et al.,*2013).

These measures have led to establish the legal basis to monitor pollutions and defined Environmental Quality Standards (EQS)[2] with a view to manage and protect aquatic environments and water resources and have consequently improved knowledge. Then, a list of 33 priority substances or groups of substances and eight other pollutants and their concentration limits have been elaborated, including for example trace elements (TE), polycyclic aromatic hydrocarbons (PAHs), polychlorobiphenyl (PCBs), polybromodiphenyls (PBDEs), pharmaceuticals, or pesticides. By definition, they are toxic, persistent and bioaccumulative or considered to be questionable (European Commission, 2008; Solaun *et al.,*2013; Besse *et al.,*2012).

In order to achieve a better ecological status of water bodies, WFD and MSFD require its member states to develop monitoring programs to assess the chemical and biological quality of those water bodies.

2. Biomonitoring using mollusks

Biomonitoring consists of the study of geographical and temporal trends of contaminants through biological responses of an organism (Chase *et al.,*2001; Rainbow, 2002; Solaun *et al.,*2013; Lacroix *et al.,*2015). It has been developed to identify sources of pollution and prevent

[2] The EQS defines "the concentration of a particular pollutant or group of pollutants in water, sediment or biota which should not be exceeded in order to protect human health and the environment" (Besse *et al.,*2012).

risks of future potential pollution accidents. Environmental contaminant analysis through biomonitoring uses biomarkers from a bioindicator species. Bioindicators are all sedentary species which can bioaccumulate pollutants and give an indication on the environment quality (Phillips, 1978; Gobert *et al.,*1992; Bonanno & Orlando-Bonaca, 2018c; Pérez *et al.,*2019). Biomarkers are biologic tools corresponding to an observable change in tissues, fluids of an organism or in the whole organism, revealing its exposition to one or several chemical contaminants. They can be assessed at molecular and cellular, individual, populational and even biocenosis levels, and are from various nature: biochemical, cytological, physiological or also behavioral (Blandin, 1986; Garric *et al.,*2010; Liu *et al.,*2012; Luna-Acosta *et al.,*2015). A distinction of molecular and intra-cellular biomarkers is commonly done: biomarkers of exposure, and biomarkers of effects. The first one indicates any presence of contaminants in the bioindicator organism, whereas the second one deals with the effect of these contaminants on it (Luna-Acosta *et al.,*2017a). This concept of biomonitoring gives many advantages compared to simple chemical water analysis. It brings a time-integrated and quantified methods to assess bioavailable[3] fraction of contaminants, and allow the evaluation of effects of contaminants on organism health (Rainbow, 2002; Bocchetti, Fattorini, *et al.,*2008; Lacroix *et al.,*2015; Luna-Acosta *et al.,*2015; Wang, 2016).

Bioindicator species have finally been used worldwide since the end of the 20th century in different biomonitoring programs, e.g. the "Réseau d'Observation de la Contamination Chimique du littoral" (ROCCH), the "Programme d'évaluation et de maîtrise de la pollution marine dans la région méditerranéenne" (MEDPOL), the Asian-Pacific Mussel-Watch program (APMW), or the Spanish Monitoring Program (JAMP) (Monirith *et al.,*2003; Besada *et al.,*2011; Solaun *et al.,*2013; Ifremer *et al.,*2018).

2.1. The mussel Mytilus sp.

Different species, such as mollusks, macroalgae, seagrasses, fish, turtles, worms and even seahorses can and have been used as bioindicators for biomonitoring (Beg *et al.,*2018; Bonanno & Orlando-Bonaca, 2018b; Santos Fraga *et al.,*2018; Orani *et al.,*2022; Barrick *et al.,*2022). They have been chosen according to their presence in the studied environment, and their capacity to accumulate bioavailable contaminants, in particular those present in too low concentration to be detected directly in seawater. Mussels, especially from the genus *Mytilus,*

[3] In ecotoxicology, bioavailability corresponds to the fraction of contaminants available for potential uptake by an organism from its environment (Wang, 2016).

is successfully considered as a useful bioindicator species for coastal pollution (Chase *et al.,*2001; Besada *et al.,*2011; Fasulo *et al.,*2012; Lacroix *et al.,*2015). They have been used as bioindicator since the mid-1970s in the well-known "Mussel Watch program" in United-States to study levels, trends of pollutants in water, and effects on organisms (Goldberg *et al.,*1978; Cossa, 1989; Gobert *et al.,*1992; Livingstone, 1993; Andral *et al.,*2004; Rodrigo *et al.,*2013; Sparks *et al.,*2014). This species exhibits many advantages. Widely distributed geographically and abundant, tolerant to variation of its environment, sedentary filter-feeder, this species is also easy to identify, sample, and handle, and it bioaccumulates and tolerates for instance heavy metals with a concentration factor going up from 1000 to 100000 according to environmental conditions (Cossa, 1989; Gobert *et al.,*1992; Andral *et al.,*2004; Besada *et al.,*2011; Lacroix *et al.,*2015; Bonanno & Orlando-Bonaca, 2018c). In summary, this species concentrates all the necessary qualities of a good bioindicator.

Over time, two different methods of biomonitoring, with their advantages, and disadvantages, have been developed: passive or active biomonitoring (table 1) (Andral *et al.,*2004; Besse *et al.,*2012; Delahaut *et al.,*2019; Lacroix *et al.,*2015).

Table 1: Summary of the differences between active and passive biomonitoring, their advantages and limitations (Andral *et al.,*2004; Besse *et al.,*2012; Lacroix *et al.,*2015).

	Passive biomonitoring	**Active biomonitoring**
Characteristics	Use of native mussels	Use of cultivated or transplanted caged mussels from reference site
Advantages	- Measurement on long-term exposure, more representative of real contamination of the environment - Simpler to process - Existing guidelines	- Precise control of several experiment factors (depth and time of exposure, exact location) - Reduction of confounding factors linked to physiological, genetical and biologic characteristics (size, age, sex, stage of sexual maturity, etc) of the sample (a single population) - Compensation for the lack of wild mussels - Repeatable - Control of cost and duration of the experiment
Limitations	- Risk of scarcity of mussel stock in some places - Depends on geographical distribution of species - Unknown time of exposure - Confounding factors linked to physiological, genetical and biologic characteristics (size, age, sex, stage of sexual maturity, etc) of the sample (a single population)	- Potential effect of the caging system on biomarkers responses

In this work, the Mediterranean mussel (*Mytilus galloprovincialis* L., 1819) was chosen through an active biomonitoring, because of its scarcity in the studied locations and all the advantages listed in table 1. This species, widely used in literature as sentinel to assess coastal water quality of the Mediterranean Sea, provide well-described biomarkers which can be used as reference.

However, the absence or high scarcity of mussels in North Corsica port areas bring the need to investigate alternative bioindicators species for biomonitoring of environmental quality in those locations (Conti & Cecchetti, 2003). In addition, Bertrand et al. (2018) remind the importance of using native or sentinel species as bioindicators, adapted to the environment, thus guaranteeing quality biomonitoring. Additional authors agreed, saying that they are more suitable to a biomonitoring of water quality, due to their ubiquity in the environment (Conti & Cecchetti, 2003; Beg *et al.,*2018; Santos Fraga *et al.,*2018; Orani *et al.,*2022; Sánchez-Marín *et al.,*2022).

2.2. The limpet Patella sp.

Another potential bioindicator are limpets (*Patella sp.).* This marine gastropod is a grazer living organisms on intertidal rocks (Bonacci *et al.,*2007; Pérez *et al.,*2019; Viñas *et al.,*2018). Because of its abundance on shoreline, its sedentary lifestyle, its consumption by humans, its large distribution or its ease of sampling, it is considered a keystone species (Campanella *et al.,*2001; Bartolomé *et al.,*2011; Prusina *et al.,*2015; Pérez *et al.,*2019).

Even if knowledge on the use of limpets as a bioindicator species remains limited, some authors describe this species as promising (Pérez *et al.,*2019; Reguera *et al.,*2018). They have been used in biomonitoring of trace elements or organic pollutants (Shefer *et al.,*2015; Viñas *et al.,*2018; Reguera *et al.,*2018; Pérez *et al.,*2019; El-Damhogy *et al.,*2019; Sánchez-Marín *et al.,*2022). Several studies have shown their high sensitivity to pollutants, sometimes even more important than the mussel sensitivity (Conti & Cecchetti, 2003; Bonacci *et al.,*2007; Reguera *et al.,*2018). For all previous reasons, *Patella sp.* was also used in this study through passive biomonitoring.

2.3. Biomarkers selected in this study

A multibiomarker method was performed to measure the exposure and assess the effect of contaminants on different metabolic functions of studied organisms.

As biomarkers of exposure, numerous well-established pollutants were selected: 18 trace elements and 43 organic pollutants (22 polycyclic aromatic hydrocarbons (PAHs), 14

polychlorinated biphenyls (PCBs) and seven pesticides) (see section 3 of materials and methods).

As biomarkers of effects, 10 enzymatic activities involved in important metabolic processes and three energetic reserves compounds were used. They were all selected depending on physiological functions which are known to be impacted by chronic and diffuse pollution (Breitwieser *et al.,*2020; Lacroix *et al.,*2015).

The immune system was represented by laccases (LAC – EC 1.10.3.2 : *p*-diphenol oxidase) as part of the phenol oxidases family. It plays a key role in phagocytosis, capsule formations, nodules, and more generally in the modulation of immune response (Luna-Acosta *et al.,*2010, 2017a; Breitwieser *et al.,*2020).

Largely involved in the transmission of the nerve impulses, the acetylcholinesterase (ACHE – EC 3.1.1.7) was taken as neurotoxicity biomarker because its inhibition is described in literature to be linked with an exposure to pollutants such as pesticides or organophosphate, and more recently with a general stress of the organism (Lehtonen *et al.,*2006; Tsangaris *et al.,*2010).

Three biomarkers of energetic metabolism: lactate dehydrogenase (LDH – EC 1.1.1.27), pyruvate kinase (PK – EC 2.7.1.40) and phosphoenolpyruvate carboxykinase (PEPCK – EC 4.1.1.49). Modulation of energetic metabolism is an important response to an external stress and is not sufficiently explored according to Lacroix *et al.* (2015). In literature, LDH activity has mostly been studied in fish organisms. Its activation occurs when a high amount of energy is required, typically in a case of oxidative stress. It is also a biomarker of tissue lesions, hypoxic conditions, and muscular harm (Amanullah *et al.,*2010; Sifi & Soltani, 2019). PK takes a key part in catalyzation of the late stage of glycolysis, consisting in conversion of phosphoenolpyruvate (PEP) into pyruvate (Sussarellu *et al.,*2012; Lacroix *et al.,*2015). Finally, PEPCK is involved in the catalyzation of the decarboxylation of oxaloacetate into PEP: an anaerobic pathway of energy production which allows the synthesis of pyruvate. It is also involved in gluconeogenesis (Lacroix *et al.,*2015).

Finally, oxidative stress has extensively been studied as part of biomonitoring. Reactive oxygen species (ROS) are formed by organisms consequently to a stress. Detrimental effects of this compounds are limited by the antioxidant defenses. If the ROS production exceeds the capacity of antioxidants to defend and repair tissues, an imbalance is created, and the organism undergoes oxidative stress, resulting for example in alteration of gene expression, tissues damage, osmoregulatory dysfunctions, or even apoptosis or necrosis (Livingstone, 2001;

Pellegrini & Baldari, 2009; Lushchak, 2011; Lacroix *et al.,*2015; Baag *et al.,*2021). Here, five enzymes involved in antioxidant defenses were evaluated. Glutathione peroxidase (GPX – EC 1.11.1.9), superoxide dismutase (SOD – EC 1.15.1.1) and catalase (CAT – EC 1.11.1.6) are directly involved in the maintenance of ROS normal levels. Briefly, after the conversion by SOD of superoxide anion (O_2 $^{-\bullet}$) into oxygen (O_2) or hydrogen peroxide (H_2O_2), CAT and/or GPX subsequently convert the latter molecules into water or oxygen molecules (Weydert & Cullen, 2010; Lacroix *et al.,*2015). Glutathione-S-transferase (GST – EC 2.5.1.18) is involved in phase-II xenobiotic detoxification process, toxin biotransformation, peroxidation and ROS inactivation (Lacroix *et al.,*2015; Breitwieser *et al.,*2020). Finally, as GST and GPX need a glutathione molecule as cofactors to process their detoxification reaction, glutathione reductase (GR – EC 1.6.4.2), involved in the reduction of oxidized glutathione (GSSG) is also considered as biomarkers of oxidative stress (Lushchak, 2011; Regoli & Giuliani, 2014).

In addition, lipids, protein, and glycogen are key energy storage compounds. Lipids metabolism is considered in the literature to be the main source of energy in anaerobic conditions, and a damage by peroxidation of membrane can be induce by high levels of ROS (Dailianis, 2011; Lushchak, 2011; Rocchetta *et al.,*2014). Protein also constitutes an important source of energy (Smolders *et al.,*2004). Glycogen is known to be rapidly consumed in response to an elevated basal maintenance linked to the exposure to contaminants (Lin *et al.,*2011; Falfushynska *et al.,*2019).

The Integrative Biomarker response (IBR) combines all biomarkers responses into a single index (Beliaeff & Burgeot, 2002). It offers a holistic and simpler interpretation of biomarkers responses to pollution induced stress. Described as a useful tool in ecotoxicology, it is therefore highly recommended (Serafim *et al.,*2012; Devin *et al.,*2014; Bertrand *et al.,*2018; Chahouri *et al.,*2022).

3. The QUAMPO project

Resulting from the collaboration of the LIENSs (Littoral Environnement et Sociétés) lab based at La Rochelle University and the STARESO (Station de Recherches Sous-Marines et Océanographiques) affiliated with the university of Liège, QUAMPO (QUality of the marine environment in the Mediterranean POrt areas) is a three-years (2019-2022) biomonitoring project. It aims to assess water quality of three North Corsican ports (Calvi, Île Rousse and St-Florent). Corsica constitutes the fourth largest Mediterranean Island (8682 km2) situated at about 170 km of continental France, 14 km of Sardinia and 90 km of continental Italy (Mouillot *et al.,*2008). Since 1950, because of a large urbanization and urban spawl of coast, agriculture

and rising coastal touristic activities, the island is marked by an exponential increase of threat directly linked with the coastal demographic pressure, especially during summer (Mouillot *et al.,*2008; Robert *et al.,*2019). For example, Cali and Île Rousse would have seen their population quadruple some summers (Renucci, 1962; Cancellieri & Maupertuis, 2016; Robert *et al.,*2019). According to Robert *et al.* (2019), they are still poorly protected. In Calvi, Île Rousse or St-Florent ports, living marine organisms face numerous anthropogenic pressures (appx. 1). This biomonitoring program was carried out through a integrative multi-biomarker approach, considered as more efficient and is increasingly used now in ecotoxicology (Rodrigo *et al.,*2013). This study was performed with a view to provide to local actors and decision makers tools and interpretation keys to better assess risks related to chemical contaminants in ports areas and better understand the damage suffered by coastal marine life exposed to chronic pollution.

4. Objectives

This master's thesis focuses on the acquisition, treatment, and analyses of the last sampling campaign of the QUAMPO project. The objectives can be divided into 3 sub-aims:

1. Provide data / knowledge on the environmental quality in three North Corsican ports for the winter 2022 to help ports local actors to improve their management of ports areas and move towards more ecological practices.
2. Continue to gather additional information on the potential of *Patella sp.* as a valuable bioindicator of coastal water quality.
3. Identify the more suitable biomarkers and bioindicators to follow as part of a North-western Mediterranean port biomonitoring.

MATERIALS AND METHODS

1. Studied area

Four ports areas of North Corsica have been chosen for this study: Calvi (N 42° 33' 59.116"; E 8° 45' 29.094"), Île Rousse (N 42° 33' 59.116"; E 8° 56' 7.778"), St-Florent (N 42° 40' 50.362"; E 9° 17' 54.373") ports and STARESO station in the Revellata bay (N 42° 34' 49.584"; E 8° 43' 31.511"), considered as reference port (fig. 1). The studied ports are marinas with a capacity of 250 places for Île Rousse (maximum boat's size = 30 m), 500 for Calvi (maximum boat's size = 65 m) and 950 places for St-Florent (maximum boat's size = 45 m). The reference site STARESO can accept 4 boats only in case of emergency. Away from any city, it is a preserved site.

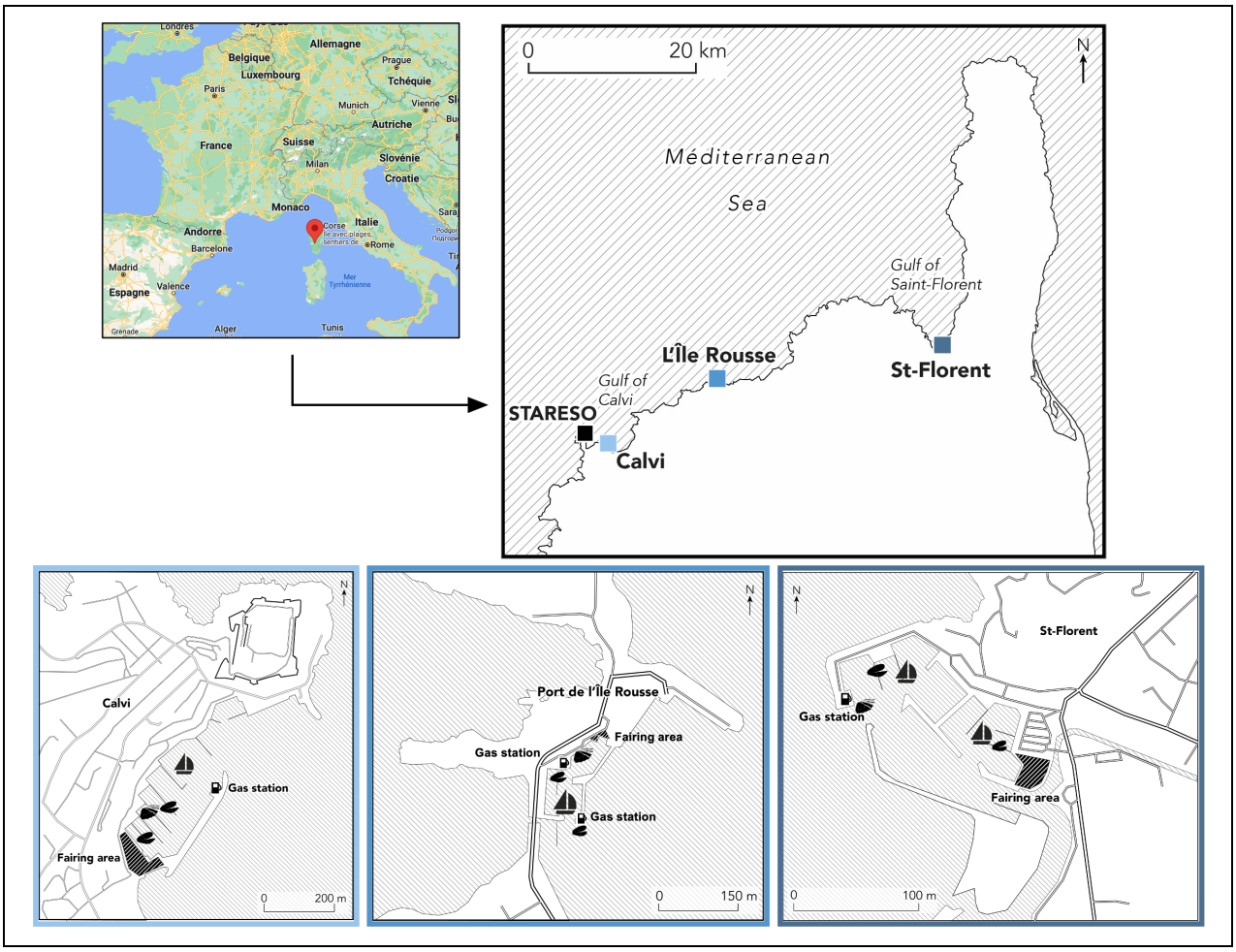

Figure 1: Map of the studied ports (Calvi, Île Rousse, St-Florent). Colored square code in the North Corsica map (top-right) distinguishes the different ports and is reported in the contour of each ports map (bottom). Different symbols allow to identify: ▨ : fairing conditions; ⛽ : gas station condition. ⛵ : presence of boats; 🦪 : mussels *Mytilus galloprovincialis*; 🐚 : limpets *Patella sp.* (modified from ©Pascal Brunello, La Rochelle Université)

2. Field sampling

During the QUAMPO project, samples were collected twice a year, in January and September. The present master thesis will focus on the last sampling collection, carried out in January 2022. It took place on 25th January 2022 at the STARESO station and Calvi port, and on 26th January 2022 at Île Rousse and St-Florent ports.

