NOVA SCHOOL OF SCIENCE & TECHNOLOGY

DEPARTMENT OF EARTH SCIENCES

SEA URCHIN (*Paracentrotus lividus*) GONADS

QUALITY AND INNOVATIVE FOOD PRODUCTS

CAROLINA RAQUEL LOPES CAMACHO

Master in Food Science and Engineering

DOCTORATE IN AGROINDUSTRIAL TECHNOLOGIES

NOVA University Lisbon

May, 2023

NOVA SCHOOL OF SCIENCE & TECHNOLOGY

DEPARTMENT OF EARTH SCIENCES

SEA URCHIN (*Paracentrotus lividus*) GONADS

QUALITY AND INNOVATIVE FOOD PRODUCTS

CAROLINA RAQUEL LOPES CAMACHO

Master in Food Science and Engineering

Adviser: Maria Fernanda Guedes Pessoa
Associate Professor with Aggregation, FCT-NOVA University Lisbon

Co-advisers: Maria Leonor Martins Braz de Almeida Nunes
Principal Researcher, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR)
Amparo Celeste Gonçalves
Auxiliary Researcher, Portuguese Institute for Sea and Atmosphere (IPMA, I.P.)

Examination Committee:

Chair: Fernando Henrique da Silva Reboredo,
Full Professor, FCT-NOVA University Lisbon

Rapporteurs: Maria João dos Ramos Fraqueza,
Associate Professor with Aggregation, Faculty of Veterinary Medicine of University of Lisbon
Isabel Palmira Joaquim Castanheira,
Principal Researcher, National Institute of Health Doutor Ricardo Jorge (INSA, I.P.)

Co-adviser: Amparo Celeste Gonçalves,
Auxiliary Researcher, Portuguese Institute for Sea and Atmosphere (IPMA, I.P.)

Members: António Manuel Barros Marques,
Auxiliary Researcher, Portuguese Institute for Sea and Atmosphere (IPMA, I.P.)
Maria do Carmo Martins Serrano,
Auxiliary Researcher, National Institute of Agriculture and Veterinary Research (INIAV, I.P.)
Helena Isabel Costa de Oliveira,
Researcher, Interdisciplinary Centre of Marine and Environmental Research (CIIMAR)
Fernando Henrique da Silva Reboredo,
Full Professor, FCT-NOVA University Lisbon
Maria Manuela Abreu da Silva,
Auxiliary Professor, FCT-NOVA University Lisbon

DOCTORATE IN AGROINDUSTRIAL TECHNOLOGIES

NOVA University Lisbon
May, 2023

Sea Urchin (*Paracentrotus lividus*) Gonads - Quality and Innovative Food Products

Ao meu pai (*in memoriam*).

AGRADECIMENTOS (*ACKNOWLEDGEMENTS*)

Perante a meta desta desafiante "maratona-PhD", o sentimento é de superação e resiliência! Estou muito grata pelo apoio que recebi nas várias etapas. Assim, quero deixar registo de algumas palavras de agradecimento.

À Doutora Maria Leonor Nunes e Doutora Amparo Gonçalves mais do que orientadoras do projeto, são verdadeiras mentoras no mundo da investigação científica; as "perguntas difíceis" foram essenciais para chegar até aqui. Expresso o meu profundo agradecimento pela constante disponibilidade, incentivo, apoio, ensinamentos científicos, e também amizade.

À Doutora Maria Fernanda Pessoa por acreditar desde o primeiro momento no valor do projeto, relembrando-me disto mesmo muitas vezes; o seu apoio e sugestões científicas bem como amizade tornaram o processo mais fácil.

Aos elementos da Comissão de Acompanhamento de Tese, Doutor António Marques e Doutora Paulina Mata pelas sugestões e críticas que me permitiram ter outras perspectivas do projeto.

À *NOVA School of Sciences and Technology*, e em especial ao Professor Fernando Lidon, Coordenador do Programa Doutoral em Tecnologias Agroindustriais e respectiva comissão científica por terem aceite a minha candidatura.

À Fundação para a Ciência e a Tecnologia (FCT, I.P.) por ter concedido e financiado a bolsa de Doutoramento (SFRH/BD/141315/2018).

Agradeço a vários projetos e respectivos recursos (Humanos, materiais, financeiros) que de forma mais direta ou indireta contribuíram para a realização dos estudos e respectivas análises.

Agradeço à Divisão de Aquacultura, Valorização e Bioprospeção do Instituto Português do Mar e Atmosfera (IPMA, I.P.; instituição de acolhimento), por proporcionar condições para a realização da maioria dos estudos no âmbito deste doutoramento.

À Doutora Carmo Serrano (INIAV, I.P.), pela colaboração, ensinamentos e resolução de problemas do HPLC durante a determinação dos carotenóides.

Agradeço ao Doutor Mário Diniz (FCT, UNL) por me ter acolhido no seu laboratório e me ter apresentado o mundo dos biomarcadores.

À Dra. Júlia Murta do Gabinete de Aconselhamento Vocacional e Psicológico (FCT, UNL), um agradecimento pelo acolhimento e empatia nas várias sessões de terapia.

Um agradecimento especial aos colegas do IPMA que constituiram o painel sensorial: Ana Bispo, Ana Braga, Ana F. Oliveira, Ana Grade, Ana Maulvault, António Marques, Carla Pires, Carlos Cardoso, Carolina Magro, Francisco Ruano, Helena Lourenço, Helena Oliveira, Henrique Ferreira, João Francisco, Luís Oliveira, Mª Conceição Almeida, Maria Pires, Margarida Saavedra, Mário Mil-Homens, Marta Santos, Miguel Carneiro, Mónica Mai, Patrícia Anacleto, Patrícia Oliveira, Paulo Fonseca, Paulo Oliveira, Pedro Costa, Ricardo Alpoim, Rui Oliveira, Susana Gonçalves e Teresa G. Pereira.

À Tatiana Correia, meu braço direito no laboratório de análise sensorial, principalmente nos ensaios de conservação. Obrigada pela preciosa ajuda, companheirismo, amizade e gargalhadas!

Agradeço à Marta Santos, Patrícia Anacleto, Ana Luísa Maulvault e Cheila Almeida pela amizade, ajuda, boa disposição e palavras de incentivo.

À Sabrina Sales, colega e amiga desta jornada de doutoramento em equinodermes. Obrigada pelo companheirismo, pelas partilhas e palavras de incentivo. Conseguimos!

Ao Miguel Baptista pela amizade, apoio e conversas verdadeiramente terapêuticas em etapas críticas da escrita da tese. Obrigada!

À Juliana Pais pelo interesse e ajuda em organizar e simplificar algumas ideias. Obrigada!

À Vera Barbosa, Helena Oliveira, Ana Bispo e Sandra Martins pela amizade e apoio a vários níveis… foram fundamentais para que este caminho fosse mais leve e fácil! Muito, muito obrigada!

Agradeço às minhas "Marias": Inês Ribeiro, Cláudia Veríssimo e Fátima Henriques. Somamos cerca de duas décadas de amizade e o carinho e boas energias nos últimos tempos foram essenciais para o meu bem-estar! Obrigada minhas meninas!

Ao Mário, meu cúmplice e companheiro, e ao nosso grande(!) lar de 4 elementos. É impagável o que também deste a estas "ouriçadas". Obrigada por estares e caminhares (sempre!) ao meu lado, com amor, dedicação, amizade e paciência que só tu tens!

Nem sei com que palavras irei agradecer à minha família… obrigada por continuarem a acreditar em mim (às vezes mais do que eu própria!). À minha mãe, Carmo, minha maior inspiração, obrigada por confiares em mim. Aos meus manos, João e Vasco, pelo amor, carinho, sorrisos e abraços reconfortantes. Muito obrigada!

A todos os que não mencionei individualmente e que de alguma forma colaboraram neste trabalho e para o meu bem-estar durante a sua realização, deixo o meu agradecimento.

Carolina

"Nothing in life is to be feared, it is only to be understood. Now is time to understand more, so that we may fear less." (Marie Curie)

ABSTRACT

The sea urchin *Paracentrotus lividus* is a seafood product of high commercial value and appreciated for the unique sensory features of its gonads (or roe), the edible part. Some of the challenges of this species are the short availability throughout the year (only a few months) associated with inconsistent overall quality and the absence of a method to assess its freshness. The approach in the present thesis was to: i) evaluate chemical contamination in the gonads; ii) study the impact of transport and storage on quality changes; iii) propose a technological processing to extend the availability of the species. The results showed that gonads from wild urchin have low levels of a wide range of emerging and persistent contaminants, as well as heavy metals (below the maximum levels in foodstuffs). It is concluded that a regular side-dish portion does not pose a risk to the consumer (**Chapter 3**). Transport of live sea urchin at 7 °C in individual compartments is more suitable for fewer losses, however it should not exceed 4 - 5 days to avoid oxidative stress with cell damage. Preservation trials, also at 7 °C, showed that the chemical index, adenylate energy charge (AEC), up to 45 % is associated with a sensory shelf-life defined in 4 - 5 days. Sensory changes in live urchin and respective gonads were thoroughly evaluated, sensory schemes based on the Quality Index Method (QIM) were proposed to evaluate the freshness degree of live sea urchin (scheme based on total of 8 demerit points) and respective gonads (scheme based on total of 10 demerit points) (**Chapter 4**). Canning is highlighted as a solution to valorise gonads of lower quality; the product was found to be stable for up to 12 months; it was well accepted by a sensory panel; and with a slight alteration in the chemical (nutritional) profile. The consumption of one portion (25 g) of canned gonads contributes to a significant daily intake of protein, EPA+DHA and selenium (**Chapter 5**). The results of the different chapters provide a significant contribution to the quality assessment (chemical and sensory) of wild sea urchins but also those from aquaculture and possibly other species.

Keywords: Seafood, Freshness, Preservation, Processing, Chemical contamination, Sensory properties, QIM

RESUMO

O ouriço-do-mar, *Paracentrotus lividus,* é um produto marinho de elevado valor comercial e apreciado pelas características sensoriais únicas das suas gónadas (ou ovas), a única parte edível. Alguns dos desafios desta espécie é a curta disponibilidade ao longo do ano (apenas alguns meses) associada a qualidade global variável e a ausência de um método para avaliar a sua frescura. A abordagem no presente trabalho foi a realização de estudos para: i) avaliar a contaminação química nas gónadas; ii) estudar o impacto do transporte e do armazenamento nas alterações da qualidade; iii) propor um processamento tecnológico que amplie a disponibilidade da espécie. Os resultados permitiram mostrar que gónadas de ouriço selvagem possuem níveis baixos de um conjunto alargado de contaminantes emergentes e persistentes, bem como de metais pesados (abaixo dos níveis máximos permitidos). Conclui-se que uma pequena porção (aperitivo) não constitui risco para o consumidor (**Capítulo 3**). O transporte de ouriço vivo a 7 °C em compartimentos individuais é adequado para menos perdas, no entanto não deve exceder 4 - 5 dias, de modo a evitar stress oxidativo com dano celular. Os ensaios de conservação, também a 7 °C, mostraram que o índice químico AEC (*adenylate energy charge*) até 45 % está associado a um ouriço com *shelf-life* sensorial de 4 - 5 dias. As alterações sensoriais no ouriço vivo e respectivas gónadas foram minuciosamente avaliadas, propondo-se esquemas sensoriais baseados no método do índice de qualidade (QIM) para avaliar o do grau de frescura do ouriço vivo (esquema com um total de 8 pontos de demérito) e repectivas gónadas (esquema com um total de 10 pontos de demérito) (**Capítulo 4**). Destaca-se a apertização como uma solução para valorizar gónadas de menor qualidade; verificou-se que o produto foi estável até 12 meses; foi bem aceite sensorialmente; e com um perfil químico (nutricional) pouco alterado. O consumo de uma dose (25 g) de gónadas em conserva contribui para uma ingestão diária significativa de proteína, EPA+DHA e selénio (**Capítulo 5**). É patente que os resultados dos diferentes capítulos proporcionam uma contribuição significativa para a avaliação da qualidade (química e sensorial) dos ouriços do mar selvagens mas também eventualmente de aquacultura e outras espécies.

Palavras-chave: Pescado, Frescura, Conservação, Processamento, Contaminação química, Propriedades sensoriais, Método do índice de qualidade

OUTLINE OF THE THESIS

This thesis is structured in a paper-style format (some already published, others under preparation for submission), except in the case of Chapters 1, 2 and 6. Each subchapter per article has its own Abstract, Introduction, Materials and Methods, Results, Discussion (together or separate from the Results) and Conclusion and can be consulted independently. Therefore, some repetition of concepts or ideas may occur throughout the thesis.

The introductory **Chapter 1** provides a contextualisation of the subject and presents the aim of the thesis with the research questions addressed. In **Chapter 2**, a bibliographic review is shown on the biology of sea urchins, its economic importance, quality features and processing through a broad contextualisation. **Chapter 3** is subdivided into two subchapters and addresses the safety evaluation in terms of persistent and emerging pollutants and macro and trace elements of wild sea urchin gonads from the Portuguese north coast. **Chapter 4** is subdivided in three subchapters focused on the freshness and quality changes of sea urchins and respective gonads, through studies of transport and preservation under refrigeration. This chapter also incorporates a proposal for a sensory scheme to assess sea urchin freshness. **Chapter 5** presents a study on the quality evaluation of canned sea urchin gonads (in light brine). And finally, the **Chapter 6** provides the main findings and final considerations of Chapters 3, 4 and 5, and answers the questions posed in the section 1.2. It also opens horizons for future research questions and ideas. All the references consulted and cited throughout the document can be found after Chapter 6 and before the Appendix.

CONTENTS

LIST OF FIGURES

List of Tables

ACRONYMS AND ABBREVIATIONS

AAS	atomic absorption spectrometry
Abs	absorbance
AEC	adenylate energy charge
AI	adequate intakes
ADP	adenosine diphosphate
AEC	adenylate energy charge, given in %
Ala	alanine
AMP	adenosine monophosphate
ANOVA	analysis of variance
AOAC	Association of Official Analytical Collaboration
AR	average requirements
Arg	arginine
Asn	asparagine
Asp	aspartic acid
ATP	adenosine triphosphate
BMDL	benchmark dose lower
BTs	butyltins
bw	body weight
CAT	catalase
CEN	Comité Européen de Normalisation
CMP	cytidine monophosphate
Cys	cystine
dp, **DP**	demerit point
DRI	dietary references intake
dw	dry weight
EC	European Commission
EDXRF	energy dispersive X-ray fluorescence
EFSA	European Food Safety Authority
e.g.	*exempli gratia* (for example)
EN	European Standard
EPA	acid eicosapentaenoic (*n*-3 fatty acid)
et al.	*et alia* (and others)
EU	European Union
EUC	equivalent umami concentration
FA	fatty acid (s)
FAA	free amino acid (s)
FAME	fatty acid methyl ester (s)
FAO	Food and Agriculture Organization of the United Nations
Gln	glutamine
Glu	glutamic acid
Gly	glycine
GMP	guanosine monophosphate
GPx	gluthathione peroxiodase
GSI	gonad somatic index
GST	glutathione S-transferase
His	histidine
Hx	hypoxanthine
HxR	inosine
i.e.	*id est* (that is; in other words)
ICP-MS	inductively coupled plasma mass spectrometry

Ile	isoleucine
IMP	inosine monophosphate
IoM	Institute of Medicine, independent and non-profit organization
IPMA	Instituto Português do Mar e da Atmosfera, I.P.
***K*-value**	chemical index of seafood freshness
Leu	leucine
LOD	limit of detection
LOQ	limit of quantification
LPO	lipid peroxidation
Lys	lysine
lw	lipid weight
Met	methionine
MLs	maximum levels
MSFD	Marine Strategy Framework Directive
MSG	monosodium glutamate
MUFA	monounsaturated fatty acids
***n*-3**	omega-3 polyunsaturated fatty acids
***n*-6**	omega-6 polyunsaturated fatty acids
NC	nutritional contribution
NP	*Norma Portuguesa* (Portuguese standard)
NW	northwest
OPFRs	organophosphate flame retardants
p	*p*-value, probability of the statistic test
PAHs	polycyclic aromatic hydrocarbons
PBDEs	polybrominated diphenyl ethers
PC	protein carbonyl
PCA	principal components analysis
PCPs	personal care products
Phe	phenylalanine
Pro	proline
PUFA	polyunsaturated fatty acids
QI	Quality index
QIM	Quality Index Method
QuEChERS	Quick, Easy, Cheap, Effective, Rugged and Safe
r	correlation
RDA	recommended dietary allowance
RUC	relative umami concentration
Ser	serine
SFA	saturated fatty acids
SOD	superoxide dismutase
sp.	single or several species within a genus
SW	southwest
Tau	taurine
TAV	taste active value, adimentional
TDI	tolerable daily intake
Thr	threonine
Trp	tryptophan
TWI	tolerable weekly intake
Tyr	tyrosine
Ub	ubiquitin
UL	tolerable upper intake level
UMP	uridine monophosphate
USA	United States of America
USDA	Unites States Department of Agriculture
Val	valine
WFD	Water Framework Directive
WHO	World Health Organization, Government Agency
ww	wet weight
WWTP	wastewater treatment plants

SYMBOLS AND UNITS

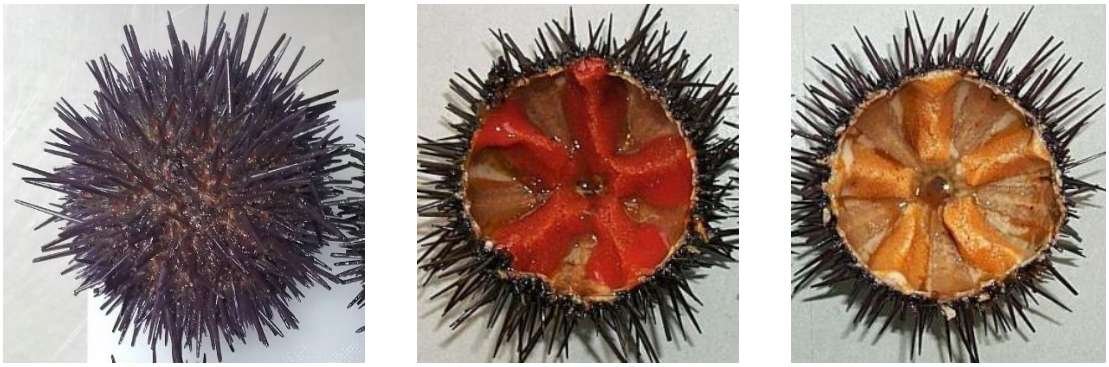
atm	atmospheres (pressure unit)
cm	centimetre
eV	electronvolt
g	gram
g	relative centrifugal force or G-force
keV	kiloelectronvolt
km	kilometre
kg	kilogram
L	litre
M	molar concentration
mA	milliampere
mg	milligram
min	minute
mL	millilitre
mm^2	square millimiters
nm	nanometer
pH	the negative logarithm (base 10) of the molar concentration of hydrogen ions
rpm	rotations per minute
s	second
t	tonne
µg	microgram
µmol	micromole
°C	degree Celsius
°Brix	degree Brix
%	percentage
±	approximately
>	more than
≥	more than or equal to
<	less than
≤	less than or equal to
Σ	summation/sum of

1

Introduction

1.1 General framework of the subject

Portugal has a long tradition in seafood consumption and sea urchins are one of the lesser-known products, but its importance has been increasing. The most relevant exploited and appreciated sea urchin in the Mediterranean and Atlantic coast areas, including Portuguese coast, is *Paracentrotus lividus* (**Figure 1.1-1 A**) which is harvested for its edible part i.e., the gonads, also called roe or *uni* (refers to both female, **Figure 1.1-1 B**; and male gonads, **Figure 1.1-1 C**). It represents one of the most valuable seafood products, being considered gourmet, a delicacy comparable to *Caviar*.



(**A**) (original) (**B**) (adapted from Pinna, 2016) (**C**) (adapted from Pinna, 2016)

Figure 1.1-1 — Whole sea urchin *Paracentrotus lividus* (**A**); mature female (**B**) and male (**C**) sea urchin with oral area removed showing the five gonads.

The demand and consumption of this delicacy have increased all over Europe, including Portugal, contrary to catch volumes. It has also been found that a significant proportion of gonads traded is not always of good quality and this is because existing data cannot portray the overall desirability and interactions of each quality trait.

Assessing the quality of wild sea urchin and its gonads is complex as some of the specific quality parameters require more data. It is therefore considered essential to improve the current knowledge to enable harvesters, consumers and markets to make more sustainable and informed choices. In this way, more studies focused on safety, biochemical features, nutritional value, freshness evaluation, best practices to preserve quality and suitable processing technologies are of utmost importance to the promotion and valorisation of the species within a competitive seafood sector. One of the main gaps with respect to the work that has been done on sea urchin quality is the absence of an objective scheme for freshness assessment.

1.2 Aims

The aim of this work was to improve the understanding of the complex interaction between the various environmental, biological, biochemical and sensory parameters that drive the quality of wild sea urchins *P. lividus* and its gonads and explore its valorisation. In a practical way, it was intended to obtain knowledge and develop tools that can be used to improve the quality evaluation and the economic viability of this fishery. To achieve this, several trials were developed to answer the following questions:

1. Is the sea urchin from Portuguese coast safe for consumption?

2. Does transport conditioning affect the quality of sea urchin?

3. Which quality changes occur during refrigerated storage of live sea urchin *P. lividus*?

4. How can the freshness of sea urchins be characterised and assessed?

5. Which is the shelf-life of live sea urchin?

6. Which changes occur in canned sea urchin gonads? Are the canned gonads accepted by consumers?

1.3 Some considerations

In the studies performed under the frame of the PhD, the wild sea urchins *P. lividus* with a minimum diameter of 50 mm (following Portuguese law Portaria n.º 82/2011 of 22nd February; MADRP, 2011) were harvested during the involved projects, or purchase to a professional with harvesting licence. In both cases, the harvesting sites did not include protected areas nor endangered/protected species and caught was realised under two conditions: absence of toxins and microbial contamination in the harvest area (reported by IPMA, I.P.) and safety conditions of the sea (low waving activity).

For sustainability reasons, the number of sea urchins was reduced to a minimum that would allow to carry out the studies, according to the 3Rs principle (Replacement, Reduction and Refinement).

The analyses were carried out mainly at the laboratories of *Divisão de Aquacultura, Valorização e Bioprospeção*, IPMA, I.P. or in collaboration with institutional partners, but always with the participation of the author of the present thesis. The exceptions were the determination of persistent and emergent contaminants (Subchapter 3.1) as well as the ICP-MS and AAS analysis (Subchapter 3.2) whose analyses were performed by project partners.

The outputs of each study were selected according to their relevance for the specific objectives within the scope of the thesis and the projects in which the author participated. The availability of resources, namely the sample amount, materials and methodologies also formed part of the criterion.

In studies involving sensory analysis, the harvesting was only done after consulting the results of the monitoring program carried out by IPMA, which attested to the absence of toxins in the water and in the shellfish and of microbiological risks (*E. coli*) (IPMA, 2023). The participation of the sensory panel in all analysis sessions was voluntary, informed and consented; the procedures to follow were conveyed in advance, as well as a description of any potential foreseeable discomfort for the panellist.

As last point, the term "urchin" refers throughout the thesis always to the sea urchin. The term gonad or roe is used to indicate the edible part of sea urchins.

2

Literature review

2.1 Sea urchins

There are archaeological records confirming that harvesting and consumption of sea urchin has been practiced during the Holocene period in some sites along the Pacific and Atlantic coasts. However, there is little information available probably due to the intrinsic fragility of sea urchin structures (Kaharudin, 2020). According to the existing records, the sea urchins were used since ancient times as food, raw materials for abrading tools and rare ornaments, and also offerings in shrines (Weisler et al., 2020). In the recent past, there has been some tradition of sea urchin consumption in many coastal areas of Asia, Polynesia, the Mediterranean and Chile. There is no unanimity on the number of sea urchin edible species, but most authors consider that the main exploited for food purposes are those indicated in **Table 2.1-1**.

Table 2.1-1 — Name and distribution of the main commercially exploited sea urchin species in the world.

Common name	Scientific name	Distribution
Japanese purple sea urchin	*Anthocidaris crassispina*	Japan, Korea, China
Black sea urchin	*Diadema setosum*	Japan
—	*Echinometra* spp.	Circumtropical
European edible sea urchin	*Echinus esculentus*	North Atlantic, Scotland
Kina	*Evechinus chloroticus*	New Zealand
—	*Glyptocidaris crenularis*	China, Japan, Korea
Purple sea urchin	*Heliocidaris erythrogramma*	West Pacific Ocean, Australia
Green sea urchin	*Hemicentrotus pulcherrimus*	Japan, Korea, China
Chilean sea urchin	*Loxechinus albus*	Chile, Peru
Variegated	*Lytechinus variegatus*	West Atlantic, Caribbean
Stony or purple sea urchin	*Paracentrotus lividus*	Atlantic, Mediterranean
Shore or green sea urchin	*Psammechinus miliaris*	Northeast Atlantic
Pink sea urchin	*Pseudocentrotus depressus*	Japan, Korea
Long spined black sea urchin	*Stomopneustes variolaris*	Tropical Indo-Pacific, Sri Lanka
Green sea urchin	*Strongylocentrotus droebachiensis*	Circumpolar (north), Pacific
Red sea urchin	*Strongylocentrotus franciscanus*	NE Pacific (Alaska to California), British Columbia
Japanese sea urchin	*Strongylocentrotus intermedius*	Japan, Russia, Korea, China
Dalian purple urchin	*Strongylocentrotus nudus*	Japan, China
—	*Strongylocentrotus pallidus*	Russia
—	*Strongylocentrotus polyacanthus*	Russia
Purple sea urchin	*Strongylocentrotus purpuratus*	NE Pacific, Alaska to California
Collector urchin; sea egg	*Tripneustes gratilla*	Circumtropical (all oceans)

(Adapted from Archana and Babu, 2016; Harris and Eddy, 2015; Kaneko et al., 2012; Stefánsson et al., 2017)

2.1.1 Biology, ecology and morphology aspects

Sea urchins belong to the taxonomic class Echinoidea (from the Greek *ekhinos* 'spines'), which together with crinoids, asteroids, ophiuroids and holothurians integrate the Echinoderms *phylum* (Agnello, 2017; Lawrence, 2013). The echinoids class includes the sea urchins (about 950 species), heart urchins and sand dollars (Hammer et al., 2013; Harris and Eddy, 2015).

2.1.1.1 Anatomy

The body of most sea urchins shows a penta-radial symmetry and tends to be spherical or globular in shape, but slightly flattened in the oral (mouth) and aboral areas (anus) (**Figure 2.1-1**). All sea urchins have a hard calcareous test or exoskeleton (typically range in diameter from 3 to 10 cm), which is covered with a thin epithelium and moveable spines, usually with few centimetres (Guidetti and Mori, 2005) (**Figure 2.1-2**). The test and the spines represent about 50 % of the live sea urchin body weight (McBride, 2005).

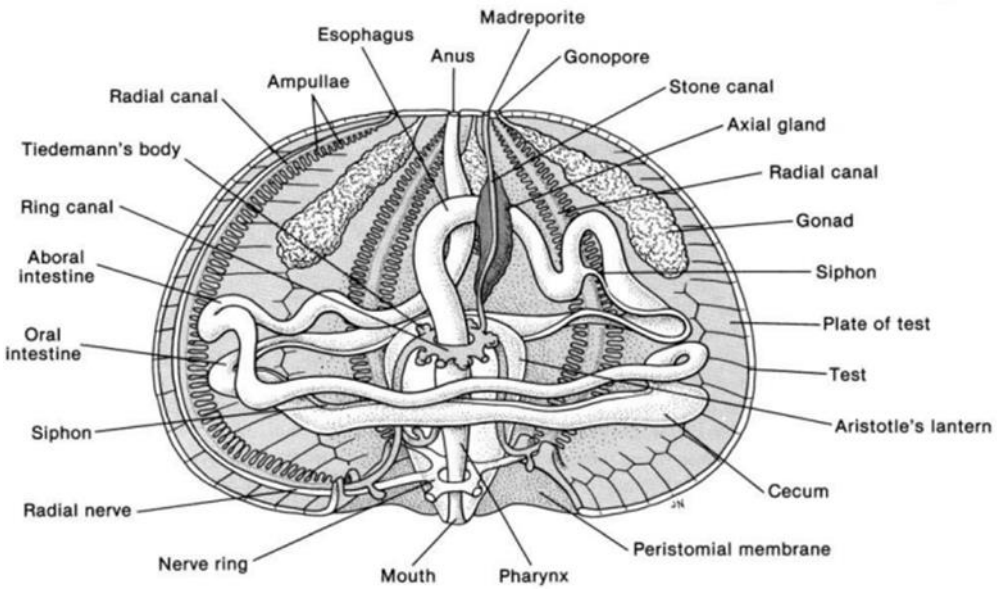


Figure 2.1-1 — Internal anatomy of sea urchin (side view).
(Adapted from Whalen, 2008)

The sea urchins live with oral area downward facing. In the oral area there are 5 pairs of gills arranged in the peristomial membrane or peristome (**Figure 2.1-2 A**), a sensitive, flat and taut region, which function is the gas exchange through the *buccal podia* (Boudouresque and Verlaque, 2013; Harris and Eddy, 2015; Meloni and Fois, 2013). In the middle of peristome is found the mouth, also known as Aristotle's lantern, with the respective 5 teeth (**Figure 2.1-2 A**).

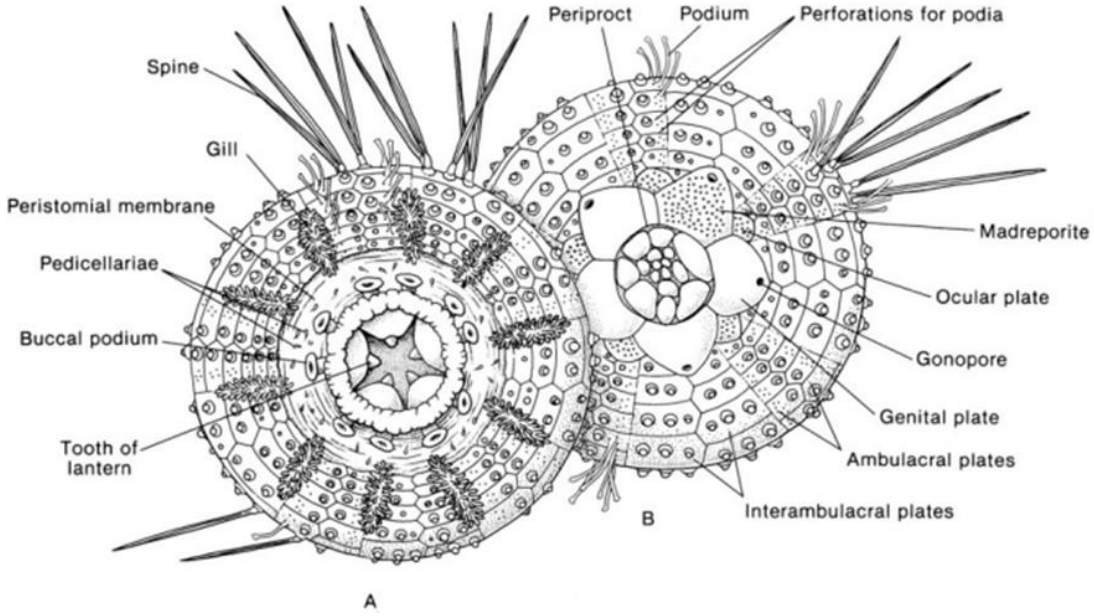


Figure 2.1-2 — External anatomy of a regular sea urchin (oral side view, **A**; aboral side view, **B**). (Image from Grosjean, 2001)

Each spine is settle on a socket joint in the test (Agnello, 2017). The spines are in axial position with a natural stiffness (Tsafnat et al., 2012), are controlled by muscles and used for protection and locomotion (vertical and horizontal movements), as well as to capture and trap floating food items (Agnello, 2017; Moureaux et al., 2010). The colour of the sea urchins spines can vary, irrespective of sex and diet. For example, in *P. lividus* species, the most common colour is purple but others such as black-purple, red-brown, yellow-brown, light brown or olive green are also frequent (Boudouresque and Verlaque, 2013).

These animals have ambulatory feet or *podia* - extensible structures with suckers on their extremities (**Figure 2.1-2 B**) used for locomotion and to attach themselves (Agnello, 2017). These structures emerge from the test through two rows of pores in each skeletal plate (ambulacral plates) which alternate with another two rows of poreless plates (interambulacral plates; **Figure 2.1-2 B**). The test is composed of 5 plates of each, alternated with each other (Ebert, 2013). *Podia* are also sensitive to chemicals and touch, absorb oxygen, catch drifting algae for food, and keep the body clean. Between the spines and also attached to the test, are *pedicellariae*, a small pincer-shape structure used for defence, to capture small prey or to clean the test surface (Lawrence, 2013; McBride, 2005).

The internal cavity (also called coelom) supports the water vascular, digestive, and reproductive systems. The vascular system is composed of canals connecting several tube feet. The digestive system contains a tube linking the down-faced mouth to the anus on the upper area

(**Figure 2.1-1**), attached to the inner wall of the body by means of a membrane (Lawrence, 2013).

The reproductive system consists of five gonads arranged between the ambulacral plates, each one connected to gonopores in the aboral area (**Figure 2.1-1**). The length of the gonads to the oral area depends on the stage of gametogenesis (Walker et al., 2013). The functional unit of the reproductive system is called ascinus where gametogenesis takes place. Each of the five gonads has a single gonoduct that shows up the test through a pore on each of the genital plates. The gonad wall is made up of 2 sacks (outer and inner with distinct characteristics), split apart by the genital coelomic sinus (GCS). The outer sac has visceral peritoneum facing the perivisceral coelom, which is connected to a connective tissue layer (CTL). Epithelial cells flank the CTL towards the GCS on the other side (Carboni, 2013; Walker et al., 2013).

The body size and growth of sea urchins are directly related to cellular processes, resulting in changes in skeletal mass that involve an expansion, calcification and tissue production (Ebert, 2013). It is still very difficult to determine accurately the age of individuals, and the usual approach is trying to evaluate it based on the diameter of their test. According to Boudouresque and Verlaque (2013), a test diameter of 2 cm corresponds to an average age of 2 years and 4 cm would correspond to 4 - 5 years old urchin. Additionally, it has been noticed that the growth is influenced by biotic and abiotic factors such as water temperature, population density, feed quality, and gonadal development (Boudouresque and Verlaque, 2013; Tomšić et al., 2010).

2.1.1.2 Distribution

Different species of sea urchins live in a variety of marine environments from intertidal shallow waters to the deep ocean and cannot survive in fresh water. Urchins are mostly sedentary and colonize rocky and sand bottoms, wave-exposed rocks and pools, coral reefs in kelp forests and sea grass beds (Agnello, 2017; Lawrence, 2013). In the intertidal, sea urchins often wear away the rock, over generations, to create holes in which they are partially protected from predators (Banks, 2014). The distribution and the density are extremely variables in space and time owing to numerous physical and biological factors, for example, heterogeneity of habitats, wave motion, slope of the bottom, light, nutrition, mortality, predation and larval survival, (Guidetti et al., 2003).

Sea urchins are mainly herbivorous and prefer areas rich in macro and microalgae. Some species feed on animal organisms such as small crustaceans and mollusks, sponges, coelenterates (e.g. jellyfishes, corals, anemones), and other sessile species (who lived mainly fixed)

(Banks, 2014; Boudouresque and Verlaque, 2013). They also feed on already dead and decaying individuals of these and other animal species. In case of feed scarcity, the adults can become detritivores (Boudouresque and Verlaque, 2013). One other characteristic of these organisms is that they are light sensitive, different behaviours and consequent movements were observed with different light intensities (Verling et al., 2002).

It has been found that increasing temperatures can lead to a decrease in abundance, a fact that was seen in the case of *P. lividus* from the south-eastern Mediterranean Sea during the period when temperatures exceeded 30.5 °C before reaching peak summer values (Yeruham et al., 2015). These results suggest that higher seawater temperatures in recent years may be the main cause for the disappearance of *P. lividus* from the southeast Mediterranean Sea, which may indicate distributional range contraction in this region (Yeruham et al., 2015).

2.1.1.3 Reproduction and life cycle

Sea urchins are found in aggregations that contain specimens of different sizes and degrees of maturity. Sea urchins are dioecious, i.e., have male and female reproductive organs (gonads) in separate individuals, however no external differences can be detected between male and female; rare case of hermaphroditism are reported in *P. lividus* (Lawrence, 2013). It is only during the period of spawning where the sexes can be identified; males release a white fluid and females release an orange fluid, or by dissection and further histological analysis.

Both male and female adult sea urchins normally spawn once per year and release their gametes (eggs or sperm) into the water column (this is called broadcast spawning), where mixing and fertilisation of the eggs occurs. According to studies on a typical sea urchin (*S. droebachiensis*) the reproductive cycle in sea urchins can be divided into four stages: (i) post spawning; (ii) storage cell growth (pre-gametogenesis and storage cell (NP) renewal); (iii) development of reproductive cells (gametogenesis and storage cell utilisation); and (iv) spawning and pre-spawning (end of gametogenesis, storage cell exhaustion and spawning) (Walker et al., 2013). However, the reproductive cycle can vary significantly between geographical locations and even within sea urchin populations. The factors that stimulate gametogenesis and spawning are not fully understood (Harris and Eddy, 2015), but there is evidence that the availability of food as well as the density of urchins can influence the development of gonads. Environmental conditions such as seasonal variation in seawater temperature and light (photoperiod) can also impact on the reproductive cycle of sea urchins, resulting in huge differences between populations and even individuals within a population (Walker et al., 2013).

The reproductive season depends on the species, and within species the distribution and biotic and abiotic factors dictate the different spawning seasons (Banks, 2014). For example, *P. lividus* from west coast of Ireland spawned between May and July (Byrne, 1990), while in the Basque and Cantabrian coast the spawning is during spring (Garmendia et al., 2010; González-Irusta et al., 2010). In Portugal coast, the spawning occurred between spring-summer (Rocha et al., 2019b).

2.1.2 Harvesting processes

Traditionally, the sea urchins were collected manually from a boat with an relatively inexpensive equipment with two long handles connected to two claws like rakes (James et al., 2016). In Mediterranean and Atlantic areas it was called "truel de vara" or "varal" and consisted of a metal ring with a net attached to a rod (Gómez, 1992). This traditional technique requires very quiet and steady weather and it was labour intensive (James et al., 2016).

Presently the dominant method of harvesting sea urchins around the world is by self-contained underwater breathing apparatus (Scuba diving; Reynolds and Wilen, 2000). In some geographical areas (e.g., in California), divers use specially equipped urchin diving vessels which average around 8 m in length, with a covered cabin in the front and large work area in the stern. Breath-hold diving is another technique that is widely used around the world to harvest benthic invertebrates including sea urchins. It involves divers equipped with snorkel, mask and flippers breath holding and diving to depths up to 15 m to collect sea urchins. This is the cheapest form of diving as it has minimal demands for equipment and it also avoids the dangers posed by breathing compressed air (James et al., 2016).

In Europe they are usually collected manually by divers in near-shore waters (James et al., 2016; James and Siikavuopio, 2014). In most cases, both professional and amateur fishermen collect sea urchins by hand with or without Scuba diving equipment (Lourenço et al., 2022a). The shift on the harvesting techniques led to higher catchability and consequent overexploitation of *P. lividus* species in NW Spain (Galicia; Fernández-Boán et al., 2012) and Portuguese coast (Bertocci et al., 2014).

2.1.3 Economic importance, markets and consumption drivers

Finding reliable figures of the size of the global market is challenging; it is generally agreed that data provided by FAO producing countries underestimate landings in some cases. In other situations accuracy is compromised because data for other echinoderms are

aggregated with those of sea urchins and FAO cannot filter out the differences (FAO, 2022). The demand significantly augmented in the second half of the last century, which led to a steadily increase of world catches from 1950 until 1995 when it peaked 110,000 tonnes (Andrew et al., 2002). This amount included all identified or unidentified sea urchins and other echinoderms in the FAO database.

The most recent data available by FAO indicates a sea urchin production of about 69,000 tonnes (live weight) in 2020 (FAO, 2022). Only the catches of two harvested sea urchin species are considered in this publication: the Chilean sea urchin *L. albus* (55.1 %) and the sea urchins of the genus *Strongylocentrotus* spp. (44.9 %). However, such amount certainly underestimates the total landings because many other sea urchin species are harvested, albeit in small quantities. Moreover, the precision is additionally compromised because the amounts of other echinoderms is sometimes aggregated with the sea urchin data (Stefánsson et al., 2017).