Temperature, salinity, pH and dissolved oxygen (DO) were measured in the water column (1 m deep). One water sample was taken in each location for chemical contamination analyses (trace elements and organic pollutants). Marine invertebrates were collected on each site to analyze the selected biomarkers of exposure and effects.

Wild limpets (n = 8-10 per site) were sampled (fig. 2, a) for biomarkers of effects analyses. Additional individuals were samples for the analyses of biomarkers of exposure (TE and organics pollutants) (n = 8 per site).

Because of their absence or scarcity in most of the ports (only founded in St-Florent), mussels *M. galloprovincialis* were purchased from the Diane salty pond (42° 07′ 52,1″ N, 9° 32′ 05,65″ E, Corse, France). They were transplanted for one month before sampling (fig. 2, b). Two sub-locations were identified in each port: one near the gas station named "gas station condition" and one near the fairing place corresponding to "fairing condition" (fig. 1). This subdivision was created to see potential impact of an exposure to oil for gas station condition and to antifouling paint for the fairing condition. Thus, 70 individuals were sampled: 20 per port (10 at the gas station locations and 10 at the fairing area location), except for STARESO (n = 10) where no gas station or fairing place is present.

Immediately after collection, limpets and mussels were placed in dry ice and transferred to the LIENSs (La Rochelle University) laboratory, in France, for further analyses (fig. 2, c). Once in the lab, they were frozen at -80°C until analyses. The samples reserved for the analyses of biomarkers of exposure (TE, organic pollutants) were kept at – 20 °C.

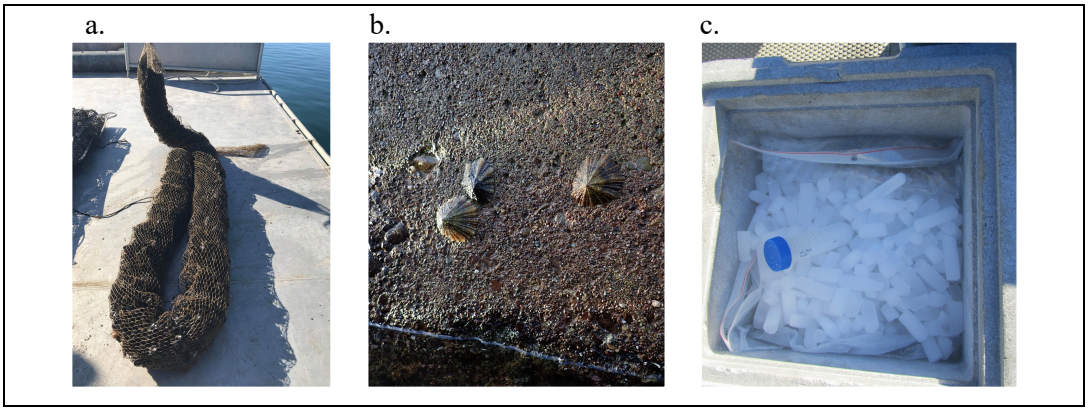

Figure 2: Photos of the sampling and transplantation procedures (© STARESO).

3. Analyses of biomarkers of exposure

All biomarkers of exposure (traces elements and organics pollutants) measurement were performed as part of the QUAMPO project, but independently of the present master's thesis. For this assessment, additional mussels and limpets were collected during the sampling period and the analyses were made on pools of individuals, one pool per species and per port, giving only one measurement. This method prevents any statistical analyses, but provides general trends of the exposition of the studied invertebrates to marine pollutants (Turle & Collins, 1992; Breitwieser *et al.,*2018).

3.1. Trace elements

Traces elements analyses were sent to the Centre d'analyses élémentaires of the La Rochelle University for analyses. A total of 18 TE contents in water and tissues were analyzed: aluminum (Al); silver (Ag); arsenic (As); barium (Ba); cadmium (Cd), cobalt (Co), chromium (Cr); copper (Cu); iron (Fe); manganese (Mn); molybdenum (Mo); nickel (Ni); lead (Pb); antimony (Sb); selenium (Se); tin (Sn); vanadium (V) and zinc (Zn).

Briefly, soft tissues of six to seven limpets per port (mean ± SD length = 36.8 ± 4.7 mm) and of eight mussels per sub-location (n_{total} =16 per port, average length = 73.3 ± 6.9 mm) were ground and pools were created per species and locations. After digestion of the samples and two references (DOLT-5, dogfish liver and TORT-3, lobster hepatopancreas, National Research Council Canada), TE bioaccumulation was analyzed thanks to an ICP-OES Vista-Pro (Varian Inc., Palo Alto, California, USA) and an ICP-MS XSeries 2 (ThermoFisher Scientific, Waltham, Massachusetts, USA). Results are expressed in $\mu g.g^{-1}$ of wet weight for organisms and in $\mu g.L^{-1}$ for water samples.

3.2. Organics pollutants

Measurements of organics pollutants were given to the "Centre de Documentation, de Recherche et d'Expérimentations sur les Pollutions Accidentelles des Eaux" (CEDRE), in Brest (France). Concentration of the following 22 polycyclic aromatic hydrocarbons (PAHs), 14 polychlorinated biphenyls (PCBs) and 7 pesticides were analyses in soft tissues of limpet and mussels: naphthalene, benzothiophene, biphenyl, acenaphthylene, acenaphthene, fluorene, dibenzothiophene, phenanthrene, anthracene, fluoranthene, 2-methylfluoranthene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(e)pyrene, benzo(a)pyrene, perylene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, PCB 7, PCB 28, PCB 52, PCB 35, PCB 101, PCB 77, PCB 135, PCB

118, PCB 153, PCB 105, PCB 138, PCB 156, PCB 180, PCB 169, hexachlorobenzene, aldrine, isodrine, 2-4-DDE, 4-4-DDE, dieldrine and endrine. The same "pools" methods as for TE measurements were chosen and samples were assessed by stir bar sorptive extraction-thermal desorption-gas chromatography-tandem mass spectrometry (SBSEGC-MS/MS) following Lacroix et al. (2014) method. The limits of quantification were calculated according to Shrivastava & Gupta (2011). Results are expressed in $ng.g^{-1}$ of dry weight.

A more precise version of protocols for biomarker of exposure analyses is available in appx. 3 for trace elements and in appx. 4 for organics pollutants.

4. Analyses of biomarkers of effects

Once at the lab, morphometrics and weights measurements were taken (length, width, thickness, weight, digestive gland weight for mussel and soft tissues weight for limpet). Then, soft tissues of limpets were used for biomarkers analyses, whereas only the digestive gland was selected for mussels. The high capacity of the digestive gland to accumulate pollutants and its participation in immune defense, detoxification, homeostatic regulation or even xenobiotic metabolism makes it an organ of choice in ecotoxicology (Fasulo *et al.*, 2012).

Tissues were ground in liquid nitrogen using a MM400 Retsch© (GmbH, Éragny, Luxembourg) mixer mill (fig. 3). Samples reserved for energetic stocks analyses (total lipids and glycogen contents) were hand homogenized on ice in 1 mL of citrate buffer (0.1 M, pH 5). Half was directly kept at - 80°C for lipids analyses, and the other half, reserved for glycogen analyses, was incubated 4 min in boiling water (~ 100°C) before being placed at - 80°C.

Samples reserved for enzymatic analyses were hand homogenized on ice in one volume of phosphate-buffered saline (PBS) (100 mM, pH 7.5) containing 0.1 % Triton X-100 and 1 mM ethylenediaminetetraacetic acid (EDTA), to limit protein degradation. They were centrifuged at 12500 g at 4°C for 15 min (Sorvall Legeng Micro 17R, ThermoFisher Scientific, Waltham, Massachusetts, USA). The supernatant was collected and divided into 8 aliquots and kept at - 80°C until further protein and enzymatic analyses.

Then, 12 biomarkers of effects were measured: 10 enzymatic biomarkers and two biomarkers of energetic stocks. Protein, glycogen, ACHE, LDH, PK, PEPCK, CAT, GPX, GST and GR analysis were performed in duplicates, and in triplicates for lipids, SOD and LAC. If necessary, dilution of homogenates was done to obtain linear slopes for 5 min minimum at 25°C. Values of total enzymatic activities measured during the lab were a posteriori normalized

by protein concentration to obtain specific activities values expressed in $U.mg^{-1}$ of protein. All chemicals used come from Sigma-Aldrich®.

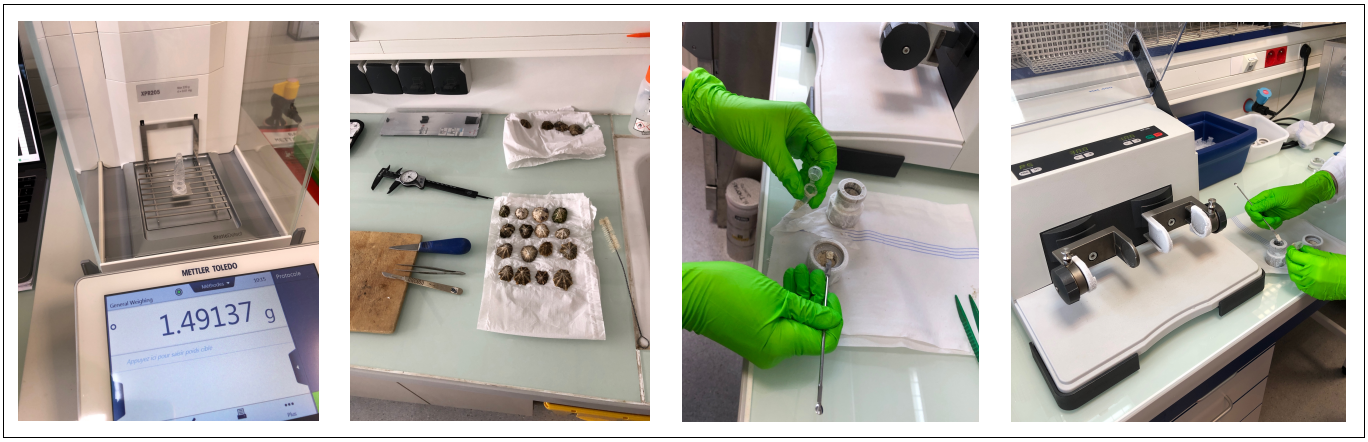

Figure 3: Photos of tissues preparation before the analyses of biomarkers of effects (©Lise LE VERN, University of Liège)

For each enzyme analyzed, all reaction equations, the associated based-literature references, and the final principle of each biomarker of effects are given in the table 2. As all enzyme activity methods were adapted for this study, a precise version of all performed protocols can be found in appx. 2. Measurements were done on a SpectroStar Nano spectrophotometer with UV microplates (BMG labtech, Champigny-sur-Marne, France).

The same methodology of sampling was carried out in January and September 2021 and in September 2020. Thus, all two-year data will be integrated into the present study.

Table 2: Reaction equations and bibliographic references of the measurement methods for each biomarker of effects.

	Reaction equation	Simplified final measurement	Bibliographic references
Protein	-	Total concentration of protein	Lowry *et al.*,1951
Glycogen	-	Total concentration of glycogen	Carr & Neff, 1984
Lipids	-	Total concentration of lipids	Frings *et al.*,1972
ACHE	acetylcholine $\xrightarrow{\text{AChE}}$ thiocholine + acetate thiocholine + DTNB $\longrightarrow$ 5-thio-2-nitrobenzoate	5-thio-2-nitrobenzoate production. Absorbance read at 405 nm, every 30 sec for 6 min	Ellman *et al.*,1961
LDH	pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ lactate + NAD^+	NADH consumption. Absorbance read at 340 nm, every 30 sec for 6 min	Childress & Somero, 1979; Bailey *et al.*,2005
PK	phosphoenolpyruvate + ADP $\xrightarrow{\text{PK}}$ pyruvate + ATP pyruvate + NADH + H^+ $\xrightarrow{\text{LDH}}$ lactate + NAD^+	NADH consumption. Absorbance read at 340 nm, every 30 sec for 6 min	Childress & Somero, 1979; Bailey *et al.*,2005
PEPCK	Oxaloacetate + H_2O + GTP $\longrightarrow$ phosphoenolpyruvate + HCO^{3-} + GDP L-Malate + NAD^- $\longrightarrow$ NADH + Oxaloacetate	NADH consumption. Absorbance read at 340 nm, every 30 sec for 6 min	Petrescu *et al.*,1979; Jamieson *et al.*,1999
SOD	$H_2O_2 : 2\ O_2{\bullet-} + H^+$ $\xrightarrow{\text{SOD}}$ $O_2 + H_2O_2$	NADH oxidation. Absorbance read at 340 nm, every 5 min for 1 h 10 min	Paoletti *et al.*,1986
CAT	H_2O_2 $\xrightarrow{\text{CAT}}$ ½ $O_2 + H_2O_2$	H_2O_2 consumption. Absorbance read at 240 nm, every 30 sec for 6 min	Aebi, H., 1984

	Reaction equation	Simplified final measurement	Bibliographic references
GPX	H_2O_2 + 2 glutathionnes (GSH) $\xrightarrow{GPx}$ 2 H_2O + glutathionne disulfide (GSSG) GSSG + NADPH + H^+ $\xrightarrow{GR}$ 2 GSH + $NADP^+$	NADPH consumption by GR. Absorbance read at 340 nm, every 30 sec for 6 min	Paglia & Valentine, 1967; Janssens *et al.*,2000
GST	GSH + 1-chloro-2,4-dinitrobenzène (cDNB) $\xrightarrow{GST}$ glutathionyl-dinitrobenzene (GS-DNB) + HCl	GS-DNB production. Absorbance read at 340 nm, every 30 sec for 6 min	Habig *et al.*,1974
GR	GSSG + NADPH + H^+ $\xrightarrow{GR}$ 2 GSH + $NADP^+$	NADPH consumption. Absorbance read at 340 nm, every 30 sec for 6 min	Calberg, I. & Mannervik, B., 1985
LAC	*p*-phenylenediamine + O_2 $\xrightarrow{LAC}$ H_2O + *p*-benzo-quinone diimine	*p*-phenylenediamine consumption. Absorbance read at 420 nm, every 5 min for 2h	Pomerantz, 1963; Luna-Acosta *et al.*,2010

5. Statistical analysis

All statistical analyses were performed using R version 4.2.0 and RStudio 2022.02.3 (see R script in appx 5). First, Pearson's correlation was used to verify good correlation between total and specific enzymatic activities. This was intended to allow the continuation of data treatment on specific activity only. Other Pearson correlation tests were done between morphometric measurements, physicochemical parameters, and biomarkers, in addition to parametric one-way analyses of variance (ANOVA), used to highlight any significant differences of these parameters between sites.

In order to compare the effect of location on enzyme specific activity, a one-way ANOVA was carried out for each species separately, followed by Tukey post-hoc. Conditions of normality and homoscedasticity of the residuals of ANOVAs were tested with Shapiro-Wilk test and Levene's test, respectively. When needed, data were log10 transformed so that normality conditions were respected. In case of heteroscedasticity of residuals even with transformed data, a Kruskal-Wallis test was performed, followed by Dunn's post-hoc test. If there were only heteroscedasticity, a Fisher post-hoc test was used after ANOVA. For mussels, a two-way ANOVA permitted firstly to test the effect of "site" and "condition" and their interaction. For all statistical tests, the level of significance was set at 0.05.

The Integrated Biomarker response index (IBR) was finally calculated according to Devin *et al.* (2014) initially developed by Beliaeff & Burgeot (2002). Calculation of this index was done thanks to the IBRtools package. Briefly, a 4 steps calculation based on each biomarker standardized mean permit to build a star plot of the different biomarker responses (from 3 up to 9 biomarkers) for each species. Computation of the star plot area gave a value: the IBR. Here, only based on biomarkers of effect, an increasing IBR value was interpreted as an increase of stress in the given environmental conditions. This tool allows easy interpretation keys for ecological managers to assess and monitor environmental quality. The IBR was calculated for each species in the 2022 sampling. To assess any differences between ports, a Kruskal-Wallis test was finally done because of heteroscedasticity of residuals, followed by Dunn's post-hoc test.

All measures were expressed as mean ± standard deviation (SD).

Finally, no statistical analysis was performed on trace element and organics pollutant 2022's data as these measurements were done on pools of individuals. Those data only provide general trends to help the interpretation of biomarkers of effect.

RESULTS

1. Morphometrics measurements

For mussel width (35.47 ± 3.15 mm) and digestive gland weight (1.06 ± 0.3 g), no significant differences between locations were found (table 3). However, mussels from Calvi were significantly longer than those from the other ports (table 3) (ANOVA, F = 4.90; p = 0.004), but also the thickest and the heaviest (ANOVA, thickness: F = 4.87; p = 0.003 and weight: F = 9.78; p = $1.999e^{-05}$, respectively). For those three parameters significantly different among locations, if individuals from Calvi always presented the most important average values, specimens from St-Florent almost always showed the lowest ones. Only width and digestive gland weight average were lowest for mussels from Île Rousse (table 3).

Regarding limpet, two of the three morphometrics measurements (width, and weight) showed significant differences between ports (AVOVA, F = 4.90; p = 0.006 and F = 4.19; p = 0.012, respectively) (table 3). For this species, individuals from St-Florent were also globally the smallest. On the contrary, organisms from STARESO were the biggest one, up to almost 3-fold heavier than those coming from Île Rousse.

Table 3: Average morphometric measurements of *M. galloprovincialis* and *Patella sp.* collected in the four North Corsican studied ports in January 2022. Grey cells highlight the data when a significant difference was found (p-value < 0.05).

	Mussels				Limpets			
	STARESO	**Calvi**	**Île Rousse**	**St Florent**	**STARESO**	**Calvi**	**Île Rousse**	**St Florent**
Length (mm)	73.73 ± 6.56^{ab}	76.83 ± 6.34^{a}	71.50 ± 5.57^{b}	70.06 ± 5.49^{b}	36.77 ± 4.43	35.37 ± 4.60	32.52 ± 4.04	31.82 ± 3.79
Width (mm)	35.64 ± 2.98	36.98 ± 3.75	34.67 ± 2.75	34.69 ± 2.53	32.07 ± 2.71^{a}	29.12 ± 5.45^{ab}	27.01 ± 3.90^{ab}	25.71 ± 2.33^{b}
Thickness (mm)	23.92 ± 2.00^{ab}	24.89 ± 2.63^{a}	23.14 ± 1.87^{b}	22.46 ± 1.12^{b}	-	-	-	-
Weight (g)	20.00 ± 4.60^{ab}	22.76 ± 4.70^{a}	17.64 ± 3.62^{b}	16.36 ± 3.20^{b}	2.93 ± 1.15^{a}	1.80 ± 1.05^{ab}	1.38 ± 0.62^{b}	1.70 ± 0.52^{b}
Digestive gland weight (g)	1.16 ± 0.23	1.15 ± 0.32	0.96 ± 0.20	1.01 ± 0.36	-	-	-	-

2. Biomarkers of exposure

In total, 18 TEs, 22 PAHs, 14 PCBs, and 7 pesticides have been analyzed in pools of organisms (table 4 and 5). As no pesticides were measured above the detection limits. They are not presented.