Most sea urchins are consumed in traditional markets (Sun and Chiang 2015; Stefánsson et al. 2017) such as Japan, Korea, Greece, France, and New Zealand. Japan has the largest market, is considered the epicenter of sea urchin with about 90 % of world demand, is also the predominant influencer of its consumption. The delicacy is served at special events and only a small portion is consumed domestically (Sun and Chiang, 2015).

Stefánsson et al. (2017) discussed with detail the key traditional markets and prices for sea urchins in many countries where the capture and consumption of sea urchins is relevant as well as the current suppliers to the market. According to this author the predominant EU countries for consuming sea urchin products are Italy, France and Spain and the markets with the greatest potential for establishing more trade are likely France and Italy.

The price of live sea urchins and gonads and that of processed products is determined by many factors, including colour, size, species, appearance, nutritional value, and type of processing. These factors are affected by time of year, temperature, photoperiod, feed (Chen et al., 2013), and the sea urchin's reproduction cycle highly influence the market conditions (Reynolds and Wilen, 2000) and availability of the product. Recently, Lourenço et al. (2019) pointed out that other sensory features such as size, colour and appearance also determine market value and consumer acceptance. In Japan and some European countries, sea urchin is considered to be one of the most valuable fisheries for the price its gonads can attain due to their unique flavour (Chen et al., 2013). Depending on species, the live sea urchins costs between 15 and 35 €·kg^{-1} in the French and Spanish market (Lourenço et al., 2021). The popularity outside of traditional markets gained special emphasis with the increase in ethnic populations and the growing status of sushi restaurants. Nowadays, both male and female gonads are

delicacies consumed in various ways, such as raw in sushi/sashimi or with lemon, onion, and olive oil, and as savoury ingredients in omelettes, scrambled eggs, fish soup, and mayonnaise (Sun and Chiang, 2015). Regarding processed gonads, it is possible to find canned *P. lividus* gonads in 50 g jars in French retailers, especially before Christmas with an average price of (100-120 €)·kg^{-1} (Monfort, 2002).

Japan is the world's largest importer and consumer of sea urchin roe. However, smaller markets exist in the United States of America, Europe, and several Asiatic countries. in Mediterranean cuisine, urchin roe has traditionally been popular for centuries, where it is eaten plain or as an ingredient in various dishes. In the old days, several coastal villages on the western shore of Portugal, such as Ericeira and Peniche, had family habits of gathering sea urchins during the Easter Spring tides (Lourenço et al., 2021). According to Baião et al. (2021a), the sea urchin market in Portugal is still in an immature stage and, in general, consumers far from the harvesting areas do not know too much about this product. To publicize and raise consumer awareness for the consumption of this delicacy, festivals are annually held (for example in *Ericeira* between March and April) (Lourenço et al., 2021). On the other hand, Portuguese chefs identify the lack of stability in the supply of a high-quality sea urchin product as a strong barrier to include it in the several dishes (Baião et al., 2021a).

2.1.4 Farming challenges

The growing demand for sea urchins and the development of new products based on sea urchin gonads, has led the processing industry to a regular supply to meet market needs based on sustainable production. This demand has led to the development of sea urchins aquaculture (echinoculture), using *in vitro* fertilisation of female and male gamete (Mendes et al., 2019). According to McBride (2005) this production process may take 2 - 5 years until commercialization. Even so, it has been proposed as a solution to meet the market demand and contribute to the recovery of overexploitation of wild stocks in some regions (Kelly, 2005).

The echinoculture turn into an interesting and valuable business all over the world (Sun and Chiang, 2015). The objective is to produce high quality gonads, and several efforts and progresses have been made. The adaptation of sea urchins to intensive culture conditions is favourable due to their biological and physiological features, however the economic viability is still unclear.

Sea urchin farming has been the topic of several projects and programs to enhance knowledge about the best abiotic factors and feed composition. To achieve high survival and culture success, the development and optimisation of mass-culture conditions and techniques,

diets (natural or processed) and feeding regimes are of critical concern (Kelly, 2005; Repolho, 2012). Because to their different phases of lifecycle and the complexity of biotic interactions that take place during the metamorphosis (Mendes et al., 2019), associated with its slow rate of somatic growth and low gonad yield (Lourenço et al., 2022a), the production of echinoderms poses a challenge to the scientific community and aquaculture sector.

The availability of affordable feeds with nutrients that promote gonad bulking and high quality gametes continues to be a barrier to the growth of echinoculture techniques (McBride, 2005; Zupo et al., 2019). In general, two strategies were proposed to extend the phase during which valuable sea urchin gonads can be harvested. One is to stimulate the growth of NPs through artificial feed rather than macroalgae. The alternative is to suppress gametogenesis by altering environmental signals that induce gametogenesis or even breeding triploid sea urchins (sterile) (Unuma and Walker, 2009).

Catching wild sea urchins and feeding is nowadays a reality in some coastland areas (McBride, 2005). Gonad enhancement is precisely harvesting wild, mature sea urchins, usually with suboptimal quality, held in land or sea-based holding systems and fed with natural or manufactured feeds to improve the roe (James et al., 2017). These can be harvested from areas with little access to feed, inadequate environmental conditions or even from barrens where urchins are abundantly found (James et al., 2017; Pert et al., 2018a). The enhancement in this topic means increasing the quality of roe, namely size (GSI; Pearce et al., 2004, 2002; Shpigel et al., 2005), colour (Shpigel et al., 2006), taste, texture of the gonads (Baião et al., 2019; Phillips et al., 2009, 2010; Siikavuopio et al., 2007), as well as maturity and viability of the gametes (Mendes et al., 2019).

Despite Chefs and consumers have not yet fully embraced aquaculture products, they are aware that only echinoculture can provide all year-round high quality sea urchins (Baião et al., 2021a).

2.2 Sea urchin quality

According to consumer studies, the key feature for purchase seafood continues to be the quality (Alasalvar et al., 2011b), which is a multidimensional term that includes some identifiable aspects (Bremner, 2002; Ólafsdóttir et al., 1997). Convenience, availability, authenticity, price, ethical and ethnic issues, nutritional value, sensory properties as well as freshness play a relevant role in the choice of seafood.

Since sea urchin gonads have been seen as a high-quality product, many studies have focused on studying the effect of several factors such as origin, season, sex (Mol et al., 2008b; Rocha et al., 2019b; Verachia et al., 2012b), environment/habitat (Pert et al., 2018b) and diet (from natural habitat or farmed; see examples of the latter in **2.1.4**) on its quality. Some authors reported changes in the chemical composition and sensory profile of the gonads. For example, Pert et al. (2018) observed that urchins with small and low-quality gonads can result from a lack of macroalgae in their habitats.

For most of the sea urchin species, gonad quality is optimal for consumption even before gametogenic differentiation, corresponding to the presence of nutritive phagocytes (NP) in association with high gonad yield (Marsh et al., 2013; Spirlet et al., 2000; Unuma and Walker, 2009; Walker et al., 2015). The **Figure 2.2-1** depicted the gonads size of *P. lividus* and some attributes during the year.

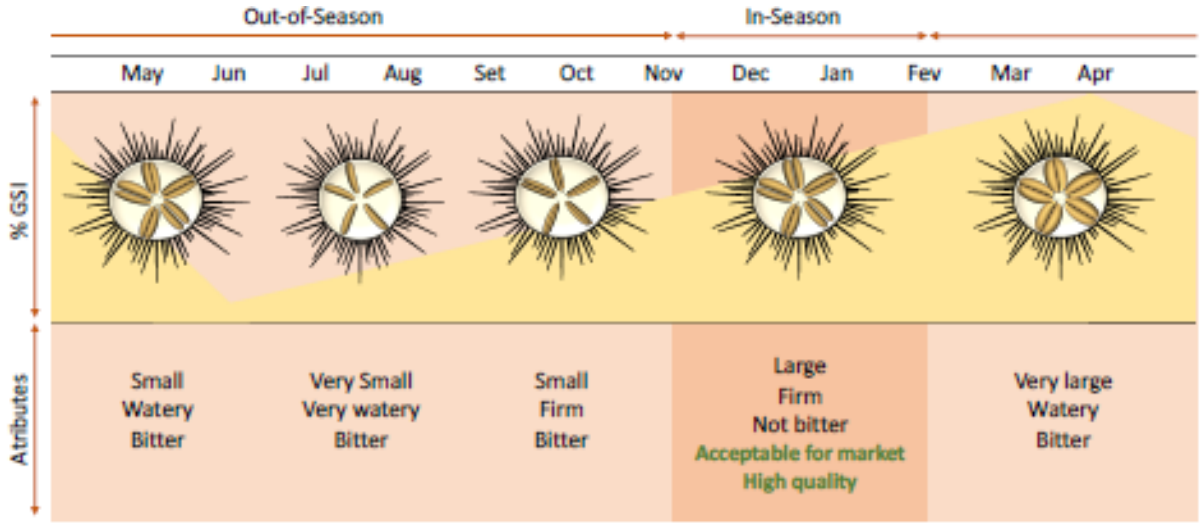


Figure 2.2-1 — Gonadosomatic index (GSI) variation of *P. lividus* from Atlantic and Mediterranean areas. (Adapted by Baião (2021) from Unuma (2002))

The perception of better quality during the pre-mature season (growth phase), corresponds to larger gonads with acceptable sensory characteristics. During this phase, the NP store glycogen as a primary source of energy, which can be broken down into glucose without the need for extra energy to break free (Taylor et al., 2017). Although glycogen has no specific taste on its own, when in the mouth, salivary amylase breaks it down into glucose, which structurally resembles sugar. When NP (and glycogen) are denser (and reproductive cells are comparatively few), it is observed that a greater proportion of gonads exhibit more palatable flavour characteristics because taste receptors recognize glucose molecules as sugar, which implies sweetness.

On the other hand, gonads out-of-season have predominance of proliferative cells and have been associated with poor quality as well as with relatively high levels of lipids, which may contribute to a bitter taste (Phillips et al., 2010). Additionally, large reproductive cells are though to impact the surface of germinal epithelium, which could lead to poor texture and graininess (Pecorino et al., 2013).

2.2.1 Safety concerns

2.2.1.1 Microbiological contaminants

The food safety of sea urchins is regulated in EU by the Regulations (EC) No. 853/2004 and 854/2004 (EC, 2004a, 2004b) that consider the presence of *Escherichia coli*, which is routinely used to test microbiological quality and to classify harvest areas into three different quality levels (A-C). On the other hand, microbiological criteria for the control of these seafood products are established in Regulation (EC) No. 2073/2005 (EC, 2005), modified by Regulation (EC) No. 2015/2285 (EC, 2015a).

Sea urchins are considered to pose minimal risk of faecal contamination because of their grazing-feeding mode, in contrast to bivalve shellfish that concentrate faecal contaminants by filter-feeding of particles suspended in the water column (Bouchoucha et al., 2015), concentrating them in their tissues. Nonetheless, the faecal risk associated to sea urchin consumption cannot be neglected since they are mainly eaten raw.

The harvesting of echinoderms is considered a primary production, therefore subjected to official regulation (EC, 2004a). Results of the monitoring plan for echinoderms in Sardinia reported absence of *Salmonella* spp. and *Vibrio parahaemolitycus*; *E. coli* levels in the gonads of the examined specimens were in compliance with EC Regulation (Terrosu et al., 2011). The Portuguese Institute for Sea and Atmosphere, I.P. (IPMA), a public institute with responsibilities on the sea dominance through a monitoring program called National Monitoring System for Bivalve Molluscs (SNMB in Portuguese) defines, monitors, and classifies production and harvested areas of shellfish in Portugal (mainland). In September 2017, the monitoring program began to include the sea urchin *P. lividus* in their sampling program for analysis of *E. coli* as well as toxins and toxic metals (Cd, Hg and Pb). Therefore, the sea urchin monitoring campaign was started 5 years ago, and during this period, the *E. coli* counts were irrelevant (less than 18 MPN[1]·(100 g)$^{-1}$ edible part, i.e., in the gonads) with a couple of few exceptions (counts up

[1] Most probable number

to 45 MPN·(100 g)$^{-1}$ gonads were reported by IPMA webpage) (IPMA, 2023). These counts were far less than the limit regulated (EC, 2015b, 2004a), demonstrating the steady good microbial quality of the product. Until now, only a single report of *E. coli* contamination of sea urchins from naturally growing areas can be retrieved from the literature (Bouchoucha et al., 2015). Recent studies screened Hepatitis E virus and Norovirus in sea urchin gonads through four seasons, where 2 batches were found positive for norovirus, despite being within the legislated sanitary limits for *E. coli* and *Salmonella* spp. (Santos-Ferreira et al., 2020). However such detections were in the seasons where the sea urchins had less quality for consumption (Rocha et al., 2019b).

2.2.1.2 Chemical contaminants

Since some historical tragic events, a vast literature on the toxicity of non-nutritive elements such as cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As) has become visible. Guidelines and regulations on the maximum levels of toxic elements have been set: maximum permissible limits (MPLs) for the presence of some chemical contaminants in seafood were defined by European Commission (Directives 1881/2006 and 420/2011; EC, 2006, 2011a).

Several investigations have shown that urchins effectively bioconcentrate toxic elements (Ablanedo et al., 1990; Augier et al., 1989; Bielmyer et al., 2012; Rouane-Hacene et al., 2018; Warnau et al., 1995), in proportion with the pollution of seawater (Bendimerad, 2014). The bioaccumulation of toxic elements varies within urchin body tissues, where the higher levels is generally found in the digestive wall (Warnau et al., 1998). Even so, the ingestion of roe hampered some risks mainly if the harvesting site is subjected to possible contamination. In addition, the gonad elemental fingerprinting hold the possibility to trace the harvesting location (Mamede et al., 2022).

The Portuguese monitoring program, through its monthly reports on the presence of the toxic elements, reported for sea urchin gonads average values of 0.13, 0.01 and 0.08 mg·kg^{-1} for Cd, Hg and Pb, respectively. These values are in respect of the 5-year monitoring campaign of sea urchin harvested in the Portuguese coastal areas (IPMA webpage; IPMA, 2023). Recently, it was stated that sea urchins from the Atlantic areas were safe for consumption and have high nutritional quality (Rocha et al., 2019b). Nonetheless, an approach of a risk-benefit perspective is still needed.

Beyond toxic metals, the butyltins (BTs), polycyclic aromatic hydrocarbons (PAHs), pesticides and emerging pollutants, such as pharmaceuticals and personal care products (PCPs) and flame retardants (FRs), are nowadays a global environmental concern (e.g., Aminot et al., 2017;

Carvalho et al., 2009; Salgado et al., 2010; Silva et al., 2014). In this sense, the European Commission published in 2008 the Marine Strategy Framework Directive (MSFD) in the field of marine environmental policy aiming to reduce or cease marine PAHs, polybrominated biphenyl ethers (PBDEs) and toxic metals (Directive 2008/56; EC, 2008).

The pharmaceuticals and PCPs are discharged into the marine ecosystems and bioaccumulate in marine organisms (Huerta et al., 2012; Serra-Compte et al., 2018), although their presence is still not regulated in the EU but are instead in the "Watch List" of emerging non-regulated aquatic pollutants (Decision 2015/495; EC, 2015a). Within the FRs, the PBDEs are listed as persistent organic pollutants by the Stockholm Convention due to their persistence, bioaccumulation and toxic effects towards organisms and humans (Gu et al., 2017). Concerning pesticides, maximum pesticide residue limits were not stipulated for seafood (in contrast to crops, milk and meat), although EC is working on the magnitude of pesticides residues in fish (EC, 2021).

The *P. lividus* has been considered a sentinel species, a suitable organism as bioindicator of environmental quality (e.g., Bouiba et al., 2023; Cunha et al., 2005; El Idrissi et al., 2020; Johnstone et al., 2019; Pinsino and Matranga, 2015), mainly due to its wide distribution, sedentary habits, easy collection, grazer-feeding behaviour and tolerance to pollution (Salvo et al., 2014; Warnau et al., 1998). A recent study provided information on the bioaccumulation of most toxic elements in the Mediterranean area, highlighting that in general the North African coasts of Western Mediterranean is the region with most contaminated *P. lividus* (Bouiba et al., 2023). Investigations on the presence of most pollutants of emerging concern in sea urchin is limited, few studies reported PAHs (Angioni et al., 2012). The accumulation of pharmaceuticals, PCPs, flame retardants and pyrethroids pesticides did not have been investigated in *P. lividus* of commercial size.

2.2.2 Chemical composition and nutritional attributes

It should be remembered that the chemical composition of seafood changes depending on the season, origin, size, sex, availability of food and stage of reproductive cycle (Oehlenschläger, 1997), thus challenging the comparison and evaluation of the benefits and drawbacks of its consumption. Nevertheless, the insights on the chemical composition of marine organisms are a remarkable contribution to understand the species quality. Several authors have studied the proximate composition of gonads of some species of sea urchins (**Table 2.2-1**).

Table 2.2-1 — Proximate composition (g·(100 g)$^{-1}$ wet weight, ww) in gonads of wild sea urchin's species.

Species	Moisture	Total lipids	Protein	Ash	Carbohydrates	Reference
A. crassispina	70.6 ± 0.2	14.0 ± 0.1	4.1 ± 0.0	1.9 ± 0.0	9.4 ± 0.1	(Lee et al., 2012)
D. setosum [1]	63.9 - 76.4	3.6 - 6.9	14.1 - 17	1.8 - 2.6	0.1 - 11.7	(Kaneko et al., 2012)
E. chloroticus [2]	78.0 - 80.6	n.r.	10.9 - 18.2	n.r.	5.3 - 10.0	(Verachia et al., 2012b)
H. pulcherrimus	74.1 ± 1.1	8.4 ± 0.7	16.3 ± 0.5	n.r.	n.r.	(Bledsoe et al., 2003)
H. pulcherrimus	74.5 ±0.9	13.2 ± 0.5	3.5 ± 0.1	2.2 ± 0.1	6.5 ± 0.2	(Lee et al., 2012)
P. lividus [3]	73.0 ± 0.5	3.8 ± 0.7	15.1 ± 1.2	n.r.	n.r.	(Cruz-García et al., 2000)
P. lividus [4]	78.3 - 82.0	2.4 - 4.3	9.26 - 11.75	1.5 - 1.7	2.0 - 2.5	(Dincer and Cakli, 2007)
P. lividus	73.4 ± 5.3	3.5 ± 1.3	15.1 ± 1.1	2.1 ± 0.3	n.r.	(Zlatanos et al., 2009)
P. lividus [5]	79.9 ± 1.4	3.1 ± 0.5	12.0 ± 1.3	2.3 ± 0.2	2.8 ± 2.4	(Mol et al., 2008b)
P. lividus	81.1 ± 1.6	3.8 ± 0.5	11.8 ± 1.1	2.1 ± 0.3	1.3 ± 0.2	(Stamatis and Vafidis, 2009)
P. lividus	85.0 ± 0.4	1.9 ± 0.1	11.0 ± 0.2	1.9 ± 0.0	0.3 ± 0.1	(Pais et al., 2011)
P. lividus [1]	n.r.	1.5 - 4.8	2.5 - 9	n.r.	0.5 - 2.5	(Arafa et al., 2012)
P. lividus	78.6 ± 1.2	3.9 ± 1.1	4.5 ± 0.2	3.5 ± 0.6	1.7 ± 0.3	(Prato et al., 2018)
P. lividus [6]	70 - 81	3 - 6	13 - 16	1 - 3	n.r.	(Rocha et al., 2019b)
P. lividus, males	80.3 ± 0.5	2.6 ± 0.4	15.6 ± 1.6	n.r.	n.r.	(Baião et al., 2019)
P. lividus, females	78.9 ± 0.6	4.0 ± 0.5	12.9 ± 0.4	n.r.	n.r.	(Baião et al., 2019)
P. lividus, males	79.3 ± 0.5	4.1 ± 2.7	12.9 ± 0.4	2.1 ± 0.1	n.r.	(Baião et al., 2022)
P. lividus, females	80.3 ± 2.0	2.7 ± 0.5	14.6 ± 0.8	2.5 ± 0.1	n.r.	(Baião et al., 2022)
P. depressus	72.1 ± 0.5	13.7 ± 0.3	3.5 ± 0.1	1.9 ± 0.0	8.8 ± 0.2	(Lee et al., 2012)
Sphaerechinus. granularis [7]	n.r.	3 - 4	9 - 12	n.r.	n.r.	(Lourenço et al., 2022a)
S. variolaris	77.5 ± 0.8	5.0 ± 0.3	12.1 ± 0.4	3.8 ± 0.3	1.6 ± 0.2	(Archana and Babu, 2016)
S. franciscanus	75.4 ± 0.5	5.0 - 0.4	13.6 ± 0.6	3.7 ± 0.1	n.r.	(Cuevas-Acuña et al., 2019)
S. nudus	74.9 ± 0.5	7.1 ± 0.2	11.1 ± 0.2	1.5 ± 0.1	n.r.	(Zhu et al., 2010)
T. gratilla [1]	82.1 ± 1.6	3.0 ± 0.6	9.0 ± 1.8	2.8 ± 0.3	3.2 ± 2.0	(Chen et al., 2013)

Values are means ± standard deviation or content range, min - max (-); n.r. - not reported; [1]obtained monthly through one year; [2]found quarterly for one year; [3]the given moisture was used to convert values from dry weight (dw) to ww; [4]observed monthly through 6 months; [5]obtained every two months for a year; [6]found quarterly for 15 months; [7]a moisture content of 80 % was considered to convert values from dw to ww.

Overall, the proximate composition of raw sea urchin gonads shown in **Table 2.2-1**, lead to consider such product as a low-calorie and healthy seafood product with high protein levels and low lipid content.

2.2.2.1 Proteins

Proteins have a structural and functional role in cellular metabolism of living organisms (Marsh et al., 2013). Like in other seafood products, proteins are, after moisture, the major components of sea urchin gonads, regardless of the species (**Table 2.2-1**). The average protein

content may attain up to 18 g·(100 g)$^{-1}$ in *E. chloroticus* in spring (Verachia et al., 2012b) and 16 g·(100 g)$^{-1}$ in *P. lividus* from Portuguese coast in fall (Rocha et al., 2019b).

The seafood protein is usually of good quality due to its high digestibility as well as the proportional amounts and availability of essential amino acids (Nunes et al., 2011), such is the case of roe of many species including sea urchin (Bledsoe et al., 2003). The most abundant amino acids (in ww) in *P. lividus* are: glycine (1.0 - 2.8 g·(100 g)$^{-1}$), glutamic acid (0.8 - 1.6 g·(100 g)$^{-1}$), aspartic acid (0.5 - 2.0 g·(100 g)$^{-1}$), lysine (0.4 - 0.8 g·(100 g)$^{-1}$) and alanine (0.3 - 0.8 g·(100 g)$^{-1}$) (Cruz-García et al., 2000; Dincer and Cakli, 2007; Mol et al., 2008b; Zlatanos et al., 2009). These are the main amino acids also reported for other sea urchin species. In general, methionine/cystine or tryptophan/tyrosine are the limiting amino acids in the roe (Bledsoe et al., 2003).

2.2.2.2 Lipids

The biological role of lipids in living organisms is well recognised and includes the storage and transfer or energy, formation of cell membrane and transport of fat-soluble vitamins (Nunes et al., 2011). Lipid content can range from less than 5 %, which is the most frequent amount in sea urchin gonads (**Table 2.2-1**), up to 20 % (10 % in average) in the case of salmon roe (Bledsoe et al., 2003).

The fatty acids (FA) are the simplest compounds of lipids and are classified in three groups: saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA), whose amounts and proportions widely vary among seafood species (Nunes et al., 2011). Although with only ≈ 5 % of total lipids, the sea urchin gonads are a good source of FA. **Table 2.2-2** presents the main groups of FA for some urchin's species.

Table 2.2-2 — Groups of fatty acids (%) in gonads of wild sea urchin's species.

Species	SFA	MUFA	PUFA	*n*-3 PUFA	*n*-6 PUFA	*n*-3/*n*-6	Reference
A. crassispina	50.3 ± 5.3	9.8 ± 1.0	39.1 ± 6.6	n. r.	n. r.	n. r.	(Lee et al., 2012)
H. pulcherrimus	50.8 ± 3.3	9.9 ± 0.2	39.4 ± 4.3	n. r.	n. r.	n. r.	(Lee et al., 2012)
P. lividus [1]	25.6 ± 4.9	13.8 ± 1.6	34.2 ± 5.5	20.3 ± 2.3	13.9 ± 3.9	1.6 ± 0.4	(Mol et al., 2008b)
P. lividus	41.3 ± 1.4	13.0 ± 0.1	19.7 ± 0.9	8.9 ± 0.1	10.0 ± 0.8	0.9 ± 0.1	(Zlatanos et al., 2009)
P. lividus	36.4	17.5	41.5	33.95 *	7.25 *	4.7 *	(Stamatis and Vafidis, 2009)
P. lividus [2]	21.0 - 29.1	11.8 - 17.6	40.0 - 46.0	n. r.	n. r.	n. r.	(Arafa et al., 2012)
P. lividus	30.0 - 32.3	23.5 - 23.8	38.9 - 41.5	17.6 - 19.1	17.8 - 18	1.0*	(Siliani et al., 2016)
P. lividus	42.2 ± 2.0	23.5 ± 2.1	34.3 ± 0.1	25.9 ± 0.1	8.4 ± 0.1	3.1 ± 0.1	(Prato et al., 2018)
P. lividus [#3]	3.6 - 10.3	3.0 - 7.6	4.7 - 14.6	2.9 - 9.9	1.8 - 4.6	0.5 - 0.7	(Rocha et al., 2019b)
P. lividus, males [#]	2.3 ± 0.5	2.2 ± 0.2	4.2 ± 0.7	2.2 ± 0.4	1.9 ± 0.3	0.9 ± 0.1	(Baião et al., 2019)
*P. lividus, f*emales [#]	6.8 ± 1.8	5.3 ± 1.3	6.3 ± 1.5	3.8 ± 1.0	2.4 ± 0.6	0.6 ± 0.0	(Baião et al., 2019)
P. depressus	37.5 ± 3.3	10.9 ± 0.6	51.7 ± 2.5	n. r.	n. r.	n. r.	(Lee et al., 2012)
S. variolaris	61.1 ± 1.0	16.9 ± 0.8	22.0 ± 2.2	11.0 ± 0.4	10.3 ± 0.8	1.1 *	(Archana and Babu, 2016)
S. nudus	25.2 - 28.1	28.4 - 30.4	34.7 - 37.4	n. r.	n. r.	n. r.	(Zhu et al., 2010)
T. gratilla [2]	16.1 ± 6.8	8.3 ± 3.0	8.7 ± 2.0	3.8 *	4.5 *	0.8 *	(Chen et al., 2013)

Values are means ± standard deviation or content range, min - max (-); [#]values in $mg \cdot g^{-1}$; *calculated using the given values; n. r. - not reported; SFA - saturated FA; MUFA - monounsaturated FA; PUFA - polyunsaturated FA; *n*-3 PUFA - very-long chain polyunsaturated omega-3 FA; *n*-6 PUFA - very-long chain polyunsaturated omega-6 FA; [1]found every two months for a year; [2]obtained monthly through one year; [3]observed quarterly for 15 months.

In general, PUFA are the dominant group or the second one (up to 50 % of total FA) in the gonads of several species (**Table 2.2-2**). Specifically in *P. lividus*, eicosapentaenoic acid (EPA, 20:5 *n*-3) is dominant, followed by arachidonic acid (ARA, 20:4 *n*-6) (Martínez-Pita et al., 2010; Rocha et al., 2019b; Siliani et al., 2016) or docosadienoic acid (22:2 *n*-6) (Mol et al., 2008b), depending greatly on the growing area and feeding habits (Angioni and Addis, 2014; Bledsoe et al., 2003; Siliani et al., 2016). A recent key finding by Farag et al. (2021) showed that lipid composition and FA profiles are more efficient than protein analysis to discriminate caviar origin and quality.

Seafood is not the major contributor of SFA (Nunes et al., 2011), even though the sea urchin gonads of some species can account great percentages in relation to total FA (**Table 2.2-2**). The major SFA reported is undoubtedly palmitic acid (16:0), followed by myristic acid (14:0) (Angioni and Addis, 2014; Mol et al., 2008b; Prato et al., 2018; Siliani et al., 2016; Stamatis and Vafidis, 2009; Zlatanos et al., 2009) or stearic acid (18:0) (Rocha et al., 2019b). The MUFA ranged between 8 and 30 % (**Table 2.2-2**), being eicosenoic acid (20:1 *n*-9), vaccenic acid (18:1 *n*-11) and gadoleic acid (20:1 *n*-11) the main MUFA in the case of *P. lividus* (Mol et al., 2008b; Rocha et al., 2019b; Siliani et al., 2016).

Although sea urchin gonads are not part of a regular diet, their consumption contributes to the intake of PUFA (mainly EPA and ARA), essential in the prevention of cardiovascular disease, hypertension, inflammation, arrhythmias and cancer (EFSA, 2017). Regarding cholesterol, high levels were discovered in *M. nudus* urchins, but the ingestion of their gonads had no impact on the contents found in the plasma of tested mice. The same authors concluded that eating sea urchins increases the amounts of ARA and docosahexaenoic acid (DHA, 22:6 *n*-3) in mice livers (Yamamoto et al., 2018).

2.2.2.3 Carbohydrates

For sea urchin, carbohydrates represent the primary source of energy (supporting the normal physiological function and gametogenesis), being stored by the nutritive phagocytes in the gonad (Marsh et al., 2013). These macronutrients are generally found in higher levels in this tissue (**Table 2.2-1**) when compared to those usually observed in muscle of the main seafood (< 0.5 %; Sikorski et al., 1990). The glycogen, which is the main carbohydrate form in invertebrates and echinoderms (e.g. sea urchins; Dincer and Cakli, 2007), may have a role in the sweet taste of gonads (Marsh et al., 2013).

2.2.2.4 Carotenoids

Animals usually are unable to synthesize carotenoids *de novo*, instead they obtain such compounds through the diet. Sea urchins are predominantly herbivorous and metabolize and accumulate carotenoids and carotenoid precursors in their tissues from dietary algae and plants (Kelly and Symonds, 2013). There are suggestions that the major site for carotenoids metabolism is the gut wall of the urchins, where dietary pigments are anabolised into echinenone before being transported to other tissues, including the gonads (Symonds et al., 2007). The biological role of carotenoids in the gonads is associated to reproduction with a positive role in fecundity (Kelly and Symonds, 2013).

The dominant carotenoids in *P. lividus* gonads are (9'-*cis*- and all-*trans*-) echinenone (accounting 50 to 60 % of total pigments) and (alfa- and beta-) carotene (De Quirós et al., 2001b; Rocha et al., 2019a, 2019b). These results have been generally reported for many urchin species, such as *A. crassispina* (Peng et al., 2012), *E. chloroticus*, *H. erythrogramma* (Brewster et al., 2018), *S. intermedius* and *S. nudus* (Borisovets et al., 2002).

Carotenoids content varies according to sex, gonad maturation stage and size (Borisovets et al., 2002; Hagen et al., 2008; Lourenço et al., 2022b; Rocha et al., 2019b, 2019a). It is accepted that the deposition of carotenoid pigment in the gonads dictates their colour (Borisovets et al.,

2002; Symonds et al., 2007), which is one of the most important criteria for indicating quality of the product. In fact, bright/golden yellow or orange colours are the only ones that receive market acceptability and greater prices (Sun and Chiang, 2015).

Several attempts have been made to improve gonads colour through modulation of carotenoids during farming (feeds). However, enhancements of the colour are still a challenge (Lourenço et al., 2019), since sex-specific strategies for carotenoid supplementation may be beneficial only in some species (Hagen et al., 2008). For instance, the astaxanthin concentration of farmed eggs of *A. lixula* was about 15 times higher than that found in eggs from wild samples (Cirino et al., 2017).

2.2.2.5 Other beneficial features

The considerable antioxidant activity of the gonads of various species indicates that the sea urchins may be an alternative source of natural antioxidants (e.g., *S. variolaris*; Archana and Babu, 2016). Such fact suggests that the gonads may be used as functional food with important role in preventing inflammatory diseases and diabetes, as in the case of *S. droebachiensis* in which cyclooxygenase-2 (an enzyme frequently expressed in inflammation cases), was inhibited at high levels (Pozharitskaya et al., 2015). The bioactive components of sea urchins and their therapeutic applications have been recently reviewed (Sibiya et al., 2021).

Moreover, the extracts of other components than gonads, such as viscera, spines, shell, have also antioxidant, antidiabetic, anti-inflammatory, and anticancer activities, thus with potential use in pharmaceutical industry to develop therapeutic treatments for these type of diseases (Chamika et al., 2021).

2.2.3 Freshness evaluation

In general, most sea urchin species are kept alive after harvesting and during transport in a way that minimize stress, exposure to excess high temperatures, direct sunlight, wind, and prolonged air exposure. Thus, sea urchins are generally kept in cold, well-oxygenated running seawater or, if removed from the water, placed in a moist and sealed atmosphere. Regarding raw gonads, freshness can have a high variability in its evolution due to the reproductive state of sea urchin and the time between harvesting and the gonads removal.

Freshness is one of the most critical aspects in determining the quality and acceptability of all aquatic products and it is therefore essential to ensure that products are safe and meet markets and consumers expectations. The physical, microbiological and biochemical changes that occur *post mortem* affect the freshness of seafood, resulting in a progressive loss of

sensory properties and safety. *Post mortem* handling, time and temperature during transport and storage are key factors that determine the final quality of the product (Alasalvar et al., 2011a; Ólafsdóttir et al., 1997).

There are many methods of estimating seafood freshness, which generally include microbiological (e.g., total viable counts), chemical (evolution of volatile compounds, protein changes, lipid oxidation and ATP breakdown), physical measurements (colour and texture) (Prabhakar et al., 2020); and sensory analysis is also applied and currently the "quality index method" (QIM) plays an important role in determining fish freshness along the fishery chain (Bernardo et al., 2020).

2.2.3.1 Chemical indicators

In the case of seafood, the nucleotides (adenosine triphosphate - ATP; adenosine diphosphate - ADP; adenosine monophosphate - AMP and inosine monophosphate - IMP) and their catabolites (inosine - HxR and hypoxanthine - Hx) have been used as the main chemical indicators of freshness. The index that reflect this is the *K*-value, which is defined as the ratio between the sum of HxR and Hx contents and the sum of ATP and all degradation products (Hong et al., 2017; Tejada, 2009). Another index, the adenylate energy charge (AEC value) is a measure of the ratio of ATP and total adenylate concentration, and it reflects the disruption in the energy production of physiological processes (Atkinson, 1968). Some studies have found that nucleotides and nucleotide-based indexes are useful indicators in the quality evaluation of invertebrates (Anacleto et al., 2013; Kawabe et al., 2010; Yokoyama et al., 1992), including sea urchin (*E. chloroticus*, Verachia et al., 2013; *S. nudus*, Wang et al., 2012) during transport and storage under refrigeration temperatures.

2.2.3.2 Sensory features

Through the analysis and interpretation of seafood characteristics by the sense of vision, olfactory, taste, hearing, and touch (Meilgaard et al., 2016), the sensory evaluation is still considered the most effective scientific discipline to assess seafood freshness and quality decline (Alasalvar et al., 2011a; Martinsdóttir et al., 2009a; Ólafsdóttir et al., 1997).

The subjectiveness of sensory methods has led to the optimisation of evaluation schemes as well and the training of a sensory panel, which allows them to become familiar with the main sensory characteristics of the product under evaluation, and to be able to objectively identify quality alterations. This training also allows the selection of the most adequate descriptors for

the evaluation of each product. The intensity of each descriptor can be evaluated by means of several scales (Martinsdóttir et al., 2009a; Meilgaard et al., 2016).

The seafood transaction has been carried out based on freshness grades as well as defects on the product, and scientific community have been working on the improvement of sensory methodologies. Sensory evaluation can be applied at different levels in seafood commercial players. It is very common in European auction sites after landing, for selection and separation of the products.

The sensory features of the sea urchins gonads are unique, being affected by many factors such as season (Phillips et al., 2010) and diet (Siikavuopio et al., 2007). The characteristic attributes and descriptors (in terms of appearance, odour, texture, and taste) and respective grading score used in the evaluation of several species (in some studies) can be found in **Table 2.2-3**.

Table 2.2-3 — Sensory features and grading scores of fresh sea urchin gonads from different species.

Sea urchin species	*S. droebachiensis*		*S. franciscanus* and *S. purpuratus*	*S. nudus*	*E. chloroticus*	*P. lividus*
Grading score	1 to 4	0-10 points (from none to high intensity of the given attribute)	Californian Uni Grades (A, B, C)	0 to 3	-	-
Appearance*	Bright yellow or orange (1), to other colours (4); Very firm (1) to very soft (4); Two distinct gonad segments halves, very smooth (1) to distinction of gonad segments halves not possible, rough/granular (4)	Very soft (slimy) to very firm (elastic); Not possible to discern single "granules" (slimy) to easily discerned "granules" (with reference to capelin roe)	Bright orange/orange (A); Pale orange (B); Pale Yellow (C) Firm, grainy, Moist (A) to broken up; soft, creamy, more liquid; Usually frozen (C)	Bright yellow (0) to discoloured (3) Firm (0) to very soft (3) Fine (0) to rough (3) Intact lobes (0) to no distinct lobes (3)	Colour (golden, sandy, salmon, peach, pale ochre, orangey brown, burnt, orange, others) grain, wetness, regularity of structure, curl of end, flaps	Firm, milky white fluid, grainy, appealing, not appealing, fresh, not fresh, soft/slimy, yellow, pale yellow, orange, vivid orange, pale orange, brownish
Odour	-	All kinds of odour.	-	Fresh odour (0); Neutral (1); Slight off-odour (2); Strong off-odour (3)	Dairy, marine, earthy, sharpness, sulphur	Inodorous, underdeveloped, sea smell, sweet, fresh/sea, tropical, pleasant, unpleasant, earthy, sulphur, rusty, mud
Texture/ mouth feel	-	Not melting (keeps its shape in the mouth) to melting (melts like butter in the mouth)	-	-	Soft, firm	Soft, creamy, firm, slimy, juicy, grainy, dry, astringent
Taste/ flavour	-	With an association to carrot and cucumber and a "touch of bitterness"; sweet; bitter; sea, seagrass, seashore; old, stale, nauseating; metallic/sour	Briny, Fresh, sweet (A); Milder, nutty, crisp (B); Not as sweet, neutral (C)	Strongly like taste (0); Like taste (1); No taste (2); Dislike taste (3)	Sweet, umami, salty, bitter, sour, earthy, herbaceous, dairy, seafood, metallic	Salty, tropical, slightly sweet, sweet, sea taste, iodine, very intense, soft, pleasant, unpleasant, tasteless; earthy, sour, bitter, metallic
Aftertaste	-	Intensity of aftertaste evaluated after the sample has been spitted out.	-	-	Sulphur, metallic, bitter, umami	Prolonged aftertaste; persistent, iodine, bitter
Reference	(Pearce et al., 2004)	(Siikavuopio et al., 2007)	(Proville, 2009)	(Wang et al., 2012)	(Phillips et al., 2010)	(Baião et al., 2021b, 2021a)

* colour, firmness, texture, shape, graininess; - not reported in the reference

Other works besides those mentioned in **Table 2.2-3** also contemplate the sensory evaluation of gonads based on the grading system with some modifications. These studies were performed with *M. nudus* (Takagi et al., 2017), *H. erythrogramma* (Pert et al., 2018a; Senaratna et al., 2005), *S. purpuratus* (Azad et al., 2011), *E. chloroticus* (Woods et al., 2008) and *P. lividus* (Prato et al., 2018; Volpe et al., 2018).

The premium quality gonads are usually golden orange to yellow, with a distinct sweet ocean taste. Those with less quality tends to be brownish and bitter (Sun and Chiang, 2015).

Sea urchin gender is a key factor impacting the sensory quality of gonads (Baião et al., 2021b; Phillips et al., 2010; Walker et al., 2015). For example, male gonads have a pronounced sweet taste (*P. lividus*; Baião et al., 2020) and female gonads a bitter and sour taste (*E. chloroticus*; Phillips et al., 2009).

Some chefs have come to describe gonads as fresh when in the mouth they feel a "pillowy sensation" that gradually disappears; leaving the "expression of the ocean in a solid ingredient" (Proville, 2009), a "dive" and the "essence of the sea" (Baião et al., 2021a) and even in terms of mouthfeel "like the ocean's answer to foie gras" (Jonze, 2018).

There are some papers that report the changes that occur during the handling of sea urchins or storage of their gonads (Kinoshita et al., 2009; Phillips et al., 2009; Verachia et al., 2013; Wang et al., 2012). Most of them are focused on the chemical outputs and others also contemplate the sensory evaluation mainly based on the already presented scoring grades (**Table 2.2-3**). For example, Phillips et al. (2009) reported a decrease of fresh odour in gonads over 10 days of storage under chilling.

One of the methodologies used for the sensory evaluation of seafood freshness is the quality index method (QIM) that seeks to overcome the difficulties arising with the application of less objective methods (Hyldig et al., 2012; Martinsdóttir et al., 2009a), such as the EU grading schemes. It has been initially developed for whole fish, stored under refrigeration, and currently is applied to fillets, frozen seafood, ready-to-eat products, and others.

The QIM scheme is based on the assessment of the attributes and descriptors that better reflect the changes that occur in the product. Like other sensory methods, it also requires the training of a sensory panel. For each attribute, the set of descriptors that best reflect the occurred change are selected, usually with linear correlation with preservation time. Attributes and descriptors related to higher freshness are scored with 0 demerit points, while those of with loss of quality are scored with 2 or 3 points (Bernardo et al., 2020; Hyldig et al., 2012). Summing up all the demerit points assigned to the attributes and descriptors, the Quality Index (QI) is obtained in a simple and well documented approach (Bernardi et al., 2013; Freitas et al.,

2021). As a result, the QI of very fresh seafood is zero and as deterioration proceeds, there is a linear evolution of this index as a function of shelf-life (Martinsdóttir et al., 2009a). Once this evolution and the maximum preservation time are established, (useful shelf-life; usually defined from the sensory evaluation in cooked product), it is possible to estimate the residual conservation time, i.e. the period in which the seafood stored in the same conditions is still acceptable for consumption. The limit of acceptability is usually based on measuring the intensity of certain attributes by trained assessors, reflecting the consumer's perception (Giménez et al., 2012).

In the last years, the development, optimisation and application of QIM schemes have been studied in seafood (please see the content of recent reviews in the field: Bernardi et al., 2013; Bernardo et al., 2020; Freitas et al., 2021). Few studies have addressed the sensory changes in the valuable sea urchin and respective gonads during preservation and to date no QIM scheme was developed to assess its freshness.

2.3 Sea urchins' processing

Sea urchin processing has been considered advantageous as it extends the shelf life and allows its consumption out of season.

2.3.1 Post harvesting and handling

Although sea urchins are relatively robust when properly handled (James and Evensen, 2018), they can be subject to stress when exposed to air for prolonged periods of time (Verachia et al., 2012). It is a common practice to hold live *E. chloroticus* in air on the deck of a fishing boat or even when they are caught near the coast. This practice can have a negative impact on gonad energy metabolism, reducing the time of subsequent shelf-life; nucleotides and *K*-value (indicators of freshness; section **2.2.3.1**) may change dramatically (Verachia et al., 2013). These observations are of utmost importance and have a great impact if gonads are further technologically processed. There is a holding system, the "SeaNest crates", especially designed for sea urchin roe enhancement (James et al., 2017), that could be used for holding sea urchins prior processing.

2.3.2 Transport strategies for live sea urchins

Usually after harvesting, sea urchins are packed for transport (live shipping) or processed to remove the gonads (Sun and Chiang, 2015). In many regions, transport is still done in bulk

(Rubilar and Crespi-Abril, 2017), with hessian sacks soaked in seawater and a tarpaulin to ensure a dark, moist and cool environment (James et al., 2017) and sometimes at inadequate temperatures (James and Evensen, 2018). Many sea urchin distributors still have concerns about the best conditions to transport live animals.

Most studies showed that sea urchins should be transported under refrigerated conditions, where spines movement is verifiable after 96 h at 4 and 8 °C (Kelly et al., 2015), highlighting the importance of a damp environment within the packaging which prolong survival.

The feasibility to transport sea urchins alive in polystyrene boxes with ice gel packs from Norway to Japan was investigated. The authors concluded that the transport can last 44 h if they were immediately consumed or processed or up to 34 h if sea urchins were stored in a holding system (James et al., 2017; James and Evensen, 2018). Other studies concluded that sea urchin kept in EPS boxes at 3-4 °C, can remain alive for 5-9 days based on the status of the oral area (Stefánsson and Ólafsdóttir, 2017). If the objective is to keep sea urchins alive for longer periods, the recirculating seawater systems may be an option. However, these systems are quite expensive, challenging and can cause difficulties due to oxygen supply, changes in pH, increase of CO_2 and ammonia accumulation (Stefánsson and Ólafsdóttir, 2019).

It is known that in other marine invertebrates, variations in natural habitat alter metabolic activity and affect the level of oxidative stress (e.g. Verlecar et al., 2008). Sea urchins are sensitive to stress and may loss quality during transport and storage. Dale et al. (2005) studied the effects of handling (gentle and rough) during harvest and transport (dry and wet) on mortality and subsequent gonad growth trials of *S. droebachiensis*, revealing a clear relationship between visual injuries and gonad index. One of the tools to assess the degree or severity of physiological stress to which aquatic organisms are exposed under sublethal conditions are the biochemical indicators (biomarkers), acting as early-warning signals (Gagné, 2014). The effectiveness of these tools in sea urchin has been reported mainly in contexts of environmental contamination (e.g. Amri et al., 2017), but its usefulness is not exploited in a perspective of potential stress due to transport.

It is important to ensure the quality of sea urchin throughout the supply chain. To this end, the stress imposed on sea urchins should be as low as possible. This will decrease their mortality, which will reduce the ecological impacts, economic losses, and food waste, but also the quality decay that have effects on distribution chain.

2.3.3 Preparation of sea urchin gonads

Fresh gonads from live sea urchin are the main marketable product, but they can also be processed. Portuguese chefs may keep the sea urchin live at 6 °C under high levels of moisture for three days and others use it for about a week (Baião et al., 2021a).

The industry usually stores live sea urchins up to 48 h prior processing, but they can be held in chilled storage for up to 2 weeks until processed (Sun and Chiang, 2015). Alternatively, it is possible to store sea urchin in saltwater, but this may raise several operational constraints.

Despite the existence of a sea urchin processing system, for shell cutting and gonad extraction, the technology was only optimised for *S. droebachiensis* (Oceatec, 2022). This work is mainly performed in the traditional way i.e., by hand which makes the final product more expensive.

The manual processing of gonads is extremely laborious. In brief, the exoskeleton or shell needs to be cracked using a special tool, a metal cracking device, or carefully opened by means of scissor from the oral area (in the bottom). In both cases, the objective is to expose the five gonad segments (**Figure 1.1-1 B**, **C**; **pp. 3**) and gently removed them with small spoons, clean off the organic matter and shell fragments with small pincers, and handle with care to maintain the structure and therefore their commercial value. There are several subsequential processing and packaging procedures according to quality categories (grading scores).

Top-grade gonads generally comes from sea urchins with more than 10 % yield (Sun and Chiang, 2015), present uniform size, firm texture, appealing colour and optimum maturation. They are generally packed into attractive wooden trays (traditional packaging, which hold 250-350 g of roe) and more recently in plastic trays. The small trays are then packed in bigger crates for shipping. Fresh roe is rushed to the Japanese market, even the one with lower quality, but packed in bulk trays and blocks to further process into other products (Reynolds and Wilen, 2000).

The processors guarantee the structure and firmness of gonads placing them in cold saltwater (≈ 2 °C and 3 %) and potassium solution (Kolbe and Kramer, 2007; Reynolds and Wilen, 2000). Other similar practices are applied in fresh *uni* with the best quality (with yellow or orange colour; whole and undamaged): they are placed in strainers previously soaked in saltwater with anhydrous potassium aluminium sulphate alum or ($KAl(SO_4)_2$) solution (Bledsoe et al., 2003) in concentrations varying between 0.4 and 0.7 %. Soak time vary between 15 min to 1 hour, till gonads becomes firm (Kato and Schroeter, 1985). The delicacy is then drained on a cloth and fine grid to remove as much of extra water as possible. The soak procedure

enhances the roe firmness, make the tissue denser and help to maintain the desirable colour (Nordin and Kramer, 1979). Although this practice is still popular (e.g. Verachia et al., 2012), it is recommended that the use of food additives containing aluminium be restricted (Regulation 1129/2011; EC, 2011b). Moreover, gonads treated with Alum had a strong off-flavour, making them unfit for consumption (Stefánsson and Ólafsdóttir, 2017). A different brining is used by some chefs in UK: dip the gonads in kombu (seaweed) water for about 10 to 15 minutes before presenting to the customer (Jonze, 2018).

Gonads with lower quality or from low-yield sea urchins are typically runny, small or broken and end up in processed products, such as frozen, steamed, dried and salted or mixed with other fish products (Reynolds and Wilen, 2000; Sun and Chiang, 2015).

2.3.4 Preservation and processing

The main markets for sea urchin products are generally far away from the capture area. In wholesale and retail gonads marketing circuits, there is a wide variety of presentations, which result from the application of different preservation and processing methods. These processes make possible, to extend the shelf life of products, add value to the gonads of sea urchins caught outside the best season and to make available more convenient products.

There is evidence that gonads begin to lose quality when stored under refrigeration for periods longer than 36 h due to water loss and discoloration (Nordin and Kramer, 1979).

Based on the work carried out by Wang et al. (2012), it was found that it is possible to extend the shelf life of *S. nudus* gonads by an additional 4-5 days at 5 °C when gonads are packed in artificial seawater with dissolved oxygen compared to those that were packed in nitrogen or air. Trials with the same species conducted by Kinoshita et al. (2009) also indicated that storage in oxygenated seawater was effective in maintaining freshness.

Freezing (Nordin and Kramer, 1979; Stefánsson and Ólafsdóttir, 2017), boiling (Chinprahast et al., 2013), baking (Kato and Schroeter, 1985), pasteurization (Stefánsson and Ólafsdóttir, 2017), salting (Shimada et al., 1993; Shimada and Ogura, 1990), marinating (Stamatis and Vafidis, 2009), fermentation (Choi and Surh, 2015), steaming (Lee et al., 2012), high pressure (Coroneo et al., 2022) and canning (Cruz-García et al., 2000; De Quirós et al., 2001a, 2001b; Lee et al., 2012) are examples of processes that have been applied to gonads of diverse species that generally are of low quality for fresh consumption. The authors studied the effect of processing, reporting differences on: appearance (colour and texture) (Choi and Surh, 2015; Coroneo et al., 2022); odour and taste (Coroneo et al., 2022); chemical composition (Coroneo et al., 2022; Lee et al., 2012), including volatile components (De Quirós et al., 2001a;

Soliman et al., 1983), FAA and ATP related compounds (Chinprahast et al., 2013; Lee et al., 2012); carotenoids (Choi and Surh, 2015; De Quirós et al., 2001b), lipids class or fatty acid composition (Lee et al., 2012; Shimada and Ogura, 1990) and vitamins (De Quirós et al., 2001b; Shimada et al., 1993). Absence of changes regarding chemical composition were reported by Cruz-García et al. (2000) and Stamatis and Vafidis (2009).

Soaking treatments before freezing have been applied, for instance: 3 to 10 % of NaCl for 10 to 15 min; magnesium chloride in seawater; 0.1 % citric acid and 0.01 % propyl gallate; KCl or CaCl 1 M; Alum solution with several concentrations (Kolbe and Kramer, 2007). According to Stefánsson and Ólafsdóttir (2017), the freezing of gonads may impair apparent texture, but did not affect flavour for frozen storage periods up to 6 months, after which a trace of off-odour was perceived. A commercial consequence is that this process lowers the price of the product for about 25-50 % (Sun and Chiang, 2015).

The work already done indicates that more process-oriented research is needed for the valorisation of sea urchin gonads considering the sustainability of this resource. According to Alishahi and Aïder (2012), the use of natural ingredients with antibacterial and antioxidant properties may be a strategy to consider to improve their shelf-life, functionality and nutritional attributes.

3

EVALUATION OF CHEMICAL SAFETY OF WILD SEA URCHIN

3.1 Bioaccumulation of persistent and emerging pollutants in wild sea urchin *Paracentrotus lividus*

Abstract

Marine pollution has been increasing as consequence of anthropogenic activities. The preservation of marine ecosystems, as well as the safety of harvested seafood, are nowadays a global concern. Here, we report for the first time the contamination levels of a large set of 99 emerging and persistent organic contaminants (butyltins (BTs), polycyclic aromatic hydrocarbons (PAHs), pesticides including pyrethroids, pharmaceuticals and personal care products (PCPs) and flame retardants) in gonads of sea urchin *Paracentrotus lividus*. Sea urchins are a highly prized worldwide delicacy, and the harvesting of this seafood has increased over the last decades, particularly in South West Atlantic coast, where this organism is harvested mainly for exportation. Sampling was performed in three harvesting sites of the NW Portuguese coast subjected to distinct anthropogenic pressures: Carreço, Praia Norte and Vila Chã, with sea urchins being collected in the north and south areas of each site. Butyltins and pharmaceuticals were not found at measurable levels. Several PAHs, four pyrethroids insecticides, four PCPs and eleven flame retardants were found in gonads of sea urchins, though in general at low levels. Differences among harvesting sites and between areas within each site were found, the lowest levels of contaminants being registered in Carreço. The accumulation of contaminants in sea urchins' gonads seemed to reflect the low anthropogenic pressure felt in the sampling sites. Nevertheless, taking into account the low accumulated levels of chemicals, results indicate that sea urchins collected in South West Atlantic coast are safe for human consumption.

Keywords: Seafood; Gonads; Coastal contamination; Bioaccumulation

This subchapter is adapted from the publication:

Rocha, A.C., **Camacho, C.**, Eljarrat, E., Peris, A., Aminot, Y., Readman, J.W., Boti, V., Nannou, C., Marques, A., Nunes, M.L., Almeida, C.M. 2018. Bioaccumulation of persistent and emerging pollutants in wild sea urchin *Paracentrotus lividus*. Environmental Research 161, 354-363. https://doi.org/10.1016/j.envres.2017.11.029

Contribution of Carolina Camacho: Data curation, Formal analysis, Visualization, Writing – review & editing.

1 Introduction

Industrial revolution contributed greatly to human welfare and paved the way for the comforts of modern living. Technological and industrial advances, over the years, and the increasing demand for goods have led to the production and use of new chemicals. The production, transportation and dumping of effluents and residues from industrial processing, sludge from sewage treatment plants, agricultural drainage and untreated sewage into aquatic environment, as well as maritime traffic, accidental spills and activity on oil rigs, have resulted in the contamination of coastal marine and freshwater environments owing to the release of toxic xenobiotics and, in many cases, persistent substances (Reuver et al., 2017; UNEP, 2005).

Several contaminants, such as metals, butyltins, polycyclic aromatic hydrocarbons (PAHs), and emerging pollutants, e.g. pharmaceuticals, personal care products, flame retardants, etc., have already been found in drinking water, seawater and sediments (Aminot et al., 2017; Carvalho et al., 2009; Salgado et al., 2010; Silva et al., 2014). Exposure to contaminants can induce deleterious effects to aquatic organisms menacing their health and affecting the ecosystem (Abdel-Shafy and Mansour, 2016; Antwi and Reddy, 2015; Torres et al., 2016). Evidence that toxicants can biomagnify from one trophic level to the next, passing along food chain, can be found in literature (Sukhn, 2013). Several contaminants were proven to find their way from aquatic environment into fish and other aquatic organisms via water or diet (Karouna-Renier et al., 2007; Kruzynski, 2004; Salvo et al., 2014), accumulating in their tissues (Gray, 2002; Mizukawa et al., 2009). Moreover, several aquatic organisms have been pointed out as good bioindicators of pollution due to their capacity to accumulate contaminants in proportion to the levels found in the environment and the period of exposure (Corcellas et al., 2015; Viñas et al., 2009). Older, carnivorous and benthic marine organisms are among those that can more often accumulate contaminants (Angioni et al., 2012; Corcellas et al., 2015; Ebele et al., 2017; Jabeen et al., 2015; Lebrun et al., 2014). The consumption of contaminated seafood (e.g. natural toxins, metals, organic pollutants) can therefore be a potential health risk.

The Marine Strategy Framework Directive (MSFD, 2008/56/EC; EC, 2008) aims to provide an integrative marine environment status assessment and considers both coastal and offshore environment, thus overlapping with The Water Framework Directive (WFD, 2000/60/EC; EC, 2000) for some parts of the marine environment. To achieve the good environmental status of European waters, several practices were proposed, including the establishment of a monitoring programme for the assessment of environmental targets and associated indicators of good quality control. Biomonitoring programmes and research focusing on accumulation of

contaminants, especially of newly introduced substances, in aquatic organisms are crucial to produce scientific knowledge, assess contamination of European waters, and support the MSFD, European Seafood Safety measures and legislation (Reuver et al., 2017).

Seafood is widely recognised as a high quality and healthy food source, providing energy and proteins and containing all essential amino acids, nutrients, minerals and trace elements humans need, as well as *n*-3 long-chain polyunsaturated fatty acids (Nunes et al., 2011). Among seafood items are sea urchins whose consumption has been rising. Sea urchins are an important prey item of predatory organisms, such as fish, seals, and whales (Ahn et al., 2009). In addition, sea urchins are a highly prized worldwide delicacy, with high gastronomic value due to distinct organoleptic features and usually consumed uncooked, but also salted, pickled or made into paste. Sea urchins are commercialised in several European countries, North and South America and Asia, especially Japan which accounts for more than 80 % of world consumption (Sun and Chiang, 2015).

Paracentrotus lividus (commonly known as purple sea urchin) is a powerful voracious grazer herbivore echinoderm commonly found in intertidal rocky and seagrass areas along the Atlantic coast, from Ireland to southern Morocco, and throughout the Mediterranean Sea (Bayed et al., 2005; Bertocci et al., 2014), being largely exploited in France, Spain and Italy, as well as Portugal (FAO, 2004), for either consumption or exportation (Bertocci et al., 2014). *P. lividus* shows a wide range of adaptive responses to environmental conditions such as temperature, food and wave action (Bayed et al., 2005). In addition, its sedentary habits and sensitivity to a variety of pollutants enables this sea urchin to be considered as a suitable biological–biochemical indicator of local pollution (Soualili et al., 2008), especially in restricted marine areas (Angioni et al., 2012). Additionally, *P. lividus* has often been used in toxicity assays in monitoring and risk assessment programmes to assess the effects of pollutants on embryonic and larval development (Soualili et al., 2008). There is growing evidence that *P. lividus* can accumulate contaminants in its body (e.g. roe, test, spine and teeth) but, to date, studies have only investigated heavy metals (Bendimerad, 2014; Pagano et al., 2002) and selected flame retardants in vitro (Danis et al., 2005).

The increased market demand for sea urchins has resulted in nonselective harvesting practices, seriously affecting the sustainability of wild stocks in some regions (Bertocci et al., 2014). The limited information available about the levels of contaminants accumulated in sea urchins gonads and the absence of specific legislation regarding the maximum limits of contaminants in this species represent a potential risk for human health in case of consumption of contaminated goods. For this reason, it is important to assess the quality and safety of sea

urchins to be consumed. Considering the increasing interest and demand for sea urchins worldwide, this work aimed at reporting for the first time the presence of a large selection of 99 persistent and emerging organic pollutants in the gonads of the sea urchin *P. lividus* to meet not only good food safety standards, but also to assess the contamination status of the Portuguese NW sea urchin production areas. The objective is not only to characterise intrinsic contamination but also to evaluate the contamination status of a sea urchin production area located in a central zone of the distribution of this echinoderm. The season of maximum maturation and prior to spawning was selected (Lozano et al., 1995). Sampling sites were chosen according to potential sources of industrial and urban activities.

2 Materials and methods

2.1 Study area and sampling procedures

The study area is located on the north-western (NW) Portuguese Atlantic coast, being limited by the Minho River estuary in Caminha in the North and by Douro River estuary in Porto in the South, and including Lima River estuary in Viana do Castelo and Ave River basin in Vila do Conde. Along the NW Atlantic coast, different urban and industrial activities are observed. Minho River estuary, included in the Natura 2000 network (Rodrigues et al., 2013), and some rocky shore beaches (e.g. Carreço, Vila Chã; Cairrão et al., 2004) show low susceptibility to human influence and low levels of environmental contamination, having been used as a reference sites. Lima and Douro River estuaries and adjacent areas are subjected to stronger anthropogenic pressure due to the presence of oil refining industries and two cargo ports (located at Leixões and Viana do Castelo), a high population density and several industrial and urban activities. Praia Norte is located nearby the mouth of Lima River estuary and Viana do Castelo harbour, being subjected to vessels traffic. Ave River basin is highly impacted by industrial effluent discharges, especially from textile industries (Vasconcelos, 2015). Furthermore, in the NW Atlantic, there are important maritime traffic routes involving chemical substances transportation ships, where accidents and spills often occur, like the Prestige ship oil spill in 2002 (Cairrão et al., 2004; CNADS, 2002). So, anthropogenic pressures include contamination due to possible discharge of urban and industrial effluents and to oil spills.

A sampling campaign was carried out in early spring, before the spawning (Garmendia et al., 2010; González-Irusta et al., 2010), which may contribute, at least partially to eliminate the contaminant load in the gonads, in NW Atlantic coast, at low tide, in three sampling sites along the NW Atlantic coast: Praia Norte, Carreço and Vila Chã (**Figure 3.1-1**).

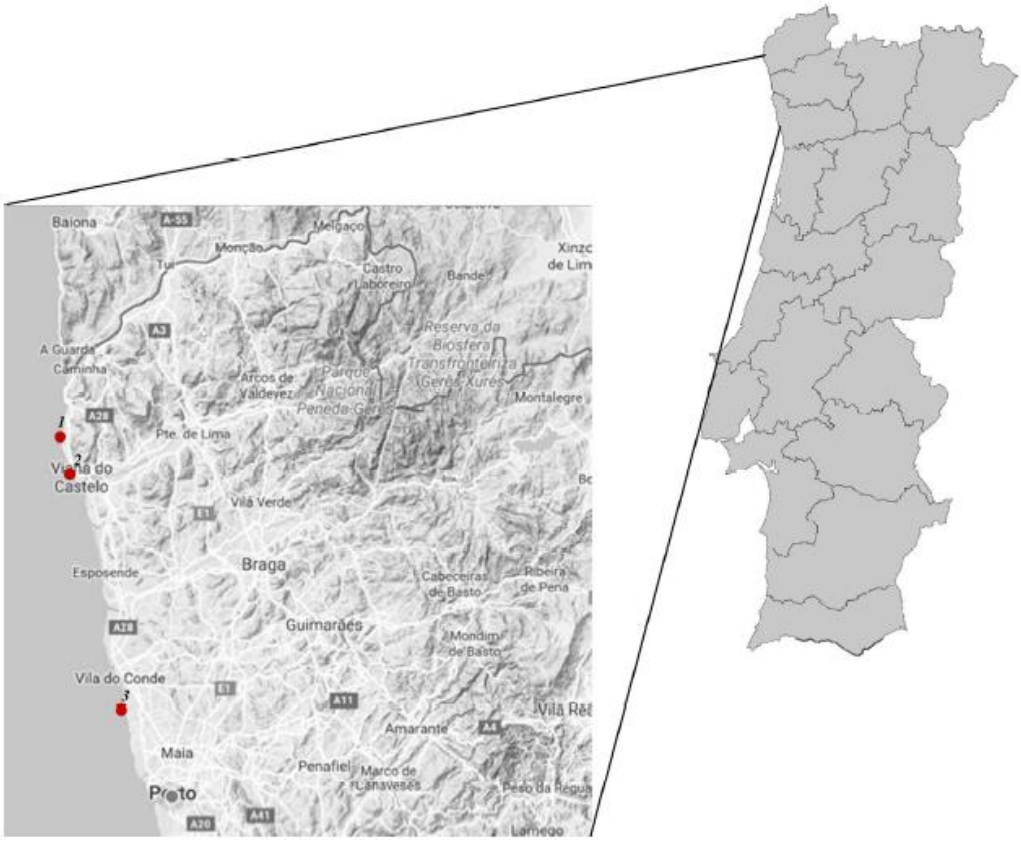


Figure 3.1-1 — Geographic location of sea urchins sampling campaigns of north-western Portuguese coast. (**1**. Carreço; **2**. Praia Norte; **3**. Vila Chã)

In Praia Norte and Carreço the harvesting of sea urchin is more intense compared to Vila Chã, which is currently used as reference site in sea urchin population abundance studies (Bertocci et al., 2014). Twenty specimens were collected in two distinct areas of each sampling site, a northern and a southern area (corresponding to 40 sea urchins per sampling site). The specimens were transported to the laboratory in cooled portable freezers. In the laboratory, sea urchins were measured, weighed, and dissected. Since sea urchins have five gonads, the individuals were pooled in five homogenised samples, each sample containing one gonad from each sea urchin sampled. Each pooled sample was stored at -20 °C and freeze-dried whenever required. Freeze-dried material was grounded by means of a mortar and pestle. Freeze-dried material or raw samples were sent to the relevant laboratories according to their extraction methods.

2.2 Chemical analysis

Three independent replicates were carried out to determine each group of contaminants in gonads of sea urchins collected in each beach.

2.2.1 Butyltins

Butyltins (BTs) were measured using an adapted procedure described in Carvalho et al. (2007). The analytes were extracted from about 0.25 g of freeze-dried gonads with 3 mL of 6 M hydrochloric acid/ethanol 1:1 (v/v) mixture, in an ultrasonic bath for 2 h, and then centrifuged at 2500 rpm for 15 min. A 50 µL aliquot of the extract was then put into a vial containing 500 µL of buffer solution of $NaCH_3COOH$ (2.0 M) and CH_3COOH (5.6 M) (pH = 4.3) and the required water to accomplish a total solution volume of 10 mL per vial. 250 µL of NaBEt4 (2 % (w/v), ABCR Gmbh Co. KG (Karlsruhe, Germany) was then added to each vial by means of a syringe properly washed with MeOH to start the derivatisation of BTs. The analytes were always quantified by the standard addition method. For the pre-concentration of BTs, a fiber of poly(dimethylsiloxane) (PDMS, Supelco) with 100 µm film thickness handled with an autosampler CombiPal model (CTC Analytics) was used. The extraction was performed at controlled temperature (40 °C) and agitated for 40 min. Analyses were performed by GC-MS in a Varian Saturn 2000 mass spectrometer. Linear response range was from 0.02 to 1260 $ng \cdot g^{-1}$ for monobutyltin (MBT), 0.07 – 1568 $ng \cdot g^{-1}$ for dibutyltin (DBT) and 0.04 – 2146 $ng \cdot g^{-1}$ for tributyltin (TBT) and relative standard derivation (RSD) < 15 % was also obtained.

2.2.2 Polycyclic aromatic hydrocarbons (PAHs)

Freeze dried tissues (between 0.5 and 1.2 g) were spiked with an internal standard solution containing the 16 deuterated PAHs in cyclohexane (LGC Standards, UK). 10 mL of LC-MS grade dichloromethane/ethyl acetate 1:1 (v:v, Rathburn Chemicals Ltd., Walkerburn, UK) was added to tissues and three sequential extraction were carried out in a sonication bath for 10 min. After evaporation with nitrogen, alumina-cleaned extracts were reconstituted in 300 µL of ethyl acetate and analysed using an Agilent Technologies 7890 A GC system coupled to an Agilent 5975 series MS. An Agilent HP-5MS (crosslinked poly diphenyl dimethylsiloxane) capillary column (30 m) with a film thickness of 0.25 µm and internal diameter 0.25 mm was used for separation, and the analysis conditions are described in Aminot et al. (2017). The accurate quantification of the 13 PAHs was ensured using their deuterated homologue molecule as an internal standard. They were spiked in the samples prior to extraction to account for potential losses occurring during sample preparation. The procedural recoveries were in a range of 88 – 132 %. The results were corrected for the PAH levels measured in triplicated procedural blanks. Limits of detection and limits of quantification ranged from 0.2 to 3.1 and from 0.6 to 9.3 $ng \cdot g^{-1}$ dry weight (dw), respectively.

2.2.3 Pharmaceuticals and personal care products (PCPs)

Thirteen pharmaceuticals, including mainly psychiatrics, were measured in sea urchin gonad. An amount of 5 g wet sea urchin was weighed into a 50 mL propylene centrifuge tube and 5 mL of cold distilled water were added to soak the matrix for 10 min. Then, 5 mL acetonitrile (Fisher Scientific, Leicester, UK) were added, the tube was tightly capped and vigorously mixed for 1 min. Addition of 6 g anhydrous magnesium sulfate ($MgSO_4$) and 1.5 g sodium acetate (NaOAc) followed, the mixture was shaken with a vortex for 1 min and then centrifuged for 5 min (4000 rpm). An 1 mL aliquot of the upper layer was transferred into a 15 mL centrifuge tube, containing 150 mg $MgSO_4$, 50 mg PSA, 50 mg C18 and 25 mg GCB, for further purification. The tube was vigorously mixed for 1 min and centrifuged for 5 min (4000 rpm). This was a modified approach for QuEChERS AOAC official method (AOAC, 2007). The final extract was evaporated to dryness under a gentle stream of nitrogen (TechneDri-Block heater, DB-3D, Staffordshire, USA) and reconstituted in methanol: water (50:50), followed by filtration through PVDF syringe-driven filters from Millipore (Cork, Ireland) and transferred to screw cap vials for injection in the UHPLC-LTQ/Orbitrap-MS (Thermo Fisher Scientific, Inc. GmbH, Bremen, Germany) (Kosma et al., 2014). To allow the accurate quantification of the different pharmaceuticals, appropriate deuterated internal standards were employed in each case. Quantification limits were in the range of 3 – 16 $ng \cdot g^{-1}$ while detection limits ranged from 1 to 5 $ng \cdot g^{-1}$. The accuracy of the method ranged from 63.1 to 90.1 %. Precision of the method expressed as repeatability and reproducibility was below 11 % and 18 % respectively, in all cases.

A solvent extraction procedure followed by a clean-up of the extract by alumina or silica SPE to remove the lipids was performed in dried tissue for extracting PCPs (the musks traseolide, galaxolide, tonalide, musk ketone, galaxolidone and UV filters 4MBC, EHMC and octocrylene, with d3-tonalide and d15-octocrylene used as internal standards). PCPs were analysed using an Agilent Technologies 7890 A GC system coupled to an Agilent 5975 series Mass Selective detector. An Agilent HP-5MS (crosslinked poly diphenyl dimethylsiloxane) capillary column (30 m) with a film thickness of 0.25 µm and internal diameter 0.25 mm was used for separation, and the analysis conditions are described in Aminot et al. (2017). Tonalide d3 and octocrylene d15 were used to quantify the structurally related musks and UV filters, with recoveries in the acceptability range of 40 – 120 %, except for EHMC, 236 %. The results were corrected for the levels measured in triplicated procedural blanks. Limits of detection and limits of quantification ranged from 0.8 to 5.7 and from 2.4 to 17.1 $ng \cdot g^{-1}$ dw, respectively.

2.2.4 Pesticides

A QuEChERS extraction procedure was optimised for urchins, being adapted from Hladik et al. (2009). An aliquot of the supernatant (1 mL) from the QuEChERS extraction was transferred to a vial, and the extract was concentrated just to dryness using a gentle stream of nitrogen. Residue was reconstituted in 1.0 mL of n-hexane followed by filtration through PVDF syringe-driven membrane filter from Millipore (Cork, Ireland). Analyses for measuring ethoxyquine, atrazine, chlorpyriphosmethyl, parathion-methyl and chlorpyriphos, were performed with a Trace GC Ultra instrument coupled to an ISQ mass spectrometer controlled by a computer running X-Calibur software. Quantification limits were in the range of 1.2 – 24.2 $ng \cdot g^{-1}$ while detection limits achieved were below 7.3 $ng \cdot g^{-1}$. The accuracy of the method ranged from 73.2 % to 100.1 %. Good precision of the method was observed for all analytes, expressed as repeatability (< 10.5 %) and reproducibility. To determine thiamethoxam, dimethoate, acetamiprid, thiacloprid, metalaxyl, fluometuron, prometryn, myclobutanil, S-metalachlor and imazalil, the modified AOAC official 2007.01 method described above for pharmaceuticals was applied (AOAC, 2007). Quantification limits were in the range of 4 – 32 $ng \cdot g^{-1}$ while detection limits achieved were below 4 $ng \cdot g^{-1}$ in all cases, except fluometuron (10 $ng \cdot g^{-1}$). The accuracy of the method ranged from 61.1 % to 91.7 %. Precision of the method expressed as repeatability and reproducibility was below 9 % and 21 % respectively, in all cases.

For measuring pyrethroids insecticides (transfluthrin, allethrin, prallethrin, imiprothrin, resmethrin, tetramethrin, bifenthrin, phenothrin, cyhalothrin, permethrin, cyfluthrin, cypermethrin, fenvalerate, fluvalinate, deltamethrin, kadethrin, flumethrin), 0.1 − 0.3 g dry weight was fortified with 1 ng of d10-chlorpyrifos, d6-trans-permethrin and d6-trans-cypermethrin (Dr. Ehrenstorfer®, Augsburg, Germany). Samples were left for 24 h to equilibrate and extracted with hexane/dichloromethane (2:1) in a sonicator, the extracts being cleaned up by SPE with basic alumina and C18 cartridges in tandem as described in Feo et al. (2011). An Agilent Technologies 7890 A GC system coupled to a 7000A MS/MS Triple Quad was used for analysis in NCI mode (Feo et al., 2011). Recovery percentages between 55 – 106 % were obtained, being always within the range of acceptability (40 – 120 %) for analytical methods based on quantification by isotopic dilution. Reproducibility percentages between 1.3 – 9.3 % were also achieved. Limits of detection and limits of quantification ranged from 0.01 to 6.43 and from 0.04 to 21.4 $ng \cdot g^{-1}$ lipid weight (lw), respectively.

2.2.5 Flame retardants

0.5–2 g freeze dried gonad was spiked with 3 ng of 13C-polybrominated diphenyl ethers (PBDEs) and 13C-syn-dechlorane (DL) (Cambridge Isotope Laboratories Inc., Andover, MA, USA). A pressurised liquid extraction was performed, which was adapted from (Barón et al., 2014, 2012), and extracts were evaporated to incipient dryness and reconstituted to a final volume of 40 µL prior to the instrumental analysis. Instrumental analysis of flame retardants was carried out by gas chromatography coupled to tandem mass spectrometry (GC-MS-MS) using an Agilent Technologies 7890 A GC system coupled to 7000 A GC/MS Triple Quadrupole. PBDEs (brominated diphenyl ethers, BDE) and emerging brominated flame retardants (BFRs, hexabromobenzene (HBB), pentabromoethylbenzene (PBEB) and decabromodiphenylethane (DBDPE)) were analysed using electron ionization mode (EI) following the protocol optimised in (Barón et al., 2014). Halogenated norbornenes were analysed by negative chemical ionization mode (NCI) using CH4 + as reagent gas (Barón et al., 2012).

Organophosphate flame retardants (OPFRs), including tris(2-butoxyethyl) phosphate (TBOEP), tris(chloroethyl)-phosphate (TCEP), tris (chloroisopropyl)-phosphate (TClPP), trihexyl phosphate (THP), tris(2-ethylhexyl) phosphate (TEHP), isodecyldiphenyl phosphate (IDPP), 2-ethylhexyldiphenyl phosphate (EHDP), dicumyl peroxide (DCP), tributyl phosphate (TBP), triphenyl phosphate (TPHP), triphenylphosphine oxide (TPPO), tris(1,3-dichloro-2-propyl)phosphate (TDCPP), tris(monochloropropyl) phosphate (TMCP) and isopropyl phenyl phosphate (IPPP) were also measured in dried gonad after an ultrasound extraction with a mixture of hexane: acetone (1:1) for organic trace analysis (J.T. Baker, Center Valley, PA, USA). Prior to instrumental analysis, 10 ng of d12-TCEP, d15-TDCPP, d27-TBP, d15-TPHP and13C2-TBOEP (Wellington Laboratories Inc., Guelph, ON, Canada) were added as internal standards. Online sample purification and analysis was performed by Thermo Scientific TurboFlow™ system consisted of a triple quadrupole (QqQ) MS, two LC quaternary pumps and three LC columns, i.e. two for purification of the sample (Cyclone TM-P (0.5 × 50 mm) and C18-XL (0.5 × 50 mm)) and one for the analytical separation (Purosphere Star RP-18; 125 mm × 0.2 mm) as described in Giulivo et al. (2016).

Recoveries between 47 – 99 % were obtained, being always within the range of acceptability (40 – 120 %) for analytical methods based on quantification by isotopic dilution, with reproducibility between 1.3 – 16 %. Limits of detection and limits of quantification ranged from 0.002 to 19.3 and from 0.008 to 24.8 $ng \cdot g^{-1}$ lw, respectively.

2.3 Statistics

Mean values and respective standard deviation (n = 3) were calculated for each contaminant analysed. Unpaired *t*-Student test ($p < 0.05$) was performed to assess differences in the levels of contaminants found in gonads from sea urchins collected at the north and south area of each selected sampling site. Data were also analysed statistically using analysis of variance (ANOVA) and the Tukey pair wise comparisons were employed to determine the significance of the differences among sea urchins from the three selected sampling sites. The statistical package used was GraphPad Prism 6 software and the confidence limit was 95 %.

3 Results and discussion

A large set of emerging and persistent contaminants was assessed herein in the edible gonads of sea urchins. The sampling campaign was performed at Carreço, Praia Norte and Vila Chã. Sea urchins were of similar size, presenting an average total weight and diameter per individual, respectively, of 79 ± 16 g and 59 ± 3 mm for Carreço north, 97 ± 25 g and 64 ± 7 mm for Carreço south, 56 ± 16 g and 56 ± 6 mm for Praia Norte north, 77 ± 26 g and 60 ± 5 mm for Praia Norte south, 115 ± 22 g and 67 ± 4 mm for Vila Chã north and 124 ± 16 g and 65 ± 4 mm for Vila Chã south. No significant differences ($p > 0.05$) were observed within each sampling site, but sea urchins from the reference site Vila Chã were slightly heavier. Each sample group contained both male and female specimens and an average fresh weight of gonad per individual of 10 ± 4 g for Carreço north, 11 ± 3 g for Carreço south, 7 ± 4 g for Praia Norte north, 11 ± 7 g for Praia Norte south, 12 ± 3 g for Vila Chã north and 12 ± 3 g for Vila Chã south was attained without significant differences ($p > 0.05$) among sites. Gonads presented an average percentage of moisture of 80 % and lipid content of 4 %.

Measurable levels of PAHs, PCPs, pyrethroids insecticides and flame retardants were found in the analysed tissues (**Table 3.1-1**, **3.1-2**, **3.1-3** and **3.1-4**). However, BTs, pharmaceuticals, pesticides frequently used in agriculture, emerging brominated flame retardants and dechloranes were all below the analytical limit of detection. The use of BTs, biocides commonly used in antifouling paints of vessels, was banned worldwide on ship hulls in 2008 (Price and Readman, 2013). Despite the persistence of BTs in the marine environment, the absence of measurable levels of BTs in gonads of sea urchins might reflect almost ten years of non-use of TBT products. Thirteen PAHs, recognised as priority pollutants by the US Environmental Protection Agency, were analysed in sea urchins gonads, with fluorene, phenanthrene,

fluoranthene, pyrene, benz(a)anthracene, chrysene,benz(a)pyrene, benzo(ghi)perylene being found (**Table 3.1-1**).

Table 3.1-1 — Levels of butyltins (BTs) and polycyclic aromatic hydrocarbons (PAHs) measured in sea urchin gonads in three harvesting sites of north-western Portuguese coast.