2.1. Trace elements

In mussel, STARESO presented the most important value only for Ba. In organisms collected at Calvi, quite important concentrations of Cu were observed compared to the other sites. A value about 3.5-fold more important in Île Rousse's faring area (2.06 $\mu g.g^{-1}$) than near its gas station (0.58 $\mu g.g^{-1}$) can be noticed. The highest values of As, Cd, Mn and Zn were found at Île Rousse. Relatively high values of Al, Fe, Mn, Ni and Se were observed at St-Florent compared to the other locations. In general, this latter port, which presented the highest values for 7 of the 18 TE measured in mussel's tissues, seemed to be the most contaminated.

In limpet, the TE pattern was completely different from the mussel one. The most contaminated port was STARESO with the highest values measured for 6 of the 18 ETs investigated (Ag, As, Ba, Cd, Mo, Sb). Relatively important Cu concentration (0.43 $\mu g.g^{-1}$) was found in this port, representing more than 2-fold the Cu concentration observed at Île Rousse and more than 5-fold those of Calvi and St-Florent. For limpet's tissues also, Calvi showed the highest Cu concentration. Île Rousse presented the highest value of the four sites for Fe, Pb and particularly Al. Finally, compared to other ports, St-Florent presented the highest concentrations only for Co, Mn, and Ni. It presented the lowest values for 11 of the 18 TEs measured and was thus the less contaminated port, consistent with IBR mean value found in January 2022 for limpet at St Florent (see section 4).

2.2. Organic pollutants

Regarding the organic pollution, no pesticides were detected in North Corsica in January 2022. PAHs were mainly found in limpet's tissues, in all ports but especially in Calvi and Île Rouse. The two principal PAHs detected everywhere were the naphthalene and the phenanthrene, with higher levels at Calvi**.** For mussels, only four PAHs were detected in very low concentration in the fairing zone of Île Rousse. In this species, PCB 153 and 138 were found in tissues collected in the four studied ports, and PBC 101, 135 and 156 were found in addition at STARESO. In limpet tissues, some PCBs were only detected at STARESO (PBC 135, 153, 138, 156, 180). Notice that values are generally very low.

Table 4: Trace elements concentration (in µg.g^{-1} of wet weight) in soft tissue of *M. galloprovincialis* and *Patella sp*. (1 pool of 8 individuals per port). Numbers in bold are the highest values for each species. Reglementary maximum levels presented only concern bivalve mollusks.

	Mussel							Limpet				Reglementary maximum levels (mg.kg^{-1})
	STARESO	Calvi		Île Rousse		St Florent		STARESO	Calvi	Île Rousse	St Florent	
		Oil	Fairing	Oil	Fairing	Oil	Fairing					
Ag	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	**0.05**	0.02	< 0.01	< 0.01	
Al	2.91	9.14	7.62	3.41	5.67	**18.07**	**13.8**	59.19	54.21	**132.08**	19.24	
As	1.63	1.51	1.27	**1.57**	**1.71**	1.62	1.12	**5.83**	3.13	5.32	2.12	
Ba	**0.06**	0.04	0.03	0.03	0.03	0.02	0.03	**0.28**	0.13	0.17	0.07	
Cd	0.11	0.08	0.09	**0.10**	**0.09**	0.08	0.04	**0.43**	0.08	0.18	0.06	**1**
Co	0.03	0.02	0.02	0.03	0.02	**0.04**	**0.02**	0.07	0.06	0.10	**0.12**	
Cr	0.07	0.11	0.14	0.1	0.11	**0.24**	**0.16**	0.43	**0.46**	0.46	0.31	
Cu	0.61	**4.97**	**4.69**	0.58	2.06	1.60	2.30	2.48	**14.97**	6.52	3.06	
Fe	7.13	19.19	15.20	7.95	12.55	**31.12**	**23.27**	356.06	286.12	**406.83**	121.87	
Mn	0.36	0.42	0.34	0.29	0.42	**0.79**	**0.75**	0.86	0.87	1.06	**2.18**	
Mo	0.04	0.03	0.03	**0.03**	**0.04**	0.04	0.03	**0.18**	0.10	0.12	0.09	
Ni	0.03	0.02	0.03	0.03	0.03	**0.16**	**0.10**	0.47	0.30	0.26	**0.47**	
Pb	0.07	**0.09**	**0.07**	0.06	0.07	0.05	0.04	0.93	0.57	**1.02**	0.05	**1.5**
Sb	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	< 0.04	
Se	0.35	0.26	0.2	0.22	0.24	**0.62**	**0.33**	**2.27**	2.03	2.05	0.93	
Sn	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	< 0.02	0.03	< 0.02	< 0.02	
V	< 1	< 1	< 1	< 1	< 1	< 1	< 1	< 1	**1.21**	< 1	< 1	
Zn	18.71	16.82	15.81	**14.04**	**17.03**	16.06	9.51	11.27	**32.55**	23.42	10.38	

Table 5: Organic pollutants concentration (ng.g^{-1} of wet weight) in soft tissue of *M. galloprovincialis* and *Patella sp*. (1 pool of 8 individuals per port). < LD = below the detection limit. < LQ = below the quantification limit. To help table reading, numbers in red represent values egal or above 1.0 ng/g.

		Mussel							Limpet				Reglementary maximum levels (µg.kg^{-1})
		STARESO	Calvi		Île Rousse		St Florent		STARESO	Calvi	Île Rousse	St Florent	
			Oil	Fairing	Oil	Fairing	Oil	Fairing					
PAHs	naphthalene	< LD	< LD	< LD	< LD	< LQ	< LD	< LD	5.4	10.5	8.2	6.6	
	benzothiophene	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	biphenyl	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LQ	< LQ	< LQ	0.5	
	acenaphthylene	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	acenaphthene	< LD	< LD	< LD	< LD	< LD	< LQ	< LQ	< LD	1.9	< LD	< LD	
	fluorene	< LD	< LQ	< LD	< LQ	< LQ	< LQ	< LQ	0.4	2.7	< LQ	< LQ	
	dibenzothiophene	< LD	< LD	< LD	< LQ	< LD	< LD	< LD	< LD	0.7	< LD	< LD	
	phenanthrene	< LD	< LQ	< LQ	< LQ	0.7	< LD	< LQ	1.2	11.5	7.1	2.9	
	anthracene	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	1.0	0.7	< LQ	
	fluoranthene	< LD	< LD	< LD	< LD	< LQ	< LD	< LD	< LD	0.8	< LD	< LD	
	2-methylfluoranthene	< LD	< LD	< LD	< LD	< LQ	< LD	< LQ	< LD	1.0	2.6	0.9	
	pyrene	< LD	0.1	0.1	< LD	0.2	< LD	< LQ	< LD	< LQ	0.2	< LD	
	benzo(a)anthracene	< LD	< LQ	< LQ	< LD	0.3	< LD	0.1	< LD	0.2	0.9	< LD	10*
	chrysene	< LD	< LQ	< LD	< LD	0.8	< LD	< LD	< LD	< LQ	3.8	< LD	10*
	benzo(b)fluoranthene	< LD	< LD	< LD	< LD	< LQ	< LD	< LD	< LD	< LD	< LD	< LD	10*
	benzo(k)fluoranthene	< LD	< LD	< LD	< LD	< LQ	< LD	< LD	< LD	< LD	0.9	< LD	
	benzo(e)pyrene	< LD	< LD	< LD	< LD	< LQ	< LD	< LD	< LD	< LQ	1.9	< LD	
	benzo(a)pyrene	< LD	< LD	< LD	< LD	< LQ	< LD	< LD	< LD	< LD	< LQ	< LD	5

		Mussel							Limpet				Reglementary maximum levels ($\mu g.kg^{-1}$)
		STARESO	Calvi		Île Rousse		St Florent		STARESO	Calvi	Île Rousse	St Florent	
			Oil	Fairing	Oil	Fairing	Oil	Fairing					
	perylene	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	indeno(1,2,3-*cd*)pyrene	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	dibenzo(a.h)anthracene	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	benzo(g.h.i)perylene	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LQ	< LD	
PCBs	PCB 7	< LD	< LD	0.0	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	75**
	PCB 28	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	PCB 52	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	PCB 35	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	PCB 101	0.7	< LQ	< LD	< LQ	< LQ	< LQ	0.4	< LQ	< LD	< LD	< LD	
	PCB 77	< LD	< LD	< LD	< LQ	< LD	< LD	< LD	< LD	< LD	< LD	< LD	
	PCB 135	0.2	< LQ	< LQ	< LQ	< LQ	< LD	< LQ	0.1	< LD	< LD	< LD	
	PCB 118	< LQ	< LQ	< LQ	< LD	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LD	
	PCB 153	2.1	0.9	< LQ	< LQ	0.8	< LQ	1.0	2.3	< LQ	< LQ	< LD	
	PCB 105	< LQ	< LD	< LQ	< LD	< LD	< LD	< LQ	< LD	< LQ	< LQ	< LD	
	PCB 138	2.5	0.9	< LQ	< LQ	0.9	< LQ	1.0	2.0	< LQ	< LQ	< LD	
	PCB 156	0.1	< LD	< LD	< LQ	< LD	< LD	< LD	0.1	< LD	< LQ	< LD	
	PCB 180	< LQ	< LD	< LD	< LQ	< LD	< LD	< LQ	1.1	< LQ	< LQ	< LD	
	PCB 169	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LD	< LQ	< LD	

* Correspond to the sum of benzo(a)pyrene. benzo(a)anthracene. benzo(b)fluoranthene and chrysene.
** Correspond to the sum of PCB28, PCB52, PCB138, PCB14 and PCB180.

3. Biomarkers of effect

For each species, biomarkers measured showed significant correlation between total and specific enzymatic activity (correlation coefficient between 0.55 and 0.94 depending on the biomarkers. $p < 0.001$), except for LDH (correlation coefficient = 0.338; $p = 0.05$) (appx. 6). Here are only presented specific activity assessments for January 2022 sampling. Total activity can be found in appx. 7.

3.1. Two-way ANOVA tests

For mussel's biomarkers, a two-way ANOVA was performed to test, in addition to the effect of sites (ports) and intra-ports conditions ("gas station" and "fairing" conditions), their interactions (table 6).

Significant effects of the two factors had been highlighted in protein concentration and GST activity (fig. 4). The concentration was higher in the fairing zone of Calvi port while the lowest values were found in the gas station zone of Île Rousse. For GST activity, on the contrary, the highest values were found in the faring zone for Île Rousse, and the lowest ones in organisms collected in the faring zone of St Florent. For the log-transformed CAT activity, Tukey post-hoc test didn't show any contrasts, and is therefore not presented.

The statistical test detected an interaction between the two factors (site + conditions) on PK ($F = 3.9698$; $p = 0.02486$), GR ($F = 3.6220$; $p = 0.03444$) and SOD ($F = 4.3671$; $p = 0.01822$) specific activity.

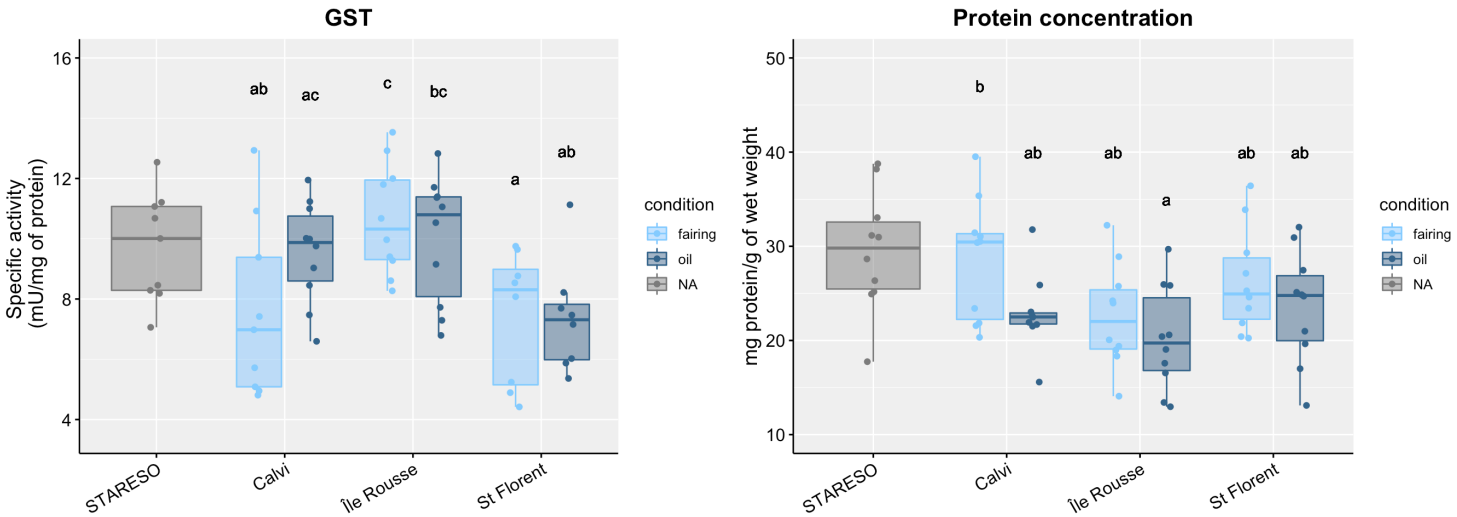

Figure 4: Significant results provided by a two-way ANOVA regarding site and conditions factors. The test was performed for the digestive gland of *M. galloprovincialis* (n = 20) and soft tissues of limpets (n = 7-10) collected in January 2022. Values are expressed as mean ± standard deviation. For each species, letters indicate significant differences between ports (ANOVA test followed by Tukey's post-hoc test, with p-value < 0.05).

Table 6: Results of two-way ANOVA performed to test the effect of sites and conditions in biomarkers of effects of *M. galloprovincialis* digestive gland collected in January 2022.

	Factor	F ratio	*p* value	Interaction p-value
Protein*	Site	3.44	**0.039***	
	Condition	6.22	**0.016***	
Glycogen	Site	0.19	0.829	
	Condition	3.76	0.059	
Lipids	Site	0.13	0.877	
	Condition	1.11	0.298	
Log 10 transformed	Site	0.14	0.871	0.025*
PK	Condition	0.66	0.19	
PEPCK	Site	2.17	0.125	
	Condition	0.05	0.818	
GPX	Site	3.06	0.061	
	Condition	0.28	0.598	
GST*	Site	9.85	**< 0.001***	
	Condition	0.91	0.345	
GR	Site	1.12	0.336	0.034*
	Condition	0.99	0.325	
LAC	Site	1.71	0.19	
	Condition	0.82	0.37	
SOD	Site	2.23	0.119	0.018*
	Condition	0.32	0.574	
Log 10 transformed	Site	4.11	**0.023***	
CAT	Condition	0.09	0.769	
ACHE	Site	0.38	0.688	
	Condition	0.03	0.865	

As the two-way ANOVAS didn't provide many results regarding the condition factors and interactions between the two factors, a one-way ANOVA for each factor separately was also performed, presented in following results. However, it is important to keep in mind that any significant results from one-way ANOVA tested for the two factors (site + conditions) on PK, GR, and SOD specific activity can't be interpreted by associating the response directly to the factor tested, as it could be the response of the interaction between factors.

3.2. One-way ANOVA tests

3.2.1. Gas station and fairing conditions

Except at STARESO, an intra-ports subdivision has been done in all the studied ports to test the effect of the two conditions (gas station and faring zone), using an active biomonitoring by mussels caging.

At Calvi, mussels located in the fairing zone were significantly more concentrated in protein (28.54 ± 6.47 $mg.g^{-1}$ of wet weight) than those placed near the gas station (22.90 ± 4.03 $mg.g^{-}$

^{1} of wet weight) (ANOVA, F = 5.48; p = 0.030). They also presented significantly higher lipids concentration near the fairing zones (12.20 ± 5.72 mg.g^{-1} of wet weight) compared to the gas station (7.03 ± 3.42 mg.g^{-1} of wet weight) (ANOVA, F = 5.68; p = 0.029).

At Île Rousse, one-way ANOVA tests didn't show any significant variation between conditions.

Finally, at St-Florent, SOD specific activity was significantly higher in mussels located in the fairing zone (15.78 ± 5.58 U.mg^{-1} of protein) compared to those placed near the gas station (9.01 ± 6.62 U.mg^{-1} of protein) (ANOVA, F = 5.85; p = 0.027).

3.2.2. Energetic stocks concentration

For each species, the energetic stocks contents (glycogen, lipids, and protein) followed a quite different trend between ports (fig. 5).

In mussels, protein concentration showed significantly higher mean values at STARESO (29.50 ± 6.39 mg.g^{-1}) (ANOVA, F = 4.58; p = 0.005). The least concentrated have been collected at Île Rousse (21.40 ± 5.48 mg.g^{-1}). For this species, lipids and glycogen contents weren't significantly different among locations. However, their highest values were found at STARESO (fig. 5).

The glycogen content of limpets from STARESO (9.45 ± 3.56 mg.g^{-1}) were almost 200% more concentrated than those from Calvi (5.59 ± 2.66 mg.g^{-1}) (ANOVA, F = 3.01; p = 0.045).

Regarding lipids concentration, no significant inter-location differences were observed in any species, However, the gap between mussels and limpet's values can be noticed. Mussel's tissues were about 2-fold more concentrated in lipids than limpet's tissues.

Furthermore, lipids and glycogen were slightly correlated to water temperature (Pearson correlation's coefficient of -0.528 for lipids and -0.518 for glycogen) (appx. 6).

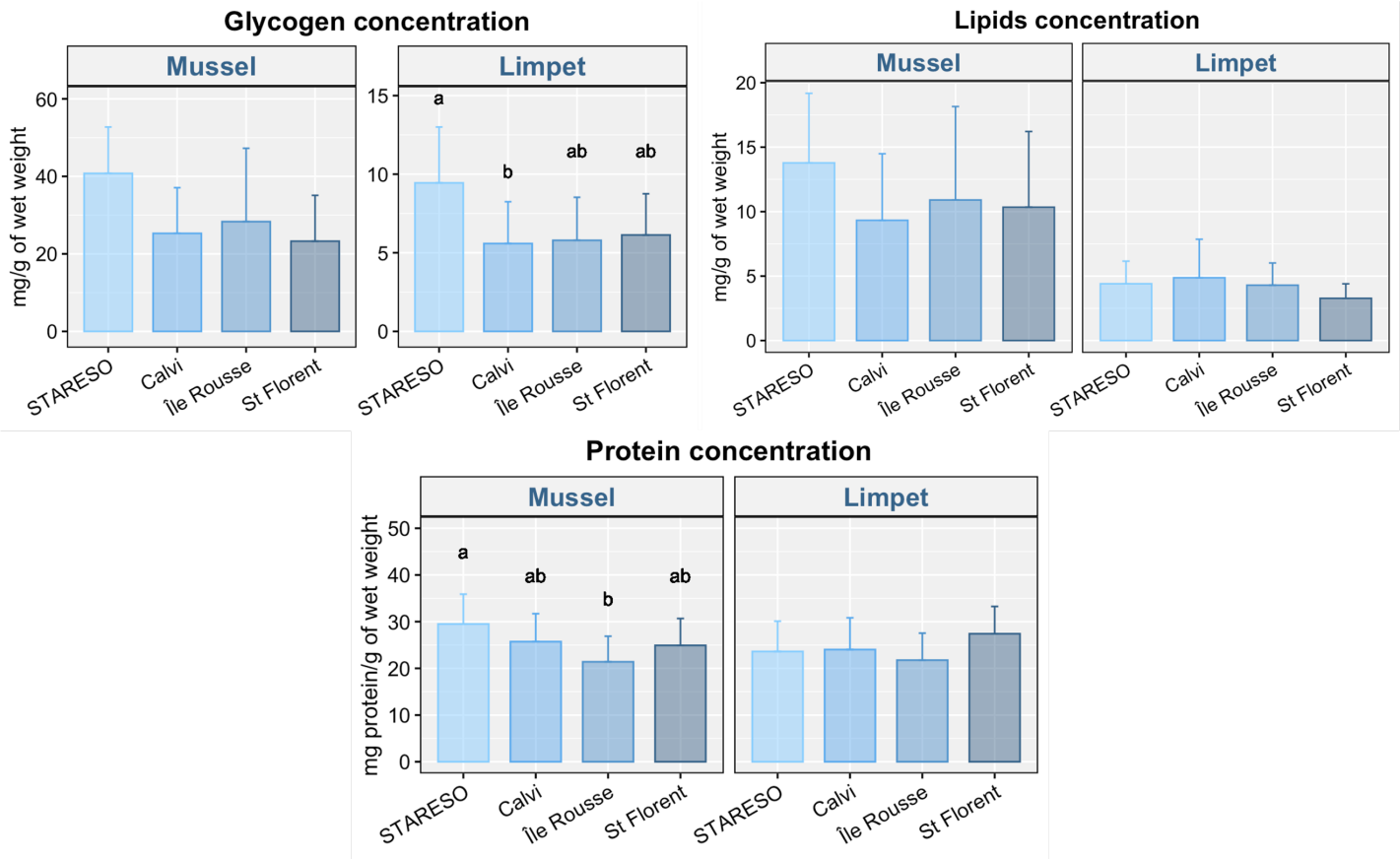

Figure 5: Glycogen, lipids, and protein concentration (mg.g^{-1} of wet weight) in the digestive gland of *M. galloprovincialis* (n = 20) and soft tissues of *Patella sp.* (n = 7-10) for each site and collected in January 2022. Values are expressed as mean ± standard deviation. For each species, letters indicate significant differences between ports (ANOVA test followed by Tukey's post-hoc test, with p-value < 0.05).