Analyte	Sampling location					
	Carreço northern	Carreço southern	Praia Norte nortern	Praia Norte southern	Vila Chã northern	Vila Chã southern
BTs (ng·g^{-1} dw)						
MBT	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)
DBT	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)
TBT	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)
PAHs (ng·g^{-1} dw)						
Fluorene Δ	2.3 ± 0.1 ^{a}	3.1 ± 0.1 ^{a}	4.0 ± 0.2 ^{a}	2.6 ± 0.1 ^{b}	2.7 ± 0.1 ^{a}	1.9 ± 0.1 ^{b}
Phenanthrene Δ	12.8 ± 0.3 ^{a}	15.6 ± 0.3 ^{b}	23.3 ± 0.5 ^{a}	7.6 ±0.2 ^{b}	32.8 ± 0.7 ^{a}	11.0 ± 0.2 ^{b}
Anthracene	< 0.8 (LD)	< 0.8 (LD)	< 0.8 (LD)	< 0.8 (LD)	< 0.8 (LD)	< 0.8 (LD)
Fluoranthene Δ	< 0.7 (LD)	1.40 ± 0.01	1.40 ± 0.01 ^{a}	1.00 ± 0.01 ^{b}	1.00 ± 0.01 ^{a}	1.70 ± 0.02 ^{b}
Pyrene Δ	0.50 ± 0.01 ^{a}	1.20 ± 0.01	1.30 ± 0.01 ^{a}	0.80 ± 0.01 ^{b}	0.80 ± 0.01 ^{a}	1.40 ± 0.01 ^{b}
Benz(a)anthracene	0.30 ± 0.04	< 0.2 (LD)	< 0.2 (LD)	0.30 ± 0.04	< 0.2 (LD)	0.50 ± 0.06
Chrysene	< 0.4 (LD)	1.0 ± 0.1	1.1 ± 0.1 ^{a}	1.1 ± 0.1 ^{a}	< 0.4 (LD)	< 0.4 (LD)
Benzo(b)fluoranthene	< 0.4 (LD)	< 0.4 (LD)	< 0.8 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)
Benzo(k)fluoranthene	< 1 (LD)	< 1 (LD)	< 1 (LD)	1.20 ± 0.01	< 1 (LD)	< 1 (LD)
Benzo(a)pyrene Δ	< 1 (LD)	2.8 ± 0.2	6.2 ± 0.4 ^{a}	2.2 ± 0.2 ^{b}	7.0 ± 0.5 ^{a}	1.7 ± 0.1 ^{b}
Indenopyrene	< 3 (LD)	< 3 (LD)	< 3 (LD)	13.0 ± 0.1	< 3 (LD)	< 3 (LD)
Dibenz(a,h)anthracene	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)
Benzo(ghi)perylene Δ	1.5 ± 0.1 ^{a}	5.0 ± 0.4 ^{b}	4.8 ± 0.4 ^{a}	4.2 ± 0.4 ^{a}	< 1 (LD)	< 1 (LD)
Total PAHs (ng·g^{-1} dw)	17	30	42	34	44	18

Mean values and respective standard deviation (n = 3) are presented; dw - dry weight; LD - limit of detection; LQ - limit of quantification;

t-Student test ($p < 0.05$) was performed for each site, north and south areas being compared, with different letters indicating significant differences ($p < 0.05$);

The Δ indicates significant difference among sampling sites (ANOVA test or *t*-Student test, $p < 0.05$).

Total PAH concentrations ranged from 17 to 44 ng·g^{-1} dw. Statistical differences were registered among sampling sites and between the north and south areas of each site. In addition, the 6-ring PAH indenopyrene was found only in gonads of sea urchins collected in Praia Norte Sul. PAHs are ubiquitous environmental pollutants which can derive from the incomplete combustion of organic materials such as coal, oil, petrol and wood (yielding preferentially to high molecular weight PAHs), and from accidental oil spillages/discharges from industries, harbour facilities and ships (yielding to low molecular weight PAHs). The sea urchins in our study were exposed to both sources with significant levels of both the low molecular weight (fluorene and phenanthrene) and the high molecular weight PAHs (benzo(a)pyrene, benzo(ghi)perylene and indenopyrene). PAHs possess toxic, mutagenic and/or carcinogenic properties, being readily absorbed and rapidly distributed within body organs (especially those with high fat content) since they are highly lipid soluble (Abdel-Shafy and Mansour, 2016). PAHs have been previously found in gonads of *P. lividus*, the bioaccumulation of these contaminants being associated to human activity (Angioni et al., 2014; Viñas et al., 2009). The concentration of PAHs in sea urchins can also reflect the human pressure exerted with an area. For instance, considerably higher levels of several PAHs, especially fluorene, anthracene and chrysene, were found in sea urchins inhabiting the very touristic Sardinia coast (Angioni et al., 2014) than those found in the less impacted NW Portuguese coast. Within the same coast, similar levels of total PAHs were found in sea urchins analysed herein in comparison to those collected in spring of 2003 and 2004, at Fisterra, Arousa and Vigo after the Prestige accident (Viñas et al., 2009). The authors verified that coastal organisms (mussels, razor shells, sea urchins and barnacles) were initially affected by the Prestige spill, but background levels of PAHs were thereafter attained for all organisms (in sea urchin case reaching 25 ng·g^{-1} dw), the levels now found in sampled beaches.

With regard to pesticides, various herbicides, fungicides and insecticides, recognised as toxic to aquatic organisms (Schäfer et al., 2011; Van Scoy et al., 2016) and often used in agriculture, were assessed in soft tissue of sea urchins collected (**Table 3.1-2**). Only some pyrethroids insecticides were quantified in sea urchin's gonads, namely, transfluthrin, cyhalothrin, permethrin, cyfluthrin, cypermethrin, fenvalerate, bifenthrin, phenothrin, fluvalinate, deltamethrin and flumethrin (**Table 3.1-2**).

Table 3.1-2 — Levels of pesticides measured in sea urchin gonads in three harvesting sites of north-western Portuguese coast.

Analyte	Sampling location					
	Carreço northern	Carreço southern	Praia Norte northern	Praia Norte northern	Vila Chã northern	Vila Chã southern
Pesticides (ng·g^{-1} ww)						
Thiamethoxam	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)
Dimethoate	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)
Acetamiprid	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)
Thiacloprid	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Metalaxyl	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Fluometuron	< 10 (LD)	< 10 (LD)	< 10 (LD)	< 10 (LD)	< 10 (LD)	< 10 (LD)
Prometryn	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)
Myclobutanil	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)
S-metalachlor	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Imazalil	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Ethoxyquine	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Atrazine	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)
Chlorpyriphos-Methyl	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)
Parathion-Methyl	< 5 (LD)	< 5 (LD)	< 5 (LD)	< 5 (LD)	< 5 (LD)	< 5 (LD)
Pyrethroids (ng·g^{-1} lw)						
Transfluthrin	0.27 ± 0.02^{a}	0.26 ± 0.02^{a}	2.7 ± 0.2^{a}	0.95 ± 0.06^{b}	<0.04 (LQ)	0.83 ± 0.05
Allethrin	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)
Prallethrin	< 6 (LD)	< 6 (LD)	< 6 (LD)	< 6 (LD)	< 6 (LD)	< 6 (LD)
Imiprothrin	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Resmethrin	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Tetramethrin	< 0.3 (LD)	< 0.3 (LD)	< 0.3 (LD)	< 0.3 (LD)	< 0.3 (LD)	< 0.3 (LD)
Bifenthrin	< 0.1 (LQ)	< 0.1 (LQ)	< 0.1 (LQ)	< 0.1 (LQ)	< 0.1 (LQ)	0.26 ± 0.01
Phenothrin	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)	320 ± 13
Cyhalothrin	< 0.2 (LQ)	< 0.2 (LQ)	0.28 ± 0.01^{a}	1.84 ± 0.04^{b}	< 0.2 (LQ)	1.38 ± 0.03
Permethrin Δ	2.6 ± 0.1^{a}	3.2 ± 0.1^{b}	23 ± 1^{a}	17 ± 1^{b}	2.3 ± 0.1^{a}	3.7 ± 0.2^{b}
Cyfluthrin	< 0.4 (LD)	< 0.4 (LD)	3.0 ± 0.1^{a}	2.2 ± 0.1^{a}	< 0.4 (LD)	1.32 ± 0.03
Cypermethrin Δ	0.330 ± 0.004^{a}	0.62 ± 0.01^{b}	3.70 ± 0.04^{a}	2.71 ± 0.04^{b}	<0.3 (LD)	1.95 ± 0.02
Fenvalerate Δ	0.96 ± 0.02^{a}	0.87 ± 0.01^{b}	5.2 ± 0.1^{a}	3.8 ± 0.1^{b}	0.40 ± 0.01^{a}	2.87 ± 0.04^{b}
Fluvalinate	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	2.0 ± 0.2
Deltamethrin	< 0.5 (LD)	< 2 (LQ)	< 0.5 (LD)	< 0.5 (LD)	< 0.5 (LD)	1.8 ± 0.1
Kadethrin	< 0.1 (LD)	< 0.1 (LD)	< 0.1 (LD)	< 0.1 (LD)	47 ± 3	< 0.1 (LD)
Flumethrin	< 0.3 (LD)	< 0.9 (LQ)	78 ± 7	< 0.9 (LQ)	35 ± 3	< 0.9 (LQ)
Σ Pyrethroids (ng·g^{-1} lw)	4.4	5.2	116	29	85	337
Chlorpyriphos	< 0.1 (LQ)	< 0.1 (LQ)	< 0.1 (LQ)	< 0.1 (LQ)	3.8 ± 0.1^{a}	5.6 ± 0.2^{b}

Mean values and respective standard deviation (n = 3) are presented; ww - wet weight; lw - lipid weight; LD - limit of detection; LQ - limit of quantification; *t*-Student test ($p < 0.05$) was performed for each site, north and south areas being compared, with different letters indicating significant differences ($p < 0.05$). The Δ indicates significant difference among sampling sites (ANOVA test or *t*-Student test, $p < 0.05$)

On the other hand, compounds moderately to highly mobile in soils and moderately soluble in water, e.g. atrazine, thiamethoxan, dimethoate (IARC, 1999; Van Scoy et al., 2016; Wypych and Wypych, 2015), were not found in samples, possibly indicating a preferential use of pyrethroids insecticides by local farmers. The use of pyrethroids has been increasing in recent years as a replacement for organophosphate pesticides. Despite being strongly hydrophobic, pyrethroid transport within aquatic systems can happen through movement of pyrethroid absorbed to suspended solids and particulates (Palmquist et al., 2012). The occurrence of pyrethroids is of concern since these pesticides are highly toxic to aquatic organisms, especially sediment-dwelling organisms (Hladik et al., 2009). The toxic effects of pyrethroids towards sea urchin embryos are reported in literature (Erkmen, 2015; Gao et al., 2008), but no information was found regarding their accumulation in roe. Accumulation of pyrethroids in fish and bivalves has been previously reported (Barsh et al., 2009; Corcellas et al., 2015; Jabeen et al., 2015). The selected sampling sites, Carreço, Praia Norte and Vila Chã, are surrounded by agriculture areas and the accumulation of pesticides in gonads of sea urchins may reflect the use of these chemicals. Significant differences were registered among sampling location (**Table 3.1-2**), Carreço beach presented the lowest levels of pyrethroids. Total pyrethroids levels were higher in Vila Chã Sul, mostly due to phenothrin (320 $ng \cdot g^{-1}$ lw). The occurrence of permethrin, cypermethrin, fenvalerate and flumethrin was significantly higher in sea urchins from Praia Norte than in those from Carreço and Vila Chã. Levels found herein in sea urchins were generally rather low, in the $ng \cdot g^{-1}$ lw range, whereas in mussels and fish, for instances, pyrethroids levels within $\mu g \cdot g^{-1}$ lw were found (Barsh et al., 2009; Jabeen et al., 2015).

Pharmaceuticals and personal care products (PCPs), a unique group of compounds used to improve our daily life quality, are of emerging environmental concern. Because they are designed to be biologically active, these contaminants can pose a risk for aquatic organisms even at low concentrations (Ebele et al., 2017; Gago-Ferrero et al., 2013). These compounds are released into the aquatic environment mostly through urban wastewater discharges after their incomplete removal in wastewater treatment plants (WWTPs) (Kosma et al., 2014). Pharmaceuticals and PCPs are therefore prone to reach environmental waters, as previously reported (Archer et al., 2017; Ebele et al., 2017). Both hydrological basins within the sampling area contain more than 250 wastewater facilities and high population density (APA, 2012a, 2012b). In Portugal, influent and effluent analysis proved that WWTPs are not capable of completely removing pharmaceuticals and PCPs (Salgado et al., 2010; Silva et al., 2014). Bioaccumulation of these contaminants has already been reported in several aquatic organisms, e.g. algae, crustaceans, mussels and fish (Ebele et al., 2017) but to our knowledge not in sea urchins. Herein, the

pharmaceuticals screened in sea urchins were not found at detectable levels in any sampling location (**Table 3.1-3**).

Table 3.1-3 — Levels of pharmaceutical and personal care products (PCPs) measured in sea urchin gonads in three harvesting sites from the north-western Portuguese coast.

Analyte	Sampling location					
	Carreço northern	Carreço southern	Praia Norte northern	Praia Norte southern	Vila Chã northern	Vila Chã southern
Pharmaceuticals (ng·g^{-1} dw)						
Paracetamol	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)
Phenazone	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Bezafibrate	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)
Budesonide	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)
Olanzapine	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Carbamazepine	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Alprazolam	< 5 (LD)	< 5 (LD)	< 5 (LD)	< 5 (LD)	< 5 (LD)	< 5 (LD)
Diazepam	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Citalopram	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Haloperidol	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Amitryptyline	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)
Paroxetine	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)	< 4 (LD)
Fluoxetine	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)
PCPs (ng·g^{-1} dw)						
Traseolide	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
Galaxolide Δ	< 6 (LD)	12 ± 3	42 ± 12	< 6 (LD)	11 ± 3^{a}	9 ± 3^{a}
Tonalide Δ	1.4 ± 0.4^{a}	4 ± 1^{b}	4 ± 1^{a}	1.7 ± 0.4^{b}	3 ± 1^{a}	1.8 ± 0.5^{a}
Musk ketone	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)
4-MBC	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	2.4 ± 0.3
Galaxolidone	< 1 (LD)	1.3 ± 0.2	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)
EHMC	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)	< 1 (LD)
Octocrylene Δ	< 1 (LD)	< 1 (LD)	6.3 ± 0.2^{a}	15.1 ± 0.5^{b}	16.0 ± 0.5^{a}	2.5 ± 0.1^{b}

Mean values and respective standard deviation (n = 3) are presented; dw - dry weight; LD - limit of detection; LQ - limit of quantification; *t*-Student test ($p < 0.05$) was performed for each site, north and south areas being compared, with different letters indicating significant differences ($p < 0.05$); The Δ indicates significant difference among sampling sites (ANOVA test or *t*-Student test, $p < 0.05$).

Within PCPs, galaxolide and tonalide (musks) are generally the most frequently detected in waters (Rosal et al., 2010; Smyth et al., 2008). Such substances have also been detected in influents of Portuguese WWTPs and, at lower concentrations, in effluents (Salgado et al., 2010). These chemicals are therefore likely to reach the aquatic environment, with toxicological effects

being induced to aquatic organisms at $\mu g \cdot L^{-1}$ or lower levels (Giraldo et al., 2017; Parolini et al., 2015). Despite the low levels attained (**Table 3.1-3**), data indicate that sea urchins can accumulate musks and UV filters. To the best of our knowledge, this is the first report regarding PCP accumulation in sea urchins' gonads. Levels found herein were rather low, probably denoting the low contamination of these compounds along the Portuguese NW coast. Wild mussel, *Mytilus galloprovincialis*, sampled in different sites on the south coast of Portugal was shown to accumulate octocrylene, EHMC and galaxolide. Depending on the season and the sampling site, levels above 500 $ng \cdot g^{-1}$ dw were reached for octocrylene and EHMC whereas galaxolide levels were lower than 12 $ng \cdot g^{-1}$ dw (Groz et al., 2014). The presence of octocrylene and EHMC was associated to discharges of WWTPs rejects and the increasing use of sunscreens, particularly in summer. Organisms inhabiting aquatic systems receiving wastewater effluents reflect in many cases the contamination of the medium. For instance, mussel *Lasmigona costata*, was shown to readily accumulate 43 pharmaceuticals and PCPs from many classes (de Solla et al., 2016). Fish fillets collected from a regional effluent-dominated stream in Texas, USA, also presented for instance considerably high levels of benzophenone, galaxolide, tonalide and triclosan (37 - 90, 234 – 970, 26 – 97 and 17 – 31 $ng \cdot g^{-1}$ wet weight (ww) respectively; Mottaleb et al., 2009).

Flame retardants are added to numerous products to reduce their flammability. Like pharmaceuticals and PCPs, these emerging contaminants are not efficiently removed from wastewaters in WWTPs, due to their low biodegradability and capacity to adsorb to sewage sludge (Covaci et al., 2011). The occurrence of these chemicals has already been reported for several environmental matrices, including tissues of aquatic organisms, e.g. mussels and fish (Aznar-Alemany et al., 2017; Covaci et al., 2011; Lebrun et al., 2014; Po et al., 2017). The consumption of contaminated seafood is reported as the main human exposure route to flame retardants (Guo et al., 2017). So, flame retardants contamination has become a global concern. To our knowledge, the accumulation of flame retardants by *P. lividus* roe has never been reported in literature. With regard to other species, the urchin *Sterechinus neumayeri* were shown not to accumulate PBDEs compared to other benthic and pelagic species collected off Adélie Land, Antarctica (Goutte et al., 2013).

Herein, PBDEs, emerging BFRs, dechloranes and organophosphate flame retardants (OPFRs), were assessed in sea urchin gonads (**Table 3.1-4**).

Table 3.1-4 — Levels of different types of flame retardants (polybrominated diphenyl ethers (PBDEs), organo-phosphate flame retardants (OPFRs), emerging brominated flame retardants (BFR), dechloranes) measured in sea urchin gonads in three harvesting sites of north-western Portuguese coast.

Analyte	Sampling location					
	Carreço northern	Carreço southern	Praia Norte northern	Praia Norte southern	Vila Chã northern	Vila Chã southern
PBDEs (ng·g^{-1} lw)						
BDE-28 Δ	< 0.04 (LD)	1.17 ± 0.02	< 0.04 (LD)	< 0.04 (LD)	1.19 ± 0.02^{a}	0.88 ± 0.02^{b}
BDE 47 Δ	4.3 ± 0.1	< 0.05 (LD)	9.6 ± 0.1^{a}	2.42 ± 0.03^{b}	4.02 ± 0.05^{a}	3.96 ± 0.05^{a}
BDE-99	4.2 ± 0.2	< 0.29 (LD)	< 0.29 (LD)	< 0.29 (LD)	< 0.29 (LD)	< 0.29 (LD)
BDE-100	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)
BDE-154 Δ	1.1 ± 0.1^{a}	0.89 ± 0.05^{b}	2.2 ± 0.2^{a}	0.91 ± 0.05^{b}	0.82 ± 0.04^{a}	0.72 ± 0.04^{b}
BDE-153	< 2 (LQ)	< 2 (LQ)	3.7 ± 0.3	< 2 (LQ)	< 2 (LQ)	< 2 (LQ)
BDE-183	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)
BDE-209 Δ	0.94 ± 0.06^{a}	0.70 ± 0.04^{b}	2.0 ± 0.1^{a}	0.92 ± 0.06^{b}	0.70 ± 0.04^{a}	0.56 ± 0.03^{b}
Σ PBDEs	12.07	2.76	17.41	5.63	7.79	7.12
OPFRs (ng·g^{-1} lw)						
TCEP	< 4 (LQ)	< 4 (LQ)	< 4 (LQ)	< 4 (LQ)	< 4 (LQ)	< 4 (LQ)
TPPO Δ	< 0.4 (LD)	4.8 ± 0.1	16.2 ± 0.4^{a}	1.43 ± 0.04^{b}	3.4 ± 0.1	< 0.4 (LD)
TClPP Δ	< 1 (LD)	8.9 ± 0.3	62 ± 2^{a}	9.1 ± 0.3^{b}	4.6 ± 0.1	< 1 (LD)
TDCPP	< 0.2 (LD)	< 0.2 (LD)	8.1 ± 0.7	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)
TPHP	< 1 (LD)	< 1 (LD)	44 ± 1	< 1 (LD)	< 1 (LD)	< 1 (LD)
TBP	< 3 (LD)	< 3 (LD)	711 ± 23	< 3 (LD)	< 3 (LD)	2659 ± 85
DCP	< 2 (LD)	< 2 (LD)	23 ± 1	< 2 (LD)	< 2 (LD)	12.5 ± 0.5
TBOEP	< 0.4 (LD)	< 0.4 (LD)	18 ± 2	< 0.4 (LD)	< 0.4 (LD)	< 0.4 (LD)
IDPPΔ	< 3 (LD)	< 3 (LD)	389 ±17^{a}	16 ± 1^{b}	10.9 ± 0.5^{a}	15 ± 1^{b}
IPPP	< 25 (LQ)	< 25 (LQ)	< 25 (LQ)	< 25 (LQ)	< 25 (LQ)	< 25 (LQ)
THP	< 0.8 (LD)	< 0.8 (LD)	< 0.8 (LD)	< 0.8 (LD)	< 0.8 (LD)	< 2 (LQ)
EHDP	< 0.5 (LD)	< 0.5 (LD)	< 0.5 (LD)	< 0.5 (LD)	< 0.5 (LD)	< 0.5 (LD)
TEHP	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)
TMCP	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)	< 3 (LD)
Σ OPFRs	-	14	1271	26	19	2687
Emerging BFRs (ng·g^{-1} lw)						
HBB	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)
PBEB	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)	< 0.2 (LD)
DBDPE Δ	0.30 ± 0.04^{a}	0.5 ± 0.1^{b}	0.5 ± 0.1^{a}	0.37 ± 0.04^{a}	0.8 ± 0.1^{a}	0.29 ± 0.03^{b}
Dechloranes (ng·g^{-1} lw)						
Dec 602	< 21 (LD)	< 21 (LD)	< 21 (LD)	< 21 (LD)	< 21 (LD)	< 70 (LQ)
Dec 603	< 7 (LD)	< 7 (LD)	< 7 (LD)	< 7 (LD)	< 24 (LQ)	< 24 (LQ)
Dec 604	< 7 (LD)	< 7 (LD)	< 7 (LD)	< 7 (LD)	< 7 (LD)	< 7 (LD)
syn-DP	< 18 (LQ)	< 6 (LD)	< 6 (LD)	< 6 (LD)	< 6 (LD)	< 6 (LD)
anti-DP	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)	< 2 (LD)

Mean values and respective standard deviation (n = 3) are presented; lw - lipid weight; LD - limit of detection; LQ - limit of quantification; *t*-Student test ($p < 0.05$) was performed for each site, north and south areas being compared, with different letters indicating significant differences ($p < 0.05$). The Δ indicates significant difference among sampling sites (ANOVA test or *t*-Student test, $p < 0.05$).

Several PBDEs, namely, BDE-28, BDE-47, BDE-154 and BDE-209 were found in sea urchins, with significant differences detected between the north and south areas of each sampling site. ANOVA tests also indicated statistical differences among sampling sites. BDE-99 and BDE-153 were only found in samples from Carreço north and Praia Norte north, respectively. PBDEs are listed as persistent organic pollutants by the Stockholm Convention due to their persistence, bioaccumulation and toxic effects towards organisms and humans (Gu et al., 2017). Dechloranes and emerging BFRs were not found in urchin gonads, apart from DBDPE. This BFR has been used for more than 20 years as an alternative to decabromodiphenyl ether which was banned in 2008 through a decision by the European Court of Justice (Ricklund et al., 2010). Nevertheless, DBDPE has been shown to be as ubiquitous as its predecessor (Ricklund et al., 2010). From OPFRs, TPPO and TClPP were found in roe collected in all sampling sites, whereas IDPP and DCP were not found in urchins from Carreço, and a considerable high level of IDPP was registered in gonads from Praia Norte north (**Table 3.1-4**). In this sampling area, a higher variety of OPFRs was registered in gonads collected (namely, TDCPP, TPHP, TBOEP, TBP and DCP) than those quantified in Carreço and Vila Chã. TBP and DCP were also found in urchins from Vila Chã south. The levels of flame retardants found in sea urchins were generally low except for IDPP and TBP. High concentrations of this OPFR were found in gonads from both sampling sites, especially in Vila Chã south (> 2000 $ng \cdot g^{-1}$ lw). In agreement to Kim et al. (2011), low levels of flame retardants have been previously registered in fish muscle tissue, whereas considerably higher concentrations of plasticisers like TBP were also detected. TBP is a very stable compound and persistent in natural environments, such as sediments and water (Nancharaiah et al., 2015). So their ubiquitous presence in the environment and, the consequent longer exposure, may explain the results obtained (Kim et al., 2011). Water contamination can occur in hydrographic network of Cávado, Ave and Leça rivers due to the high industrialisation of the area (APA, 2015). One of the main industries in this area is the textile production, which has been proven to severely affect the aquatic environments in this region (Gonçalves and Alpuim, 2011; Gonçalves et al., 1992). Among others, TBP has several applications, including the coating of textiles (Horrocks et al., 2007), which can likely explain the significantly high amount of OPFRs in Vila Chã urchins.

The current legislation only sets the maximum levels for some contaminants like benzo(a)pyrene and the sum of benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene and chrysene for smoked fish, crabs and molluscs (EC, 2011c). According to this, benzo(a)pyrene levels determined in the current study in sea urchin gonads were well below the maximum level set for smoked fish (2 $\mu g \cdot kg^{-1}$ ww) and smoked molluscs (6 $\mu g \cdot kg^{-1}$ ww) regardless of the

sampling site. Codex Alimentarius from Food and Agriculture Organisation of the United Nation (FAO) and World Health Organisation (WHO) stipulates maximum pesticide residue limit for crops, milk and meat but not for seafood. For flame retardants, the levels found in sea urchins were below those reported for foodstuff in Scientific Opinion on Emerging and Novel Brominated Flame Retardants (BFRs) in Food prepared by the European Food Safety Authority (EFSA) (e.g. HBB up to 5 $ng \cdot g^{-1}$ lw, DBDPE up to 7 $ng \cdot g^{-1}$ lw, PBEB up to 10 $ng \cdot g^{-1}$ lw) (EFSA, 2012a).

Significant differences found herein among sampling locations evidence the importance of biomonitoring contamination at a local scale since bioaccumulation of contaminants vary according to the anthropogenic pressure in the area, marine currents, and transportation of pollutants from urban centres, and the nature of seabed. Even within the same sampling site, the selection of the sample collection area is important, as the shape and dynamics of the sampling site may also contribute to the variability of contaminant accumulation pattern in marine organisms. So, care should therefore be taken when choosing the sampling location. Carreço is delimited by a very fragile dune system due to intense erosion and presenting a pier perpendicular to the shoreline. Similarly, Praia Norte has a narrow sand strip bordered by an urban waterfront and a pier perpendicular to the shoreline. Vila Chã, on the contrary, is an open beach parallel to the shore. The three sampling sites are urban beaches which have received blue flag and present excellent bathing quality water. Praia Norte and Vila Chã are inserted within an urban parish, while Carreço is considered as a rural area (Barroco et al., 2007). Human activities nearby are mainly small-scale traditional agriculture and fishery. Severe contamination by urban, agricultural and industrial effluents in Carreço and Vila Chã areas is unlikely to occur, as these sites are often used as reference sites (Bertocci et al., 2012). In terms of sea urchin harvesting, Vila Chã beach is also recognised as a reference shore since Carreço and Praia Norte beaches are shores frequently used for commercial harvesting of *P. lividus* (Bertocci et al., 2014). However, the three locations are not safe from pollution. For example, in 2002, Carreço beach was menaced by oil contamination due to its proximity to the region where the oil spill of Prestige occurred (Cairrão et al., 2004). Praia Norte is located nearby Viana do Castelo harbour, so this area is frequently subjected to intense ship traffic and can be exposed to oil spills. The sampling area is inserted within two hydrographical basins with well-developed industrial sectors (e.g. food, textile, paper, rubber and metallomechanic industries) (APA, 2012a, 2012b). Industries nearby the sampling areas were identified as industries that can potentially discharge priority substances and other pollutants, such as several metals, phenols, sulphide, fluoride and cyanide to the hydrographical network of Minho and Lima Rivers (APA, 2015),

which can contaminate these areas. Cadmium, mercury, nickel, lead, dichloromethane, benzene, trichloroethylene can be discharged into the hydrographical network of Ave, Cávado and Leça Rivers. Several industries have inclusively been identified as SEVESO plant (APA, 2012b). Several urban wastewater treatment plants are also present in these areas which can contribute for water degradation (APA, 2012a, 2012b) and the release of emerging contaminants (Silva et al., 2014), representing a significant anthropogenic pressure. The presence and activity of these industries have possibly contributed for the registered results. Sea urchins from Praia Norte generally contained a larger number and higher levels of contaminants, followed by Vila Chã and Carreço, even though, in general, the levels observed were low. Results seem to reflect the anthropogenic pressure in each area (effluents discharges and/or oil spills), Carreço appearing to be the least impacted area.

4 Conclusion

Within the large set of contaminants assessed, some persistent and emerging contaminants were found at measurable levels in sea urchins' gonads (e.g. PAHs, pyrethroids, some PCPs and flame retardants), but most of them were not detected or found in very low levels.

Sampling location is an important parameter to consider when planning a biomonitoring campaign, as great differences were found in contaminant levels among different areas within the same sampling site. The current data indicates that Carreço site is the less impacted area for harvesting sea urchins. There is a growing interest in the commercial exploitation of the purple sea urchin *P. lividus* in SW Atlantic coast. Given their abundant distribution in this area and aiming at the commercialisation of a safe and healthy product, it is important to monitor harvesting areas and develop measures to preserve SW Atlantic coastal ecosystems.

This study shows that sea urchins inhabiting NW Portuguese coast are of good quality and safe for consumption. Care should be taken however with regards to PAHs, phenothrin, TBP and IDPP. Despite being found in gonads at considerably lower levels than those established in European regulation, these contaminants should be monitored to guarantee the food safety and the quality of this marine resource.

Acknowledgements

Authors acknowledge INSEAFOOD colleagues that carried out sea urchin samplings. This work has been financially supported by the European project 'Real time monitoring of SEA contaminants by an autonomous lab-on-a-chip biosensor (SEA-on-a-CHIP; No. 614168) and the research line INSEAFOOD within the Structured Program of R&D&I INNOVMAR – Innovation and Sustainability in the Management and Exploitation of Marine Resources (Reference NORTE-01–145-FEDER-000035) is funded by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF). António Marques acknowledge FCT for the IF2014 contract. Carolina Camacho acknowledges MARINALGAE4aqua Project (ERANETCOFASP/004/2015) for the research grant. Part of this work was conducted in the Unit of Environmental, Organic and Biochemical high-resolution analysis-Orbitrap-LC-MS analysis of the University of Ioannina, Greece and the authors would like to thank the Unit for providing access to the facilities.

3.2 Macro and trace elements in *Paracentrotus lividus* gonads from South West Atlantic areas

Abstract

Sea urchin represents one of the most valuable seafood products being harvested and explored for their edible part, the gonads or roe. This species is generally considered a sentinel organism for ecotoxicological studies being widely used in monitoring programs to assess coastal aquatic environments quality, because is directly exposed to anthropogenic contaminants in their habitat. In this context, the aim of this study is to evaluate the concentrations of macro (Cl, K, P, Ca, S) and trace (Zn, Br, Fe, Sr, I, Se, Rb, Cu, Cr, Ni, As, iAs, Cd, Pb, Hg) elements in *Paracentrotus lividus* gonads from three South West Atlantic production areas subjected to distinct environmental and anthropogenic pressures. In all studied areas, the elements profile in sea urchin gonads was Cl > K > P > Ca > S > Zn > Br > Fe > Sr > I > Rb > Cu > Se > Cr > Ni, suggesting an element guide profile with special interest for sea urchin farming development. Concerning toxic elements, the profile was the following: As > Cd > Pb > Hg > iAs. The results evidenced higher levels of Pb and Hg in open areas. Distinct area characteristics and anthropogenic pressures of production areas evidence the importance of biomonitoring contaminants, particularly toxic elements. In general, the levels of these elements were below maximum levels in foodstuffs (MLs) which pose a minimal health risk to consumers.

Keywords: Sea urchin; Macro elements; Trace elements; Environmental determinants; Risk/benefit assessment

This subchapter is adapted from the publication:

Camacho, C., Rocha, A.C., Barbosa, V.L., Anacleto, P., Carvalho, M.L., Rasmunssen, R.R., Sloth, J.J., Almeida, C.M.R., Marques, A., Nunes, M.L., 2018. Macro and trace elements in *Paracentrotus lividus* gonads from South West Atlantic areas. *Env. Res.* 162, 297-307. doi.org/10.1016/j.envres.2018.01.018

Contribution of Carolina Camacho: Data curation, Validation, Formal analysis, Visualization, Writing – original draft, review & editing.

1 Introduction

Seafood has a great importance in a well-balanced diet, providing several nutritional benefits and being a healthy source of proteins, lipids, *n*-3 polyunsaturated fatty acids and other important nutrients (EFSA, 2012b). Oligoelements are among the most relevant nutrients provided by seafood like iodine, selenium, zinc, calcium, phosphorus, iron and copper (EFSA, 2014) since they participate in many physiological processes. The essentiality of elements has been intensively discussed, being recommended that daily necessities should be fully covered but avoiding excessive intake since it can lead to toxic effects (e.g. Berdanier, 1998; IoM, 2001). Fish and shellfish products are increasingly promoted as functional foods and nutraceuticals. Yet, sea urchins are one of the least known seafood products, despite their globally increased consumption. Sea urchin gonads represent the edible part of this organism and is one of the most valuable seafood products, being a delicacy like *Caviar*. It is a good source of proteins, lipids, carbohydrates, fatty acids, vitamins and minerals (De Zoysa, 2014; Dincer and Cakli, 2007). The high demand for this delicacy product has led to a great pressure over sea urchin stocks all over the world. Therefore, special attention must be paid to regulate this species harvest activities (Dincer and Cakli, 2007) since their populations are decreasing (Bertocci et al., 2014). Moreover, it is important to understand its nutritional composition to improve the farming of this species.

The purple sea urchin *Paracentrotus lividus* (Lamarck 1816) is distributed in Mediterranean areas and in the north-eastern Atlantic coasts, in rocky intertidal and shallow subtidal environments (Boudouresque and Verlaque, 2013). One of the main harvested areas in Europe is the north-western coastal areas of Portugal, where *P. lividus* populations have been commercially harvested to supply nearby region markets, such as Spain (Bertocci et al., 2014). These highly explored areas are inserted within two hydrographical basins where industry sector is well developed and so subjected to possible urban and industrial contamination (Cairrão et al., 2004; Vasconcelos, 2015). In addition, Carreço site is located nearby where the Prestige oil spill occurred in 2002 (Cairrão et al., 2004) and Praia do Norte has an harbour nearby where ship traffic is quite intense, therefore, exposure to oil spills can occur. Leather tanning, metal plating and textile industries are the main sources of toxic metal contamination in the Ave river (Gonçalves et al., 1992), once considered the most polluted river in Europe (Soares et al., 1999), that drain less than 10 km north from Vila Chã, one of the harvesting sites in study. Several urban wastewater treatment plants are also present in these areas which can contribute for water degradation (APA, 2015). All these anthropogenic pressures can contribute for distinct

elements bioaccumulation patterns in *P. lividus*, including persistent and emerging pollutants (Subchapter 3.1).

Toxic element contamination is still a potential problem, since elements are frequently found in aquatic environments as an outcome of anthropogenic inputs, mainly from industrial activities (Bielmyer et al., 2012; Radenac et al., 2001), leading to a change in marine communities' structure and in specimen composition (e.g. Rouane-Hacene et al., 2018). Due to its wide distribution, sedentary habits, easy collection, grazer-feeding habits and tolerance to pollutants, the sea urchin *P. lividus* may become contaminated, thus being widely used in monitoring programs to assess coastal aquatic environments quality (Salvo et al., 2014; Warnau et al., 1998). In addition, its gonads are consumed mainly raw or lightly cooked. In this way, consumers are easily exposed to environmental contaminants accumulated in sea urchins. Although marine organisms and humans need trace amounts of elements, such as copper (Cu), zinc (Zn), nickel (Ni) and iron (Fe), they can also play a deleterious effect. Chronic Cu toxicity in humans, for instance, has its most pronounced effects on liver function whilst acute effects of Cu toxicity are primarily observed in the gastrointestinal tract, as a local intestinal irritation effect (EFSA, 2006). Toxic elements, like mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As), may accumulate in marine organisms and humans potentially causing toxicity (Bielmyer et al., 2012). In areas subjected to industrial, agricultural and mining activities, the levels of toxic elements can increase significantly (Olmedo et al., 2013). The consumption of sea urchin gonads is still hampered by some health risks, since several field studies demonstrate that sea urchins effectively bioconcentrate toxic elements (Ablanedo et al., 1990; Augier et al., 1989; Bielmyer et al., 2012; Rouane-Hacene et al., 2018; Warnau et al., 1995). The accumulation of toxic elements is different within sea urchin body tissues, showing higher concentration levels in the digestive wall (Warnau et al., 1998). In addition, seasonality changes in edible tissues also influence pollutants accumulation, and consequently element bioavailability (Rouane-Hacene et al., 2018). Worldwide, food safety authorities established tolerable upper intake levels (UL) for some elements Cu (1 – 4 $mg \cdot day^{-1}$), Zn (7 – 22 $mg \cdot day^{-1}$), Ni (1.0 $mg \cdot day^{-1}$) and Fe (45 $mg \cdot day^{-1}$) in food products (EFSA, 2006; USDA, 2015). Other indicators are tolerable weekly intake (TWI) levels for Cd (2.5 $\mu g \cdot kg^{-1}$ bw; EFSA, 2009a, 2011), benchmark dose levels for inorganic arsenic (iAs; 0.3 – 8 $\mu g \cdot kg^{-1}$ bw day^{-1}, EFSA, 2009b; 3 $\mu g \cdot kg^{-1}$ bw day^{-1}, FAO/WHO, 2011) for seafood products. Maximum limits of toxic elements in sea urchins, however, have not yet been established in the EU. So, the assessment of essential and toxic elements in sea urchins is necessary to evaluate possible environmental contaminations and prevent risks associated with their consumption.

There are some reports on the occurrence of macro and trace elements in commercial sea urchins from production regions like Morocco, France, Spain, Chile, Sardinia, Sicily (Salvo et al., 2016), Algeria coast (Bendimerad, 2014; Rouane-Hacene et al., 2018). However, and to the best of our knowledge, there is no information about the assessment of macro and trace elements in sea urchin gonads from production areas in the South West (SW) Atlantic coast. So, in this context, the present study intends to assess the macro and trace elements content in *P. lividus* gonads in the harvesting season in different areas subjected to distinct environmental and anthropogenic pressures.

2 Materials and methods

2.1. Study areas and sample preparation

This study was carried out in three sea urchin harvesting areas along the NW Atlantic coast of Portugal: Carreço (C), Praia Norte (PN) and Vila Chã (VC), (**Figure 3.1-1**, page **44**).

The areas were chosen due to their relevance in the exploitation of sea urchins and due to the diversity of environmental and anthropogenic pressures (industrial and urban) as previously characterized in the introduction section. Sea urchins were collected during low tide in early spring, when gonads are well mature and before spawning (Garmendia et al., 2010; González-Irusta et al., 2010). A total of twenty sea urchins were collected in two sites (northern and southern) of each area, to obtain representative sample. Live animals were transported to laboratory facilities under refrigeration. Biometric data was registered, where the gonad index represents the percentage of the gonad wet weight in relation to the individual's wet weight (gonad index (%) = gonad weight / total weight × 100).

For each site, ten individuals were separated in two pools of five specimens each. Each pool was composed by one gonad of each sea urchin and a second set of gonads was taken for the same sea urchins to have a pool duplicate. Each sea urchin was cracked by the mouth, the Aristotle's lantern was discarded, the coelomic fluid and gut was also discarded and gonads were collected with a spoon. After gender identification, pooled samples were frozen and freeze-dried for 24 h at −45 °C and low pressure (approximately 10^{-1} atm). Afterwards, samples were powdered by means of a mortar and pestle, divided, and stored at −20 °C until analysis in, at least, duplicate. Moisture content was determined using gonads mass differences before and after freeze-drying.

2.2. Analytical methods

Three analytical methods were applied in sea urchin samples and **Figure 3.2-1** presents which elements were determined by analytical methods.

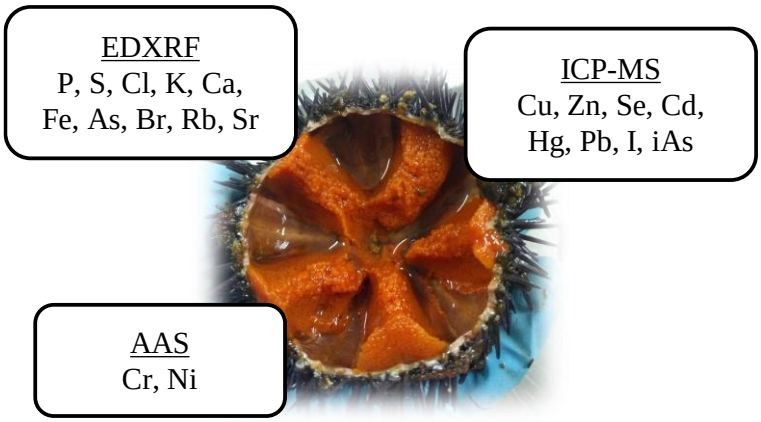


Figure 3.2-1 — Image of cracked sea urchin with gonads and indication of which elements were determined by Energy dispersive X-Ray fluorescence (EDXRF), Atomic Absorption Spectrometry (AAS) and Inductively coupled plasma mass spectrometry (ICP-MS) for all samples.