3.2.3. Biomarkers of energetic metabolism (LDH, PK, PEPCK)

Specific activity of LDH, PK and PEPCK of mussels and limpets collected in North Corsica ports in January 2022 are displayed in figure 6.

In mussels, PK and PEPCK specific activities remained stable between locations, with respective values of 14.74 ± 4.86 mU.mg^{-1} of protein, and 11.23 ± 6.18 mU.mg^{-1} of protein.

In limpets, only PK varied significantly between ports (ANOVA, F = 3.98; p = 0.018). The highest PK specific activities were found for specimens sampled at STARESO (49.25 ± 13.00 mU.mg^{-1} of protein), while the lowest values were calculated at Calvi (28.29 ± 11.46 mU.mg^{-1} of protein) and St-Florent (24.12 ± 12.68 mU.mg^{-1} of protein). No significant differences of LDH (mean of 6.58 ± 1.44 mU.mg^{-1} of protein) and PEPCK (3.39 ± 0.95 mU.mg^{-1} of protein) among locations were observed for this species.

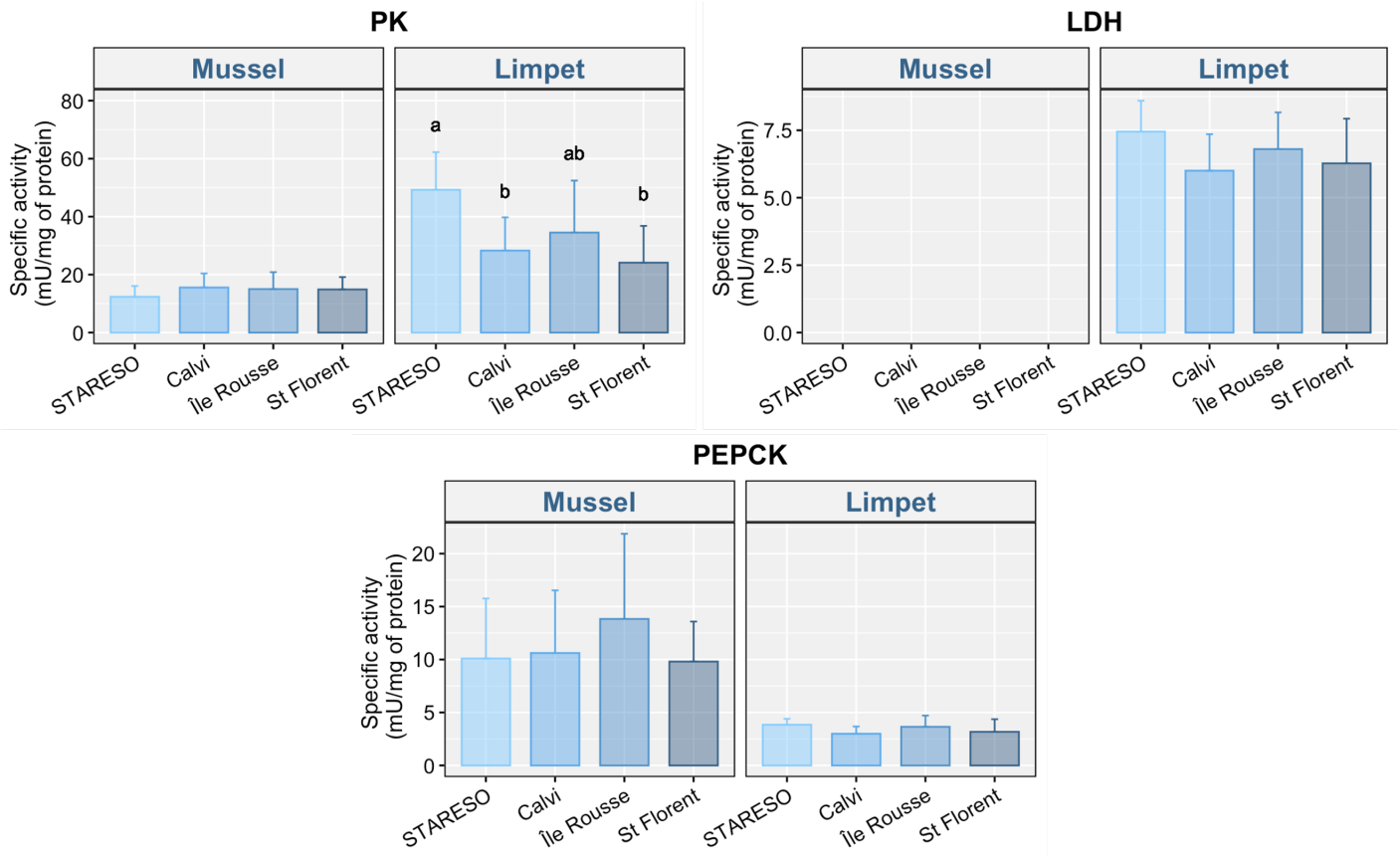

Figure 6: Biomarkers of energetic metabolism assessed in the digestive gland of *M. galloprovincialis* (n = 20) and soft tissues of *Patella sp.* (n = 7-10) collected in each port in January 2022. Pyruvate kinase (PK), lactate dehydrogenase (LDH), and phosphoenolpyruvate carboxykinase (PEPCK) specific activities are expressed as mean ± standard deviation. in mU/mg of protein. For each species, letters indicate significant differences between ports (ANOVA test followed by Fisher's post-hoc test, p-value < 0.05).

3.2.4. Biomarkers of antioxidants defenses (GR, GPX, GST, SOD, CAT)

Five biomarkers of antioxidants defenses were measured for each species in North Corsica ports in January 2022 (fig. 7).

Two biomarkers responded significantly in mussel's tissues. The GST activity showed its highest values at STARESO and Île Rousse (9.72 ± 1.81 mU.mg^{-1} of protein and 10.32 ± 1.95 mU.mg^{-1} of protein. respectively), and its lowest ones at St-Florent (7.15 ± 2.13 mU.mg^{-1} of protein) (ANOVA. F = 7.16; p = 0.0003). Regarding CAT specific activity, highest values were found at Île Rousse (0.69 ± 0.21 U.mg^{-1} of protein) whereas STARESO and St-Florent presented the lowest mean (0.49 ± 0.22 U.mg^{-1} of protein, and 0.50 ± 0.19 U.mg^{-1} of protein, respectively) (ANOVA, F = 3.74; p = 0.016). Statistical analyses didn't detect any significant differences among ports for GR (5.96 ± 1.32 U.mg^{-1} of protein), GPX (2.38 ± 1.04 mU.mg^{-1} of protein), and SOD (15.88 ± 8.86 U.mg^{-1} of protein) specific activities.

In limpets, SOD was the only antioxidative enzyme of this category to provide significantly different responses among ports. Here, St-Florent specimens showed notable lower specific

activity (2.14 ± 2.15 U.mg^{-1} of protein) than those from STARESO, Calvi and Île Rousse (9.31 ± 4.80; 10.68 ± 5.06 and 9.31 ± 2.39 U.mg^{-1} of protein, respectively) (ANOVA, F = 8.376; p = 0.0003). CAT activity's trend remained stable even after log10 transformation (0.63 ± 0.23 U.mg^{-1} of protein). In general, lower values were observed at St-Florent for GPX, GST, SOD and CAT specific activity of mussels, and for GR, GPX and SOD specific activity of limpets, so a large part of the tested biomarkers of antioxidants defenses (fig. 7).

SOD and GST were correlated to water temperature (Pearson's correlation coefficient of -0.510 for SOD and -0.629 for GST) (appx. 6).

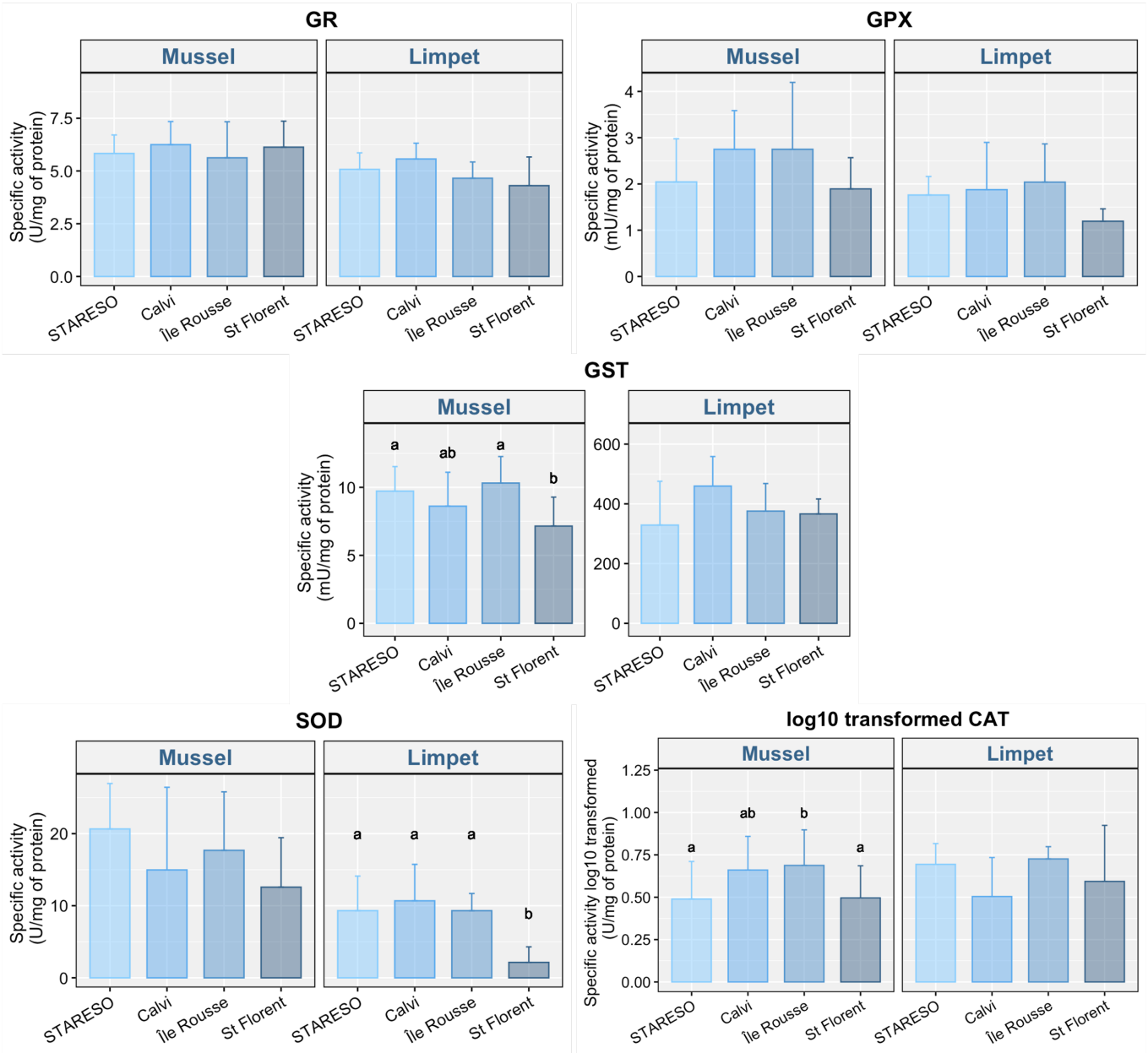

Figure 7: Biomarkers of antioxidants defenses assessed in the digestive gland of *M. galloprovincialis* (n = 20) and soft tissues of *Patella sp.* (n = 7-10) collected in each port in January 2022. Specific activities of glutathione peroxidase (GPX) and glutathione-s-transferase (GST) (mU/mg of protein), glutathione reductase (GR), superoxide dismutase (SOD) and log transformed catalase (CAT) (U/mg of protein) are expressed as mean ± standard deviation. For each species, letters indicate significant differences between ports (ANOVA test followed by Tukey's post-hoc test, p-value < 0.05).

3.2.5. *Biomarkers of immune system (LAC) and neurotoxicity (ACHE)*

The two last biomarkers of effects assessed during this project were the LAC and the ACHE, respectively involved in immune system and neurotoxicity.

ACHE specific activity stayed stable among locations for both species (1.76 ± 0.55 mU.mg^{-1} of protein for mussels and 6.56 ± 3.12 mU.mg^{-1} of protein for limpets) (fig. 8).

LAC specific activity also remained stable between ports in limpet's tissues. For mussels, specific activity of this enzymes was higher at Calvi (0.79 ± 0.15 mU.mg^{-1} of protein) and St-Florent (0.79 ± 0.16 mU.mg^{-1} of protein) than STARESO (0.59 ± 0.13 mU.mg^{-1} of protein) (ANOVA, F = 4.35; p = 0.007).

Furthermore, LAC was DO and temperature correlated (correlation coefficient DO = 0.466; Temperature = -0.525) (appx. 6).

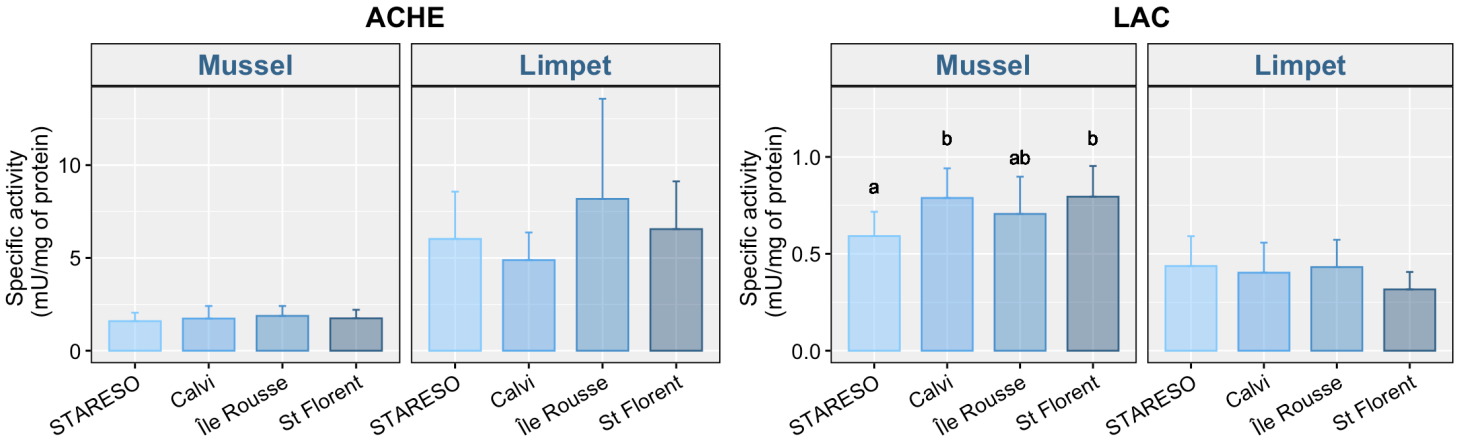

Figure 8: Biomarkers of immune system, acetylcholinesterase (ACHE) and neurotoxicity, laccase (LAC) assessed in the digestive gland of *M. galloprovincialis* (n = 20) and soft tissues of *Patella sp.* (n = 7-10) collected in each port in January 2022. Values represent specific activities expressed as mean ± standard deviation, in mU/mg of protein. For each species, letters indicate significant differences between ports (ANOVA test followed by Tukey's post-hoc test, p-value < 0.05).

4. The integrated biomarker response (IBR)

4.1. *January 2022 sampling*

The IBR was assessed through seven biomarkers of effects for mussel (Glycogen, ACHE, CAT, SOD, LAC, GST, PK) and eight for limpet (Lipid, Glycogen, ACHE, SOD, LAC, GST, LDH, PK) (fig. 9.a and 9.b). Mean IBR values per port for the sampling period of January 2022 are presented in fig. 9.

For each species, St-Florent displayed the lowest IBR value (1.168 ± 0.7 for mussels and 0.236 ± 0.1 for limpets).

Mussel seemed to be more stressed at Île Rousse port (7.658 ± 0.2), and the most stressed limpet were collected at STARESO (7.466 ± 0.4). Finally, both species coming from Île Rousse presented higher IBR mean than those coming from Calvi.

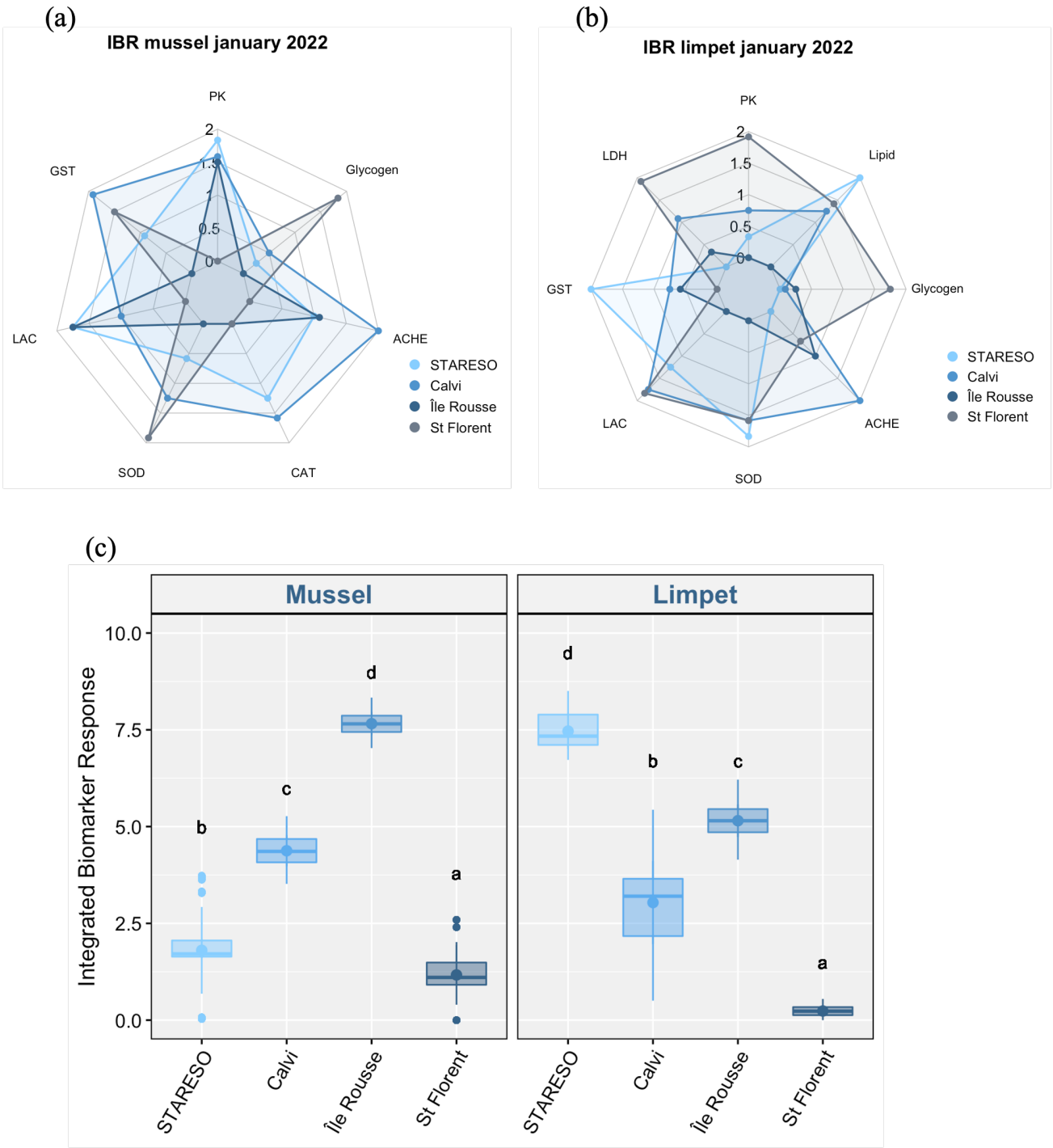

Figure 9: (a). (b). Star plots of standardized response of a selection of biomarkers of effect in *M. galloprovincialis* and *Patella sp.*, collected in North Corsica ports in January 2022. (c). Mean Integrated Biomarkers Responses (IBR, mean ± standard deviation). For each species, letters indicate significant differences between ports (Kruskal-Wallis test followed by Dunn's post-hoc test. p-value < 0.05).

4.2. All sampling periods of the QUAMPO project

Finally, the table 7 gathers IBR mean values calculated on data acquired throughout the QUAMPO project.

Mussel's IBR provided a completely different trend for each sampling period. STARESO presented the highest value in September 2020, the lowest one in January 2021, and a decrease can be observed since September 2021. Individuals collected at Calvi always provide quite high value of IBR and were the most stressed in September 2021. If Île Rousse was the most stressful port for mussel in January 2022, it was not always the case. Since the beginning of the project, it presented values among the lowest, compared to the other ports. Finally, St-Florent appeared twice less stressful for this species (September 2020 and this year).

For limpet, since January 2021, the highest values of IBR have been calculated for individuals collected at STARESO, with values exceeding seven and increasing with time. In September 2020, it was at Calvi that limpet seemed to be the most stressed. At Calvi, limpet showed higher IBR values after summer (September months) than during winter (January months). Individuals coming from St-Florent also provided the lowest IBR value in January 2022, which means that the port would be a less stressful environment than the others this year, for this species. However, this was not always the case since he was second to last in the first two sampling of the project (September 2020 and January 2021). A downward trend of IBR value has been observed for this port since January 2021.

Table 7: Integrated biomarkers response (IBR, mean ± SD) calculated for *M. galloprovincialis* and *Patella sp*. using biomarkers of effects analyzed since the beginning of QUAMPO project (data from September 2020, January 2021 and September 2021 communicated by Pillet, M.). For each species, different colors permit an easier ready read of the table, from the lightest (white) to the darkest values going from the lowest to the highest respectively.

	Mussels				**Limpets**			
	STARESO	**Calvi**	**Île Rousse**	**St Florent**	**STARESO**	**Calvi**	**Île Rousse**	**St Florent**
September 2020	3.978 ± 0.62^{a}	/	2.386 ± 0.50^{b}	0.533 ± 0.64^{c}	2.128 ± 0.81^{b}	4.748 ± 0.49^{d}	1.026 ± 0.48^{a}	3.563 ± 0.78^{c}
January 2021	0.556 ± 0.42^{a}	5.976 ± 0.49^{c}	1.643 ± 0.91^{b}	6.674 ± 0.41^{d}	7.288 ± 0.74^{d}	0.989 ± 0.32^{a}	1.158 ± 0.71^{b}	3.997 ± 0.61^{c}
September 2021	2.950 ± 0.83^{c}	6.144 ± 0.76^{d}	1.978 ± 0.63^{a}	2.509 ± 0.57^{b}	7.388 ± 0.14^{d}	3.270 ± 0.85^{c}	1.206 ± 0.43^{a}	2.981 ± 1.23^{b}
January 2022	1.803 ± 1.12^{b}	4.376 ± 0.4^{c}	7.659 ± 0.3^{d}	1.168 ± 0.77^{a}	7.466 ± 0.46^{d}	3.038 ± 1.07^{b}	5.152 ± 0.42^{c}	0.236 ± 0.13^{a}

DISCUSSION

As part of the QUAMPO project, this master thesis provides supplementary data on the first biomonitoring applied in Calvi, Île Rousse and St-Florent using a multibiomarker approach of two different species. A combination of native (limpet) and transplanted (mussel) organisms was used to better integrate real concentrations of contaminants and their biological effects, as recommended by several authors (Nigro *et al.,*2006; Marigómez *et al.,*2013; Lacroix *et al.,*2015).

1. Active biomonitoring of mussels in North Corsican ports

1.1. Inter-ports chemical contamination (ET, organics pollutants)

A "pool" sampling technique has been used to determine element traces and organics pollutants concentrations in the two studied models. This technique, allowing to have reliable general trend of contamination of the different sites at lower costs, were validated by other study and even tested and validated at the start of the QUAMPO project (Martincié *et al.,*1984; Turle & Collins, 1992; Breitwieser *et al.,*2018). Notice that this technique still present inconvenient as extreme or abnormal values can't be detected.

In most studies, only principal TE (Cu, Ni, Cd, Cr, Pb or Zn, Fe, V) known as highly toxics if present in tissues in large concentrations (Conti & Cecchetti, 2003; Serafim *et al.,*2011; Cravo *et al.,*2012; Marigómez *et al.,*2013; Breitwieser *et al.,*2020) are monitored. In this study, the concentration of 18 TE was measured. In mussel, Ag, Sb, Sn and V was under the minimum detection limit. Ba, Co and Mo concentration values were also very low. The mean bioaccumulation pattern of most concentrated TE was Fe > Zn > Al > Cu > As > Mn > Se > Cr > Cd > Pb > Ni. Others studies observed a pattern of the most widely monitored TE equal to Fe > Zn > Mn > Cu > Ni > Cr > Pb > Cd (Roméo *et al.,*2003; Andral *et al.,*2004; Lafabrie *et al.,*2007; Richir & Gobert, 2014; Caricato *et al.,*2019). The observed pattern tends to show a possible contamination in Al and Cu of North Corsican ports in January 2022, since they are placed at higher ranks. Richir & Gobert (2014) already brought data from Corsican coast (table 8). When comparing their values with those measured as part of this master thesis, a notable increase of some TE concentrations since 2014 can be observed. A contamination in Al appeared at St-Florent and Calvi (measured values showed an increase of about 350% and 190%). In all sites except STARESO, Cu showed particularly high levels compared to the previous study (our mussels from Calvi presented Cu levels close to 15-fold higher, with mean concentration of 44.67 $\mu g.g^{-1}$ dry weight, against average of 3.05 $\mu g.g^{-1}$ of dry weight for Richir

and Gobert's study). The same conclusion can be made for Fe, with a mean concentration of 241.36 µg.g^{-1} of dry weight observed at St Florent, representing more than 2.7-fold higher than in mussels of 2014. The concentration in Cr also increased, whit a multiplication of about 8.5 in the most Cr concentrated site (St-Florent). The latter ports also showed a peak of Ni and Se, especially near its gas station, while a decrease trend was observed for these TE in recent years. Finally, Mn and Zn are also concerned by the increase of their levels since 2014. Mn concentrations jumped by a factor 3 at St-Florent, and concentration of Zn was for example 141.15 µg.g^{-1} dry weight at STARESO, the reference site. In all sites, neither Cd or Pb exceed the maximum levels fixed by Commission regulation (CE) No 1881/2006, but Cd didn't tend to decrease since 2014, and even reach concentration of 0.91 µg.g^{-1} dry weight in Ile Rousse this year.

Several studies also provided TE contamination data of mussel in Mediterranean Sea in general. Higher concentrations of Cu and Zn was observed in Cannes harbor on the French coast (Roméo *et al.,*2003). Similarly, Moroccan coast showed in 2018 Fe concentration 2-fold higher than St-Florent, and the Sicilian coast presented particularly high levels of Al, Ni, Pb and Cr compared to our measurements.

Table 8: Example of mean bioaccumulation of trace elements (mean, µg.g^{-1} of dry weight) in soft tissue of *Mytilus galloprovincialis* recorded by recent studies. < LOQ: below limit of quantification.

	Corsican Coast (Richir & Gobert, 2014)	**French Mediterranean coast (Roméo et al., 2003)**	**Northern Moroccan coast (Azizi et al., 2018)**	**North-western Mediterranean coast (Lafabrie et al., 2007)**	**Sicilian coast (Caricato et al., 2019)**
Ag	0.02	-	-	-	-
Al	40.0	-	-	-	621.5
As	13.55	-	-	-	-
Ba	-	-	-	-	-
Cd	0.75	0.76	0.75	1.4	1.67
Co	0.48	-	< LOQ	0.75	-
Cr	0.26	-	2.6	1.7	5.15
Cu	3.05	102.7	5.7	-	2.8
Fe	87.5	-	506.5	-	387
Mn	2.25	-	-	-	2.09
Mo	5.3	-	-	-	-
Ni	0.94	-	2.9	1.89	3.17
Pb	0.92	-	< LOQ	1.25	2.12
Sb	0.012	-	-	-	-
Se	3.15	-	-	-	-
Sn	0.02	-	-	-	-
V	1.4	-	-	-	-
Zn	119	261	162	-	184.5

In summary, throughout the TE assessment of January 2022, higher concentrations were observed for 8 out of 18 TE. Mussels from Calvi seemed to be more contaminated in Cu than in 2014, but value remain in general lower than in some highly polluted sites. St-Florent, especially near its gas station presented mussels generally the most contaminated. This port was involved in almost all the peaks of contamination observed this here in North Corsica, except the Cu one. Further fields investigations, maybe by communication with marina managers, would be necessary to find the precise sources of these contaminations in North Corsican ports. Nevertheless, levels of TE found in the four studies ports remain low when compared with other site of the Mediterranean Sea.

PAHs and metallic trace elements are of concern because of their high toxicity, and their facility to be accumulated in tissues, especially of marine organisms such as mollusks (Lima *et al.,*2007; Lacroix *et al.,*2015). 16 of them are listed as priority pollutants by the United States Environmental Protection Agency (OFR, 1982; Yan *et al.,*2004; Lacroix *et al.,*2015). When accumulated in living tissues, they participate in the increase of ROS. This causes lipids peroxidation, protein oxidation or DNA damages, enzymes inhibition, change in gene regulation (Livingstone, 2001; Lima *et al.,*2007; Honda & Suzuki, 2020). PAHs can be carcinogenic, mutagenic and teratogenic and can disrupt endocrine equilibrium (Yan *et al.,*2004; Liu *et al.,*2012; Honda & Suzuki, 2020). In January 2022, Calvi's seawaters were the most contaminated in PAHs, with total concentration of 52.3 $ng.L^{-1}$ (appx. 9). The lowest concentration of PAHs in seawater was found in the reference site, STARESO (15.0 $ng.L^{-1}$).

In general, mussel's tissues showed really low PAHs values (concentration of 2 $ng.g^{-1}$ for the most contaminated site, the fairing area of Île Rousse). This makes it difficult for this species to reliably translate the general trend observed in water samples. It could be explained by the fact that organisms, used through active monitoring, were placed into ports only for one month. So, they provide only recent pollutions episode without integrate chemical pollutants over long periods. They may not have enough time to accumulate these compounds in sufficient concentration to be interpreted. According to several authors, three to four weeks would however be enough to reach an equilibrium between the levels of environmental and tissues pollutants (metals and organics pollutants) (Bolognesi *et al.,*2004; Andral *et al.,*2004; Nigro *et al.,*2006; Orbea & Cajaraville, 2006; Caricato *et al.,*2010). But these results concerned highly polluted areas. In his study, Marigómez et al. (2013) find higher PAHs and PCBs concentrations in native mussels than in caged ones, and explain this difference by the possibility that it takes more times to reach such equilibrium and that the time to obtain it would be positively correlated with the pollution level. This conclusion was supported by Serafim et al. (2011). At

STARESO, the absence of PAHs above the instrument detection limit in mussel's tissues tends to confirm that it is an appropriate reference site for the study of PAHs contamination. When detected, PAHs contamination were observed mainly in the fairing area, and especially in Île Rousse, representing most PAHs polluted site (total concentration of 2 $ng.g^{-1}$ wet weight). Only 0.1 $ng.g^{-1}$ wet weight of pyrene was found near the gas station of Calvi. This is interesting because the presence of this type of pollutants in ocean is often reported as coming from oil spills, so their observation next to the gas station of each site was expected (Honda & Suzuki, 2020). One more time, the values measured were so small makes their interpretation complicated or even biased. For example, concentration of 2139 $\mu g.g^{-1}$ wet weight of the 16 priority PAHs was measured in 2013 in mussels from the Sicily eastern coastline (Italy) (Cappello *et al.,*2013), and of 2025 $\mu g.g^{-1}$ wet weight in Barcelona in 2001 for the same species. Finally, the most contaminated port (Île Rousse) is about one million-fold less polluted than those two sites.

Regarding PCBs, mussels situated in the reference site accumulated 5 different PCBs (PCB 101, 135, 156 are particularly PCB 138 and 153). Trace of the two latter were also found in the three other sites but in less quantity. Those two PCBs have commonly been reported as the majors congeners among the 209 already known that contribute to total PCBs accumulation (Andral *et al.,*2004; Scarpato *et al.,*2010; Bajt *et al.,*2019). Higher values have already been recorded, as in the coastal water of the Adriatic Sea in 2019, where PCBs 138 and 153 reach 18.9 and 46.4 $\mu g.kg^{-1}$ of dry weight, respectively (Bajt *et al.,*2019). In 2004, the French Mediterranean sea also exposed higher value of those tow congeners, in the same order, in particular at the Marseille basin, with values about 6.5 and 4-fold higher (Andral *et al.,*2004; Scarpato *et al.,*2010). In general, it is most common to measured larger amount of PCBs near large ports, marinas, estuaries (Bajt *et al.,*2019). They are mainly derived from commercial mixture (Aroclor 1260 and 1254 were the most widely used) used in European country (Ivanov & Sandell, 1992; Bajt *et al.,*2019). They can come from accidental spill or untreated wastewater, but principally from atmospheric deposition (Bajt *et al.,*2019; Villeneuve *et al.,*1999). Even if residues of PCBs, as long-term persistent compounds, are still present in living tissues, they globally tend to decrease with times (Villeneuve *et al.,*1999).

Overall, regarding the results of all biomarkers of exposure in transplanted mussels, and compared with others location, differences in the nature and concentrations of pollutants can be observed between each port. The mussels from St-Florent would be more subject to pressures related to peaks of several TE (Al, Fe), those from STARESO and Ile Rousse would mostly suffer from the presence of organic pollutants (PCBs and PAHs, respectively). Although the

presence of these contaminants can have an impact on the physiological function of organisms, their concentration levels seem low enough not to impact their overall state of health. Furthermore, they are below the prescribed limits fixed by Commission regulation (CE) No 1881/2006 for human consumption.

1.2. Effects of chemical contamination on caged mussels

The measurement of physiological functions of the bioindicators species facing pollution pressures is important to evaluating the impact of pollutants on the health. To do this, a battery of 13 biomarkers has been assessed in the digestive gland of mussels collected in the three studied North Corsican ports and the reference site, STARESO. This organ, involved in pollutant detoxification and homeostasis maintenance, has been highly recommended for bioaccumulation assessment and was therefore chosen in this study (Cappello *et al.,*2013; Milinkovitch *et al.,*2015; Luna-Acosta *et al.,*2017b; Breitwieser *et al.,*2020).

Antioxidant defenses are among the most used biomarkers in biomonitoring. They are considered as relevant biomarkers because of their short-term response to external stress (Nasci *et al.,*2002; Vlahogianni & Valavanidis, 2007; Fernández *et al.,*2010; Ozkan *et al.,*2017). Regarding results, response of GST, CAT and SOD activities was found. Mussels showed higher GST activity at Île Rousse and STARESO than St-Florent, and higher CAT activity at Île Rousse than STARESO and St-Florent. The higher activity of GST at Île Rousse and STARESO and of CAT at Île Rousse fit with the presence of HAPs and PCBs in mussel's tissues, although in small quantity. The activation of these enzymes is commonly involved in polluted site (with TE, organic pollutants, pollution mixture) (Lima *et al.,*2007; Vlahogianni & Valavanidis, 2007; Bocchetti, Lamberti, *et al.,*2008; Cappello *et al.,*2013; Lacroix *et al.,*2015). Indeed, the exposure of marine organisms to contaminant can increase their levels of ROS. CAT activity can increase in response to an higher amount of H_2O_2, itself generated by the previous reaction of SOD to trap the superoxide radicals (O_2 $^{-\bullet}$) of these ROS (Fernández *et al.,*2010; Lacroix *et al.,*2015). On the contrary, lower activity of CAT and GST was observed at St-Florent. This is surprising, as the port was the most TE contaminated. According to these results, the two enzymes seem to be more sensitive to organics pollutants, especially PAHs. In the literature, no specific trend of sensitivity of GST and CAT to a particular pollutant clearly emerged. May be, CAT could be a little more susceptible to increase in presence of TE, as reported by Duarte *et al.* (2011). This would explain its low activity at STARESO where almost no TE contamination was detected in mussel. But this remains a hypothesis and doesn't explain its low activity in St-Florent. For this case, even if GST and CAT activities tend to increase with the concentration pollutants, some studies suggested that these enzymes may be

temporarily inhibited when it face acute pollution, resulting in opposite responses depending on pollutants concentration or exposure time (Regoli & Principato, 1995; Duarte *et al.,*2011; Benali *et al.,*2015; Ozkan *et al.,*2017). The disparate responses of CAT and GST to pollutants found at St-Florent could be the results of an acute pollution, the presence of unmeasured pollutant, or disturbances in others environmental factors. For example, the established correlation between GST and temperature was also illustrated in this study (appx. 6) (Duarte *et al.,*2011).

At St-Florent, significant difference of SOD activity between condition was revealed. Mussels placed near the fairing zone (Fz) showed more than 1.5-fold higher SOD activity than those next to the gas station (Gs). SOD activity is supposed to increase in response to pollutants, especially TE, but also organic pollutants (Fernández *et al.,*2010; Lacroix *et al.,*2015). Then, its response was not consistent with the levels of TE observed, as peaks of its activity was found near the less TE concentrated zone (Gs). Nevertheless, the only HAPs and PCBs of St-Florent were measured in the fairing zone. So, mussels undergo a mixture of pollutants in this area particularly, which could explain a higher levels of SOD activity. Also, correlation between SOD and nitrite, nitrate, ammonia, phosphates and Hg has already been illustrated, but those compounds were not analyzed here (Fernández *et al.,*2010; Lima *et al.,*2007). This contradictory results between contaminants measured and biomarkers of effect can also be explained by the presence of others source of stress or pollutants not analyzed as part of this study (e.g., petrochemical products, emerging pollutants).