2.2.1. Energy dispersive X-Ray fluorescence (EDXRF)

Each freeze-dried sample powder (approximately 500 mg in duplicate) was pressed with a 10 t mechanical press and a pellet of 2 cm diameter was obtained without any chemical treatment. Pellets were glued onto Mylar films, in sample holders, and placed directly in the X-Ray beam. EDXRF method (Panalytical, Netherlands) was used to quantify total concentrations of macro and trace elements in sea urchin gonads: phosphorus (P), sulphur (S), chloride (Cl), potassium (K), calcium (Ca), iron (Fe), arsenic (As), bromide (Br), rubidium (Rb) and strontium (Sr). The spectrometer was a self-constructed system with a Philips X-Ray generator (PW 1140/00/60). The technique consists in an XRay tube equipped with a molybdenum secondary exciter. The specific radiation of the elements was detected by a Lithium drifted Silicon [Si(Li)] detector, with an active area of 30 mm^2 and 8 μm beryllium window. The energy resolution was 135 eV at 5.9 keV acquired by a Nucleus PCA card (The Nucleus, Inc.). The X-Ray generator was operated at 50 kV, 20 mA and a typical acquisition time of 1000 s. Quantitative calculations were conducted according to fundamental parameters method (Carvalho et al., 2005; Custódio et al., 2003). Certified material was used to check accuracy (SRM-1571, TORT-2 and DORM-2). Satisfying values for the duplicate analysis of reference materials were obtained (see Appendix, **Table S1**).

2.2.2. Atomic Absorption Spectrometry (AAS)

Chromium (Cr) and nickel (Ni) concentrations in pooled sea urchin gonads were quantified by Atomic Absorption Spectrometry (AAS) with stoichiometric flame atomization or electrothermal atomization, depending on metal levels. Freeze dried samples were previously digested with concentrated nitric acid (HNO_3) in a microwave at high-pressure in PARR vessels following the methods previously reported (Reis et al., 2012, 2009). Briefly, three independent replicates per sea urchin gonad sample were analysed. Concentrations were expressed in $\mu g \cdot kg^{-1}$ or $mg \cdot kg^{-1}$, dry basis, quantification being carried out by external calibration. Certified materials (DORM-2 and TORT-2) were used to check accuracy. Concentrations were expressed in $\mu g \cdot g^{-1}$ or $mg \cdot kg^{-1}$, dry weight basis (dw). Satisfying values for the triplicate, analysis of reference materials was obtained (see Appendix, **Table S1**).

2.2.3. Inductively coupled plasma mass spectrometry (ICP-MS)

Total copper (Cu), zinc (Zn), selenium (Se), cadmium (Cd), mercury (Hg) and lead (Pb) were determined by inductively coupled plasma mass spectrometry (ICP-MS) after acid pressure digestion. Subsamples (0.13 − 0.18 g) of sea urchin gonads were digested by nitric acid (HNO_3) and hydrogen peroxide (H_2O_2) mix (4 mL:1.5 mL). All samples were adjusted to a final volume of 25 mL with milli-Q water. Prior to analysis, sample aliquots further diluted at least 5 times and with internal standards were prepared [tellurium (Te), bismuth (Bi), indium (In) and rhodium (Rh)] to obtain ~2 % HNO_3, 1 % HCl (c/v) aqueous solutions. Quantification was performed in no gas and helium mode with an iCAP Q ICP-MS from Thermo Fisher Scientific (Bremen, Germany) using external calibration ($ng \cdot mL^{-1}$) for Cu (2 − 12), Zn (4 − 240), Se (0.16 − 9.6), Cd (0.02 − 1.2), Hg (0.08 − 4.8) and Pb (0.03 − 0.9). This gave satisfying recoveries (85 – 128%) and relative standard derivations (1 – 18 %) for the duplicate extractions of reference materials TORT-2 and SRM 2976, except for Hg in TORT-2 include had high recovery (143 %), but 96 % of the Hg target value for SRM 2976 was obtained.

Inorganic arsenic (iAs) determination followed the principles settled in the standard EN 16802 (CEN, 2016). Subsamples of sea urchin gonads (0.30 − 0.31 g) were extracted by 10 mL (aqueous 0.1 M HNO3, 3 % H_2O_2) at 90 °C for 1 h and centrifuged (2500 *g*, 10 °C) for 10 min. The addition of hydrogen peroxide ensured a quantitative oxidation of arsenite (iAsIII) to arsenate (iAsV) (Rasmussen et al., 2012). The extracts were filtered (0.45 µm Mini-UniPrep HPLC vials, Whatman International, Maidstone, Kent, UK), injected (5 µl) onto a strong anion exchange HPLC column (10 µm, 2×250 mm and Dionex Ionpac AS7) and isocratic eluted for 9 min with 0.2 $mL \cdot min^{-1}$ mobile phase (40 mM ammonium carbonate, 3 % (v/v) aqueous methanol

and pH 10.3 adjusted by 25 % (v/v) aqueous ammonia). Quantification on 1100 HPLC coupled to 7500ce ICP-MS from Agilent Technologies (Waldbronn, Germany) was performed in time-resolved analysis mode and no gas mode (75As) with external calibration for iAs (0.20 − 20 ng·mL^{-1}). Repeated injections of quality control samples (70405a certified reference material) gave satisfying recoveries (93 – 104 %) and relative standard derivations (3 – 15 %).

Iodine (I) determination followed the principles of the European standard (EN 11511; CEN, 2007), in which iodine is determined by ICP-MS after alkaline extraction with dilute tetramethylammonium hydroxide (TMAH). Subsamples of homogenized freeze died sea urchin gonads (0.20 − 0.22 g) and bladderwrack reference material CD200 (0.30 g) were mixed thoroughly with 1 mL of TMAH (25 % w/w, 99.9999 %, Thermofisher, Germany) and with 5 mL ultrapurified water (< 18 MΩ cm, from a Milli-Q-Integral system, Merck, Germany) before being incubated at 90 ± 3 °C during 3 h. After cooling, the extracts were diluted to 25 mL with water. Before analysis, extracts were further diluted (sea urchin gonads: 2-fold; bladderwrack: 200-fold) and added internal standard to obtain 0.5 % TMAH. Quantification was performed in no gas mode, monitoring iodine (127I) and tellurium (125Te) with an iCAP Q ICP-MS from Thermo Fisher Scientific (Bremen, Germany) using external calibration for iodine (0.15 − 100 ng·mL^{-1}). Satisfying recovery (93 %, n = 1) was obtained for CD200, for includeh a target iodine value has recently been established in a collaborative trial; 631 ± 47 mg·kg^{-1} (mean ± standard deviation, n = 12 laboratories; Sloth, 2017).

Reference materials for quality assurance included SRM 2976 (mussel) from National Institute of Standards and Technology (NIST, Gaitherburg, MD, USA), TORT-2 (lobster hepatopancreas), DORM-2 and DORM-4 (fish proteins) from National Research Council of Canada (Ontario, Canada), BC211 (rice) and CD200 (powdered bladderwrack; *Fucus vesiculosus*) from Institute of Reference Materials and Measurements (Geel, Belgium) and 7405-a (seaweed; Hijiki) from National Metrology Institute of Japan (NMIJ, Tokyo, Japan). Water was ultra-purified (< 18 MΩ cm) using a Milli-Q-Integral system (Merck, Germany) and all reagents used were of per analysis quality or better. External linear calibration was applied for quantification and prepared in a solvent, matching the samples. Blank samples were analysed together with the samples and were subtracted to all results before reported. Limit of quantification (LOQ) and detection (LOD) were reported as the lowest calibration standard and 3× standard deviation (σ) of reagent blanks (n = 4), respectively. For quality control details see Appendix, **Table S2**.

2.3. Risk-benefit balance

According to Dietary References Intakes (DRIs), like recommended dietary allowances (RDA) or adequate intakes (AI) provided by the Institute of Medicine (IoM, 2001) and European Food Safety Authority (EFSA, 2014, 2012b, 2012c, 2011, 2010, 2009a, 2009b), percentages of the average requirements (AR) or AI for P, K, Ca, Fe, Cu, Zn, Se (using EFSA references), Cl, Cr and I (using IoM references) were calculated. Elements content in samples were previously converted to wet weight (mg·(100 g)$^{-1}$ ww) according to samples moisture (**Table 3.2-1**) and considering a meal composed by 20 g or 50 g of sea urchin gonads consumed by individual adults (> 18 years old) with body weight of 70 kg. The portions were considered given the fact that this product is usually consumed as a side dish. Calculations of the percentage of reference values for iAs (benchmark dose lower, $BMDL_{01}$ = 0.3 μg·kg^{-1} bw day^{-1}; EFSA, 2009b), Cd (TWI = 2.5 μg·kg^{-1} bw; EFSA, 2011, 2009a), Hg (TWI = 4 μg·kg^{-1} bw; (EFSA, 2012c), Pb (BMDL = 0.5 μg·kg^{-1} bw·day^{-1}; (EFSA, 2010) and Cr (tolerable daily intake, TDI = 0.3 μg·kg^{-1} bw·day^{-1}; (EFSA, 2014) were also conducted.

2.4. Statistical analysis

The elements average values from each area (samples from north and south sites were considered as representatives of each area) were calculated. Differences in elements concentrations were analysed by one-way ANOVA with the significance level set at 1 % after checking for variance homogeneity and normality. Statistical analysis was conducted using STATISTICA data analysis software system (version 8.0., StatSoft, Inc. (2007), USA).

3 Results and discussion

The biometric parameters of *P. lividus* gonads are displayed in **Table 3.2-1**.

Table 3.2-1 — Composition and biometric data of sea urchin.

Parameters	Sampling area					
	Carreço north	Carreço south	Praia Norte north	Praia Norte south	Vila Chã north	Vila Chã south
Gender composition (Males:Females)	3M:7F	4M:6F	6M:4F	6M:4F	5M:5F	5M:5F
Shell diameter (mm)	62 ± 3	64 ± 5	55 ± 5	62 ± 5	68 ± 5	67 ± 3
Total mass (g)	84.3 ± 16.6	107.0 ± 16.7	63.0 ± 15.8	73.5 ± 31.7	114.1 ± 22.2	125.2 ± 14.8
Gonads (g)	5.7 ± 3.1	7.1 ± 1.6	4.9 ± 2.8	6.5 ± 4.1	7.0 ± 2.1	7.1 ± 1.5
GSI (%)	6.9 ± 3.5	6.7 ± 1.5	7.3 ± 2.4	9.3 ± 5.2	6.4 ± 2.2	5.8 ± 1.3
Gonad moisture (%)	82.6 ± 0.1	81.2 ± 0.3	83.1 ± 0.1	81.5 ± 0.2	82.8 ± 0.1	79.9 ± 0.2

Mean values and respective standard deviation (n = 3) are presented.

The pooled samples were balanced as much as possible in terms of gender (males and females), since sea urchin do not present sexual dimorphism. The average sea urchin shell diameter was always above the permitted harvesting size (50 mm) in Europe. Sea urchins from Vila Chã were slightly heavier than those from the remaining two areas. The mean gonad index was higher in Praia Norte (7.3 − 9.3 %), followed by Carreço (6.7 − 6.9 %) and Vila Chã (5.8 − 6.4 %). Nonetheless, the highest gonad index registered in Praia Norte corresponded to the lowest gonads average weight.

3.1 Macro elements

The concentrations (mg·kg^{-1} dry weight, dw) of macro elements in sea urchin gonads from the three areas are shown in **Figure 3.2-2**.

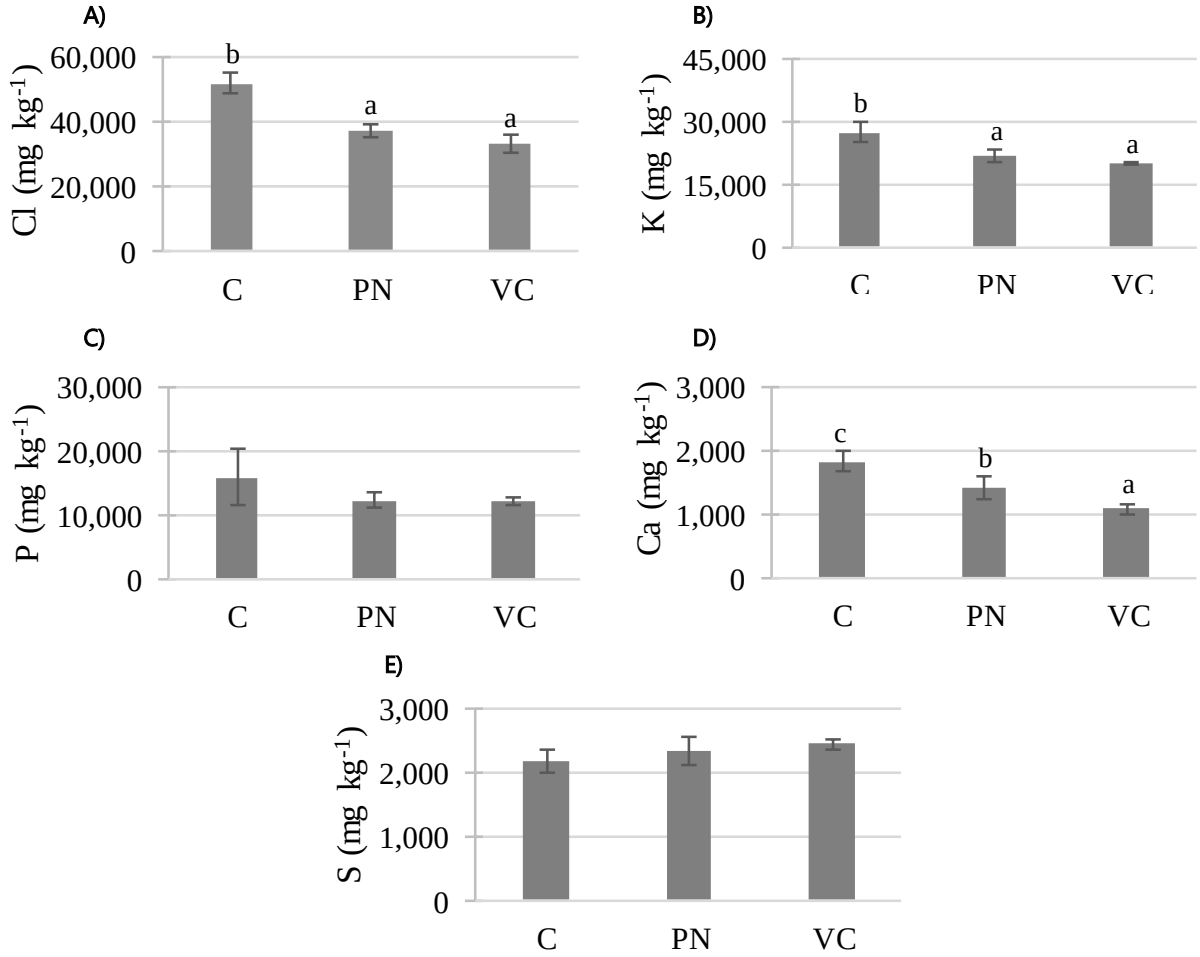


Figure 3.2-2 — Concentration of macro elements (mg·kg^{-1} dw).
A) Cl - chlorine; **B**) K - potassium; **C**) P - phosphorus, **D**) Ca – calcium and **E**) S - sulphur in sea urchin gonads from each sampling area. C - Carreço; PN - Praia Norte; VC - Vila Chã.

For each element, different letters represent significant differences ($p < 0.01$) between sea urchin gonads from the three sampling areas.

Statistical differences ($p < 0.01$) between the three harvesting areas were identified for each element. Chlorine was the most abundant macro element, followed by K and P. These macro elements have a vital function in fish as they contribute to maintain the homeostasis and acid-base balance (Lall, 2003). Calcium levels varied between 1000 and 2000 $mg \cdot kg^{-1}$ dw, being statistically different ($p < 0.01$) between areas. Generally, macro elements content diminished from Carreço area to Vila Chã, however no significant differences were registered in P and S levels in sea urchin gonads between the three harvesting areas. Besides, gonads from Vila Chã were bigger, had similar concentrations in Cl and K elements compared to Praia Norte. Samples from Carreço area had significantly higher ($p < 0.01$) concentrations compared to the other areas, for Cl and K elements.

3.2 Trace elements

Regarding trace elements, Zn showed the highest levels, followed by Br, Fe, Sr, I, Rb, Cu, Se, Cr and Ni (**Figure 3.2-3**).

Indeed, Praia Norte showed statistically the lowest levels of Fe and Se, but statistically higher levels of Rb; Vila Chã revealed significantly lower levels ($p < 0.01$) of Sr and significantly higher levels of iodine and Cr (this last element only from Praia Norte area); gonads from Carreço area revealed significant higher levels ($p < 0.01$) of Br than those found in Praia Norte and Vila Chã. No differences were found for Cu and Ni between the three areas. The Cr levels found in Vila Chã area may be due to the major proximity to leather companies discharges that act along Ave river (Gonçalves et al., 1992), which is located less than 10 km north from Vila Chã. Generally, the differences found in the present study may be due to elemental concentration in the body of an aquatic organism varies according to species, feed source, stage of development, size and animal physiological status and environmental exposure. In fact, organisms accumulate and retain trace elements from the environment, but their incorporation is highly selective (Lall, 2003). For instance, the accumulation in which sea urchin species (*Strongylocentrotus droebachiensis*) may occur even with long-term exposure to low elemental levels (Bielmyer et al., 2012). As mentioned before, the three harvesting areas might have different anthropogenic pressures which can result in the input of different loads of pollutants into the environment.

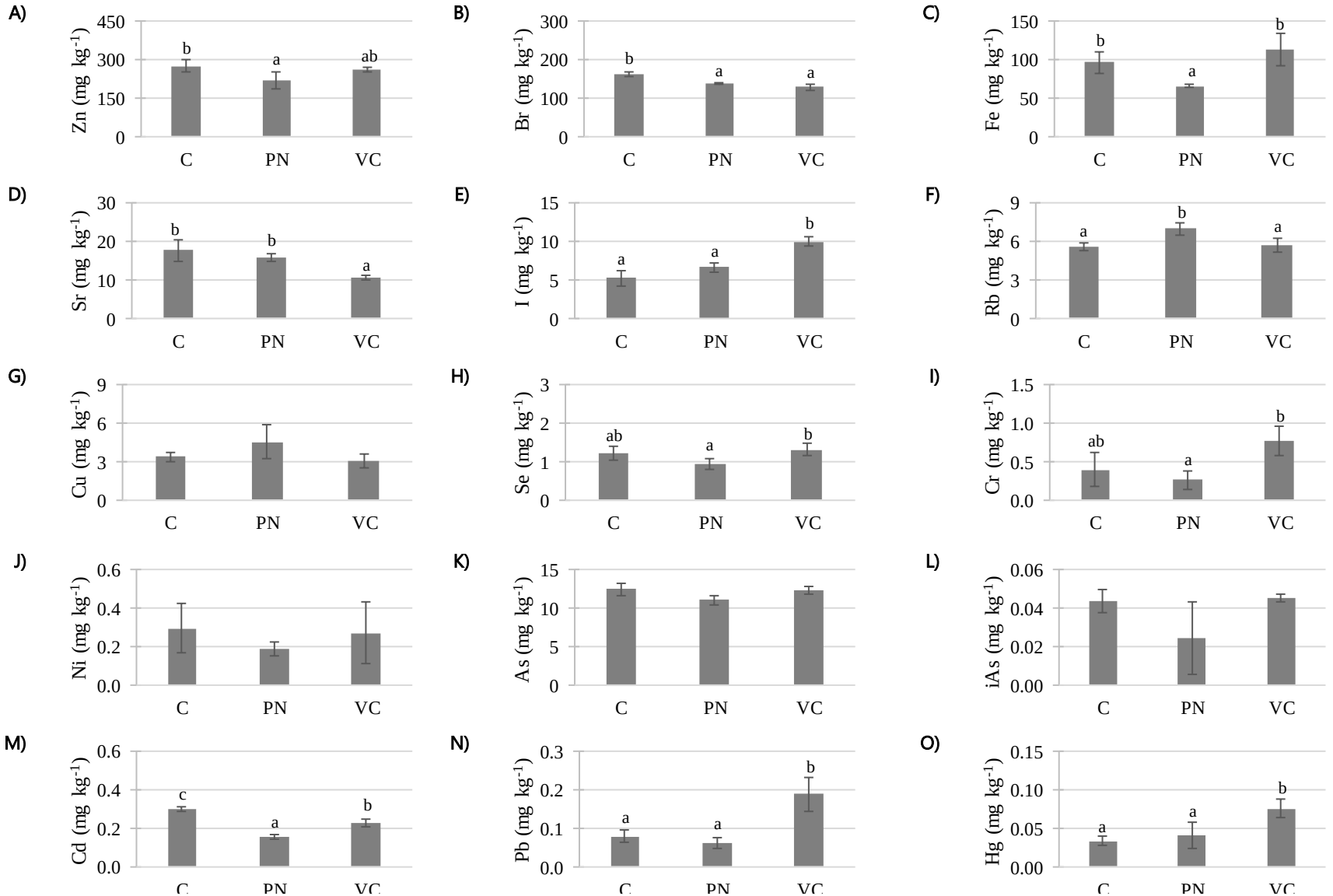


Figure 3.2-3 — Concentration of trace elements (mg·kg^{-1} dw).

A) Zn -zinc; **B**) Br - bromine; **C**) Fe - iron; **D**) Sr - strontium; **E**) I - iodine; **F**) Rb - rubidium; **G**) Cu - copper; **H**) Se - selenium, **I**) Cr – chromium; **J**) Ni – nickel; **K**) As - total arsenic; **L**) iAs - inorganic arsenic; **M**) Cd - cadmium; **N**) Pb - lead and **O**) Hg - mercury in sea urchin gonads from each sampling areas. C - Carreço; PN - Praia Norte; VC - Vila Chã; different letters represent significant differences (p < 0.01) between sea urchin gonads from the sampling areas.

Despite Fe and Zn levels generally decrease during female somatic organs (Lall, 2003), in the present study, pools from Praia Norte area with less females showed significantly lower levels ($p \leq 0.01$) in Fe than sea urchin gonads from the other two areas, as well as significantly lower levels of Zn ($p < 0.01$) than Carreço area, contrarily to what was expected. However, Soualili et al. (2008) observed that female gonads, from Algeria coast, had lower levels of Fe than male gonads. The differences may thus be associated with the bioavailability and with different patterns of accumulation of these elements, according to gender needs.

Strontium concentration in seafood has a positive correlation with fish containing high Ca levels and is considered as an essential element (Qin et al., 2015) that has an important role in homeostasis (Nielsen et al., 2004). Herein, Ca and Sr showed similar levels in Carreço area, which did not occur in the other two areas. Concerning Rb levels and distribution, few studies reported its occurrence in marine organisms (Barbosa et al., 2017; Campbell et al., 2005), but since it is a metal that biomagnifies in diverse food webs, it should be included, in multi-element biomagnification studies (Campbell et al., 2005).

When compared to other harvesting sites (**Table 3.2-2**), the present study revealed higher concentrations of Cu (slightly higher), Zn (5 times higher) and Cr (10 times higher) in harvested sea urchin, than those found in sea urchin species from the French market, as reported by (Guérin et al., 2011).

Table 3.2-2 — Concentrations of trace elements (mg·kg^{-1} ww) in the present and from previous studies.

Area	Elements (mg·kg^{-1} ww)										Reference
	Zn	Cu	Cr	Fe	Se	Ni	Cd	Pb	Hg	As	
SW Atlantic [a]	45.4 ± 6 (32 - 54)	0.66 ± 0.19 (0.5 - 1.1)	0.09 ± 0.05 (0.02 - 0.21)	16.6 ± 4.6 (11.4 - 25.0)	0.01 ± 0.01 (0.01 - 0.05)	0.05 ± 0.02 (0.02 – 0.10)	0.04 ± 0.01 (0.03 - 0.06)	0.021 ± 0.012 (0.01 - 0.05)	0.009 ± 0.004 (0.005 - 0.016)	2.12 ± 0.2 (1.9 - 2.5)	Present study
French market	7.6	0.44	0.005	81.2	0.65	0.29	n.r.	0.01	n.r.	n.r.	Guérin et al. (2011)
Morocco	n.r.	1.23 ± 0.11	0.07 ± 0.23	n.r.	n.r.	0.22 ± 0.05	0.10 ± 0.06	0.14 ± 0.03	9.85 ± 0.05	0.57 ± 0.30	Salvo et al. (2016)
France	n.r.	0.41 ± 0.10	0.38 ± 0.06	n.r.	n.r.	0.11 ± 0.04	0.31 ± 0.03	0.011 ± 0.01	3.08 ± 0.01	5.03 ± 0.85	Salvo et al. (2016)
Spain	n.r.	0.50 ± 0.13	0.17 ± 0.30	n.r.	n.r.	0.09 ± 0.10	0.10 ± 0.02	0.06 ± 0.04	11.20 ± 0.02	1.15 ± 0.89	Salvo et al. (2016)
Chile	n.r.	0.80 ± 0.13	0.46 ± 0.05	n.r.	n.r.	0.30 ± 0.13	0.61 ± 0.01	0.04 ± 0.02	5.52 ± 0.02	6.54 ± 0.20	Salvo et al. (2016)
Sardinia	n.r.	1.58 ± 0.09	0.30 ± 0.02	n.r.	n.r.	0.07 ± 0.01	0.44 ± 0.05	0.14 ± 0.01	0.06 ± 0.29	0.54 ± 0.30	Salvo et al. (2016)
Sicily	n.r.	1.50 ± 0.20	< 0.031[c]	n.r.	n.r.	< 0.040 (LD)	< 0.023 (LD)	0.09 ± 0.08	0.02 ± 0.01	0.06 ± 0.04	Salvo et al. (2016)
Algerian west [b]	14.2 ± 5.7	0.87 ± 0.6	n.r.	n.r.	n.r.	n.r.	0.13 ± 0.02	0.87 ± 0.2	n.r.	n.r.	Rouane-Hacene et al. (2018)

[a] Values presented as mean ± standard deviation (range) of the three studied areas from SW Atlantic Coast (present study);

[b] Mean ± standard deviation of three values presented from the reference. Moisture mean content in sea urchin gonads from present study (82 %) was used to transform data dw to ww;

n.r. - not reported.

Higher levels were also registered for Zn (2 – 5 times higher) than sea urchin from Algeria west coast (Rouane-Hacene et al., 2018). Salvo et al. (2016) reported the occurrence of essential and potentially toxic elements in commercial sea urchins collected in different production countries. Indeed, in the present study, Cu levels in sea urchin gonads were lower than levels found in specimens collected in Morocco, Sardinia, Sicily, slightly higher than in France, and in the same range as Spain (Salvo et al., 2016). Chromium levels in sea urchin gonads in the present study were lower than those found in France, Chile and Sardinia, but in the same range of specimens from Morocco and Spain (Salvo et al., 2016). Nickel concentrations in the present study were below values reported by Salvo et al. (2016). So, the concentrations of trace elements in sea urchin gonads from the three selected Portuguese harvesting areas were in general similar or even lower than those found in sea urchin gonads from other harvesting locations. To our knowledge this is the first report of this trace elements in sea urchin from SW Atlantic coast. Still comparing with seafood data available in the French market (Guérin et al., 2011): a) Cr in sea urchins presented similar levels than those found in angler fish, coalfish, seabass, seabream, whiting, cuttlefish, oyster, shrimp and swimcrab; b) Cu levels were similar to those found in pilchard and scallops; c) Fe concentration was in the same range as pilchard, sardine, crab and shrimp; d) Ni levels were in the same order of magnitude as eel, haddock, hake, halibut, salmon, sole and cuttlefish; e) Se levels were in the range of grenadier and cuttlefish and f) Zn presented similar levels to crab and swimcrab. The elemental variability found in sea urchin gonads compared to other seafood may be explained by the fact that concentrations of most trace elements have the highest increase in the first trophic level (seawater-phytoplankton) (Lall, 2003).

Measurable levels of total As, iAs, Cd, Pb and Hg were found in gonads of sea urchins collected in all sampling areas (**Figure 3.2-3** K to O). Sea urchin gonads from Carreço and Vila Chã registered slightly higher total As content (p-value ≈ 0.01) than Praia Norte. The levels of this toxic element can be due to sea grass leaves that constitute the basis of sea urchin feed (Warnau et al., 1998), since macroalgae species like *Laminaria digitata* and *Saccharina latissima*, may present high levels of As (41 – 43 mg·kg^{-1}; Maulvault et al., 2015). It is generally accepted that As organic species, are excreted more quickly from animals and generally considered less toxic. The main As organic forms are arsenobetaine, arsenosugars and arsenolipids, with seafood being the major sources of exposure (Giri et al., 2016).

Regarding iAs, low levels were found in sea urchin gonads (0.2 – 0.05 mg·kg^{-1} dw), no significant differences being found between sampling areas. Aquatic animals, have the ability to absorb some As inorganic forms from their external environment in both fresh and seawater

(Lall, 2003), but in the present study less than 1 % of the total arsenic was in its toxic inorganic form. Concerning human exposure to the main inorganic As species, a sequence of reduction and oxidative/methylation steps occur in liver, being arsenite (trivalent) and arsenate (pentavalent) the most toxic As species (Vahter, 2002).

Cadmium registered the highest content in sea urchin gonads from Carreço, followed by Vila Chã and Praia Norte, being all statistically different ($p < 0.01$). According to Lall (2003), the concentration of Cd reach higher levels in crustaceans and the lowest in fish. Lead reached the highest levels in sea urchins gonads collected in Vila Chã, being statistically higher than samples from Carreço and Praia Norte areas. At last, Hg showed the highest level in samples from Vila Chã, being statistically different ($p < 0.01$) from Praia Norte and Carreço. Some extractions of methylmercury (MeHg), the most toxic Hg species, that highly bioaccumulate and biomagnifies along the aquatic food chain (Bustamante et al., 2006; Murphy et al., 2008), was conducted in these samples with levels below the limit of detection.

The Commission Regulation No. 1881/2006 (EC, 2006), amended by 420/2011 (EC, 2011a), set maximum levels (MLs) for certain contaminants in foodstuffs, including in bivalve molluscs: Pb (1.5 $mg \cdot kg^{-1}$ ww), Cd (1.0 $mg \cdot kg^{-1}$ ww) and Hg (0.5 $mg \cdot kg^{-1}$ ww) and for fish muscle: Pb (0.3 $mg \cdot kg^{-1}$ ww), Cd (0.05 $mg \cdot kg^{-1}$ ww) and Hg (0.5 $mg \cdot kg^{-1}$ ww), but no limits have been established yet for sea urchins in the EU. A comparison of levels obtained in the present study (on a fresh weight basis; moisture content shown in **Table 3.2-1**) revealed that Hg levels in sea urchin gonads were by far lower than MLs (35 – 80 times lower) set for fish muscle (EC, 2011a, 2006). As far as Pb levels are concerned, levels were 8 – 30 times lower than MLs set for fish muscle. Concerning Cd, levels in sea urchin gonads were well below the MLs set for bivalves (18 – 37 times below). However, compared to Cd MLs for fish muscle, gonads match the MLs in Carreço area, are close to ML in Vila Chã and below ML in Praia Norte (0.027 ± 0.002 $mg \cdot kg^{-1}$ ww).

When compared to other harvesting sites (**Table 3.2-2**), Cd levels in sea urchin gonads in the current study were lower (2 – 22 times) than values reported in Salvo et al. (2016) and 3 times lower than sea urchin gonads from Algeria west coast (Rouane-Hacene et al., 2018).

Concerning Pb, the levels found in the current study were lower than those registered in specimens from Morocco, Spain, Sardinia, Sicily (Salvo et al., 2016) and Algeria west coast (Rouane-Hacene et al., 2018), or in the same range as those reported for Chile (Salvo et al., 2016) and France specimens (Guérin et al., 2011; Salvo et al., 2016). The same trend, i.e., lower concentrations in Hg (200 – 800 times lower), was registered in the present study in other studied sites (Salvo et al., 2016). Only Sardinia and Sicily presented levels in the same range for Hg.

Concerning As levels, the present study reported higher values compared to Spain (2 times higher), Morocco (4 times higher), Sardinia (4 times higher) and Sicily (around 30 times higher), but lower concentrations than in France (2 times lower) and Chile (3 times lower). So, the concentrations of toxic elements in sea urchin gonads from the three selected Portuguese harvesting areas were, in general, lower or similar to those found in sea urchin gonads from other harvesting locations. To our knowledge this is the first report of toxic elements in sea urchin from SW Atlantic production areas.

3.3 Elements in sea urchin gonads and the environmental area

The selected sampling sites are surrounded by several industries, which have proven to affect the aquatic environment in this region, like textile (Gonçalves and Alpuim, 2011; Gonçalves et al., 1992), food, paper, rubber and metallomechanic industries, as well as several urban wastewater treatment plants (APA, 2015). Water contamination can occur through the hydrographic network of Lima, Cávado, Ave and Leça rivers (APA, 2015).

Studies indicate that a relationship can exist between metal content in water and the tissues of organisms (Reis, 2013). Warnau et al. (1997) also found that bioconcentration of Cd in *P. lividus* body compartments is directly proportional to Cd concentration present in seawater. The same study revealed that Cd displays a long biological half-life in echinoid tissues (Warnau et al., 1997), thus being suggested that *P. lividus* can be a valuable species to biomonitor Cd contamination in the marine environment. Sea urchins are not filter-feeders as bivalves, but a grazer herbivore echinoderm (Boudouresque and Verlaque, 2013). *P. lividus* feeds mainly on algae and suspended organic particles (Bulleri et al., 1999; Gianguzza et al., 2006), and generally does not take up metals (e.g. Cd) from its food (Warnau et al., 1998).

Spatial and seasonal variation of elements concentrations have been previously found along the north-western Portuguese coast (Reis, 2013). The trends might be associated to coast dynamics, nature of sediment geology of the region and the surrounding anthropogenic pressure. In fact, higher metal concentrations have been generally found near urban areas, reflecting their proximity to anthropogenic sources (Reis, 2013). Nevertheless, elements levels found were generally lower compared to data from other areas like southern Spain (Morillo et al., 2008), Algeria coast – Mediterranean Sea (Soualili et al., 2008), Egypt (Sharaf and Shehata, 2015) and Bangladesh (Ali et al., 2016). In fact, a survey carried out between 2009 and 2011 revealed that all sampled locations along NW coast of Portugal could generally be classified as "class I–background level" or "class II–good" (Reis, 2013), according to Norwegian Pollution Control Authority guidelines for metals in coastal seawaters (SFT, 2007), although with variability in

trace and toxic elements concentrations among locations. A sampling campaign carried out in 2015 in north-western Portuguese coast (with two common sampling points, Carreço and Vila Chã) also showed low concentrations of dissolved and particulate metals, once again with variability among location (Costa et al., 2015).

3.4 Risk-benefit for consumers

The percentages of DRI (set by IoM), AR and AI (set by EFSA) are presented for the different essential elements in 20 g and 50 g portions (**Table 3.2-3**) of sea urchin gonads (consumption per week).

Table 3.2-3 — Percentages of the recommended dietary reference intake (DRI) and limit reference values accomplished with the consumption of 20 and 50 g (presented as interval) of sea urchin gonads per week from each sampling area.

Element	Reference values ($mg \cdot day^{-1}$)		% Reference values range per area		
	IoM	EFSA	Carreço	Praia Norte	Vila Chã
P [a]	700	550 (AI)	7.5 - 34	7.0 – 22	7.5 – 22
Cl [b]	2300	N.R.	7.4 - 22	5.6 – 15	4.6 – 15
K [a]	4700	3500 (AI)	2.5 – 8.0	2.1 - 5.7	1.9 - 5.8
Ca [a]	1000	750 (AR)	0.8 – 2.5	0.6 - 1.8	0.5 - 1.5
Cr [b]	0.035	N.R.	3.6 – 11	1.6 – 9.2	6.7 – 24
Fe [a]	18	6 (AR)	5.3 - 16	3.7 – 10	6.2 - 19
Cu [a]	0.9	1.3 (AI)	0.9 - 2.4	1.1 - 3.4	0.8 - 2.2
Zn [a]	11	6.2 (AR)	16 - 41	11 - 34	15 – 41
Se [a]	0.055	0.07 (AI)	6.1 – 16	4.4 - 12	5.9 – 20
I [b]	0.15	0.6 (UL)	11 - 47	15 - 40	23 - 70
iAs [cd]	$BMDL_{01}$ = 0.3 $\mu g \cdot kg^{-1}$ bw day^{-1}		0.1 - 0.4	0.1 - 0.3	0.1 - 0.4
Cd [c]	TWI = 2.5 $\mu g \cdot kg^{-1}$ bw		0.7 - 1.9	0.3 - 0.9	0.5 - 1.4
Hg [c]	TWI = 4 $\mu g \cdot kg^{-1}$ bw		0 - 0.1	0.1 - 0.1	0.1 - 0.4
Pb [cd]	BMDL = 0.5 $\mu g \cdot kg^{-1}$ bw day^{-1}		0.1 - 0.4	0.1 - 0.3	0.3 – 1.1
Cr [cd]	TDI = 0.3 $\mu g \cdot kg^{-1}$ bw day^{-1}		1.0 - 3.2	0.4 - 2.6	1.8 – 6.8

[a] Reference values from European Food Safety Authority (EFSA) were taken for calculations; [b] Reference values from Institute of Medicine (IoM) were taken for calculations; [c] Reference value from EFSA. Calculations were conducted considering an adult average body weight of 70 kg; [d] Daily limit references converted to a weekly basis; AI- Adequate intakes; AR- Average requirements; UL- Upper limit; N.R.– No recommendation; bw- body weight of an individual; $BMDL_{01}$- Benchmark dose lower confidence limit; TWI- tolerable weekly intake; BMDL- Benchmark dose level; TDI- tolerable daily intake.

The highest contribution is from iodine element (up to 70 % in 50 g portion), followed by Zn (41 %), P (34 %), Cr (24 %), Cl (22 %), Se (20 %) Fe (19 %), K (8 %), Cu (5 %) and Ca (3 %).

Whenever seafood species are harvested from areas subjected to strong anthropogenic pressures involving discharges of contaminants from industrialization areas, its consumption can represent an important exposure to toxic elements or high concentrations of trace elements that, despite being required at low concentrations, can be toxic at high concentrations. Besides MLs, food safety agencies set guidelines for element intakes, namely TWI for Cd and Hg (2.5 and 4 $\mu g \cdot kg^{-1}$ body weight (bw), respectively), TDI for Cr (0.3 $\mu g \cdot kg^{-1}$ $bw \cdot day^{-1}$), BMDL for Pb (0.5 $\mu g \cdot kg^{-1}$ $bw \cdot day^{-1}$), to reduce human risk exposure to toxic elements. For total As, there are no MLs established by EU regulation, but EFSA (2009b) established a $BMDL_{01}$ of 0.3 $\mu g \cdot kg^{-1}$ bw per day for the most toxic form, i.e. iAs. Using a semideterministic approach, percentages of each toxic element contribution were calculated considering an adult average body weight of 70 kg. Reference values and the approach of one meal a week of 20 g and 50 g of sea urchin gonads is given in **Table 3.2-3**. With respect to toxic elements contributions to the scenario previously presented, iAs and Hg reached only 0.4 % of its BMDL, Pb reached only 1.1 % of its BMDL and Cd reached only 1.9 % of its TWI. Concerning Cr, a major contribution may occur, especially for sea urchins from Vila Chã area (up to 6.8 % of its TDI). Therefore, results indicate that the risk for consumers is low regarding sea urchins harvested in SW Atlantic areas and they represent a good source of essential elements for consumers, specially iodine, with important biological functions as an obligatory constituent of thyroid hormones (EFSA, 2014).

4 Conclusion

The present study provides a dataset on macro (Cl, K, P, Ca and S) and trace (Zn, Br, Fe, Sr, I, Se, Rb, Cu, Cr, Ni, As, iAs, Cd, Pb and Hg) elements in sea urchin gonads (*P. lividus*) from highly explored production areas in the SW Atlantic coast. Sea urchin may constitute an effective sentinel species to biomonitor environment contamination, particularly due to the levels of Cd and Cr found. Higher levels of Pb and Hg were also registered in sea urchin gonads from exposed areas. The distinct area characteristics evidence the importance to biomonitor contaminants at local scale, particularly for toxic elements. Overall, the studied areas seem to be suitable locations for the sustainable exploitation of sea urchins.

In terms of seafood safety, the levels of toxic elements were generally below MLs (except for Cd levels that were similar to fish muscle). In this sense, the current legislation should be revised to include this specific product. Nonetheless, consumers of this delicacy from the

sampling area are protected, since toxic elements were always below the maximum levels set for regulated contaminants as well as UL set for essential elements if a weekly consumption of 50 g is considered. Sea urchin gonads from the assessed areas are a good source of oligoelements, particularly for iodine when consuming one meal a week. These results are of utmost importance for the feasibility of sea urchin aquaculture, which would be strongly promoted, as the species availability in the natural environment is decreasing. Future studies should be conducted involving environmental sampling (e.g. seawater, sediments, algae) to link the environmental element concentrations with those found in sea urchin gonads.