Regarding energetic reserves, a difference in protein concentration among port was noticed. Lower amount of this compound was revealed in mussels from Île Rousse. A decrease of protein contents has been discovered by several authors in *Daphnia magma* and *Chlorella algae* who faced high levels of pollutants (De Coen & Janssen, 2003). This is consistent with the levels of antioxidant defenses in Île Rousse. It showed the highest GST and CAT activity, indicating higher levels of stress, probably by pollutants. In addition, it is well known that high ROS levels can lead to the oxidation of proteins (Vlahogianni & Valavanidis, 2007; Lushchak, 2011). The lower amount of protein and lipids contents in Fz of Calvi could be linked to a general higher accumulation of TE of the organisms in this area. If it is sufficient to bring higher stress conditions to mussels in this zone, the same conclusion as above can be given.

Finally, in this species, the immune system also responds. Higher activity of laccase was found at Calvi and St-Florent, compared to STARESO. Regarding contaminants, the Cu-dependence of this enzyme is now well established (Luna-Acosta *et al.,*2017b). This is consistent with our study, where the response of LAC activity clearly reflected the Cu

accumulation pattern in each site. As for LAC, Calvi and St-Florent faced higher levels of Cu, and STARESO, where LAC activity presented the lowest values, was the least contaminated. Others studies reported significant correlation with Co, Fe, Ni or V (Breitwieser *et al.,*2017). This correlation has been verified in this study for Fe, the others TE being too low concentrated. Recent studies determined that laccases can be involved in antioxidant and detoxification processes in addition to immune system (Luna-Acosta *et al.,*2017b). This type of enzymes can be considered as a biomarker of choice for pollution biomonitoring.

1.3. Limits

An active biomonitoring method had to be used for mussel because of its absence in all sites except in St-Florent. This scarcity could be due to the oligotrophic characteristic of water in North Corsica, providing limited availability of nutrients (Sara *et al.,*1998). The low level of phytoplankton biomass present in this type of waters is considered as unsuitable for their well growth, even in some recent studies have demonstrated the possibility for mussel to feed also detrital organic particles found in oligotrophic waters (Hawkins *et al.,*1999; Sarà, 2007; Sarà & Pusceddu, 2008). At St-Florent, primary production is enhanced by nutrients in freshwater inputs by Aliso River, creating meso- or eutrophic waters with higher food supply and then explaining the presence of mussel. As they may be starving in most of the studied sites, it is difficult to ensure that all biomarkers of effect responses reflect only pollution.

2. Passive biomonitoring of limpets in North Corsica ports

2.1. Inter-ports chemical contamination

One of the objectives of this master thesis, as part of the QUAMPO project, was to test the potential of limpet as suitable bioindicator species for North Corsican waters.

Limpet contamination trend seems to well fit with water one, as the contamination in Pb found in water samples of Île Rousse correspond to their accumulation (appx. 8). As mussel, the contamination in Mn observed at St-Florent was also detected in limpet, but this species accumulates much more.

The concentrations of TE accumulated in limpet decrease in the order Fe > Al > **Zn** > **Cu** > As > Se > Mn > **Pb** > **Cr** > Ni > **Cd** > Ba > Mo > Co. In comparison, Conti & Cecchetti, (2003) observed a decreasing order of Zn > Cu > Cd > Pb > Cr in *P. cerulea* in Italy. Regarding these trends, a contamination of Pb and Cr could be suggested, as those two compounds are placed in higher range. But limpet collected in central Italy were highly concentrated in Cd, compared to limpet of the present study. This must explain the observed change in accumulation

range because Pb concentration measured in North Corsica showed lower concentration of this contaminant that in Conti & Cecchetti (2003) study for most sites, except for Île Rousse.

Compared to other ports, a relatively high value of Cd has been found in STARESO. Richir & Gobert, (2014) have already found high values of this contaminant in mussels located in North-west Corsica (table 8). Furthermore, Cd, As, Pb, Cu or Zn, are commonly found in higher levels in limpet than mussel (Conti *et al.,*2010; Mbandzi *et al.,*2022; Pérez *et al.,*2019). The observed data are consistent with this trend for Cd, As, Pb, Cu. They have linked those amounts with potential upwelling of deep water that would bring Cd to the surface, the difference in variation physico-chemical characteristic of Ligurian Sea and Tyrrhenian Sea, or to a potential source in south-west Corsica, carried north by currents (Andral *et al.,*2004; Lafabrie *et al.,*2007, 2008). STARESO presented the highest concentrations of Ag, As, Ba, Cd, Mo, Se, meaning that it is not a very reliable reference site. Nevertheless, Cd value remains lower at STARESO than in Italian studies (table 9). Finally, our study showed that ports was not as clean as in Canary Islands, with higher levels of Cd at STARESO, of Cr, Fe and Cu in every sites, of Ni at STARESO and St-Florent, of Pb in all site except St-Florent, of V at Calvi, and particularly of Zn in all site and Al at Île Rousse (713 $\mu g.g^{-1}$ of wet weight found this year), suggesting a potential contamination of organisms.

Table 9: Example of mean bioaccumulation of trace elements (mean, $\mu g.g^{-1}$ of dry weight) in soft tissue of *Patella sp.* recorded by recent studies. < LOQ: below limit of quantification.

	Gaeta Gulf (Central Italy) (Conti & Cecchetti, 2003)	**Sicily (Italy) (Campanella et al., 2001)**	**Canary Islands (Spain) (Lozano-Bilbao et al., 2021) (in $mg.kg^{-1}$ of wet weight)**
Al	-	-	9.44
Cd	3.54	4.41	0.26
Cr	0.85	0.30	0.22
Cu	14.3	1.7	1.16
Fe	-	-	80.8
Ni	-	-	0.37
Pb	0.95	0.20	0.21
V	-	-	0.32
Zn	100.8	5	3.5

The total quantity of PAHs found in limpet well reflected the measurements in water samples. As for water, the highest quantities were found in limpet from Calvi, and the lowest ones in those from STARESO. But the latter port still showed HAPs traces (naphthalene, phenanthrene, fluorene). As native organisms of the studied ports, they had more time to

accumulate contaminants in tissues, and revealed long-term contamination of the sites. The concentrations discovered in limpet's tissues may be the results of past episodes of pollution. In this species, PAHs contents tend to decrease with times, whit the highest levels occurring immediately after an oil spill. They also have been reported to decrease with the distance to oil spill sources (Lewis *et al.,*2010; Bartolomé *et al.,*2011; Reguera *et al.,*2018).

As for mussels, PCBs were found in limpet tissues coming from STARESO. PCB 153 and 138 are again the most concentrated congeners, but 1.1 $ng.g^{-1}$ of PCB 180 was also detected. Even in mussels was describe as a better accumulator of PBCs than limpets, the latter one still well accumulate some PBCs, mostly congeners 28, 138 or 180.

Again, all organics pollutants measured in the different ports was remained under the reglementary maximum levels settled by the Commission regulation (CE) No 1881/2006 for bivalve mollusks.

2.2. Effects of chemical contamination of native limpet

Among antioxidant defenses, a response of SOD was observed in limpet. This enzyme constitutes an important defense against oxygen toxicity by catalyzing the conversion of superoxide anion (O_2 $^{-\bullet}$) to oxygen and hydrogen peroxide (Fernández *et al.,*2010; Lacroix *et al.,*2015). In North Corsica, limpet from St-Florent were significantly lower concentrated in SOD than in the other ports. This biomarker is particularly interesting as it integrates well the response of organisms to all type of external stress, as previously describe for mussels (Fernández *et al.,*2010; Zaidi *et al.,*2022). Indeed, here both STARESO, Calvi and Ile Rousse were concerned by several pollutants, and St-Florent appeared to be the less stressful port. This trend is clearly confirmed by the SOD, which constitute a biomarker of choice for biomonitoring of limpet.

Regarding energetic stock and metabolism, limpet showed higher PK activity and glycogen content at STARESO, whereas the lowest levels of theses biomarkers were detected at Calvi. Only few studies investigated the energy metabolism, but its variation could be a key response to chemical stress. Energy metabolism pathway can be impacted when organism is exposed to pollution, through an increase of metabolic costs and a perturbation in ATP-synthase pathway (Lacroix *et al.,*2015). As STARESO face the most important mixture of pollutants, it is consistent that higher PK activity was found there. This would mean that higher ATP is needed to process antioxidant respond. Several studies defined limpet as a good accumulator of some TE, including Cd (Conti & Cecchetti, 2003; Lozano-Bilbao *et al.,*2021). This also confirm in this study, where higher stress of the organisms corresponds to higher concentration of the

compound. On the contrary, the high level of glycogen is not consistent with the previous result. This energy storage should be mobilized by the organism facing pollution as a rapid usable substrate to support high energy flux induce by high basal maintenance coasts. To supply requested energy, lower amount of glycogen stock was expected at STARESO, in organisms that activate PK, the enzyme responsible for the glycolysis (conversion of glucose into pyruvate) (Falfushynska *et al.,*2019; Lacroix *et al.,*2015). The high amount of glycogen in this port could be explain by the PCBs. These compounds are generally found in places highly concerned by wastewater discharge and mainland run-off. These water intakes are commonly rich in nutrients, which could explain higher energy stock in limpet. In addition, the species were significantly bigger in this port (table 3). This can also explain why limpets were more concentrated in glycogen than in other port. Further investigation on wastewater treatment at STARESO should be done to bring more answers. Limpet situated at St-Florent also showed among the lowest PK activity, similarly to those from Calvi. At St-Florent, this responds correspond with correlate with low level of pollution. But the trend of Calvi is quite interesting since this port showed among the highest levels of organics pollutants. It is possible, on more time, that external parameters (i.e., emergent pollutants, strong variation of physico-chemical characteristics of water) not analyzed in the study could have had an non negligible impact organisms.

2.3. Potential for further biomonitoring

A good understanding of natural seasonal variations linked to the biological cycle (reproductive cycle, spawning period, etc) of the bioindicators species from pollution-induced effects is crucial before adopting a multibiomarker approach in environmental risk assessment. It is therefore important to know precisely the basal response of used biomarkers (Cravo *et al.,*2012). The present study participates in providing additional data on limpet as a potential bioindicators. Several studies highlight the high potential of this species as cosmopolitan bioindicators species for traces elements (Conti *et al.,*2010; Reguera *et al.,*2018). Even in relatively low polluted area such as ours, several responses, in agreement with literature, was observed for limpet, making it a species with high indicator potential.

3. The integrated biomarker response (IBR)

The multibiomarker approach brings a more holistic assessment of the biological responses to possible pollution and a better understanding in environmental risk assessment, as it allow to assess the impacts of multiple contaminants (Beliaeff & Burgeot, 2002; Cravo *et al.,*2012; Devin *et al.,*2014; Ozkan *et al.,*2017). However, in biomonitoring study leading to the

development of concrete action in field, it seems important to integrate the response of both biomarkers to obtain a global overview of the quality of the environment. Bringing all these results separately to ports managers would be inefficient. It is therefore needed for this approach to be coupled with a tool summarizing all biomarker responses to simplify their interpretation and acting like a decision-support tool for field actors. The IBR index has been developed in this sense.

For mussels, IBR results goodly correlated with biomarkers responses. Higher value for Île Rousse was calculated, and fit with antioxidant defenses responses for this port, which showed higher stress.

For limpet, IBR results were also consistent with the general trend of biomarkers responses (section 4). The lower levels of stress of antioxidant biomarkers responses and biomarkers of exposure observed in St-Florent explain lower IBR value found for these ports. On the other hand, as illustrated above, the higher value for STARESO clearly correspond to the higher level of stress found by all significant biomarker responses.

Regarding IBR results, it can be considered as a good and easy tool to summarize biomarker responses in a general "stress-index" and can help to communicate with leaders and ports managers about water quality.

Regarding previous years, it is quite hard to understand trends among seasons or locations. The calculation of this index induces the comparison of factors. Then, the calculation of IBR where all ports were compared for a given sampling period didn't provide the same values as the calculation where all sampling periods were compared for one given port. Devin *et al.* (2014) warn about the importance of considering the IBR value as for comparison between site but not as an absolute index giving a biological stress. Also, it is important to keep in mind that this study was carried out *in situ*. Indeed, any significant variation of bio-physico-chemical parameters and processes that play an important role in the environmental quality can had an impact on the results obtained in this study. Finally, the QUAMPO project took place during the COVID 19 pandemic. The cessation of touristic activity and the decline of ports frequentation by visitors induced by this pandemic may have altered the seasonality of chronic pollution. Indeed, higher stress level was expected in summer due to high touristic pressure.

4. Interspecific variability

The difference in effect biomarker responses and bioaccumulation pattern of contaminants between the two species highlight the fact that they integrate differently the pollutants. This makes even more relevant to combine the use of more than only one bioindicator as it allows

to scan more precisely the impact of a wider set of pollutants. This clearly shows the importance of keeping investigate and describe new biomarkers of environmental quality in other species.

As mussels were absent in most studied sites, this species can be considered as not well adapted to North Corsican environmental conditions. Using this species only in complement to other bioindicators would be recommended in this context. In this study, limpet constituted an interesting bioindicator species in oligotrophic waters as it bioaccumulates more TE than mussel.

5. Limitations and perspectives

In situ environmental studies, such as this one, are marked by complex interaction of numerous parameters (contaminants, environmental conditions) which makes it difficult to analyze the nature and sources of pollution. Many parameters remain out of control but can have an impact on the measurements.

Furthermore, this study highlights the fact that each species exposed different reactions to various pressures of contaminants present in seawaters. Their complementarity can be considered as a strength in biomonitoring. If possible, the use of several bioindicators simultaneously would be recommended in *in situ* biomonitoring of waters currently affected by cocktails of pollutants.

In addition, it is difficult to compare to two model specie, as they were no used through the same method (active and passive biomonitoring). Mussels could only integrate short-term pollution. This might explain the lower concentration of contaminant observed in general in this species, compared to limpet.

Conclusion

This master's thesis was part of the first biomonitoring study carried out in Calvi, Île Rousse and St-Florent ports. Results obtained highlight difficulties of *in situ* biomonitoring, as many parameters must be considered. The use of limpet as promising bioindicators was also confirmed. Besides, the complementary of the mussel and limpet responses was showed, and that can be considered as an advantage because it makes the information provided by the biomonitoring more precises and holistic.

In general, trace of some contamination has been found in every site, from various nature depending on the species, the site, and the biomarker. But values remained overall quite low and did not exceed quality standards established by WFD or MSFD. The presence of PCBs detected in the tissues of the two species in STARESO constitute a problem as the sites was supposed to be a reference place, but the source of the pollution is being identified with local managers.

To conclude, a global good quality of waters bodies in the studied North Corsica ports was showed by this three years biomonitoring. This work and the QUAMPO project helped ports managers and leaders of Calvi, Île Rousse and St-Florent ports to fill a gap between field management and water quality data availability and therefore constitute a starting point for sites wishing to obtain the label "port propre". Nevertheless, it must be kept in mind that the study was performed during the COVID 19 pandemic situation. Results could reflect in a biased way the quality of water bodies in each ports studied, since a significant slowdown in human activities was recorded at this period. Then, a prolongment of this biomonitoring would be highly recommended to reveal the temporal trends of contaminants over a longer period and under real existing anthropogenic pressures.

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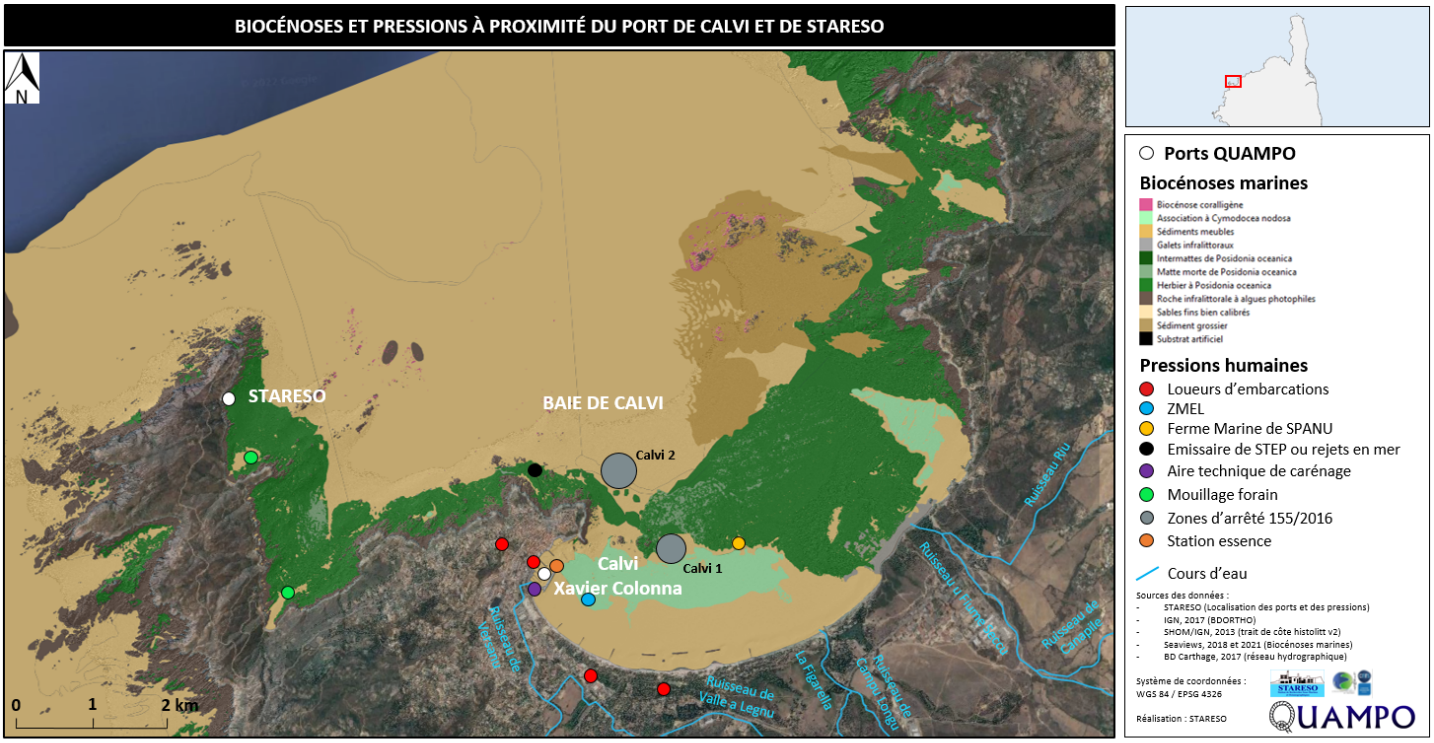
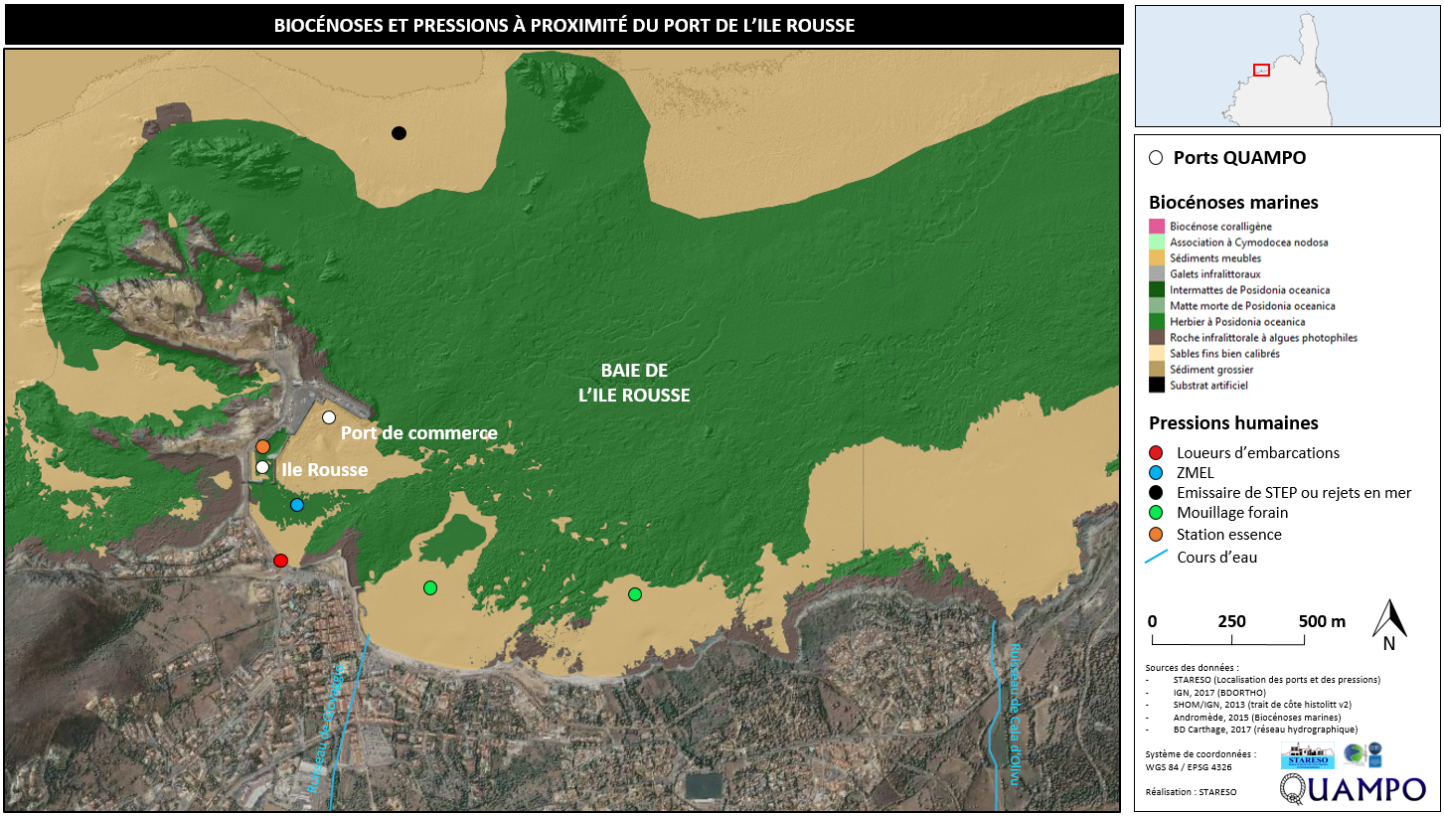
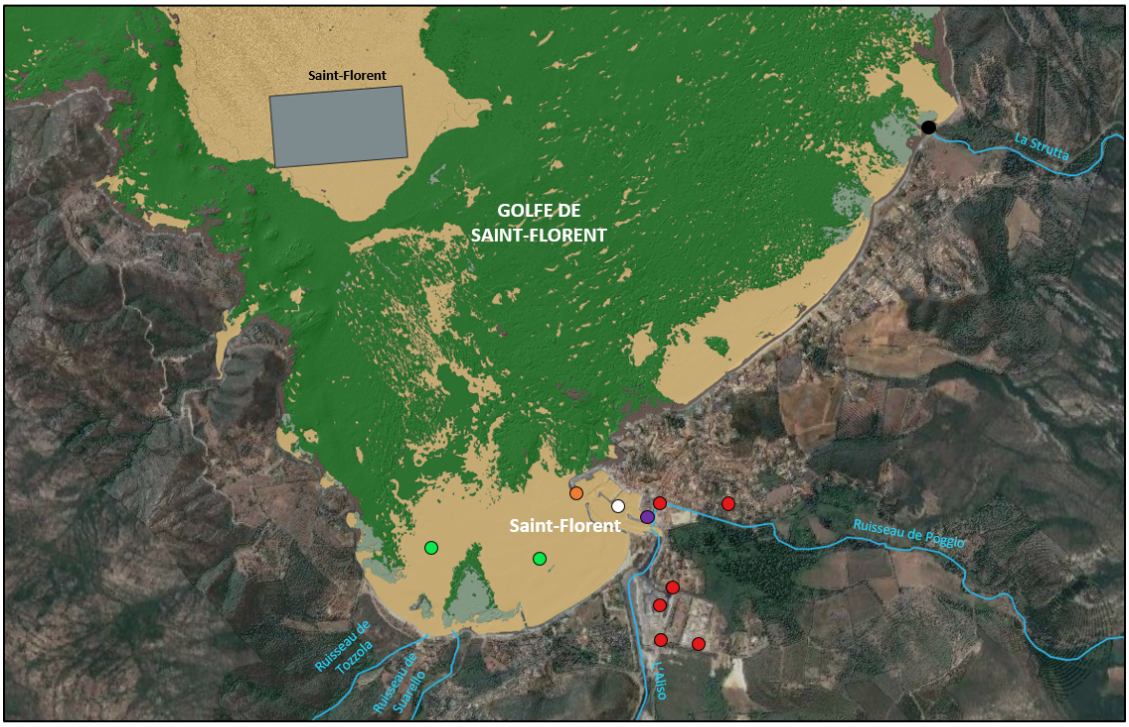
APPENDIXES

Appendix 1: Map of the biocenose and pressures found near each north-Corsican port.





BIOCÉNOSES ET PRESSIONS À PROXIMITÉ DU PORT DE SAINT-FLORENT



Ports QUAMPO

Biocénoses marines

- Biocénose coralligène
- Association à Cymodocea nodosa
- Sédiments meubles
- Galets infralittoraux
- Intermattes de Posidonia oceanica
- Matte morte de Posidonia oceanica
- Herbier à Posidonia oceanica
- Roche infralittorale à algues photophiles
- Sables fins bien calibrés
- Sédiment grossier
- Substrat artificiel

Pressions humaines

- Loueurs d'embarcations
- Emissaire de STEP ou rejets en mer
- Aire technique de carénage
- Mouillage forain
- Zones d'arrêté 155/2016
- Station essence

Cours d'eau

0 1 2 km

N

Sources des données :
- STARESO (Localisation des ports et des pressions)
- IGN, 2017 (BDORTHO)
- SHOM/IGN, 2013 (trait de côte histolitt v2)
- Andromède, 2014 et 2015 (Biocénoses marines)
- BD Carthage, 2017 (réseau hydrographique)

Système de coordonnées : WGS 84 / EPSG 4326

Réalisation : STARESO

Appendix 2: Protocols of trace elements assessments performed in March and April 2022 at LIENSs laboratory. as part of the QUAMPO project.

- Trace elements in organism's tissues

The soft tissues of limpets and mussels were hand grinded with a ceramic mortar and pestle and freeze dried for 48-72h. About 0.25 mg of the samples and the reference materials (DOLT-5, dogfish liver and TORT-3, lobster hepatopancreas. National Research Council Canada) were digested by addition of 6:2 (v/v) 67-70% HNO3 / 34-37% HCl mixture (Fisher, trace metal quality). After for one night of digestion process at ambient temperature, samples also underwent a microwave digestion (30 min with constantly increasing temperature up to 120 °C, and 15 min at this temperature). They were finally completed with ultrapure water until 50 mL. A total of 18 traces elements (aluminum Al; silver Ag; arsenic As; barium Ba; cadmium Cd. cobalt Co. chromium Cr; copper Cu; iron Fe; manganese Mn; molybdenum Mo; nickel Ni; lead Pb; antimony Sb; selenium Se; tin Sn; vanadium V and zinc Zn) bioaccumulation were analyzed thanks to an ICP-OES Vista-Pro (Varian Inc., Palo Alto, California. USA) and an ICP-MS XSeries 2 (ThermoFisher Scientific, Waltham, Massachussetts, USA). Means recovery rates were 88% (Al), 82% (Ag), 100% (As), 91% (Cd), 106% (Co), 95% (Cr), 96% (Cu), 92% (Fe), 87% (Mn), 91% (Mo), 92% (Ni), 81% (Pb), 108% (Se), 89% (V) and 100% (Zn). Quantification limits were 19.28 (Al), 0.01 (Ag), 0.1 (As), 0.1 (Ba), 0.01 (Cd), 0.01 (Co), 0.1 (Cr), 0.96 (Cu), 3.86 (Fe), 0.01 (Mn), 0.1 (Mo), 0.04 (Ni), 0.01 (Pb), 0.04 (Sb), 0.39 (Se), 0.02 (Sn), 0.96 (V) and 3.86 (Zn) $\mu g.g^{-1}$ of dry weight. Results are expressed in $\mu g.g^{-1}$ of wet weight.

- Trace elements in seawater

Same instruments were used to analyze water samples, with a 1/5 dilution for water samples and seawater standards in 2% nitric acid for ICP-OES, and a 1/20 dilution in 2% nitric acid for ICP-MS. Results are expressed in $\mu g.L^{-1}$.

Appendix 3: Protocols of organic pollutants assessments performed in March and April 2022 at CEDRE laboratory. Brest. France. as part of the QUAMPO project.

- Organics pollutants in organism's tissues

The concentration of 22 polycyclic aromatic hydrocarbons (PAHs). including the 16 Environmental Protection Agency (EPA) PAHs. 14 polychlorinated biphenyls (PCBs) and 7 pesticides were measured in soft tissues of limpet and mussels. They are assessed by stir bar sorptive extraction-thermal desorption-gas chromatography-tandem mass spectrometry (SBSEGC-MS/MS) following Lacroix *et al.* (2014) method. Firstly. tissues were digested by saponification and analytes were extracted for 16h at 700 rpm using polydimethylsiloxane stir-bar (Twister 20 mm x 0.5 mm. Gerstel). Secondly. bars were analyzed using a gas chromatography system Agilent 7890A coupled to an Agilent 7000 triple quadripole mass spectrometer (Agilent Technologies) and equipped with a Thermal Desorption Unit combined with a Cooled Injection System (Gerstel). The GC column was a Restek Rxi-5 ms (30 m. 0.25 mm. 0.25 µm). Then. a calibration curve ranging from 0.01 ng to 30 ng per bar were used to quantify analytes relatively to deuterated compounds. Quantification limits. calculated by the calibration curve method from Shrivastava and Gupta (2011) were 25 (naphthalene. phenanthrene and PCB 138). 5 (dibenzothiophene. fluoranthene. 2-methylfluoranthene. chrysene. benzo(b)fluoranthene. benzo(k)fluoranthene. benzo(e)pyrene. benzo(a)pyrene. perylene. indeno(1.2.3-cd)pyrene. dibenzo(a.h)anthracene. benzo(g.h.i)perylene. PCB 153 and 4-4-DDE). 2.5 (benzothiophene. biphenyl. acenaphthylene. acenaphthene. fluorene. anthracene. pyrene. benzo(a)anthracene. PCB 7. PCB 28. PCB 52. PCB 35. PCB 101. PCB 77. PCB 135. PCB 118. PCB 156. PCB 180. PCB 169. aldrine. isodrine. dieldrine and endrine) and 0.5 (PCB 105. hexachlorobenzene and 2-4-DDE) $ng.g^{-1}$ dry weight. Results are expressed in $ng.g^{-1}$ of dry weight. The Standard Reference Materials 1974c "Organics in Mussel Tissues (*Mytilus edulis*)" provided by the National Institute of Standards (SRM) and Technology (NIST. Gaithersburg. USA) was used to make analytical quality control.

- Organics pollutants in seawater

The sampling and analysis methods describe above were also carried out in January 2021 ("winter period"). and in September 2020 and 2021 ("summer period"). Differences in the methodology arose in January 2020. Thus. all two-year data except those corresponding to the latter sampling (January 2020) will be integrated into this study

Appendix 4: Enzymatic activities protocols performed in March and April 2022 at LIENSs laboratory. as part of last sampling of the QUAMPO project.

Protein concentration

Total protein concentration was carried out using the bicinchoninic acid protein assay kit #BACl and #B9643 (Sigma-Aldrich®) thanks to Lowry *et al.* (1951) method. In microplate wells. 25 µL of sample or standard were mixed with 200 µL of the reacting solution. This latter was made by mixing between 77 mL of bicinchoninic acid and 1.54 mL of copper sulfate. Then. the microplate was covered by aluminum and incubated 30 min at 37°C on a shaker tray. Absorbance was read at 562 nm. Protein concentration was finally calculated using standard range:

$$protein\ concentration\ (mg.g^{-1}\ of\ tissues) = (\frac{A_{sam}}{a}) \times d$$

Where:
A_{sam} = sample or standard absorbance
a = constant "a" in the expression slope of the standard range ax + b
d = homogenate dilution

Energetic stocks

Lipids concentration

Protocol of determination of total lipids concentration is taken from Frings *et al.* (1972). In glass tubes and under fume hood. 20 µL of sample or standard were added to 200 µL of sulfuric acid 96%. After vortexing. they were incubated at 95°C for 10 min and placed for 5 min at ambient temperature. 1 mL were added. tubes were again vortexed and placed in an incubator at 37°C for 15 min. 200 µL of samples were then deposited in microplate wells and absorbance was read at 540 nm after 45 min. The total lipids concentration was calculated as followed:

$$lipids\ concentration\ (mg.g^{-1}\ of\ tissues) = \frac{(\frac{OD_{sam} - OD_{Blank}}{S}) \times V}{m} \times d$$

Where:
DO = absorbance (nm)
S = standard curve slope
V = total volume of homogenate at departure (lipids and glycogen. in mL)
m = weight of sample at departure (g)
d = sample dilution

Glycogen concentration

Total glycogen concentration measurement followed Carr and Neff (1984) method. 10 µL of amyloglucsidase was added on 1 out of 2 microtubes already containing 400 µL of standard or sample. They were vortexed and incubated at 55°C for 2h. Microtubes with the only sample or standard stayed in the fridge at 4°C during these 2h. All microtubes were centrifuged 10 min at 12000 rpm at 4°C. On ice. 20 µL of supernatant was placed in glass tubes with 200 µL of citrate buffer (0.1 M. pH 5). 1.6 mL of glucose oxidase peroxidase (GOD-POD) were added. and tubes were immediately vortexed and incubated at 20°C for 40 min. The GOD-POD solution was prepared by added 59.2 mg of peroxidase type I. 153.65 mg of glucose oxidase type II and 25 mL of O-dianisidine-di-HCl solution (60 mg of O-diazine-di-HCl + 25 mL of ultrapure water) to 875 mL of Tris-HCL buffer (0.5 M. pH 7). To stop the reaction. 60 µL of HCl 5N were added. 200 µL of samples were then deposited in microplate wells and absorbance was read at 420 nm.

The total glycogen concentration was calculated as followed:

$$glycogen\ concentration\ (mg.g^{-1}\ of\ tissues) = \frac{(\frac{\Delta_{sam} - \Delta_{samN1}}{S}) \times V}{m} \times d$$

Where:

Δ_{sam} = incubated sample OD – blank OD (absorbance in nm)

Δ_{samN1} = non incubated sample OD – blank OD (absorbance in nm)

S = standard curve slope

V = total volume of homogenate at departure (lipids and glycogen. in mL)

m = weight of sample at departure (g)

d = sample dilution

Enzymatic total activity

Biomarkers of neurotoxicity

Acetylcholinesterase (ACHE) (EC 3.1.1.7)

The determination of ACHE total activity was conducted according to Ellman *et al.* (1961). 20 µL of sample or blank (PBS buffer) were put in microplate wells with 160 µL of the reacting solution (RS). RS was made by mixing 42 mL of phosphate buffer (100 mM. pH 7.4). 6 mL of $NaHCO_3$ (0.55 mM) and 6 mL of DTNB (0.3 mM). Reaction was initiated by 20 µL of acetylthiocholine (0.5 mM). Absorbance was read at 405 nm. Total activity of ACHE was measured through the following calculation:

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 10 \times d}{\varepsilon_{anion} \times l}$$

Where:
Activity = $mmol.L^{-1}.min^{-1} = \mu mol.mL^{-1}.min^{-1} = \mu mol.g^{-1}.min^{-1} – U.g^{-1}$
slope = slope value (a from its equation **a**x+b). $mOD.min^{-1}$
10 = dilution in well
d = sample dilution
ε = molar extinction coefficient of 5-thio-2-nitrobenzoate = 13600 $mol.cm^{-1}$
l = optical pathlength

Biomarker of immune systems

Laccase (LAC) (EC 1.10.3.2: *p*-diphenol oxidase)

Protocol for LAC activity assessment was performed according to Luna-Acosta *et al.* (2010). by following the oxidation of the substrate p-phenylenediamine (PPD). As this substrate may oxidize itself without any enzyme's presence. non-enzymatic autoxidation of substrate control was performed in addition to the blank (PBS). Thus. in microplates. 2 types of mixture were done: substrate (133 µL of PBS + 7 µL of PPD) and sample (10 µL of samples. 123 µL of PBS and 7 µL of PPD). PPD was made by mixing 216 mg of the product with 2 mL of methanol. Microplates were read at 420 nm every 5 min for a 2h kinetic.

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 14 \times d}{\varepsilon_{NADPH} \times l} \times 10^3$$

Where:
Activity = $mmol.L^{-1}.min^{-1} = \mu mol.mL^{-1}.min^{-1} = \mu mol.g^{-1}.min^{-1} – U.g^{-1}$
slope = slope value (a from its equation **a**x+b). $mOD.min^{-1}$
10 = dilution in well
d = sample dilution
ε = molar extinction coefficient of NADPH = 6.22 $mol.cm^{-1}$
l = optical pathlength

Lactate dehydrogenase (LDH) (EC 1.1.1.27)

LDH total activity was determined by measuring the NADH consumption (Childress *et al*.. 1979 with modification as in Bailey *et al*.. 2005). 20 µL of sample or blank (PBS buffer) were put in microplate wells with 180 µL of the reacting solution (RS). RS was made by mixing 26.4 mL of Tris/HCl buffer (80 mM. pH 7.5). 3 mL of KCl (100 mM). 300 µL of NADH (150 µM) and 300 µL of sodium pyruvate (2 mM). Absorbance was read at 340 nm. The total LDH activity was calculated as followed:

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 10 \times d}{\varepsilon_{NADH} \times l}$$

Where:
Activity = $mmol.L^{-1}.min^{-1}$ = $\mu mol.mL^{-1}.min^{-1}$ = $\mu mol.g^{-1}.min^{-1}$ – $U.g^{-1}$
slope = slope value (a from its equation **a**x+b). in $mOD.min^{-1}$
10 = dilution in well
d = sample dilution
ε = molar extinction coefficient of NADH = 6220 $mol.cm^{-1}$
l = optical pathlength

Pyruvate kinase (PK) (EC 2.7.1.40)

To assess the pyruvate kinase total activity. measurement of NADH consumption was made according to Childress *et al.* (1979) as modified by Bailey *et al*. (2005). The only difference from the LDH protocol was the composition of the reacting solution. It was made by mixing 39.6 mL of Tris/HCl buffer (80 mM. pH 7.5). 6 mL of $MgSO_4$ (10 mM). 6 mL of KCl (100 mM). 6 mL of ADP (5 mM). 600 µL of fructose bisphosphate (0.1 mM). 600 µL of phosphoenolpyruvate (1 mM). 600 µL of NADH (150 µM) and 600 µL of LDH solution. The latter one was made of 135 µL of LDH and 465 µL of Tris/HCL buffer.