Acknowledgements

This work was supported by the research line INSEAFOOD within the Structured Program of R&D&I INNOVMAR – Innovation and Sustainability in the Management and Exploitation of Marine Resources (Ref. NORTE-01-145-FEDER-000035) that is funded by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF). The Portuguese Foundation for Science and Technology (FCT) supported the contract of António Marques in the framework of IF2014 program, as well as the post-doc Grant of Patrícia Anacleto (SFRH/BPD/100728/2014). Carolina Camacho acknowledges MARINALGAE4aqua Project (ERA-NET COFASP/004/2015) for the research grant.

4

FRESHNESS AND QUALITY CHANGES OF SEA URCHIN

4.1 Effect of live transport conditioning on the quality of sea urchin *Paracentrotus lividus*

Abstract

Sea urchin gonads are a highly valued product and a delicacy appreciated worldwide, mainly traded as a live product. Therefore, the transport of live sea urchins is of utmost importance for the overall quality of this resource. To determine the effects of live transport on the external quality and physiological condition of *Paracentrotus lividus*, different conditions were simulated, addressing two animal conditionings: bulk and individual compartments, both at 7 °C. Bulk conditioning caused the highest mass loss (from day 6). In general, the quality was maintained in both conditionings, as evidenced by the position/firmness of spines and odour, for up to 4-6 days. The physiological biomarkers showed some oxidative stress with cellular damage, which became more evident on day 6. Therefore, live transport of sea urchins at 7 °C in a moist environment should not exceed 4-5 days.

Keywords: live seafood; animal vitality; sensory quality; gonads' biochemical responses; physiological biomarkers

This subchapter is adapted from the publication:

Camacho, C., Maulvault, A.L., Santos, M.T., Marques, A., Diniz, M.S., Pessoa, M.F., Gonçalves, A., Nunes, M.L., 2022. Effect of live transport conditioning on the quality of sea urchin *Paracentrotus lividus. EJFA* (accepted)

Contribution of Carolina Camacho: Investigation, Data curation, Validation, Formal analysis, Visualization, Writing – original draft, review and editing.

1 Introduction

The global market for sea urchins is still very traditional (Sun and Chiang 2015; Stefánsson et al. 2017) and the European market is estimated to be around 3500 t per year (Stefánsson et al., 2017). Sea urchin gonads are a highly appreciated product (Sun and Chiang 2015) and are considered a delicacy. The purple sea urchin *Paracentrotus lividus* is distributed mainly in the Mediterranean and north-eastern Atlantic (Boudouresque and Verlaque, 2013), and is the most consumed sea urchin species in Europe (Stefánsson et al., 2017). *P. lividus* from Atlantic areas is safe for consumption and has high nutritional quality, including bioactive compounds, such as carotenoids (Rocha et al., 2019b).

In Europe, sea urchins are usually collected manually by divers in near-shore waters (James et al., 2016) and traded as a live product. They are an expensive product, not only because of intrinsic characteristics of their gonads, namely colour, sweet taste and sea flavour (Sun and Chiang, 2015), but also because they are a scarce and seasonal resource.

It is important to ensure the quality of sea urchin throughout the supply chain. In doing so, the stress on sea urchins should be as low as possible. This will decrease their mortality, which will reduce the ecological impacts, economic losses, and food waste, but also the loss of product quality that can affect commercialisation along the distribution chain.

Although sea urchins are relatively robust animals when properly handled (James and Evensen, 2018), they can be subject to stress when exposed to air for extended periods of time (Verachia et al., 2012). It is known that in other marine invertebrates, variations in natural habitat alter metabolic activity and affect the level of oxidative stress (Verlecar et al., 2008). Therefore, live sea urchins may experience quality loss during transport, storage, and processing. Most sea urchin distributors still have concerns about the best conditions for transport of live animals. In many regions, transport is still done in bulk which is not recommended (Rubilar and Crespi-Abril, 2017) at inadequate temperatures (James and Evensen, 2018).

Furthermore, several studies evaluated the effects of transport conditions on the quality of live marine invertebrates such as clams (Anacleto et al., 2013), and some have led to the development of a "best practice guide" such as for crabs (Barrento et al., 2013). However, as far as transport of live sea urchins is concerned, the available information is limited. In fact, to the authors' best knowledge, only one scientific paper (Dale et al., 2005) and one report (James and Evensen, 2018) have addressed this issue, limited to a single species of sea urchin (*S. droebachiensis*). Furthermore, both publications focused on the visual attributes of the gonads (e.g.,

size and colour) and on the most extreme physiological stress endpoint, i.e., mortality, and did not consider the potential biochemical changes that could occur during transport.

Several biochemical tools (biomarkers) can act as early-warning signals to assess the degree or severity of physiological stress to which aquatic organisms are exposed under sublethal conditions, such as antioxidant defense mechanisms (e.g., catalase (CAT), superoxide dismutase (SOD), glutathione S-transferases (GST)) activated in various tissues during free radical formation (Lesser, 2006) and the resulting lipid peroxidation (LPO), as well as the expression and synthesis of proteins (e.g., ubiquitin (Ub) content) (Gagné, 2014). Although these biomarkers are widely used in ecotoxicology and environmental studies (echinoderms are bioindicators of environmental quality, e.g., Cunha et al., 2005; Johnstone et al., 2019), their potential as a tool for assessing the quality of live seafood is still underexploited.

In this context, this study aimed to evaluate for the first time, the effects of live transport conditioning on the external quality and physiological state of the wild sea urchin *P. lividus*. One of the conditionings studied was bulk, where sea urchins could interact with each other. The other involved individual compartments where sea urchins could not interact. Both conditionings were conducted under refrigerated conditions during 8 days of transport. Different tools were employed: sensory evaluation (external appearance, indicators of fitness/vitality of the animals, and odour), physical-chemical (mass loss and properties of the coelomic fluid) and biochemical responses in the gonads. This study will contribute to estimate the shelf-life of this product.

2 Materials and methods

2.1 Sea urchin sampling and conditioning

Wild sea urchins *P. lividus* from coastal areas were caught in winter prior to their maximum maturity and before spawning (Rocha et al., 2019b). The number of sea urchins used for the experiments was minimised according to the 3Rs principle (Replacement, Reduction and Refinement). All researchers involved in the handling and sampling of live animals were certified by the Federation of European Laboratory Animal Science Associations (FELASA) in laboratory animal sciences. Sea urchins were transported live inside plastic containers filled with seawater to the laboratory facilities (approximately 40 min from the harvesting point). The physiological condition of the sea urchins at the beginning of the experiment (day 0) was assessed on five randomly sampled sea urchins. The remaining sea urchins were casually distributed, into two polystyrene boxes (n = 30 per box; each box contained 50 mL of seawater) to

simulate two different transport conditions in a moist environment: i) conditioning in bulk at 7.1 ± 0.3 °C (sea urchins could interact with each other; density = 5 sea urchins·dm^{-2} to ensure two layers of animals) - treatment LTB; ii) individual conditioning at 7.0 ± 0.2 °C (individual compartments 8 × 8 cm, sea urchins could not interact) - treatment LTIC. The transport temperature was selected based on a difference of 10 °C regarding the average seawater temperature of the harvesting area to avoid excessive cold and thus additional stress (James and Evensen, 2018).

Temperature was recorded throughout the 8-day experiment using a data logger (Express Thermo, Eclo Solutions, Portugal). During the transport trials, five animals from each treatment were sampled every two days (days 2, 4, 6 and 8) to assess quality through different analyses: sensory evaluation (external appearance and odour), physical-chemical determinations (coelomic fluid properties) and biochemical responses/endpoints in the gonads (antioxidant defence mechanisms, lipid peroxidation, protein degradation).

2.2 Sensory evaluation - external appearance and odour

Three judges, previously trained, evaluated the external appearance by animal fitness/vitality/quality indicators, as well as the odour and colour of the water used to simulate the moist environment. A demerit point (dp) scale (Martinsdóttir et al., 2009) was used ranging from 0 to 3, except for the mouth, where a 2-point scale was chosen. The following characteristics were evaluated: position of spines (0-all upright to 3–completely fallen), firmness of spines (0-all firms and moving to 3–all soft, partially without spines), odour (0–odourless, slightly marine/algal, fresh to 3–strong off-odours, sickly-sweet, fermented), colour of water (0–colourless to 3-greenish) and reaction of mouth to external stimuli (0–reactive and 1–no reaction, open). Taste evaluation of the gonads was not possible due to the limited sample amount.

2.3 Physical-chemical determination - coelomic fluid properties

Mass loss was calculated by weighing the same group of fifteen sea urchins individually (the last sampling point coincides with the weighting group) in each treatment. Coelomic fluid was collected from the sea urchins (n = 5 from each day of sampling and conditioning), pH was measured using a pH probe (pH meter 539, WTW, Weilheim, Germany), while the solids content (°Brix) and turbidity were determined according to Verachia et al. (2012).

2.4 Gonads' biochemical responses – physiological biomarkers

The sea urchins were dissected, the gonads were removed (n = 5 on each sampling day and treatment), washed in cold artificial seawater (4 °C), drained and homogenised individually in cold-ice with phosphate-buffered saline solution (140 mM NaCl, 3 mM KCl, 10 mM KH_2PO_4, pH = 7.40 ± 0.03; Sigma Aldrich Co., St. Louis, MO, USA), using an Ultra-Turrax® device (Ika, Germany). Each homogenate was centrifuged (15 min, 4 °C at 10 000 *g*; Model 5804R, Eppendorf AG, Germany), the supernatant was collected and stored at -80 °C until further analysis. Each sea urchin was considered as one sample unit. All biochemical assays were performed in duplicate. Biomarker values were normalised to mg total protein (Bradford, 1976) using bovine serum albumin (BSA; Sigma) as a standard.

CAT activity (CAT; EC 1.11.1.6) was carried out according to Johansson and Borg (1988) based on decomposition of H_2O_2 and adapted (Madeira et al., 2016). A calibration curve was built using formaldehyde standards (Sigma). GST activity (EC 2.5.1.18) was determined according to the principle of Habig et al. (1974) and adjusted (Madeira et al., 2016). GST from equine liver (Sigma) was used as a positive control. GPx activity (EC 1.11.1.9) was determined as described by Paglia and Valentine (1967) and adapted (Lopes et al., 2018). The results of CAT, GST and GPx were expressed in $nmol \cdot min^{-1} \cdot mg^{-1}$ total protein.

SOD activity (EC 1.15.1.1) was determined according to McCord and Fridovich (1969) with modifications (Lopes et al., 2018). The results were expressed as the percentage of enzyme inhibition, using the following equation: SOD (% inhibition) = ((Abs550/min negative control– Abs550/min sample)/(Abs550/min negative control)) × 100.

Lipid peroxidation (LPO) was determined by malondialdehyde (MDA) quantification (Madeira et al., 2016), adapted from the method of thiobarbituric acid reactive substances (Uchiyama and Mihara, 1978). The by-products of lipid damage (i.e. peroxides) were quantified with MDA bis(dimethyl acetal) as a standard (Merck, Darmstadt, Germany). Results were expressed as $nmol \cdot mg^{-1}$ total protein.

Protein carbonyl (PC) content was determined following Alamdari et al. (2005) and adapted (Lopes et al., 2018). Primary and secondary antibodies were used, anti-DNP (Acris Antibodies Co., San Diego, USA) and alkaline phosphatase-conjugated anti-mouse IgG (Fab specific, Sigma). Results were expressed as $Abs \cdot mg^{-1}$ total protein.

The Ub content was determined using a direct ELISA (Madeira et al., 2017). Horseradish peroxidase conjugated primary antibody Ub P4D1 (sc-8017; Santa Cruz Biotechnology, Inc., USA) was used. A calibration curve was constructed using purified ubiquitin (E-1100; UbpBio

Co., Dallas, USA), and results were expressed as μg·mg^{-1} total protein. In all methodologies, 96-well microplates were used, as well as a microplate reader (BioRad, Benchmark, USA).

2.5 Statistical analysis

Statistical analysis was performed using STATISTICA™ version 7.0 software (StatSoft Inc., USA). The biochemical data was tested for normality distribution and homogeneity of variances (Zar, 2014) and parametric methods was employed. The presence of significant differences between treatments over time was investigated using factorial ANOVA at different conditioning, i.e., the effect of conditioning and time up to 8 days. Tukey multiple comparison test was then applied. Finally, possible correlations between sea urchin appearance, mass loss, pH characteristics and gonads' biochemical responses, were investigated using Pearson's correlation analysis. All statistical analyses were performed at a significance level of 0.05.

3 Results

3.1 Effects of transport conditioning on mass loss, external appearance, and properties of coelomic fluid

In general, mass loss significantly increased ($p < 0.014$) with time during simulated live transport. Conditioning affected this parameter, as the lowest mass loss was observed in urchins kept in individual compartments (LTIC treatment, **Figure 4.1-1**).

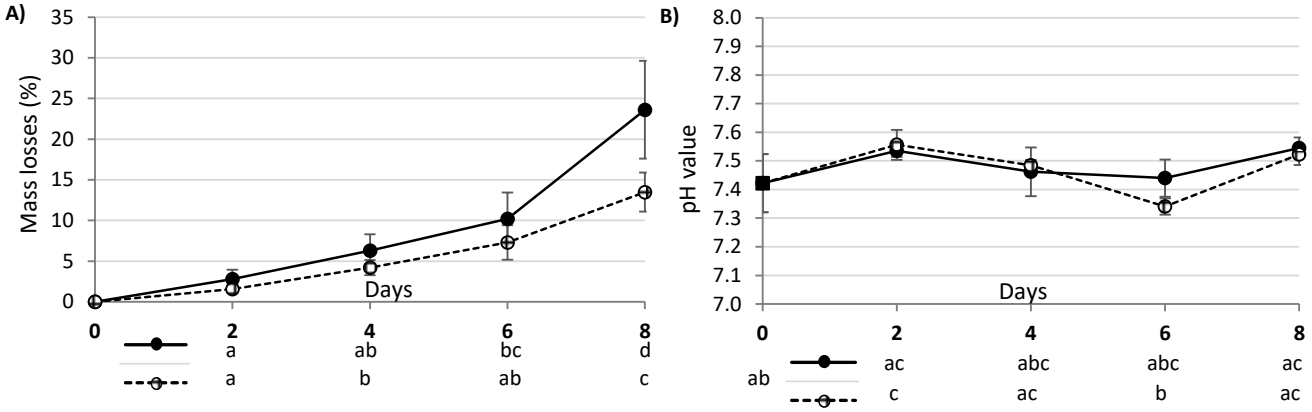


Figure 4.1-1 — Mass loss (**A**) and coelomic fluid pH (**B**) of sea urchin over time of simulated transport at 7 °C.

Continuous black line - bulk conditioning (LTB treatment); discontinuous line - individual compartments (LTIC treatment). Values represents mean ± standard deviation (n = 5). Different letters denote significant differences among treatments.

The scores of sea urchins along the transport are shown in **Table 4.1-1**.

Table 4.1-1 — Sensory scores of vitality/quality (in demerit points, dp) of sea urchin over time of simulated live transport at 7 °C.

Parameters	Beginning	Conditioning	Day 2	Day 4	Day 6	Day 8
Position of spines [a]	0.0 ± 0.0	Bulk	1.4 ± 0.6	1.4 ± 0.6	2.0 ± 0.0	3.0 ± 0.0
		Individual compartments	1.0 ± 0.0	1.4 ± 0.6	1.4 ± 0.6	3.0 ± 0.0
Firmness of spines [b]	0.0 ± 0.0	Bulk	1.0 ± 0.0	1.4 ± 0.6	1.4 ± 0.6	3.0 ± 0.0
		Individual compartments	1.0 ± 0.0	1.4 ± 0.6	1.4 ± 0.6	3.0 ± 0.0
Mouth reaction [c]	0.0 ± 0.0	Bulk	0.0 ± 0.0	0.2 ± 0.5	0.6 ± 0.6	1.0 ± 0.0
		Individual compartments	0.0 ± 0.0	0.2 ± 0.5	0.4 ± 0.6	1.0 ± 0.0
Colour of the water [d]	0.0 ± 0.0	Bulk	1.0 ± 0.0	2.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
		Individual compartments	1.0 ± 0.0	2.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Odour [e]	0.0 ± 0.0	Bulk	1.0 ± 0.0	2.0 ± 0.0	2.5 ± 0.5	3.0 ± 0.0
		Individual compartments	1.0 ± 0.0	1.0 ± 0.0	2.1 ± 0.2	3.0 ± 0.0

Values are mean ± standard deviation (n = 5 sea urchins assessed by three judges);

[a] from 0 dp- all upright to 3 dp – completely fall off;

[b] from 0 dp - all firms and moving to 3 dp – all soft;

[c] from 0 dp – reactive and 1 dp – without reaction;

[d] from 0 dp – colourless to 3 dp - greenish;

[e] from 0 dp – odourless, slight marine/algae, fresh to 3 dp – strong off-odours, sickly-sweet, fermented.

Initially, all sea urchins had firm and upright spines (0.0 dp), which softened from the second day onwards, scored with 1.4 dp on days 4 and 6, and 3.0 dp on day 8, regardless of conditioning. Strong and positive correlations were found between spines position and firmness ($r \geq 0.84$, $p < 0.0001$). At the beginning of the study, the water was colourless (0.0 dp), then changed to greenish, being scored with 3.0 dp on day 6, regardless of conditioning. Regarding the mouth reaction (**Table 4.1-1**), all sea urchins were reactive up to the 2nd day (0.0 dp); 4 were reactive until the 4th day (0.2 dp), and at the end of the trial no one of the 5 sea urchins tested showed any response, regardless of conditioning (1 dp; **Table 4.1-1**).

Concerning the odour, at beginning a slight fresh/marine odour was perceived (0.0 dp), which changed to a slight sickly-sweet and fermented at day 4 in the conditioning in bulk (2.0 dp) and at day 6 in the individual compartments (2.1 dp). On day 8, strong off-odours were perceived (3.0 dp) regardless of conditioning (**Table 4.1-1**). Strong and positive correlations were observed between odour and spines position/firmness ($r \geq 0.80$, $p < 0.0001$), fluid colour ($r \geq 0.90$, $p < 0.0001$) and mass loss ($r \geq 0.82$, $p < 0.0001$). Significant positive correlations were found between the position/firmness of spines and fluid colour ($r \geq 0.80$, $p < 0.0001$) and mass loss ($r \geq 0.84$, $p < 0.0001$).

The initial pH of the coelomic fluid was 7.42 ± 0.10 units, with non-significant variations in the following days until the 8th day (**Figure 4.1-1** B). There were no significant differences in solids content, ranging from 4.86 ± 0.11 (beginning) to 4.92 ± 0.13 and 4.88 ± 0.13 °Brix on day 8 for both conditionings. However, turbidity values increased significantly ($p < 0.0002$) from 0.06 ± 0.01 (day 0) to 0.22 ± 0.02 (day 8) in LTIC treatment.

3.2 Effect of conditioning and time on the biochemical responses of the gonads

The impact of animal conditioning over time at 7 °C up to day 8 on enzyme activities of CAT, GST and GPx and LPO of sea urchin gonads are shown in **Figure 4.1-2**.

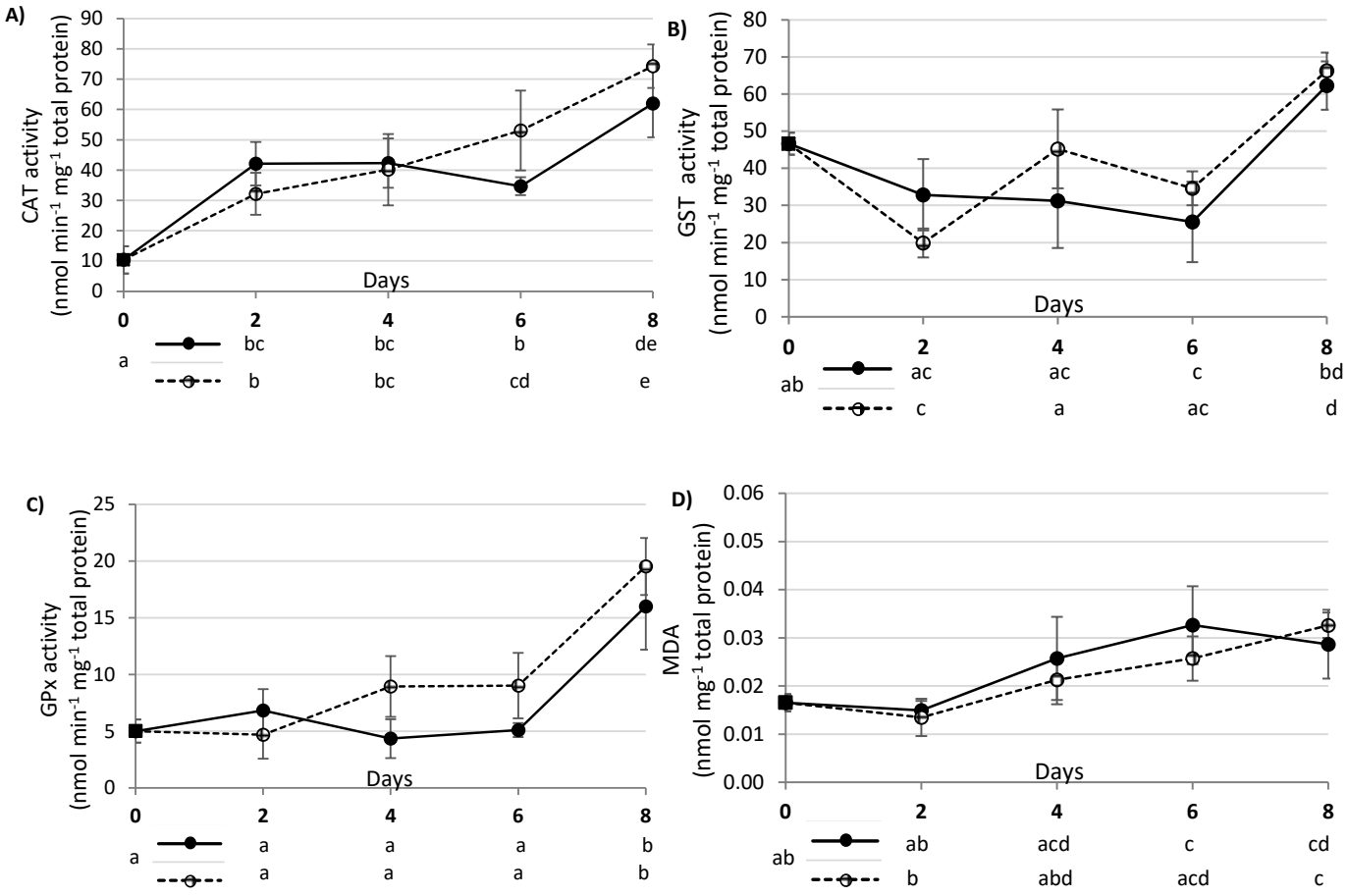


Figure 4.1-2 — Enzymatic activity and lipid peroxidation (on gonads) over time of a simulated live transport of sea urchins at 7 °C.

A) Catalase activity (CAT); **B**) Glutathione S-transferase activity (GST); **C**) Glutathione peroxidase activity (GPx) and **D**) Lipid peroxidation (LPO), as malondialdehyde (MDA) concentration. Values are mean ± standard deviation (n = 5). Different letters indicate significant differences between treatments; Continuous line - bulk conditioning (LTB); discontinuous line – individual compartments (LTIC).

The activity of CAT increased significantly over time ($p < 0.0095$), regardless of animal conditioning, and reached maximum values on day 8 (68.14 nmol·min^{-1}·mg^{-1} total protein). GST activity registered unstable values up to day 6 followed by a significant increase ($p < 0.0094$) on day 8 in both conditionings, corresponding to an average increase of 38 % compared to the beginning. GPx activity remained almost constant until day 6, followed by a significant increase ($p < 0.0002$) in both treatments, corresponding to a 3.4-fold increase compared to the beginning.

Regarding SOD (**Table 4.1-2**), no significant changes were observed during the transport.

Table 4.1-2 — Superoxide dismutase (SOD) inhibition, protein carbonyl (PC) and ubiquitin (Ub) content (on gonads) over time of a simulated live transport of sea urchin at 7 °C.

	Beginning	Conditioning	Treatment (Code)	Day 2	Day 4	Day 6	Day 8
SOD							
(% inhibition)	44.38 ± 5.27	Bulk	LTB	40.45 ± 8.69	46.87 ± 3.98	46.63 ± 6.60	45.41 ± 4.15
		Individual compartments	LTIC	42.46 ± 8.47	49.55 ± 1.85	50.36 ± 2.21	43.28 ± 9.23
PC							
(Abs·mg^{-1} total protein)	0.22 ± 0.06 abc	Bulk	LTB	0.20 ± 0.05 ^{abc}	0.20 ± 0.05 ^{abc}	0.17 ± 0.03 ^{ab}	0.28 ± 0.05 ^{bc}
		Individual compartments	LTIC	0.16 ± 0.03 ^{a}	0.14 ± 0.02 ^{a}	0.24 ± 0.13 ^{bc}	0.29 ± 0.03 ^{c}
Ub							
(µg·mg^{-1} total protein)	0.20 ± 0.04 a	Bulk	LTB	0.14 ± 0.03 ^{ab}	0.11 ± 0.03 ^{bc}	0.05 ± 0.02 ^{c}	0.06 ± 0.02 ^{c}
		Individual compartments	LTIC	0.12 ± 0.03 ^{bc}	0.11 ± 0.02 ^{bc}	0.14 ± 0.07 ^{ab}	0.07 ± 0.03 ^{bc}

Values are mean ± standard deviation (n = 5);

Different letters indicate significant differences between treatments;

LTB - bulk conditioning;

LTIC - individual compartments.

Overall, there was a significant increase in LPO from day 2 onwards, for both conditionings (**Figure 4.1-2**). Compared to the beginning, increases of 74 and 82 % were registered after day 6 and 8, respectively. Regarding protein modification and damage, the values of PC recorded the same range of values compared to the beginning, with some fluctuations (**Table 4.1-2**). For Ub, a decrease in content over transport was observed compared to initial values, which was more pronounced on day 8 (p = 0.0002) in both conditionings (**Table 4.1-2**).

4 Discussion

The greater mass loss of the sea urchin in bulk than in individual compartments may be attributed to the physiological and mechanical stress caused by direct contact between the sea urchins. The duration of transport also contributed to mass loss regardless of conditioning. James and Evensen (2018) reported an average mass loss of 8 % during transport of green sea urchin *S. droebachiensis* over a period of 39 h at 14 °C. Therefore, transport conditions and duration are crucial to ensure quality in distance and avoid mass loss, especially when delivered to demanding markets (James et al., 2016).

The spines of sea urchins undertake a fall off position during transport. In fact they play an important functional role in protecting the animal body and other activities (Moureaux et al., 2010). Spines are naturally stiff and exhibit an axial position that provide sufficient elasticity to withstand mechanical and physical-chemical stress in the marine environment (Tsafnat et al., 2012). These characteristics are mainly attributed to the composition of the organic matrix of sea urchin spines (Merino et al., 2017). Under severe stress conditions, these organic macromolecules are degraded/depleted, which explains the progressive loss of integrity/firmness and position of sea urchin spines. These phenomena could be a clue to stress in echinoderms (Rubilar and Crespi-Abril, 2017).

Odour is a fundamental attribute of fresh seafood. However, a breakdown of various components and the formation of new compounds are responsible for the quick alterations, that are triggered by the metabolic activity of microorganisms (Di Natale and Ólafsdóttir, 2009), endogenous enzymatic activity (autolysis) and by the chemical oxidation of lipids. The characteristic fresh odour of fish and shellfish (including echinoderms) is very slight, almost odourless, with minor marine/algae scents in some species, as detected at the beginning of the present study. The perceived off-odours are related to the formation of volatile organic compounds such as trimethylamine, sulphur compounds, aldehydes, and hypoxanthine over transport time (Di Natale and Ólafsdóttir, 2009). The undesirable greenish colour developed in the water may be attributed to the excretion and deterioration of some metabolites and coelomic fluid.

The composition of the coelomic fluid is similar to seawater, i.e., as the production of carbon dioxide increases during exposure to air, hydrogen ions also increase, resulting in a decrease in pH levels (Smith et al., 2010). Some authors stated that sea urchins are unable to compensate for acidosis (Burnett et al., 2002). Acidified values were reported for *E. chloroticus* when exposed to air under conditions of 72 h/15 °C and 144 h/4 °C (control values from 7.57 to 6.77 and 6.65, respectively; Verachia et al., 2012) and for *A. punculata* exposed to high temperatures (7.25 at 24 °C (control) and 7.19 at 32 °C; Johnstone et al., 2019). In turn, other authors reported that the coelomic fluid has a high buffering capacity, and the carbonate buffer system, coelomocyte cells, and other unknown compounds seem to be the basis for such ability (Collard et al., 2013). This fact could explain the maintenance of the pH of the coelomic fluid over 8 days of transport in the present study. The loss of coelomic fluid may affect the physiological functions of echinoderms, since this fluid is in direct contact with internal cells and tissues (Pinsino and Matranga, 2015), which probably explains the loss of vitality, which was assessed mainly by the position and firmness of the spines.

Previous studies reported that adverse external conditions can source an increase in the percentage of solids in the coelomic fluid (Pinsino et al., 2008). Other studies have shown that this response is absent in some stressful situations (Smith et al., 2010). Although solid contents were not affected in the present study, an increase in turbidity was recorded, as was found in other species under similar stress conditions (Verachia et al., 2012a).

The main enzymes acting against reactive oxygen species (classified as free radicals involved in many physiological disorders), i.e. CAT, SOD, GST and GPx (Lesser, 2006) were assessed, to determine whether sea urchins could protect themselves from potential oxidative damage induced by conditioning. Overall, the activity of CAT increased over time, while SOD showed no significant changes. Thus, the primary protective mechanisms against oxidative stress showed distinct patterns, although their activity is strongly linked (Johansson and Borg, 1988). SOD is an important antioxidant scavenger responsible for the dismutation of superoxide anions formed during oxidative stress into H_2O_2 and oxygen. SOD and CAT form the first line of defense against oxidative stress (Lesser, 2006) by preventing the accumulation of free radicals and subsequent tissue damage. The increase in CAT activity over time evidenced that some response to oxidative stress has occurred and is positively correlated with loss of vitality, namely spines position/firmness ($r \geq 0.68$, $p \leq 0.0009$), suggesting tissue fragility, even when sea urchins were transported in individual compartments.

The GST levels may indicate a certain type of ROS cleanse and the ability of this enzyme to catalyse reactions that detoxify harmful compounds (Lesser, 2006). Under other potential

stress conditions, there is evidence of an increase in GST activity in *P. lividus* after exposure to PAHs (Cunha et al., 2005) and even with an increase in feeding on invasive algae (Tejada et al., 2013). Environmental changes may also lead to changes in GST activity in other important marine species, such as clams (Anacleto et al., 2013). In the present study, the maximum activity of GPx and GST, registered at the end of transport, could reflect a borderline stress condition, corresponding to poor vitality/external quality of the sea urchins at this stage. In fact, GPx activity was correlated with external indicators of vitality/quality (firmness/position of spines, $r \geq 0.73$, $p \leq 0.0003$) and mass loss ($r \geq 0.67$, $p \leq 0.0013$). GST activity was also correlated with mass loss ($r \geq 0.75$, $p \leq 0.0002$).

The increase in LPO suggests that antioxidant defences could not prevent some cellular damage, even with increased activity of CAT and even GST, which plays an important role in preventing lipid peroxidation (Cunha et al., 2005).

The stability of the levels of PC suggests that this impairment has not occurred even after 8 days of transport. This is consistent with the trend reported for sea urchins under other potential stress conditions, such as the ingestion of invasive algae (Tejada et al., 2013). On the contrary, Johnstone et al. (2019) found a positive relationship between the content of PC and elevated water temperatures of 28 °C and 34 °C in Atlantic sea urchin *A. punculata*. The different responses to stress highlight the complexity of the regulatory mechanisms of carbonylation, including the promotion and elimination of protein carbonyls (Moskovitz and Oien, 2010).

When severe protein abnormalities occur, the ubiquitination pathway is activated to eliminate irreversibly damaged proteins (Tomanek, 2010). In a study with fish species, it was reported that concentration of Ub in *D. labrax* muscle remained unchanged after 28 days in warmer and acidified seawater (24 °C, pH=7.6 units), while these stressors combined with contaminants, inhibited the synthesis of Ub (Maulvault et al., 2018). Some decrease in Ub might be related to the induced stress level, which did not cause major changes in ubiquitination. On the other hand, this process Is high energy demanding (Mykles, 1998) and a progressive loss of vitality was observed In the sea urchins (higher demerit scores over time).

5 Conclusion

The biochemical responses of the gonads, especially the antioxidant system (CAT, GPx and GST) indicate some oxidative stress with cell damage, which became more evident on day 6, following the degradation of external appearance and odour.

Although bulk conditioning (smaller volume for the same mass compared to individual compartments) is the most used in the transport and commercialisation of sea urchins, it

should be avoided as it results in greater mass loss. To ensure the highest quality (including animal welfare) and the best valorisation along the trade chain, recognised by external appearance and odour, live transport of sea urchin in a moist ambient at 7 °C should not exceed 4-5 days. Further studies, including other practices (such as immersion in aerated seawater) preferably in the same temperature range as in the natural habitat, should be carried out to evaluate the benefit to the overall quality of whole sea urchins. Furthermore, a complete sensory assessment of the gonads must be considered.

Acknowledgements

This research was supported by national funds of the Portuguese Foundation for Science and Technology (FCT) through the strategic projects UIDB/04423/2020, UIDP/04423/2020 (CIIMAR) and UIDB/04378/2020 (UCIBIO); the research line INSEAFOOD within the Structured Program of R&D&I INNOVMAR – Innovation and Sustainability in the Management and Exploitation of Marine Resources (Ref. NORTE-01-145-FEDER-000035), funded by the Northern Regional Operational Programme (NORTE2020) through the European Regional Development Fund (ERDF). Carolina Camacho also acknowledges the FCT for the PhD Grant (SFRH/BD/141315/2018).

4.2 Nucleotides and free amino acids in sea urchin *Paracentrotus lividus* gonads: Contributions for freshness and overall taste

Abstract

This study aims to report the chemical changes, specifically the nucleotides and free amino acids (FAA) during refrigerated storage of live sea urchin *Paracentrotus lividus* (7 °C) and packed gonads (2 °C) and respective contributions to overall taste. Results showed that the adenylate energy charge (AEC) is an adequate indicator for live sea urchin through storage, while *K*-value does not distinguish freshness. Changes of both indexes were not clear in packed gonads. The FAA profile were related with maturation stage where amino acids associated to sweet (Gly, Ala), umami (Glu) and bitter (Arg, Lys, His, Val) had significant contribution to overall taste. In general, the storage did not significantly affect the FAA content nor Its contribution to overall taste in contrast to nucleotides that seemed to induce potential changes after day 5. The findings provide insights that will complement a full study on quality index scheme development of the products.

Keywords: sea urchin; gonads; live seafood; chemical indicators; freshness; refrigerated storage

This subchapter is adapted from the publication:

Camacho, C., Correia, T., Teixeira, B., Mendes, R., Valente, M.L.P., Pessoa, M.F., Nunes, M.L., Gonçalves, A. 2023. Nucleotides and free amino acids in sea urchin *Paracentrotus lividus*: contributions for freshness and overall taste. *Food Chem*. 404, 1234505. https://doi.org/10.1016/j.foodchem.2022.134505

Contribution of Carolina Camacho: Investigation, Data curation, Validation, Formal analysis, Visualization, Writing – original draft, review & editing.

1 Introduction

The sea urchin *Paracentrotus lividus* is a high-value commercial seafood species with increasing importance due to the intrinsic sensory features of its gonads (Rocha et al., 2019b), also called roe or *uni*, the only edible part. This valuable species is, in general, marketed alive and their gonads consumed mainly raw. The commercial harvesting of *P. lividus* has increased and considering the actual consumers demand for convenient fresh seafood, presentations such as packed refrigerated or frozen gonads Is also Important, even to restaurants. The quality of raw sea urchin gonads (in *Strongylocentrotus nudus* species) during refrigerated storage has been studied (Kinoshita et al., 2009; Wang et al., 2012) and the packaging plays an Important role regarding the quality preservation of the packed goods. Specifically for sea urchin gonads, the appearance is one of the sensory key attributes for choice and preference (Baião et al., 2021b), so the product must be easily seen and protected from mechanical damages, i.e., clear packages that assure its physical integrity should be preferred.

The quality of the *P. lividus* gonads is highly dependent on harvesting season (Dincer and Cakli, 2007; Rocha et al., 2019b) which has a pivotal influence on sensory profile and acceptance (Baião et al., 2021b). For this reason, quality assessment of this species and the development of schemes to discriminate quality grades are a challenge. The changes related to seafood quality are known to be species dependent and vary with some factors, namely the biological status, feeding habitats, harvesting methods and storage conditions. The freshness of seafood is deeply linked to sensory properties and raw appearance in general determines its commercial value. Despite sensory evaluation being the most important method for assessing the seafood freshness, some chemical methods can provide complementary information and correlations between sensory and chemical data may occur (Freitas et al., 2021). Among chemical parameters, the nucleotides (adenosine triphosphate - ATP; adenosine diphosphate - ADP; adenosine monophosphate - AMP and inosine monophosphate - IMP) and their catabolites (inosine - HxR and hypoxanthine - Hx) have been widely used as chemical indicators of seafood freshness, as well as the *K*-value. This index which represents the extension of nucleotides degradation, has been considered the best chemical predictor of freshness during storage and is defined as the ratio between the sum of HxR and Hx contents and the sum of ATP and all Its degradation products (Hong et al., 2017; Tejada, 2009).

The linear measure of the ratio of ATP to total adenylate concentration and changes In its concentrations is given by the AEC index and reflects a disruption in the energy production of physiological processes (Atkinson, 1968), being specially important when the product is

commercialised alive. The usefulness of nucleotides and nucleotide based indexes have been reported in live invertebrates during transport and storage under refrigeration temperatures (Anacleto et al., 2013; Kawabe et al., 2010; Verachia et al., 2013; Yokoyama et al., 1992).

Nucleotides along with free amino acids (FAA) are the main non-volatile taste-active compounds in marine invertebrates including sea urchin (Fuke and Konosu, 1991; Komata, 1964; Sarower et al., 2012). The relative importance of a compound on taste could be given by the measure of taste active value (TAV) (Engel et al., 2002). Specifically for the evaluation of umami taste of foods, the equivalent umami concentration (EUC) has been widely used (Yamaguchi et al., 1971), despite the mechanisms of umami perception may be altered through peptides Interaction (Liang et al., 2022).

The main objective of the present work was to study the contribution of nucleotides and FAA to the freshness and taste of the gonads from wild *P. lividus* as well as their changes during the live refrigerated storage (7 °C) over 11 days. Additionally, the effect of air packaging under refrigerated storage (2 °C) on sea urchin gonads was also evaluated. For this, nucleotides and their catabolites (chemical indicators of freshness) and FAA, both chemical compounds responsible for taste, were analysed. The taste indexes TAV and EUC were also calculated. This work was carried out in the frame of a wider study aiming the development of an objective sensory scheme, the quality Index method (QIM), to freshness discrimination of *P. lividus* and its gonads (**Subchapter 4.3**). The approach outlined here intends to be the chemical basis of that scheme. As far as the authors are aware, this is the first study combining these outputs for this kind of product.

2 Materials and methods

2.1 Sea urchins

Commercial sized wild live sea urchins *P. lividus* (exosqueleton diameter ≥ 5 cm) were used in all the experiments. After harvesting, live sea urchins were transported under refrigeration (with cold accumulators) to laboratory facilities within 1 to 4 hours from the capture point. In the storage experiments of live sea urchins the storage temperature (7 °C) was chosen based on the difference of 10 °C in relation to the average temperature of seawater in the harvest zone (James and Evensen, 2018), avoiding excessive cold and additional stress. The 3Rs principle (Replacement, Reduction and Refinement) was applied and the number of sea urchins used in the trials was minimised.

2.2 Live sea urchin storage experiments

2.2.1 Previous experiments

Three batches of sea urchins were caught in two harvesting points (Ericeira and Zambujeira area, Portugal) in February and March of 2020, in three consecutive weeks and kept in boxes without overlapping at 7 °C in a moist environment (achieved by adding about 50 mL of seawater) for 9 days. Sampling was done at days 1, 2, 5, 6, 7 and 9 for sensory evaluation and nucleotides quantification. After sensory assessment, for panellists training and selection of descriptors (**Subchapter 4.3**), several gonads from different individuals were collected to compose pools.

2.2.2 Final experiments

Three different batches of sea urchins were harvested from Zambujeira coastal area (SW of Portugal) in different periods: January (trial I), February (trial II) and March of 2021 (trial III). Upon arrival they were randomly placed in boxes (ensuring minimal contact among sea urchins) where they were kept under refrigerated conditions (Fiocchetti, Luzzara, Italy) at 7 °C and moist environment. During refrigerated storage, sea urchins from the different boxes were taken for sampling: trial I, sampling after 1, 3, 6, 8 and 9 days; trial II, sampling after 1, 3, 4, 5, 6, 7 and 11 days; trial III, sampling after 1, 3, 5 and 6 days. In the beginning of each trial (day 1), sea urchin and respective gonads were weighed, test height and diameter were recorded using a caliper and the gonad somatic index (GSI) calculated: [(g gonads wet weight·g^{-1} total wet weight) × 100] (Rocha et al., 2019b). On all sampling days, after sensory evaluation of live animals (olfactory and visual examination; **Subchapter 4.3**), they were dissected, gonads carefully collected with a spoon, washed with cold artificial seawater (4 °C), drained during 5 min and two-three gonads from each sea urchin were immediately stored at -80 °C for chemical analyses. The remaining gonads were evaluated by the trained panellists (**Subchapter 4.3**). Spawned sea urchins and those with brownish gonads, or another unusual colour than those commonly accepted for this species, were discarded and another specimen was sampled. Whenever possible, a balanced sampling, in terms of males and females' gonads were used.

2.3 Packed gonads storage experiments

Two subgroups of sea urchins from the final experiments, one from the January catch and other from February were used to perform a stability study of packed gonads stored under refrigeration at 2 °C. In brief, washed and drained gonads (as described in **2.2.2**) were

distributed in cylindric packages (55 mm diameter and 20 mm height) of transparent and rigid polystyrene. Five gonads were placed in each package, minimizing the ratio product/air while the physical Integrity of the product was guaranteed. All the packages were kept at 2 °C up to days 9 (gonads from January, trial PI) and 11 (gonads from February, trial PII). Sampling of at least 3 packages was performed for sensory (**Subchapter 4.3**) and chemical analyses, after 2, 6 and 9 days (trial PI) and after 3, 6 and 11 days (trial PII).

2.4 Chemical analyses

Pools were made with a minimum of three gonads from different sea urchin (per trial in each sampling day). Proximate composition was determined on day 1 of each final experiment (trial I, II and III). The nucleotides and catabolites were determined in the samples from all the experiments. The FAA were determined in the samples from final experiments as well as in the packed gonads from trial PI. Results were expressed in wet weight.

2.4.1 Proximate composition

Moisture was determined according to the air-drying method during 16 h at 104 ± 2 °C in an air oven according to standard procedures. The total lipid content was quantified gravimetrically according to Folch et al. (1957). Briefly, the extraction was carried out using a mixture of chloroform: methanol (2:1), the organic phase collected after centrifugation and evaporated under nitrogen. The protein content was determined in nitrogen analyser (FP-528 LECO, St Joseph, EUA) previously calibrated with EDTA and according to Dumas method (Saint-Denis and Goupy, 2004). Total nitrogen was converted into total protein using 6.25 as the conversion factor.

2.4.2 Nucleotides and their catabolites

Extracts were prepared and nucleotides separated according to Mendes et al. (2001). The pools of sea urchin gonads (2-5 g) were homogenised (60 s; 6500 rpm; Ultraturrax T25, IKA®, Labortechnik, German) in cold 0.6 M perchloric acid (1:5, m/v), centrifuged (20000 *g*, 10 min/0 °C; Kubota 6800, Kubota Corp., Tokyo, Japan), and 10 mL of supernatant was neutralized with potassium hydroxide to a pH of 6.90. After 30 min at 4 °C, the extract was filtrated through sintered glass to remove the potassium perchlorate precipitate. A second filtration was conducted using a 0.22 µm pore size syringe filter. The extracts were kept at -80 °C until injection. After a 20 µL aliquot injection, the separation was conducted in a reverse-phase column (LiChrosorb RP-18; 250 × 4.6 mm; 10 µm; VDS Optilab) in a high-performance liquid

chromatograph (HPLC) (HP Agilent 1050 series; Agilent, USA) operating isocratically at 1.6 mL·min^{-1} with a mobile phase composed of potassium dihydrogen phosphate (0.04 mol·L^{-1}) and dipotassium hydrogen phosphate (0.06 mol·L^{-1}), with a pH of 6.90. The column temperature was set to 30 °C and detection was done at 254 nm. Compounds were identified and quantified by comparison with specific standards (all from Sigma) through calibration curves. The IMP coeluted with guanosine monophosphate (GMP), therefore those results were presented as IMP/GMP. Furthermore, a good resolution between cytidine 5′-monophosphate (CMP) and uridine monophosphate (UMP) was not achieved, thus these compounds were reported as CMP+UMP. Results were expressed as μmol·g^{-1} gonad.

The Indices *K*-value and adenylate energy charge (AEC value) were calculated according to the following formulas:

K-value (%) = [(Hx+HxR)/(ATP+ADP+AMP+IMP+Hx+HxR)] × 100 (Tejada, 2009)

AEC (%) = [(ATP+0.5ADP)/(ATP+ADP+AMP)] × 100 (Anacleto et al., 2013; Atkinson, 1968)

When nucleotides were below the limit of quantification (0.085 μmol·g^{-1}), this limit value was considered for calculations and statistical analyses.

2.4.3 Free amino acids

The extraction procedure began with sample homogenization (using a Polytron) in 10 % trichloroacetic acid (1:5 m/v), then the extracts were centrifuged (25000 *g*, 20 min/4 °C), filtered (0.2 μm) and stored at -80 °C. The injection was in HPLC equipment (Agilent 1100 HPLC, Agilent, Palo Alton, USA) with precolumn derivatization using o-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC) and a Phenomenex Gemini ODS C18 110 Å column (4.6 × 150 mm, 5 μm; Phenomenex Inc., Torrance, USA). The mobile phase and gradient conditions were done according to Henderson et al. (2000), detection was done by fluorescence measurement (Ex/Em = 340/450 nm and 266/305 nm). Twenty-one amino acids were quantified: aspartic acid (Asp), glutamic acid (Glu), asparagine (Asn), serine (Ser), glutamine (Gln), histidine (His), glycine (Gly), threonine (Thr), arginine (Arg), alanine (Ala), taurine (Tau), tyrosine (Tyr), cystine (Cys), valine (Val), methionine (Met), tryptophan (Trp), phenylalanine (Phe), isoleucine (Ile), leucine (Leu), lysine (Lys) and proline (Pro). The amino acid hydroxyproline was always detected below analytical thresholds levels. The quantification was performed with the software Agilent ChemStation (Agilent, USA) by comparison with the retention times and peak areas of standard amino acids and using norvaline and sarcosine (Sigma-Aldrich) as internal standards.

2.5 Taste active value

From the results of some nucleotides and FAA, the TAV was calculated. The TAV reflects the contribution of a single compound to the overall taste and is widely used in the evaluation of food taste, being the ratio between the compound concentration in the product (C_1) and its threshold (i.e., the minimum concentration at which can be perceived C_2): $TAV = C_1/C_2$ (Engel et al., 2002). The compounds whose TAV is greater than 1, it means that the substance is considered active in food taste and higher values correspond to a greater contribution. A main reference regarding taste thresholds was used (Kato et al., 1989), however others were also used (Duan et al., 2020; Dunkel and Hofmann, 2009; Liu and Qiu, 2016; Rotzoll et al., 2006) in the absence of threshold values in the first reference. In the case of IMP/GMP as well as CMP+UMP, the TAV calculations were done with the averaged thresholds. The same thresholds references were used to associate and group the compounds according to their main taste descriptor: sweet (Gly, Ala, Thr, Ser and Pro), bitter (His, Arg, Val, Met, Iso, Leu, Tyr, Phe, Lys, Trp and Tau) and umami (Glu, Asp, IMP, GMP, AMP, CMP, UMP). To calculate the TAV of nucleotides, the concentrations found in the samples were converted to $mg \cdot g^{-1}$ according to their respective molar mass.

2.6 Equivalent umami concentration

The equivalent umami concentration (EUC) refers to the concentration of monosodium glutamate in g MSG·$(100\ g)^{-1}$, which is equivalent to umami intensity, calculated by the following formula: EUC (g MSG·$(100\ g)^{-1}$) = $\Sigma\ a_i\ b_i + 1218\ \Sigma\ a_i\ b_i\ (\Sigma a_j\ b_j)$ (Bi et al., 2021; Yamaguchi et al., 1971).

In which: a_i is the concentration of each umami amino acid (Asp and Glu); b_i is the relative umami concentration (RUC) for each umami amino acid versus MSG (Asp = 0.077 and Glu = 1); a_j is the concentration of each umami 5'-nucleotide (IMP, GMP and AMP); b_j is the RUC for each umami 5'-nucleotide versus IMP (IMP = 1; GMP = 2.3 and AMP = 0.18) and 1218 is a synergistic constant. The RUC adopted for this calculation was 1.65 in the case of IMP/GMP Which corresponds to an average between both.

2.7 Data analysis

The biometric and proximate composition data, as well as the results of previous experiments and packed gonads were subjected to analyses of variance. Exploratory data analysis was performed following multivariate parametric methods by principal component analysis (PCA) on nucleotides and FAA data from final storage experiments (trials I, II and III) to find

possible trends/similarities within the sea urchin gonads. Subsequently, analyses of variance or *t*-Student test were applied on data according to PCA outputs. The post-hoc Dunnett test was used as follows: a) one-sided test - each storage day lower or higher compared to the beginning of storage (day 2 in the previous experiments, day 1 in the final experiments and packed gonads); b) two-sided test whenever one-sided was not applicable. Linear regression analysis was performed for indices AEC and *K*-value and Pearson's correlation was tested for some data. Data analysis was done using the software STATISTICA TM (StatSoft, Inc., Tulsa, USA), statistical significance of all tests was at $\alpha = 0.05$ (p-value < 0.05).

3 Results

3.1 Biometrics and proximate composition

The sea urchins used in the final experiments had in average: 85 g of total body weight; 62.5 mm of test diameter; 32.8 mm of test height and 6.7 % of GSI. Concerning composition, the moisture ranged from 65 (February) to 72 % (March); total lipids and protein averaged 4 and 19 %, respectively (**Table 4.2-1**). Significant differences were not found among the trials, for each of these parameters.

Table 4.2-1 — Biometric data of sea urchins and proximate composition of sea urchin gonads of the final experiments.

	January (trial I)	February (trial II)	March (trial III)
Biometric data			
Total body weight (g)	82.30 ± 19.81	91.12 ± 13.95	80.32 ± 10.14
Test diameter (mm)	62.44 ± 6.75	64.17 ± 4.07	60.92 ± 1.65
Test height (mm)	33.49 ± 2.78	32.92 ± 2.69	31.98 ± 3.20
GSI (%)	7.21 ± 2.22	6.16 ± 1.89	6.58 ± 1.82
Gonad proximate composition, g·(100 g)$^{-1}$			
Moisture	67.72 ± 0.13	65.27 ± 0.38	71.62 ± 3.20
Total Lipids	4.41 ± 0.75	3.84 ± 0.27	3.76 ± 0.41
Protein	18.08 ± 1.43	19.14 ± 1.27	19.95 ± 1.81

Results are average ± SD; N = 10 for biometric data and N = 2 pool/trial for proximate composition.
No significant differences were found among trials for each parameter ($p > 0.05$).

3.2 Live sea urchins

3.2.1 Previous storage experiments – nucleotides content

Due to the lack of standards, the quantification of CMP and UMP was not possible in these experiments. The total nucleotides amount ranged between 3.57 and 9.05 μmol·g^{-1} (Appendix, **Table S3**). In the gonads from trial A, the ATP with initial values of 0.81 μmol·g^{-1} (day 2), decreased over storage, being significant lower at days 4, 6 and 9 ($p < 0.0052$). The AMP increased from 0.57 (day 2) to 1.44 μmol·g^{-1} at day 6, registering higher concentrations at day 4 and 6 ($p < 0.0114$), and attaining at day 9 a value similar to the beginning. The IMP/GMP averaged 3.44 μmol·g^{-1} throughout the storage. Both Hx and HxR ranged from non-detected or below quantification limits (day 2) up to 0.20 and 0.76 μmol·g^{-1}, respectively (day 9). Specifically, HxR was significantly higher at days 6 and 9 ($p < 0.0080$). The AEC was about 55.85 % at day 2, being significant lower in the following days ($p < 0.0047$).

A significant positive linear correlation between the *K*-value and the storage time was reported ($Y_{K\text{-value}} = 0.2503 + 1.4494 * x$; $r = 0.8166$, $p = 0.0001$; $r^2 = 0.6668$; $N = 8$), with values between 1.53 and 18.20 % at day 2 and 9, respectively. Concerning gonads from trial B and C, which most of them have been sensory evaluated (visual and with a fork; **Subchapter 4.3**), i.e., have been handled prior analysis, the nucleotides did not vary as much as those of trial A (Appendix, **Table S3**). Specifically, the ATP concentration were 0.17 μmol·g^{-1} and the HxR was quantified in 2 out of 4 pools from day 1, evidencing higher *K*-value (3.99 %) and lower AEC (19.74 %) in comparison to day 2 of trial A.

3.2.2 Final storage experiments – nucleotides and free amino acids contents

The projection of variables and cases is presented in **Figure 4.2-1**, with Factor 1 and Factor 2 explaining 59.66 % of the variance.

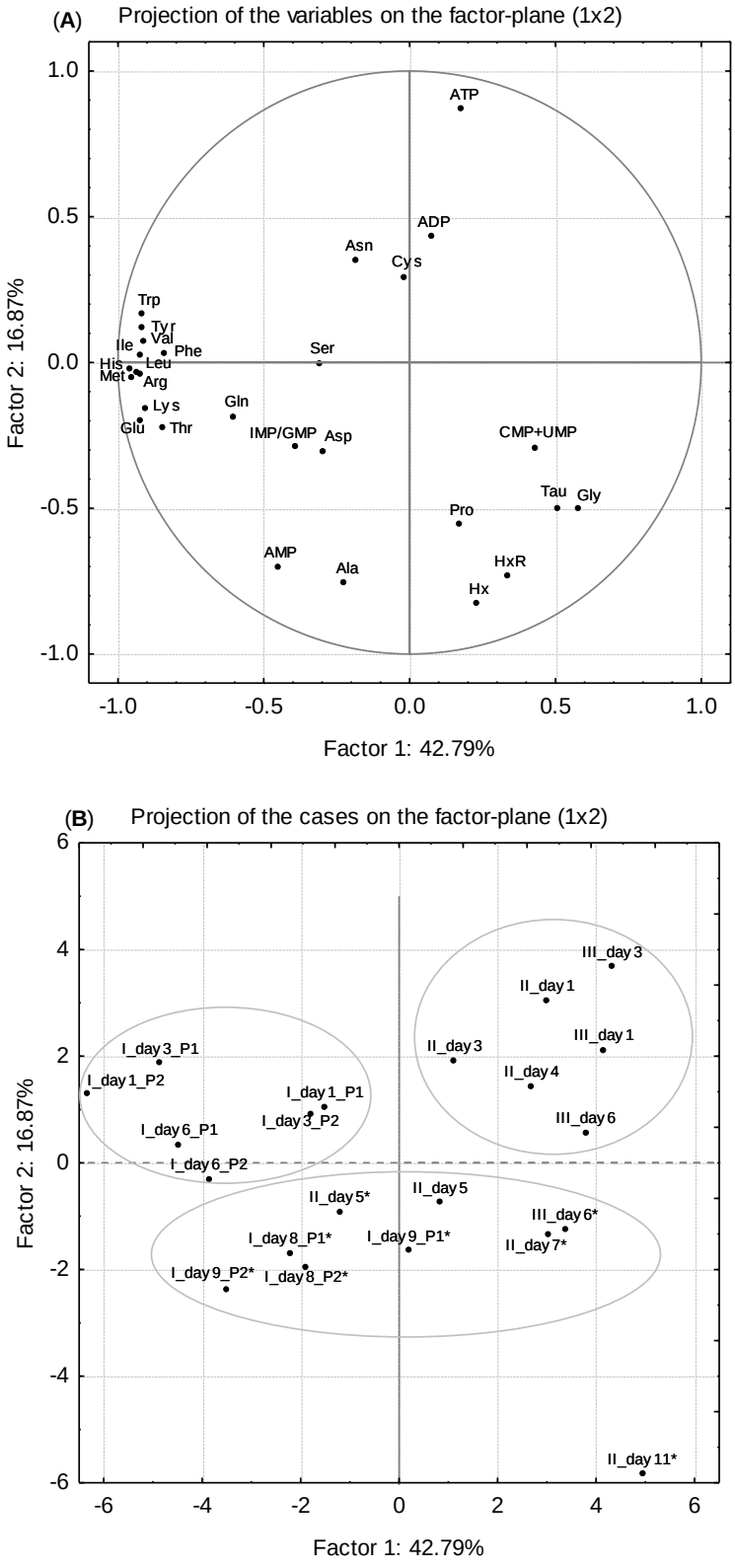


Figure 4.2-1 — Projection of variables (A) and cases (B) of dimensions 1 and 2 of principal component analysis applied to the data (nucleotides and FAA) of the gonads from live sea urchin final experiments (trials I, II and III) at 7.1 ± 0.8 °C.

Factor 1 and Factor 2 explain 59.66 % of total variance; Cases identification: I, II and III refers to trial I, II and III, respectively; P1 and P2 refers to the pools within the same day (when applicable); *sea urchins less reactive.

In summary, F1 allows to distinguish two groups, trial I and trials II-III based on FAA and F2 allows distinguish days of storage, a third group is related to more advanced days of storage associated with low concentrations of ATP and higher values of AMP, Hx, HxR, as well as Ala, Pro, Tau and Gly.

Thus, regarding nucleotides and catabolites the storage time was the factor tested for differences by ANOVA one-way followed by Dunnett test. The total amount of nucleotides ranged between 6.15 and 11.80 μmol·g^{-1} (Appendix, **Table S4**), without differences in relation to day 1 ($p > 0.05$). A gradual decrease of the ATP was observed, with significant differences between day 1 (0.99 ± 0.12 μmol·g^{-1}; Appendix, **Table S4**) and day 5 onwards ($p < 0.0441$). For ADP the lowest value was registered at day 11 ($p = 0.0138$). The AMP concentrations at day 1 had a great variability, followed by an increase to day 5 ($p = 0.0413$) until day 9 ($p < 0.0383$). The IMP/GMP as well as CMP+UMP concentrations remained without trends throughout the storage period between 2.47 - 6.26 and 1.41 - 4.24 μmol·g^{-1}, respectively (Appendix, **Table S4**).

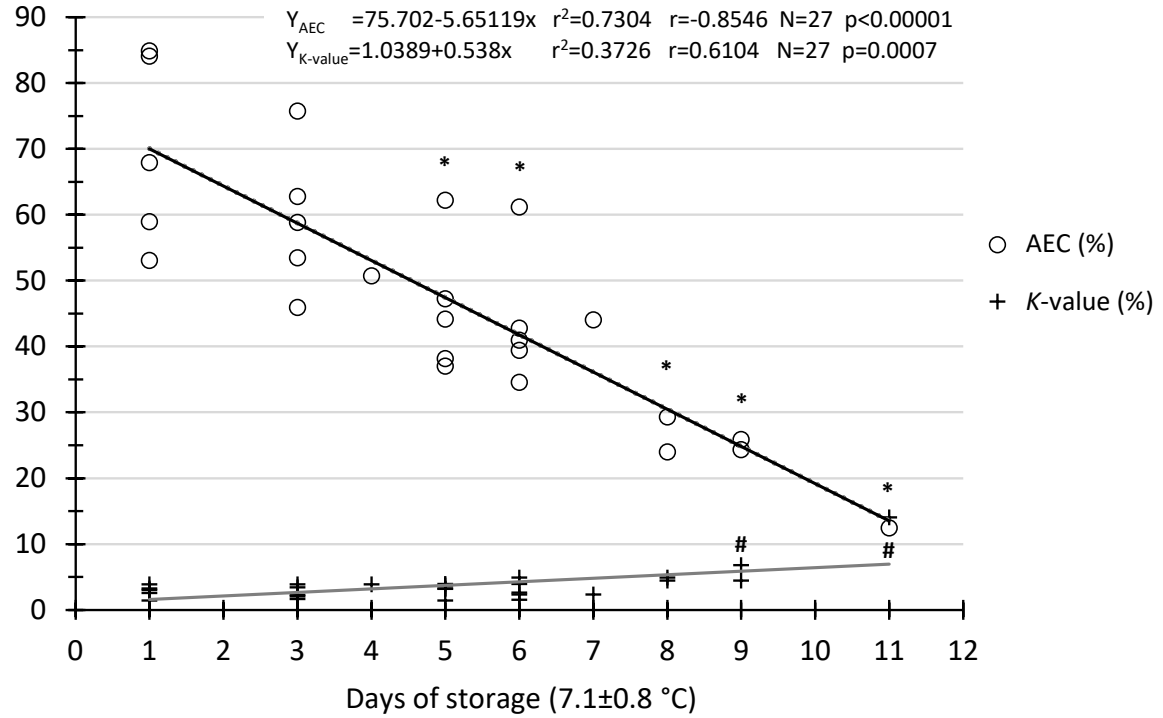


Figure 4.2-2 — Evolution of adenylate energy charge (AEC) and *K*-value (%) in the gonads of live sea urchin during the storage time (final experiments I, II and III).

* Significant lower values of AEC in relation to day 1 ($p < 0.0097$; Dunnett one-sided test);

Significant higher values of K-value in relation to day 1 ($p < 0.0197$; Dunnett one-sided test).

Regarding AEC index, a significant negative time-dependent linear regression was observed (**Figure 4.2-2**). The initial AEC index (69.77 ± 14.42 %) decreased over the storage time attaining a minimum of 12.44 % at day 11, however this decline was only significant lower after the fifth day ($p < 0.0097$).

In the case of *K*-value, a linear correlation was achieved ($p = 0.0007$) (**Figure 4.2-2**). The *K*-value was quite low up to day 5 (< 3.84 %; **Figure 4.2-2**), where HxR and Hx were below quantification limits (Appendix, **Table S4**). This index reached 5.61 and 14.01 % at day 9 and 11, respectively (**Figure 4.2-2**).

Regarding FAA according to PCA output (**Figure 4.2-1**), the differences between trial I and trials II-III were tested by *t*-Student test for independent samples. The total amount of FAA, at the beginning of the storage (day 1), was in average 3.33 ± 0.19 and 2.38 ± 0.62 g·(100 g)$^{-1}$ of gonads from trial I and II-III, respectively ($p < 0.0001$). Twenty-one FAA were quantified during storage (Appendix, **Table S5**), being Gly the most abundant FAA in all trials [1.15-2.02 g·(100 g)$^{-1}$], accounting for 40.1 and 55.8 % of the total FAA in trial I and II-III, respectively. The averaged levels of Arg [trial I: 548.73 mg·(100 g)$^{-1}$; trial II-III: 366.39 mg·(100 g)$^{-1}$], Lys [trial I: 291.74 mg·(100 g)$^{-1}$; trial II-III: 148.96 mg·(100 g)$^{-1}$] and Glu [trial I: 207.21 mg·(100 g)$^{-1}$; trial II-III: 92.94 mg·(100 g)$^{-1}$] were the ones with highest concentrations after Gly, representing together 32 and 23 % of total in trial I and trials II-III, respectively.

For the remaining FAA in trial I, concentrations of less than 160 mg·(100 g)$^{-1}$ were found for Ala, Tyr and Gln; less than 75 mg·(100 g)$^{-1}$ for Ser, Thr, His, Val, Met, Leu, and, finally, less than 40 mg·(100 g)$^{-1}$ for Pro, Ile, Tau, Phe, Trp, Asp, Cys. The levels of seven FAA (Gly, Ala, Ser, Asp, Pro, Cys, Asn) were identical in trials I and II-III ($p > 0.05$), while the levels of the remaining were statistically different ($p < 0.0032$), presenting higher concentrations in trial I (Appendix, **Table S5**).

The effect of storage time was tested (comparing with day 1) for both trials by one side Dunnett test. Most of the FAA did not show significant differences, except Ala and Pro in trial II-III, being significantly higher at day 5 onwards ($p < 0.0273$ and $p < 0.0464$, respectively).

3.3 Packed gonads – nucleotides and free amino acids content

Results regarding nucleotides quantification of packed gonads is presented in **Table 4.2-2**.

The total amount of nucleotides was in average 8.05 μmol·g^{-1} in the first day and remained with similar concentrations during the 11 days of storage. A wide range concentration of ATP was found between day 1 and day 9 (mean values of 1 and 0.7 μmol·g^{-1}, respectively),

however a significant lower concentration was observed in day 11 (p = 0.0418). ADP and AMP concentrations remained with averaged concentrations below 0.8 μmol·g^{-1} throughout the storage. The IMP/GMP as well as CMP+UMP concentrations ranged between 3.2-7.5 and 2.1-3.2 μmol·g^{-1}, respectively. Low values of HxR (< 0.09 μmol·g^{-1}) and Hx (< 0.13 μmol·g^{-1}) were registered up to day 9, attaining the highest concentration on day 11 (< 0.25 μmol·g^{-1}).

Table 4.2-2 — Nucleotides content (μmol·g^{-1}) of packed sea urchin gonads through the refrigerated storage (2.1 ± 0.4 °C).

			Days of storage									
μmol·g^{-1}	Trial	Pool	**1**		**2**		**3**	**6**		**9**		**11**
	PI	1	4.68		5.44		-	5.43		3.68		-
IMP/GMP	PI	2	3.31	**3.84**	4.16	**4.80**	-	4.94	**4.73**	3.28	**3.48**	-
	PII	1	3.54		-		4.04	3.83		-		3.27
	PI	1	0.99		1.24		-	1.13		0.60		-
ATP	PI	2	0.97	**1.03**	1.06	**1.15**	-	0.80	**0.82**	0.77	**0.68**	-
	PII	1	1.12		-		0.58	0.54		-		0.35 *
	PI	1	0.68		0.58		-	0.43		0.83		-
ADP	PI	2	0.49	**0.55**	0.82	**0.70**	-	0.64	**0.50**	0.78	**0.80**	-
	PII	1	0.49		-		0.46	0.43		-		0.35
	PI	1	0.29		ND		-	ND		0.58		-
AMP	PI	2	0.83	**0.37**	0.40	**0.20**	-	0.31	**0.10**	0.44	**0.51**	-
	PII	1	ND		-		ND	ND		-		0.42
	PI	1	2.03		3.01		-	3.08		1.92		-
CMP+UMP	PI	2	1.41	**2.25**	2.30	**2.65**	-	2.51	**3.15**	2.18	**2.05**	-
	PII	1	3.32		-		3.02	3.86		-		2.99
	PI	1	< LQ		< LQ		-	< LQ		< LQ		-
Hx	PI	2	< LQ	**< 0.09**	< LQ	**< 0.09**	-	< LQ	**< 0.09**	< LQ	**<0.09**	-
	PII	1	< LQ		-		< LQ	< LQ		-		0.14
	PI	1	< LQ		< LQ		-	< LQ		< LQ		-
HxR	PI	2	< LQ	**<0.09**	< LQ	**< 0.09**	-	< LQ	**< 0.11**	0.17	**< 0.13**	-
	PII	1	< LQ		-		0.11	0.17		-		0.25
	PI	1	8.67		10.26		-	10.07		7.61		-
Total amount	PI	2	7.00	**8.05**	8.74	**9.50**	-	9.20	**9.37**	7.61	**7.61**	-
	PII	1	8.47		-		8.21	8.83		-		7.78
	PI	1	< LQ		< LQ		-	< LQ		< LQ		-
***K*-value (%)**	PI	2	< LQ	**<2.92**	< LQ	**<2.46**	-	< LQ	**<3.35**	4.66	**< 3.80**	-
	PII	1	< LQ		-		3.70	5.13		-		8.00 #
	PI	1	67.92		83.95		-	86.26		50.55		-
AEC (%)	PI	2	53.07	**68.61**	64.36	**74.16**	-	63.91	**76.02**	58.21	**54.38**	-
	PII	1	84.85		-		77.89	77.89		-		46.95

ND: not detected; < LQ: above limit of quantification (0.085 μmol·g^{-1}); Values in bold represent the mean value; * indicate significant lower value in relation to day 1 (p = 0.0418; Dunnett one-sided test); # indicate significant higher value in relation to day 1 (p = 0.0110; Dunnett one-sided test).

The AEC index remained almost constant between days 1 and 6 (respectively 68.6 and 76.0 %), following the ATP trend, especially in trial PI (**Table 4.2-2**). The AEC index correlated positively with ATP (r = 0.811, p = 0.015) and negatively with AMP (r = 0.934, p = 0.001). The *K*-value remained low throughout the refrigerated storage, reaching 3.8 % on day 9 (**Table 4.2-2**) and was negatively correlated with ATP (r = -0.758, p = 0.029). On the 11th day, the AEC was 47.0 % and *K*-value 8.0 %. The packed gonads showed concentrations of FAA within the same range of those observed in the live sea urchin in trial I (Appendix, **Table S5**), without significant differences through storage time (p > 0.05).

3.4 Taste active value (TAV) and Equivalent umami concentration (EUC)

The taste active value of each compound and the associated main descriptor is given in **Table 4.2-3**. Since the TAV and EUC values were not significantly affected by the storage period in both trials I and II-III, the data were compared by means of *t*-Student test. The Gly and Ala, both associated to sweet, presented the highest TAV, without significant differences between trial I and II-III (p = 0.0714 in the case of Gly). Minor contributions (TAV < 1) to sweet taste were given by Ser, Thr and Pro.

Regarding those related to bitter taste in trial I, the higher TAVs were found for Arg, followed by Lys, His, Val and Tyr, significant higher of those in trial II-III (p < 0.0001). The compounds Met, Ile, Phe, Leu, Trp, Tau, Hx and HxR had TAV below 1. In the case of Cys, Asn and Gln, non-significant contributions were reported.

Table 4.2-3 — Taste active values (TAV, adimentional) of free amino acids and nucleotides of gonads from live sea urchins through refrigerated storage (7.1 ± 0.8 °C).

Main taste descriptor		Taste threshold, mg·(100 mL)$^{-1}$	Trial I - Days of storage					Trial II-III - Days of storage						
			1	3	6	8	9	1	3	4	5	6	7	11
Sweet														
Gly		130 (1)	9.70±1.11	10.07±0.33	10.25±0.16	10.49±0.81	9.81±0.89	10.51±0.74	10.34±1.56	10.23	10.63±0.53	12.12±0.37	11.27	15.50
Ala		60 (1)	2.28±0.52	1.89± 0.25	2.56±0.00	3.34±0.02	2.78±0.62	1.51±0.03	1.57±0.18	1.78	2.75±0.55	3.00±0.14	2.62	2.65
Ser		150 (1)	0.45±0.04	0.3±0.06	0.37±0.02	0.49±0.04	0.38±0.19	0.40±0.15	0.32±0.10	0.29	0.36±0.08	0.24±0.01	0.32	0.28
Thr	*	260 (1)	0.25±0.04	0.21±0.03	0.21±0.04	0.26±0.05	0.19±0.09	0.04±0.03	0.08±0.06	0.11	0.10±0.06	0.03±0.00	0.07	0.11
Pro		300 (1)	0.09±0.00	0.06±0.01	0.08±0.02	0.1±0.00	0.08±0.01	0.07±0.01	0.07±0.03	0.06	0.17±0.05	0.13±0.01	0.16	0.16
Bitter														
Arg +	*	50 (1)	11.44±1.66	11.46±0.86	10.41±0.2	10.63±0.32	10.94±1.99	7.08±1.49	6.64±0.91	6.70	9.48±2.19	5.77±1.68	7.46	4.86
Lys +	*	50 (1)	6.36±0.69	5.06±0.24	5.75±0.32	5.86±0.48	6.14±1.35	2.68±1.12	2.65±0.82	3.08	4.17±1.3	2.02±0.70	3.14	1.89
His	*	20 (1)	4.38±1.63	3.60±0.95	4.54±0.68	3.07±0.28	3.04±1.11	1.58±0.75	1.71±0.69	1.93	2.62±1.05	1.18±0.16	1.67	1.59
Val +	*	40 (1)	1.68±0.97	1.82±1.16	1.90±0.39	0.97±0.19	1.02±0.50	0.32±0.01	0.49±0.28	0.38	0.73±0.48	0.34±0.09	0.33	0.42
Tyr	*	96.6 (2,3)	1.64±1.26	1.59±0.43	1.78±0.34	1.10±0.33	0.99±0.39	0.43±0.29	0.67±0.55	0.49	0.71±0.51	0.23±0.11	0.27	0.25
Met +	*	30 (1)	0.87±0.39	0.90±0.37	0.92±0.05	0.58±0.07	0.60±0.31	0.16±0.04	0.23±0.09	0.22	0.44±0.25	0.22±0.05	0.30	0.30
Ile	*	90 (1)	0.35±0.18	0.42±0.22	0.43±0.02	0.23±0.01	0.25±0.12	0.08±0.02	0.13±0.07	0.11	0.21±0.13	0.10±0.03	0.11	0.11
Phe +	*	90 (1)	0.31±0.18	0.33±0.21	0.29±0.05	0.22±0.02	0.19±0.06	0.11±0.01	0.14±0.06	0.11	0.20±0.09	0.11±0.03	0.11	0.11
Leu +	*	190 (1)	0.28±0.14	0.36±0.12	0.33±0.01	0.20±0.03	0.23± 0.12	0.06±0.01	0.10±0.05	0.08	0.18±0.11	0.08±0.02	0.10	0.10
Trp +	*	90 (1)	0.28±0.12	0.25±0.1	0.32±0.02	0.21±0.01	0.18±0.02	0.13±0.01	0.15±0.06	0.12	0.18±0.08	0.08±0.05	0.10	0.07
Tau		1877 (3)	0.02±0.00	0.01±0.00	0.02±0.00	0.02±0.00	0.03±0.01	0.03±0.01	0.02±0.00	0.02	0.04±0.00	0.03±0.00	0.04	0.04
HxR		2575 (3)	<0.0009	<0.0009	<0.0009	0.0023	0.0026	<0.0009	<0.0009	<0.0009	<0.0009	<0.0016	<0.0009	0.0061
Hx		122.5 (2)	<0.0009	<0.0009	<0.0009	0.0011	<0.0009	<0.0009	<0.0009	<0.0009	<0.0009	<0.0016	0.0016	0.0059
Umami														
Glu	*	30 (1)	6.98±1.42	6.17±0.43	7.70±0.41	7.25±0.03	6.44±0.94	2.28±0.05	2.76±0.76	3.26	4.06±0.44	3.20±0.35	3.61	2.32
IMP+GMP a)	*	25 (4)	5.56±1.35	8.31±0.56	6.83±0.74	6.69±1.01	5.61±0.25	5.08±1.01	4.08±0.37	4.47	3.69±0.72	4.90±0.71	5.62	7.61
AMP	*	12.5 (5)	1.55±1.06	1.44±0.52	2.55±0.17	3.01±0.13	2.51±0.30	1.28±0.00	1.05±0.42	1.23	2.09±0.15	1.43±0.70	2.12	2.00
Asp		53 (1)	0.18±0.01	0.10±0.02	0.14±0.09	0.11±0.00	0.22±0.00	0.06±0.01	0.10±0.04	0.13	0.15±0.07	0.19±0.05	0.15	0.07
CMP+UMP a)		9.22 (3)	0.06±0.02	0.09±0.01	0.10±0.01	0.09±0.01	0.08±0.01	0.12±0.02	0.08±0.01	0.09	0.07±0.03	0.10±0.03	0.11	0.15
Other														
Cys		23.4 (2)	0.44±0.02	0.62±0.19	0.81±0.06	0.81±0.04	0.44 ±0.03	0.79±0.01	0.65±0.24	0.63	0.47±0.08	0.28±0.02	0.58	0.51
Asn		100 (1)	0.14±0.02	0.11±0.02	0.12±0.01	0.14±0.02	0.11 ±0.04	0.15±0.03	0.11±0.03	0.08	0.12±0.02	0.10±0.00	0.08	0.07
Gln		730 (3)	0.18±0.06	0.12±0.06	0.19±0.10	0.20±0.08	0.2 ±0.11	0.08±0.02	0.06±0.01	0.05	0.10±0.03	0.08±0.02	0.10	0.04

Compounds in bold had TAV > 1; Numbers between parentheses are the reference on taste thresholds: (1) Kato et al. (1989); (2) Rotzoll et al. (2006); (3) Dunkel & Hoffman (2009); (4) Liu & Qiu (2016) and (5) Duan et al. (2020). Threshold values from reference (2) and (3) were transformed according to molar mass of each compound. a) the taste threshold is the average value of both compounds; +may elicit sweet taste depending on compound configuration (Nishimura & Kato, 1988); *indicate significant differences between trials I and II-III (*t*-Student test, $p < 0.05$).

In what concerns umami taste, Glu, IMP/GMP and AMP were the most relevant compounds, but with different contributions between trials ($p < 0.05$). The EUC in gonads from trials I and II-III are shown in **Figure 4.2-3**.

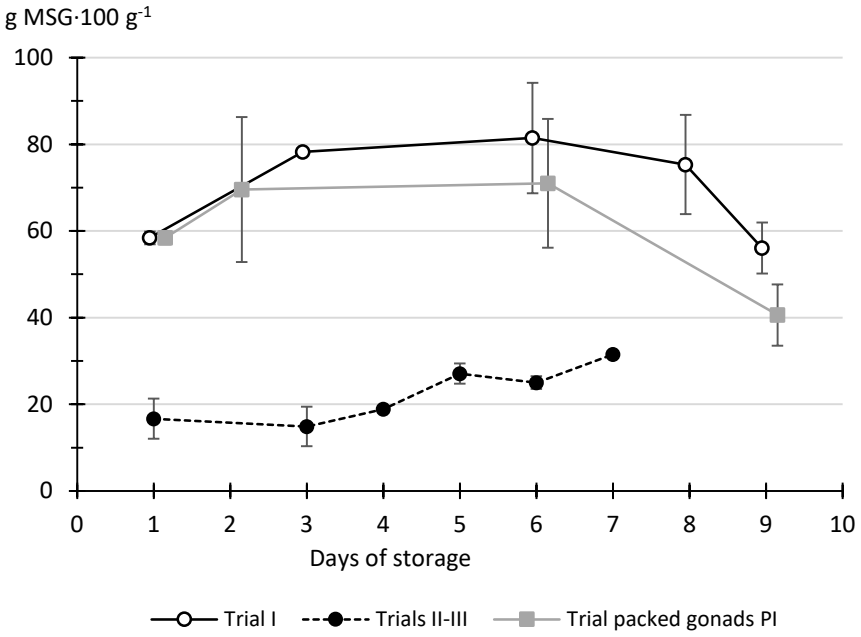


Figure 4.2-3 — The equivalent umami concentration (EUC) in monosodium glutamate concentration (g MSG·(100 g)$^{-1}$) in the gonads of live sea urchin during storage.
Trials I, II-III performed at 7.1 ± 0.8 °C and trial PI performed at 2.0 ± 0.1 °C.

In both cases the evolution during storage was not significant ($p > 0.05$), and the average values were respectively 69.90 ± 12.66 (trial I) and 22.25 ± 6.35 g MSG·(100 g)$^{-1}$ (trial II-III). However, the concentrations of trial I and trials II-III were significant different ($p < 0.0001$). Globally, the EUC was positively correlated with Glu and IMP/GMP ($r > 0.728$, $p < 0.0003$) as well as with AMP ($r = 0.511$, $p = 0.0214$), although it was a weak correlation.

For packed gonads the results of TAV (Appendix, **Table S6**) as well as EUC concentrations (**Figure 4.2-3**) were similar of those of trial I, without significant differences through storage time ($p > 0.05$). The EUC was positively correlated with IMP/GMP ($r = 0.833$, $p = 0.0103$).