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 10 \times d}{\varepsilon_{anion} \times l}$$

Where:
Activity = $mmol.L^{-1}.min^{-1}$ = $\mu mol.mL^{-1}.min^{-1}$ = $\mu mol.g^{-1}.min^{-1}$ – $U.g^{-1}$
slope = slope value (a from its equation **a**x+b). in $mOD.min^{-1}$
10 = dilution in well
d = sample dilution
ε = molar extinction coefficient of 5-thio-2-nitrobenzoate = 13600 $mol.cm^{-1}$
l = optical pathlength

Phosphoenolpyruvate carboxykinase (PEPCK) (EC 4.1.1.49)

PEPCK analysis followed the same principle of measurement that LDH and PK: assessing NADH consumption (Petrescu *et al.*. 1979 as adapted by Jamieson *et al.*. 1999). Here. microplates were filled with 20 µL of sample or blank (PBS). 160 µL of the reacting solution (RS) and 20 µL of deoxyguanosine-5'-diphosphate (dGDP) to initiate the reaction. RS was made by mixing 42 mL of Tris/HCl buffer (50 mM. pH 7.5). 5.4 mL of $MnCl_2$ (1 mM). 5.4 mL of $NaHCO_3$ (50 mM). 540 µL of NADH (0.25 mM). 540 µL of phosphoenolpyruvate (1 mM). and 120 µL of MDH solution. MDH solution and dGDP resulted from a mix between respectively 6.13 mg and 78 µL of products. . respectively. and Tris/HCL buffer (42 µL and 9.566 mL respectively). Absorbance was read at 340 nm. Total activity was calculated as followed:

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 10 \times d}{\varepsilon_{anion} \times l}$$

Where:
Activity = $mmol.L^{-1}.min^{-1}$ = $\mu mol.mL^{-1}.min^{-1}$ = $\mu mol.g^{-1}.min^{-1}$ – $U.g^{-1}$
slope = slope value (a from its equation **a**x+b). in $mOD.min^{-1}$
10 = dilution in well
d = sample dilution
ε = molar extinction coefficient of 5-thio-2-nitrobenzoate = 13600 $mol.cm^{-1}$
l = optical pathlength

Superoxide dismutase (SOD) (EC 1.15.1.1)

SOD activity was determined by measuring the rate of NADH oxidation based on the inhibition of a superoxide driven NADH oxidation. This inhibition is a function of SOD activity. One unit of SOD corresponds to fifty percent inhibition of oxidation. The protocol followed the method developed by Paoletti *et al.* (1986). 20 µL of sample or standard (SD1. ≈ 100 $U.mL^{-1}$) were put in microplate wells with 173 µL of the MIX. The latter was made by mixing 31.6 mL of PBS (1100 mosm). 1580 µL of NADH (7.5 mM) and 987.5 µL of EDTA 100mM / $MnCl_2$ 50 mM solution (100 µL of EDTA 200mM plus 100 µL of $MnCl_2$ 100 mM). Reaction was initiated by 20 µL of 2-Mercaptoethanol (10 mM). made with 3 µL of products and 4.26 mL of ultrapure water. After 15 min latency. microplates were read at 340 nm for a 1h10 kinetic. Total SOD activity was calculated as followed:

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope_{sam} - slope_{blank}}{slope_{standard}} \times d$$

Where:
Activity = $mmol.L^{-1}.min^{-1}$ = $\mu mol.mL^{-1}.min^{-1}$ = $\mu mol.g^{-1}.min^{-1}$ – $U.g^{-1}$
$slope_{sam}$ = sample slope (a from its equation **a**x+b). in $mOD.min^{-1}$
$slope_{blank}$ = blank slope (a from its equation **a**x+b). in $mOD.min^{-1}$
$slope_{standard}$ = standard slope (a from its equation **a**x+b). in $mOD.min^{-1}$
d = sample dilution

Catalase (CAT) (EC 1.11.1.6)

Thanks to the protocol of Aebi (1984). total CAT activity was assayed by recording the consumption of H_2O_2. Simply. 20 µL of sample or blank (PBS). 160 µL of reacting solution (RS) and 20 µL of hydrogen peroxide (H_2O_2) to initiate the reaction were placed in microplate wells for a lecture at 240 nm. RS was made only with phosphate buffer (50 mM. pH 7). and H_2O_2 with 1 mL of H_2O_2 3% mixed with 9 mL of ultrapure water. Absorbance was read at 240 nm. This is the calcul of catalase total activity

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 10 \times d}{\varepsilon_{H2O2} \times l}$$

Where:
Activity = $mmol.L^{-1}.min^{-1}$ = $\mu mol.mL^{-1}.min^{-1}$ = $\mu mol.g^{-1}.min^{-1}$ – $U.g^{-1}$
slope = slope value (a from its equation **a**x+b). $mOD.min^{-1}$
10 = dilution in well; d = sample dilution
ε = molar extinction coefficient of H_2O_2 = 39.4 $mol.cm^{-1}$
l = optical pathlength

Glutathione peroxidase (GPX) (EC 1.11.1.9)

GPX total activity was assessed through the method of Paglia and Valentine (1967) as adapted for microplates by Janssens *et al.* (2000). GPX can reduce hydrogen peroxide (H_2O_2) to 2 molecules of water H_2O. but this reaction also causes oxidation of 2 molecules of glutathione (GSH). Its activity is determined by measuring the consumption of NADPH by GR when the latter regenerates 2 molecules of GSH. On microplates wells. to the 15 µL of sample of blank (PBS) were added 120 µL of reacting solution (RS) and 15 µL of tert-butyl hydroperoxide solution (tBHP-2) permitted to initiate the reaction. To make tBHP-2. a first solution was made: a mix of 10 µL of tBHP (0.2 mM) and 990 µL of Tris/HCl buffer (50 mM. pH 7.6). 160 µL of that solution was secondly mixed with 7.84 mL of the same Tris/HCL buffer. RS was a mix of 45 mL of the Tris/HCl buffer. 480 µL of NADPH (0.14 mM). 480 µL of EDTA (1 mM). 480 µL of GSH (1 mM) and 1.560 mL of GR solution (315 µL of GR plus 1.245 mL of Tris/HCl buffer). Absorbance was read at 340 nm.

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 10 \times d}{\varepsilon_{NADPH} \times l}$$

Where:
Activity = $mmol.L^{-1}.min^{-1}$ = $\mu mol.mL^{-1}.min^{-1}$ = $\mu mol.g^{-1}.min^{-1}$ – $U.g^{-1}$
slope = slope value. $mOD.min^{-1}$
10 = dilution in well
d = sample dilution
ε = molar extinction coefficient of NADPH = 6.22 $mol.cm^{-1}$
l = optical pathlength

Glutathione-S-transferase (GST) (EC 2.5.1.18)

GST was determined by measuring the production of glutathionyl-dinitrobenzene (GS-DNB) (Habig *et al.*. 1974). 20 µL of sample or blank (PBS) and 160 µL of reaction solution (RS) were put in microplate wells. Reaction was initiated by 20 µL of GSH (10 mM). RS was a mix of 540 µL of 1-chloro-2.4-dinitrobenzene (cDNB. 1 mM) and 53.46 mL of phosphate buffer (100 mM. pH 6.5). Absorbance was read at 340 nm.

$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 10 \times d}{\varepsilon_{GS-DNB} \times l}$$

Where:
Activity = $mmol.L^{-1}.min^{-1} = \mu mol.mL^{-1}.min^{-1} = \mu mol.g^{-1}.min^{-1} – U.g^{-1}$
slope = slope value (a from its equation **a**x+b). $mOD.min^{-1}$
10 = dilution in well
d = sample dilution
ε = molar extinction coefficient of NADPH = 9600 $mol.cm^{-1}$
l = optical pathlength

Glutathione reductase (GR) (EC 1.6.4.2)

GR total activity was assessed according to Carlberg and Mannervik (1985). measuring NADPH consumption. Here. microplate wells were filled with 20 µL of sample or blank (PBS) and 180 µL of reacting solution (RS). a mix of 58.2 mL of phosphate buffer (100 mM. pH 7). 600 µL of NADPH (0.1 mM). 600 µL of EDTA (1 mM). and 600 µL of GSSH (1 mM. 50.32 mg of product for 739 µL of the phosphate buffer). Absorbance was read at 340 nm.

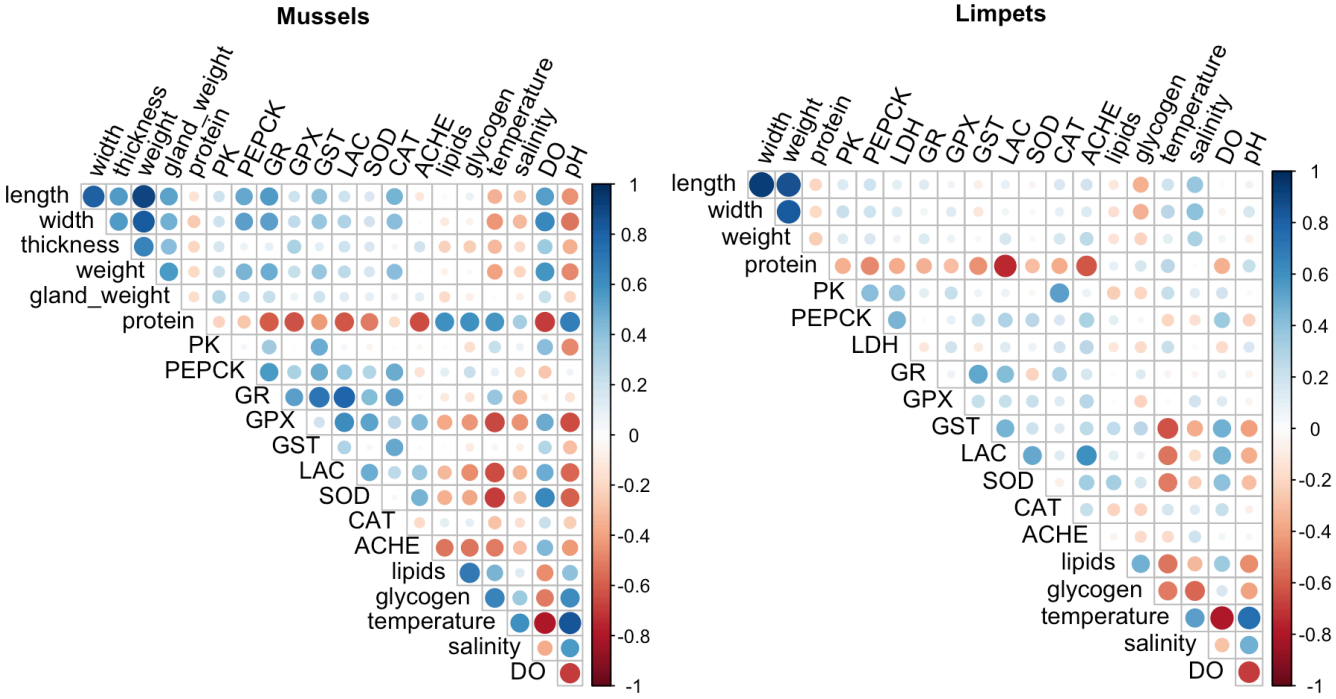
$$Activity\ (U.g^{-1} of\ tissues) = \frac{slope \times 10 \times d}{\varepsilon_{NADPH} \times l}$$

Where:
Activity = $mmol.L^{-1}.min^{-1} = \mu mol.mL^{-1}.min^{-1} = \mu mol.g^{-1}.min^{-1} – U.g^{-1}$
slope = slope value (a from its equation **a**x+b). $mOD.min^{-1}$
10 = dilution in well
d = sample dilution
ε = molar extinction coefficient of NADPH = 6.22 $mol.cm^{-1}$
l = optical pathlength

Appendix 5: Script R used for statistical analyses on data acquired during the QUAMPO project (R version 4.2.0 and RStudio 2022.02.3)

https://we.tl/t-efD98sOP5w

Appendix 6: Pearson's correlograms between morphometric measurements. biomarkers of effects and environmental parameters collected since September 2020 for the QUAMPO project.



Appendix 7: Total enzymatic activity (mean ± standard deviation. sample size in parentheses) of biomarkers of effect measured in digestive gland of mussels and soft tissues of limpets. both collected in the different ports in January 2022. Letters indicates significant differences between locations (p-values < 0.05) within the same species.

	Mussel				Limpet			
	STARESO	**Calvi**	**Île Rousse**	**St Florent**	**STARESO**	**Calvi**	**Île Rousse**	**St Florent**
PK	360.47 ± 112.57 (10)	404.22 ± 132.16 (18)	317.37 ± 133.65 (20)	365.53 ± 112.54 (20)	1199.34 ± 442.13 (6) [a]	675.08 ± 249.07 (9) [b]	661.02 ± 281.14 (8) [b]	610.31 ± 250.91 (7) [b]
LDH	/	/	/	/	173.47 ± 44.01 (7)	137.09 ± 28.14 (9)	141.30 ± 37.65 (9)	159.23 ± 24.49 (9)
PEPCK	280.86 ± 162.61 (9)	270.20 ± 149.90 (18)	287.96 ± 174.65 (18)	256.32 ± 128.33 (18)	89.36 ± 22.62 (7)	66.55 ± 15.91 (9)	73.17 ± 15.04 (9)	80.53 ± 17.05 (9)
GR	168.80 ± 28.37 (10) [a]	152.57 ± 28.64 (15) [a]	118.19 ± 39.68 (19) [b]	149.98 ± 35.73 (19) [a]	123.44 ± 36.21 (6)	133.57 ± 39.41 (8)	92.40 ± 16.21 (8)	117.14 ± 30.76 (9)
GPX	56.91 ± 23.62 (6)	2.75 ± 0.84 (13)	53.10 ± 34.00 (11)	48.15 ± 22.33 (14)	42.43 ± 14.08 (4)	40.69 ± 24.93 (7)	37.47 ± 18.16 (4)	32.04 ± 8.48 (7)
GST	277.85 ± 71.76 (9) [a]	218.20 ± 70.08 (19) [ab]	217.49 ± 58.24 (20) [ab]	181.76 ± 64.59 (17) [b]	7845.89 ± 4017.19 (7) [ab]	10605.96 ± 2046.03 (10) [a]	7772.27 ± 1172.49 (10) [b]	9918.62 ± 1882.71 (10) [ab]
SOD	613.61 ± 211.29 (10) [a]	376.43 ± 269.24 (17) [b]	356.74 ± 156.46 (17) [b]	321.71 ± 191.92 (19) [b]	202.76 ± 98.70 (7)	261.48 ± 156.52 (10)	188.97 ± 36.14 (9)	52.38 ± 41.89 (8)
CAT	95.26 ± 44.35 (8)	129.80 ± 64.20 (18)	116.99 ± 62.11 (18)	91.75 ± 43.11 (15)	133.83 ± 63.28 (6)	85.84 ± 46.87 (5)	121.44 ± 26.66 (6)	142.21 ± 108.13 (9)
ACHE	45.80 ± 10.99 (9)	41.95 ± 13.01 (19)	38.31 ± 9.09 (20)	40.84 ± 8.21 (17)	131.89 ± 44.48 (7)	112.61 ± 32.95 (7)	145.75 ± 52.29 (8)	169.64 ± 43.45 (10)
LAC	17.03 ± 3.38 (10) [ab]	20.00 ± 5.01 (20) [a]	14.89 ± 5.12 (20) [b]	19.58 ± 5.11 (20) [a]	9.59 ± 1.14 (7)	8.93 ± 1.71 (10)	8.70 ± 0.87 (10)	8.24 ± 0.74 (10)

Appendix 8: Trace element concentration (in µg.L-1) in water samples collected in the different locations in January 2022. Values above the detection limit are colored in red on grey background.

	STARESO	Calvi	Île Rousse	St Florent
Ag	< 10	< 10	< 10	< 10
Al	< 200	< 200	< 200	< 200
As	< 10	< 10	**14.84**	< 10
Ba	< 10	< 10	< 10	< 10
Cd	< 2	< 2	< 2	< 2
Co	< 1	< 1	< 1	< 1
Cr	< 10	< 10	< 10	< 10
Cu	< 2	**5.92**	**2.98**	**13.04**
Fe	< 500	< 500	< 500	< 500
Mn	< 1	< 1	< 1	**12.84**
Mo	< 10	< 10	< 10	< 10
Ni	< 100	< 100	< 100	< 100
Pb	< 1	< 1	**1.34**	< 1
Sb	< 1	< 1	< 1	< 1
Se	**83.12**	**85.16**	**49.34**	**47.82**
Sn	< 2	< 2	< 2	< 2
V	< 100	< 100	< 100	< 100
Zn	< 500	< 500	< 500	< 500

Appendix 9: Organic pollutant concentration (in ng.L^{-1}) in water samples collected in the different locations in January 2022. <LD = below the detection limit. <LQ = below the quantification limit.

	STARESO	Calvi	Île Rousse	St Florent
Naphtalène	< LQ	**8.0**	< LQ	< LQ
C1-Naphtalène	< LD	**13.2**	< LQ	< LQ
C2-Naphtalène	< LQ	**14.3**	**5.6**	**5.3**
C3-Naphtalène	**5.6**	**10.8**	**9.0**	**7.2**
Benzothiophène	< LD	< LD	< LD	< LD
C1-Benzothiophène	< LD	< LD	< LD	< LD
C2-Benzothiophène	< LD	< LD	< LD	< LD
C3-Benzothiophène	< LD	< LD	< LD	< LD
Biphényl	< LD	< LD	< LD	< LD
Acénaphtylène	< LD	< LD	< LD	< LD
Acénaphtène	< LD	< LD	< LD	< LD
Fluorène	< LQ	< LQ	< LQ	**7.2**
C1-Fluorène	< LQ	< LQ	< LQ	< LQ
C2-Fluorène	**7.6**	**6.0**	**9.2**	**5.4**
C3-Fluorène	< LD	< LD	< LD	< LD
Phenanthrene	< LQ	< LQ	< LQ	< LQ
Anthracene	< LD	< LD	< LD	< LD
C1-Phenan/anthra	< LD	< LD	< LQ	< LD
C2-Phenan/anthra	< LD	< LQ	< LD	< LD
C3-Phenan/anthra	< LD	< LD	< LD	< LD
Dibenzothiophène	< LD	< LD	< LD	< LD
C1-Dibenzothiophène	< LD	< LD	< LD	< LD
C2-Dibenzothiophène	< LD	< LD	< LD	< LD
C3-Dibenzothiophène	< LD	< LD	< LD	< LD
Fluoranthène	< LQ	< LQ	< LD	< LQ
Pyrène	< LQ	< LD	< LD	< LD
C1-Fluoranthènes/Pyrènes	< LD	< LD	< LD	< LD
C2-Fluoranthènes/Pyrènes	< LD	< LD	< LD	< LD
C3-Fluoranthènes/Pyrènes	< LD	< LD	< LD	< LD
Benzo[a]anthracène	< LD	< LD	< LD	< LD
Chrysène	< LQ	< LD	< LD	< LD
C1-Chrysènes	< LD	< LD	< LD	< LD
C2-Chrysènes	< LD	< LD	< LD	< LD
C3-Chrysènes	< LD	< LD	< LD	< LD
Benzo[b+k]fluoranthène	**1.9**	< LD	< LD	< LD
Benzo[e]pyrène	< LD	< LD	< LD	< LD
Benzo[a]pyrène	< LD	< LD	< LD	< LD
Pérylène	< LD	< LD	< LD	< LD

	STARESO	Calvi	Île Rousse	St Florent
Indéno(1.2.3-cd)pyrène	*< LD*	*< LD*	*< LD*	*< LD*
Dibenzo(a.h)anthracène	*< LD*	*< LD*	*< LD*	*< LD*
Benzo(g.h.i)pérylène	*< LD*	*< LD*	*< LD*	*< LD*
Total PAHs (ng.L^{-1})	15.0	52.3	23.8	25.2