4 Discussion

4.1 Biometrics and proximate composition

The sea urchins used in this study had body weight, diameter and height of the test, in the same range of values as those reported in other studies with wild *P. lividus* from European Atlantic shores (Rocha et al., 2019b). The GSI values were also in the range as those reported

in the same harvested areas and for other geographic zones, such as Turkey (Dincer and Cakli, 2007). The moisture content was lower than values found by Rocha et al. (2019) that studied the seasonal effect In nutritional quality of wild sea urchin *P. lividus*. The highest moisture content registered were close to spawning (trial III), which is in accordance with Rocha et al. (2019). The highest protein values (in average) were observed close to spawning period (i.e., trial III, March), contrasting the trend reported by other authors (Dincer and Cakli, 2007; Rocha et al., 2019b). A possible explanation for the differences in moisture and protein contents may be related to the sampling process, which may influence the values found. Moreover, it has been widely described that the harvest area and season clearly affect the chemical composition of seafood. In sea urchin the differences are known to be related with maturation stage of the gonads where nutritive phagocytes (somatic cells) are gradually replaced by germ cells as spawning approaches (Murata et al., 2020).

4.2 Contributions to freshness

4.2.1 Nucleotides

Data from the previous experiments showed that *K*-value could be a clue of the physiological status of live sea urchin and freshness of its gonads, highlighting that handling may have an impact on nucleotides levels and subsequently biases its usefulness. In general, the evolution of nucleotides concentrations of the final trials was like those of trial A (previous experiments).

In the final experiments some variability in the nucleotides levels and indexes was observed, reflecting the heterogeneity regarding physiological status within specimens, even with the same time of storage. Still, it was possible to observe some trends and discuss the findings. The total amount of nucleotides (8.41 $\mu mol \cdot g^{-1}$ in average) was in the same range or slight higher than that found in gonads of *S. nudus* (Kinoshita et al., 2009), as well as for other live marine Invertebrates, namely Pacific oyster (Kawabe et al., 2010; Yokoyama et al., 1992) and clams (Anacleto et al., 2013). On the other hand, considerably lower nucleotides total content was reported (0.7 $\mu mol \cdot g^{-1}$) for sea urchin *Evechinus chloroticus* (Verachia et al., 2013).

The initial concentration of ATP in *P. lividus* (1 $\mu mol \cdot g^{-1}$ in average) was about twice as high as that found in live *E. chloroticus* (Verachia et al., 2013). These authors also mentioned that the procedures in the post-harvest may negatively affect the cell energy metabolism in that species. Specifically, a 38 % decrease in ATP concentration was observed after 6 days at 4 °C. The differences found for *P. lividus* were more pronounced, around 67 %, for the same

storage period. Besides being other species, the temperature difference of 3 °C between both studies likely explains such difference in ATP changes. In studies with live Pacific oyster *Crassostrea gigas* exposed to air at 4 °C (Kawabe et al., 2010) and ice storage (Yokoyama et al., 1992), the initial ATP values were similar or much smaller, respectively. Other studies, reported lower levels in live clams (0.10 $\mu mol \cdot g^{-1}$; Anacleto et al., 2013) or higher levels in live mussel (3.6 $\mu mol \cdot g^{-1}$; Díaz-Enrich et al. , 2002) in the beginning of their studies.

The ATP degradation generally follows a pattern with successive dephosphorylation of nucleotides and formation of ADP and AMP, according to their role in metabolism and energy supply to physiological processes, although with different rates according to species and its biological condition (Hong et al., 2017; Tejada, 2009). The decrease of ATP in *P. lividus* was linear and slow like other live marine invertebrates (Díaz-Enrich et al., 2002; Kawabe et al., 2010; Yokoyama et al., 1992), indicating a slow dephosphorylation of ATP.

The concentration of IMP/GMP In *P. lividus* at 7 °C was almost constant during the storage time as well as observed for IMP in *E. chloroticus* until the six day at 4 °C (Verachia et al., 2013). This stability shows that there was neither major degradation of IMP nor successive reactions producing HxR and Hx (below the LOQ up day 5). Analogous values were found for *E. chloroticus* during 6 days (Verachia et al., 2013). Lower concentrations of HxR (0.1 $\mu mol \cdot g^{-1}$) and slight higher of Hx (0.2 $\mu mol \cdot g^{-1}$) were found in clams after 4 days of transport at 4 °C (Anacleto et al., 2013), in oysters after 10 days of air exposure at 4 °C (Kawabe et al., 2010) and after 1 day under ice storage (Yokoyama et al., 1992).

The rate of nucleotide degradation and formation of Hx and HxR Is usually shown by *K*-value, which has been considered a valuable chemical indicator of seafood freshness (Tejada, 2009). In the present study the *K*-value evolved very slowly, with values below 6 % at day 9 reaching 14 % on the 11th day of storage. Such low index values were associated mainly to the limited formation of HxR and Hx in the period of clear vitality of sea urchins. Verachia et al. (2013) reported values of 8 and 18 % for the *K*-value, associated with day 0 and day 6, respectively.

The AEC index, which was positively correlated with ATP showed a linear relationship with the storage time. In the first days, the AEC presented the highest percentages (70 %), indicating that sea urchins were between the limit of optimal (80 - 90 %) to sub-optimal physiological condition (50 - 70 %) (Bayne et al., 1985). Clearly from day 5 onwards, the values of AEC index below 50 % were associated to a less vitality (sensory evaluated, **Subchapter 4.3**). It is known that the AEC In Invertebrates varies due to Internal or external stressors and similar initial AEC was reported In oyster (Kawabe et al., 2010; Yokoyama et al., 1992) and mussel (Díaz-Enrich et

al., 2002) and considerably lower in clams (Anacleto et al., 2013). The more stressed an animal becomes, the more energy it uses to overcome the induced stress, thus decreasing the AEC.

The AEC index and the vitality of the sea urchins up day 4 evidence the slow formation of HxR and Hx suggesting also a limited spoilage activity, since these compounds are associated with bacterial growth (Hong et al., 2017; Tejada, 2009). Thus, the AEC index can give information regarding freshness and physiological indicator of live sea urchin. For live bivalves, the AEC index was not a valuable indicator of physiological condition during 11 days transport at 4 °C (Anacleto et al., 2013). Additionally, these observations here reported are possibly associated to oxidative stress and cell damage, as studied in a transport study (at 7 °C) with stress physiological biomarkers as endpoints (**Subchapter 4.1**).

Regarding packed gonads, it was observed that the concentrations of nucleotides remained stable throughout the days of storage. Surprisingly, even the more labile triphosphate nucleotide, i.e. ATP, maintain steady concentrations until the 6th day, which was reflected by the maintenance of AEC index through storage. Such result may be related to the low refrigeration temperature of 2 °C used in the preservation. The relative maintenance of ATP, ADP, AMP and IMP levels (despite with lower concentrations) was also stated by Verachia et al. (2013) in the gonads of *E. chloroticus* kept in air at 4 °C over 10 days.

Other authors reported similar ATP (1.2 μmol·g^{-1}) and AEC values (70 %) in gonads of sea urchin at the beginning of their study (day 0), but after 6 days of storage in artificial seawater at 5 °C the values were considerable lower (Kinoshita et al., 2009), while in the present study they remained high after the same time. Moreover, the ADP, AMP and IMP concentrations decreased during storage in artificial seawater (Kinoshita et al., 2009). Previous studies described a decrease in relative ATP content (from 50 to 0 %) throughout twelve days of storage of packed gonads of *S. nudus* kept in artificial seawater saturated with air, oxygen and nitrogen at 5 °C (Wang et al., 2012).

Similar or slight higher HxR and Hx were found in sea urchin gonads by other authors after 10 days of storage (Kinoshita et al., 2009; Verachia et al., 2013). With regards to *K*-value, the values remained below 3.8 % up day 9, contrary of what was reported in other studies, referring an useful *K*-value associated to increasing values: from 7 - 15 % (beginning of storage - day 0) to 30 - 55 % after 10 days of storage (Kinoshita et al., 2009; Verachia et al., 2013; Wang et al., 2012). Furthermore, it is expected a freshness maintenance of the packed gonads throughout conservation due to good hygienic procedures during preparation.

4.2.2 Free amino acids

The total levels of FAA in trial I and II-III were different, and this was evident by the fact that 14 out of the 21 FAA had higher levels in trial I. Such difference can be attributed to different states of maturity, as suggested by Murata et al. (2020). The relationship was not possible to be confirmed by histological analyses in the frame of the current study, however the relationship is acceptable once there is evidence about the increasing transition of gonadal maturation In *P. lividus* from fall to spring (Rocha et al., 2019b). Additionally, FAA concentrations also decreased with the development of the maturation stage in *Anthociadiris crassispina* (Osako et al., 2007).

Few studies have been published FAA profile in *P. lividus* (Prato et al., 2018; Volpe et al., 2018), but none was focused on changes in these compounds through storage nor their contributions to taste. It is worth mentioning that the levels of total FAA found in the trials II-III were in the same range of those reported by Volpe et al. (2018) (a mean moisture content found in the present study was considered for comparison purposes).

The absence of changes on the total FAA in gonads from live sea urchin through storage period is certainly related to the reduction of metabolic rate, an usual mechanism in marine invertebrates exposed to several stress conditions (such as air exposure, temperature changes, food availability) to avoid the utilisation of reserves (e.g. Anacleto et al., 2013). This is particularly important in the case of mature sea urchins close to spawning which compounds (mainly amino acids and lipids) will be needed to larval development. Even so, Ala significantly increased after day 5 which could be explained as an intracellular regulation, a coping mechanism observed in marine invertebrates exposed to stress (Abe et al., 2005). In fact, stress in live sea urchins was evidenced by oxidative stress with cell damage, mainly after 6 days at 7 °C (**Subchapter 4.1**).

Also, for packed gonads, changes in the levels of FAA were not observed over the 9 days of storage at 2 °C. Similar results were reported in studies concerning the storage (*post-mortem*) of marine invertebrates (reviewed by Sarower et al., 2012); only slight changes of total FAA through storage at 0 and 5 °C and more pronounced at 10 °C. Proper handling and washing of the gonads as well as the low storage temperature limited the enzymatic and microbial activities on the low molecular weight compounds (such as nucleotides and FAA), allowing the preservation of their Initial levels.

In the current study, Gly was the FAA of higher concentrations, followed in general by Arg, Lys, Glu, Ala, Gln, Tyr, His, Ser, Tau, Val, Thr, Leu, Pro, Ile, Phe, Trp, Met, Cys, Asn and Asp. Similarly, Gly was commonly the most abundant FAA in the same species (Prato et al., 2018;

Volpe et al., 2018), as well as in other wild sea urchins like *A. crassispina*, (Osako et al., 2007), *Tripneustes gratilla* (Chen et al., 2013), *Heliocidaris crassispina*, *Strongylocentrotus Intermedius*, and *Mesocentrotus nudus* (Murata et al., 2020). Other FAA such as Ala, Arg and Lys were also found In high concentrations after Gly In the cited references, while Komata (1964) referred Leu as a major FAA after Gly and Ala. The increasing trend of Gly and decreasing trend of Arg and His from trial I and trials II-III was also verified in *Hemicentrotus pulcherrimus* and *Pseudocentrotus depressus* with the progression of gametogenesis (Murata et al., 2020).

4.3 Contribution to overall taste - TAV and EUC

The sea urchin has been recognized as a distinctive product, with a complex sensory profile (Baião et al., 2021b). The FAA and nucleotides, both non-volatile compounds, have been described as those that actively contribute to taste if their levels are above a certain threshold (Engel et al., 2002).

The gonads of sea urchin have been described as a product with sweet taste (Baião et al., 2021b; Phillips et al., 2009), like other marine invertebrates (Bi et al., 2021). The high concentration of Gly and the respective TAV value suggest a strong sweet taste of *P. lividus* gonads, which is in agreement with the sensory results (**Subchapter 4.3**); gonads were scored with a moderate to strong sweet taste at the beginning of each trial. According to the TAV values, the sweet taste was also associated to Ala. Some authors suggest that Gly and Ala may widely contribute to the sweetness of various species of sea urchins (Murata et al., 2020; Phillips et al., 2009). According to Fuke & Konosu (1991), when Gly or Ala were omitted from a synthetic mixture that simulates the taste of sea urchin gonads, a weak sweet and strong bitter taste occurred as well as a decrease of the characteristic gonad flavour. Moreover, both cause a strong sweet taste by binding to sweet taste receptors (Kato et al., 1989). Sweet taste in invertebrates has also been associated with the relatively high concentration of glycogen, such is the case of *P. lividus* (up to 2.53 %; Dincer & Cakli, 2007), which enhances the complexity of the taste evaluation. The reduction of AEC through storage, associated to stress and loss of vitality, probably led to a depletion in metabolic reserves such as glycogen, which is known to be the first biochemical reserve to be consumed (Bayne et al., 1985). Therefore, the sweet taste from a possible contribution of glycogen may also have a contribution to the decrease in sweet taste during live storage.

The bitter taste in *P. lividus* was mainly associated to Arg, Lys, His, Tyr and Val due to their significant contributions, i.e., TAV > 1. For instance, Val is necessary to produce bitterness in the ovaries of sea urchin (Fuke and Konosu, 1991), but Arg, on the other hand, was not

important for the taste of *H. pulcherrimus* gonads (Komata, 1964). Some of the FAA related to bitter may also be responsible for sweet taste, depending on molecule configuration (Nishimura and Kato, 1988), such as Arg, Lys and Val. Although the contribution of Met is almost significant in trial I (TAV close to 1) and negligible in trial II-III, this compound has been associated with the group of sweet (Dunkel and Hofmann, 2009), salty (Rotzoll et al., 2006), as well as sulphurous compounds (Liu and Qiu, 2016). Likewise it has been mentioned as essential for the characteristic flavour of sea urchin (Fuke and Konosu, 1991; Nishimura and Kato, 1988; Sarower et al., 2012). The HxR and Hx levels found in gonads from both storage trials were far much lower than the respective taste thresholds. At least theoretically, may not be enough to impart a bitter taste in the gonads. Yet, Hx and HxR, like other nucleotides, even with low concentrations may have a significant role in enhancing the overall off-flavour (especially after days 5-6) through synergistic action with free amino acids and volatile compounds. In fact, sensory results evidenced that the bitter taste was more intense in advanced days of storage (Subchapter 4.3).

The contributions of Gly, Arg, Ala and Glu to taste seemed to be also relevant in other studies with *P. lividus* (Volpe et al., 2018) and other sea urchin species (Chen et al., 2013; Osako et al., 2007) (TAV calculated through the FAA levels reported). Moreover, Glu, Gly and Ala had an evident impact in oyster flavour (Bi et al., 2021).

The *palatable* umami taste is commonly induced by MSG-like substances, not being palatable by itself. The Glu, IMP, GMP and AMP are the main substances responsible for the umami taste (Yamaguchi et al., 1971), abundant in seafood (Hong et al., 2017). They may exert a significant impact on umami taste in *P. lividus* as their TAVs were greater than 1. It has been also reported that AMP contributes to sweetness, giving umami continuity, complexity and fullness in scallop extracts (Liu and Qiu, 2016). Due to the higher EUC, a higher intensity regarding umami taste is expected in gonads of trial I. Similarly, the bitterness is expected to decrease in trials II-III.

When nucleotides appear in combination with FAA, the taste is not simply overlaid. There is an enhancement of umami taste caused by a strong synergism effect between 5'-nucleotide and MSG-like amino acids (Glu and Asp) compounds (Yamaguchi et al., 1971). Moreover, if Glu and Asp were in the dissociated state may be perceived as sour (Nishimura and Kato, 1988). In the present study, Glu was the main FAA contributing to the umami taste in sea urchins. Additionally, Ala, Pro, Gly, Val, Cys, His and Met have a synergistic effect in umami taste (Nishimura and Kato, 1988). Furthermore, some taste active peptides (due to Imino acids sequence), may modify the perception of the umami taste (Liang et al., 2022).

Glycine, Ala, Val, Glu and Met, along with IMP and GMP, were those with significant role in taste perceptions in *H. pulcherrimus* (Komata, 1964). All the mentioned compounds presented significant contributions (or near significant in the case of Met) to the overall taste in the gonads of *P. lividus* of the present study. Despite the low contribution of Cys to overall taste (TAV < 1), it has been associated to bitter, sweet, sulphurous (Liu and Qiu, 2016) and even salty taste (Rotzoll et al., 2006).

Since the FAA with significant contribution to taste were generally preserved throughout the storage of live sea urchin and packed gonads, it is expected, that their contributions were maintained in trials I and II-III. On the other hand, taste might be affected by AMP and changes in both HxR and Hx (despite the low concentrations), mainly after day 5. Furthermore, Phillips et al. (2009) reported changes in the taste of *E. chloroticus* gonads stored at 4 °C (with most air excluded) for 10 days. Moreover, the complex interaction with volatile molecules can enhance or suppress the expected taste or flavour given by TAV and EUC.

5 Conclusion

It was concluded that, in general, the changes in nucleotides through days of storage were clear on gonads from live sea urchin trials but not in the packed gonads. The nucleotides indicate two phases through storage of live *P. lividus*: one until day 4 and another from day 5 onwards. In the first, the changes in nucleotides levels were not significant, the ATP levels were the highest, whereas Hx and HxR were negligible (below the limit of quantification). In the second phase, there was a significant decrease in ATP, accompanied by a significant accumulation of AMP, HxR and Hx, while IMP/GMP remained steady. The *K*-value was generally of low values, not enabling a distinction of freshness levels. The AEC seems to be an adequate indicator to assess the vitality and freshness of live *P. lividus* preserved at 7 °C, since its evolution resulted in a linear and negative trend throughout the storage period. Regarding FAA, no noticeable changes were recorded over storage of live sea urchin. However, it is possible to highlight the effect of season and maturation stage on these compounds which are closely related to taste. The differences between trial I (January) and II-III (February and March) were mainly associated to FAA with significant contribution for the sweet (Gly, Ala), umami (Glu) and bitter (Arg, Lys, His, Val) taste (TAV > 1), as also indicated by EUC concentrations. In the case of packed gonads refrigerated at 2 °C, the evolutions of nucleotides, *K*-value and AEC were not clear and somewhat unexpected, and more evidence is needed to assess their interest as indicators of freshness. These results provide insights regarding the freshness of sea urchin which are useful for the optimisation and validation of QIM scheme for these products.

Acknowledgements

This research was supported by the CAVIAR project - Market valorisation of sea urchin gonads through dietary modulation (FA_05_2017_015), financed through Fundo Azul programme and by Portuguese Foundation for Science and Technology (FCT) through the strategic projects IDB/04077/2020, UIDB/04423/2020 and UIDP/04423/2020 (CIIMAR). Carolina Camacho acknowledges the FCT for the PhD Grant (SFRH/BD/141315/2018). B. Teixeira also acknowledges the FCT, the European Social Fund (FSE) and the Ministry of Education and Science for supporting a grant (SFRH/BPD/92929/2013).

4.3 Freshness and estimated shelf-life of live sea urchin *Paracentrotus lividus*: sensory criteria and quality index method development

Abstract

Sea urchin (*Paracentrotus lividus*) is a high-value and perishable species with increasing importance within seafood products. The aim of this work was to study the quality changes of this species (live and respective gonads) during refrigerated storage at 7 °C. Sensory methods were employed, contemplating a panel training with attributes and descriptors related to appearance, odour (live sea urchin and gonads) as well as flavour and mouth feeling (gonads). The sensory shelf-life was defined in 4 - 5 days and a proposal of a quality index method (QIM) schemes based on a total of 8 and 10 demerit points (DP) for live sea urchin and gonads, respectively. The sensory limit of acceptability corresponds to an AEC value of 45 % and an average QI up to 6 DP in the live sea urchin. Further work is necessary to improve and validate the QIM schemes.

Keywords: gonads; quality changes; texture profile analysis; colour measurements; TAV

This subchapter is under submission:

Camacho, C., Correia, T., Valente, M.L.P., Pessoa, M.F., Nunes, M.L., Gonçalves, A. 2023. Freshness and estimated shelf-life of live sea urchin *Paracentrotus lividus*: sensory criteria and quality index method development

Contribution of Carolina Camacho: Investigation, Data curation, Validation, Formal analysis, Visualization, Writing – original draft, review & editing.

1 Introduction

The sea urchin *Paracentrotus lividus* has the popular name "purple sea urchin", due to the most frequent colour of its spines. However, other colours such as black-purple, red-brown, yellow-brown, light brown or olive green are also common (Boudouresque and Verlaque, 2013). It is a product with high commercial value, marketed mostly alive and highly appreciated for the unique sensory characteristics of its gonads (Baião et al., 2021b).

Although it is a highly perishable product, there are few studies that report the changes in the quality of sea urchins, especially the most common species on the Portuguese coast, *P. lividu*s. The deterioration can occur, as for other products, at any stage between harvest and consumption. These stages are diverse and include transport (**Subchapter 4.1**) processing and storage temperature. As a live product, extra care is needed once stress can lead to quality loss and accelerate deterioration.

Quality may have a wide meaning, but there are several dimensions to evaluate the quality of seafood products, where sensory analysis has special emphasis and importance. One of the fastest and most objective methods that was developed to assess the freshness of seafood products is the quality index method (QIM), which has been used as a reference in the harmonisation of sensory evaluation of seafood products in Europe (Martinsdóttir et al., 2009a). Within a QIM development, the shelf-life establishment is of extreme importance. In general terms, for fresh seafood, it can be defined as a limited period after harvesting during which the product maintains an acceptable quality, still suitable for human consumption, under well-defined storage conditions. The limit of acceptability is usually based on the measuring the intensity of a specific attributes reached a certain predetermined value by trained panellists (Giménez et al., 2012).

QIM schemes constitutes a useful tool in the quality assessment and have been developed for several fish species and products (e.g. Bernardo et al., 2020), however to date there is no scheme dedicated to the sea urchins and their gonads. The study of the effect of transport on quality changes in live sea urchin (**Subchapter 4.1**), provides useful information for the development of a QIM scheme for freshness and quality evaluation of live sea urchin and its gonads. To date, sea urchin gonads have been evaluated based on grade scores which then determine the possible uses (Azad et al., 2011; Pearce et al., 2004; Proville, 2009).

Thus, the objective of this work was: 1) to study the sensory changes of live sea urchin *P. lividus* and its gonads during refrigerated storage (7 °C) in order to (i) develop a QIM scheme for freshness discrimination of live *P. lividus* and respective gonads, (ii) establish the shelf-life

of gonads and 2) to correlate the sensory results (sensory criterion) and chemical criteria (**Subchapter 4.2**) to validate the QIM scheme as well as the shelf-life.

2 Materials and methods

2.1 Sea urchins' source

In all experiments, commercial sized wild live *P. lividus* sea urchins were used (exosqueleton diameter ≥ 5 cm). After harvesting, live sea urchins were transported in bulk conditioning with cold accumulators to laboratory facilities within 1 to 4 hours from the harvesting point. Sea urchins were obtained as previously described (**Subchapter 4.2**).

2.2 Live sea urchin storage experiments and sampling

The temperature chosen for storage was 7 °C, based on the difference of 10 ºC from the average seawater temperature in the harvesting zone (James and Evensen, 2018), limiting cold and additional stress. In both previous and final experiments, the sea urchins were disposed (without overlapping) in boxes in a moist environment by adding ≈ 50 mL of seawater in the beginning of storage.

On all sampling days, after sensory evaluation of live animals (described in section below), they were dissected, and Aristotle's lantern removed. Gonads were gently removed with a spoon, washed with cold artificial seawater (4 °C), drained for nearly 5 min and separated for chemical analyses (**Subchapter 4.2**) and sensory assessment. Sea urchins that just spawned, had brownish gonads or another unacceptable colour were excluded, and a new specimen was sampled.

2.2.1 Previous experiments - training of sensory panel and selection of descriptors

The first goal of the previous experiments was to identify the attributes/parameters and descriptors, from a qualitative point of view, which could be used for drawing up the sensory profile of sea urchin and respective gonads during refrigerated storage. Special focus was given to sensory terms/descriptors related to lower freshness levels, i.e., associated to more advanced days of storage. The previous experiments were also necessary to train the panel on this specific product.

Between the end of February and middle March of 2020, three batches of sea urchin were harvested in Ericeira and Zambujeira coastal areas (Portugal), in three consecutive weeks to assure simultaneous sensory evaluation of sea urchins with different storage time (at 7 °C) at the same day; sea urchins with one day after harvesting was used as control sample. Sensory

evaluation of live sea urchin and respective gonads was carried out at days 1, 2, 5, 6, 7 and 9 of storage.

After sensory assessment of live sea urchin (olfactory and visual examination/appearance), several gonads from different individuals were collected to compose pools for chemical analyses (**Subchapter 4.2**). The remaining gonads were taken to visual examination (appearance), odour, and mouth test (taste and sensations).

2.2.2 Final experiments

The main goal of the final experiments was to establish the shelf-life and build a specific sensory method to freshness discrimination of live *P. lividus*. Three batches of wild sea urchins *P. lividus* were harvested from Zambujeira (SW of Portugal) in January, February and March. Live sea urchins from the different boxes were sampled during the refrigerated storage: January, sampling after 1, 3, 6, 8 and 9 days; February, sampling after 1, 3, 4, 5, 6, 7 and 11 days; March, sampling after 1, 3, 5 and 6 days. Upon sensory evaluation of live sea urchin (section **2.3.1** below), an incision on peristomial membrane allowed the collection of around 5 mL of coelomic fluid. Then, each sea urchin was dissected, and gonads prepared as previously described (section **2.2** above). Gonads were separated for sensory evaluation.

2.3 Sensory analysis and QIM development

The sensory evaluation was carried out in an air-conditioned test room equipped with 5 individual booths (with pipped water and white light) according to ISO 8589 (ISO, 2007). The participation of panellists was voluntary and consented, and they were fully informed about the procedures, as well as about any possible discomfort. The sensory panel was initially composed by 6 panellists (previous experiments), whereas the final panel (final experiments) was composed by 3 (live sea urchin) and 4 trained panellists (gonads from live sea urchin). This reduction in the number of panellists was due to Covid-19 lockdown. In all trials and sampling days, the samples (live sea urchins and gonads) were placed in a petri box coded with two random digit numbers prior assessment. Coffee was used as olfactory cleanser (in more advanced days of storage) and water, cream crackers were served as palate cleanser between samples assessment.

The general approach used to develop the QIM scheme was based on the methods described earlier (Hyldig et al., 2012; Hyldig and Green-Petersen, 2005; Martinsdóttir et al., 2009a). For the reduction of the number of descriptors, the international standard ISO 11035 was followed with adaptations (ISO, 1994).

2.3.1 Assessment of live sea urchin

Firstly, a check-all-that-apply (CATA) method was applied with a total of 24 terms, based on previous work experience (**Subchapter 4.1**), to evaluate the changes of live sea urchins during the previous storage experiments. Some sensory terms/descriptors were added, mainly those related with lower freshness grade (more advanced days of storage).

Four to eight sea urchins per trial were assessed in each sampling day. Each panellist assessed the batch of live sea urchin, in terms of appearance (spines position, spines firmness and mouth position). To assess the odour, the sea urchins were divided by the assessors to ensure that the odour of each sea urchin was assessed by only one panellist, to minimise potential Covid-19 transmission. The selected attributes and descriptors from the previous experiments and the study in **Subchapter 4.1** were considered to design an initial QIM scheme used in the final experiments with a scale ranging from 0 to 2 or 3 DP, depending on the attribute under evaluation (Martinsdóttir et al., 2009a). Slight adjustments were made during the storage experiments based on the comments and suggestions given by the sensory panel.

2.3.2 Assessment of gonads and shelf-life establishment

For gonads, the first draft of CATA list used in previous experiments was based on team experience as well as previous published data on *P. lividus* (Baião et al., 2021b), other species, *Evechinus chloroticus* (Phillips et al., 2010, 2009), as well as the standard terminology (ISO 5492, 2008) with a total of 67 descriptors. The gonads from live sea urchin were firstly subjected to appearance evaluation (including colour and apparent texture) followed by odour and tasting. Each sea urchin was evaluated by at least two panellists (one gonad for each panellist).

Each panellist evaluated, in each sampling day, 4 to 6 gonads from different live sea urchin. In the final experiments, samples were evaluated using a 5-point category scale (0 - absent, 1 - slight, 2 - moderate, 3 - strong, 4 - extreme; Meilgaard et al., 2016) to rate the intensity of the following attributes: appearance, odour, taste and mouth sensations. The limit of acceptability of the sea urchins (sensory criteria) was established when slight off-odours (≥ 1), off-flavours (≥ 1), as well as slight typical sweet flavour (≤ 1) were detected in the gonads by the sensory panel.

2.4 Physical-chemical determinations

The mass losses were monitored, throughout the storage during the final experiments, by weighing each of 10 sea urchins. Concerning coelomic fluid, the pH was measured (pH

meter 539, WTW, Weilheim, Germany) and the turbidity was determined according to Verachia et al. (2012).

Colour determination in samples of trial I was performed in individual gonads by CIELab system using a Chroma Meter CR-400 (Konica Minolta Camera, Co, Osaka, Japan) at standard illuminant D_{65} and 2-degree observer. In this system, L^* values indicate lightness on a scale of 0 (black) to 100 (white); a^* values describe the intensity from green (-) to red (+); and b^* values from blue (-) to yellow (+) (Sant'Anna et al., 2013). The equipment was previously calibrated with a white standard tile (L^*=97.79; a^*=-0.02; b^*=1.84) and then three measurements (replicates) were taken for each gonad. The L^*, a^* and b^* coordinates were recorded. The hue angle (observable/characteristic/dominant colour, h) and chroma (saturation, C) were also determined according to the following expressions (Kalinowski et al., 2005): $h_{a,b}^* = \arctan(b^*/a^*)$; $C_{a,b}^* = (a^{*2} + b^{*2})^{1/2}$.

Texture profile analysis (TPA, double compression test) and cut test of samples of trial I was carried out on a TA.XTplus texture analyser and the respective software (Stable Micro Systems, Surrey, UK) using a 5 kg load cell. Firstly, individualized gonads were compressed twice up to 50 % of the original height with a cylindrical probe of 50 mm diameter (P50) at 1 $mm \cdot s^{-1}$. The time between compressions was 5 seconds. The primary characteristics obtained included hardness (N), adhesiveness ($g \cdot cm^{-1}$), springiness, and cohesiveness. The secondary characteristic chewiness was also calculated. Afterwards, the samples were subjected to cut test with a blade set at 1.5 $mm \cdot s^{-1}$. The cutting strength (firmness; $N \cdot mm^{-1}$) and work of shear (toughness; $N \cdot mm \cdot s^{-1}$) was calculated.

2.5 Data analysis

Data analysis was performed using Microsoft Excel and the software STATISTICA TM (StatSoft, Inc., Tulsa, USA); statistical significance of all tests was at $\alpha = 0.05$ (p-value < 0.05). Data from previous trials were calculated to be present in relative percentage of each descriptor (in relation to the sea urchin evaluated in each sampling day). Data from final experiments were submitted to linear regression analysis. Thereafter, analysis of variance followed by the post-hoc one-sided Dunnett test (each storage day compared to the beginning of storage, day 1: lower or higher values depending on the parameter under analysis) was performed. Pearson's correlation was performed: between different sensory parameters (live sea urchin and gonads), and between sensory and chemical data.

3 Results and discussion

3.1 Selection of descriptors

3.1.1 Live sea urchin

The relative frequency of attributes and descriptors linked with the live urchin during storage is presented in Appendix, **Table S7**. Overall, it was observed that during storage the relative frequency of firmness of the spines decreased and marine-associated odours disappeared and instead off odours were detected.

Sea urchin spines are naturally rigid and confer protection in the marine environment (Tsafnat et al., 2012), but under stress conditions such as many hours out of water, the matrix of molecules that make up the spines may undergo, compromising the firmness and its position (Rubilar and Crespi-Abril, 2017). The appearance of a live sea urchin is associated to bright and upward facing spines that move (under external pressure). After some time of capture, the spines gradually drop, become matt and fall off with moderate traction; the membranes become dark and flaccid, which may be associated to physical sign of stress (Meloni and Fois, 2013).

Parameters associated with the appearance of the spines (such as activity, position, firmness and wetness) and oral area (mouth position) were selected. The descriptors related to odour such as marine (ocean-air), fresh, clean seaweed, air-exposed seaweed, ammonia-like, cloying/sickly sweet, yeasty, putrid/rotten and sewer were selected to integrate the initial QIM scheme (**Table 4.3-1; pp. 137**).

3.1.2 Gonads

At the end of the previous trials, the list comprised more 16 descriptors than the initial, mainly related to odour and taste. The relative frequency of attributes associated with gonad appearance, odour (Appendix, **Table S8**) and mouth test (Appendix, **Table S9**) are presented in supplementary material. Overall, descriptors identified at the beginning of storage with higher relative frequency can be associated with higher freshness levels. On the other hand, absent descriptors or with low relative frequency at the beginning are associated with freshness loss. The emergence of the latter can integrate the criteria of product rejection, when occur at a specific intensity.

In the initial assessment of appearance, high relative frequencies were obtained, with a clear decline through the storage time. Those were associated with surface texture of the

gonads – firmness, halves distinction and graininess, showing that the gonad structures in the beginning were characteristic of the product.

At the first days of storage, some descriptors of appearance, odour as well as taste were similar to those previously characterised by Baião et al. (2021b). In most fishery and farmed products (after slaughter), off-odours usually result from the formation of volatile organic compounds such as trimethylamine, sulphur compounds, aldehydes and also hypoxanthine (Di Natale and Ólafsdóttir, 2009). This can explain the occurrence of off-odours after the 4th day of storage, although the hypoxanthine had been quantified below 0.085 μmol·g^{-1} (**Subchapter 4.2**).

The descriptors of seafood off-odours are related to the species and the origin, however the most commonly mentioned ones are rancid, metallic, fermented, acidic, sour, sulphurous and putrid (Freitas et al., 2021). For example, sulphur compounds are well accepted indicators of lack of freshness in different seafood species after slaughtering (Wang et al., 2022) and influence the overall aroma of the product even at low concentrations (Wu et al., 2022).

The perception of bitter taste may be indicative of an advanced stage of maturation that is common in some urchin species. For instance, in *Strongylocentrotus droebachiensis* and *Pseudocentrotus depressus*, female gonads develop a bitter taste as oogenesis progresses (Unuma and Walker, 2009). In the case of *E. chloroticus*, the bitter taste of female gonads was detected throughout the year, although with less intensity during autumn (Phillips et al., 2010). Some of the gonad descriptors identified in the present work were also previously used in descriptive analysis of the species *E. chloroticus* (Phillips et al., 2009).

The changes in the relative percentages of the gonad attributes over the previous trials reflected the panel's agreement. In the end, descriptors with a relative frequency equal to or greater than 50 % or indicating some change in their relative frequency during the storage were considered. Thus, the descriptors selected for the 5-point scale form of assessment of the gonads in the final experiments were: i) visual assessment (appearance) - halves distinction, firmness and graininess as well as oval shape, very often mentioned by the panel; ii) odour - underdeveloped, marine (ocean-air), seaweed, fresh, tropical, fruity, sweet, acid, sour, earthy, alcohol, sulphur, pungent and sewage; iii) mouth test: flavours sweet, salty, acid, bitter and those associated to fruity, tropical, marine flavours as well as sulphur and cloying very often mentioned by the panel in the form of notes in the evaluation sheet. Creaminess, firmness, succulency, sliminess, spiciness, astringency, and meltiness were selected as the most relevant mouth sensations.

3.2 QIM development

3.2.1 Physical-chemical characteristics of live sea urchin

The mass losses as well as the changes of the pH and turbidity of coelomic fluid are presented in **Figure 4.3-1**.

Mass losses were around 5 % after 3 days of storage and significantly higher on day 6 compared to day 2 ($p < 0.05$, Dunnett test). The higher losses were reached after 10 days (30 %) (**Figure 4.3-1 A**). The stress associated with the extended time the urchins were out of water led to coelomic fluid losses that were reflected in total mass losses. Transport studies with live urchins revealed losses of about 13 to 24 % after 8 days at 7 °C (*P. lividus*; **Subchapter 4.1**) and 8 % after a 39 h at 14 °C (*S. droebachiensis*; James and Evensen, 2018).

The physical-chemical characteristics of the coelomic fluid of different urchins changed over time, thus the pH values significantly lower values were recorded after 5 days of storage compared to day 1 ($p < 0.05$, Dunnett test; **Figure 4.3-1 B**) while for turbidity significantly higher values were observed after the same period ($p < 0.05$, Dunnett test; **Figure 4.3-1 C**). Positive correlations were recorded between mass loss and turbidity of coelomic fluid ($r = 0.7825$; $p < 0.0001$) and negative correlation between mass loss and pH as well as between both parameters of the coelomic fluid ($r < -0.6519$; $p < 0.0001$), considering the data of the 3 trials together.

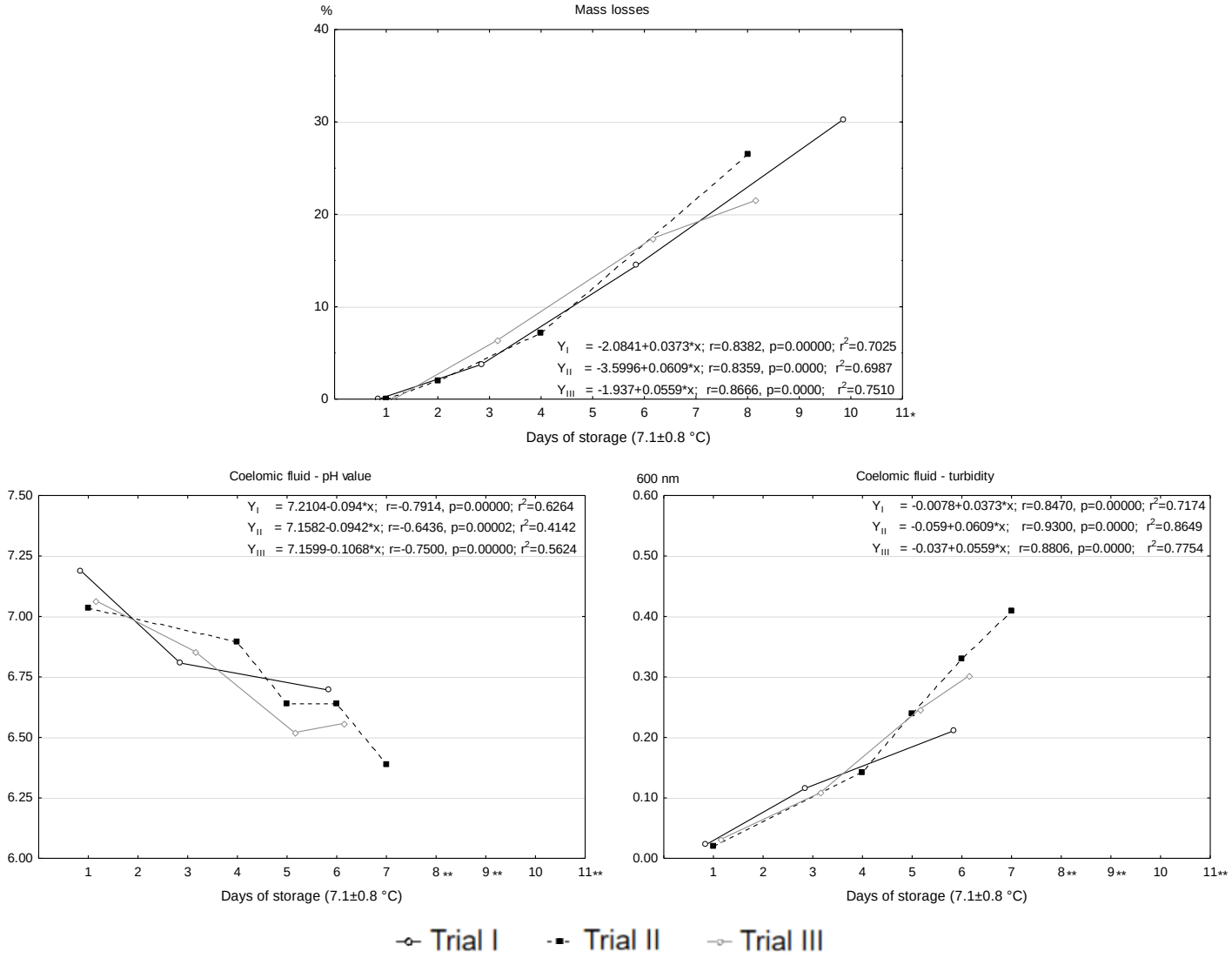


Figure 4.3-1 — Mass losses (%), pH value and turbidity of coelomic fluid of commercial size live sea urchin during the refrigerated storage.

Each point is the average value; N=5 for mass losses (0.8 %<SD<10.8 %) and N=6 to 10 for coelomic fluid (pH value: 0.09<SD<0.23; turbidity: 0.01<SD<0.08); *Mass loss was not calculated due to intense fall of the spines; ** Not determined due to insufficient amount of coelomic fluid.

Although some authors had reported the buffering capacity of urchin coelomic fluid (Collard et al., 2013), there are studies that reveal that the pH of coelomic fluid decreases with exposure to air and high temperatures (Johnstone et al., 2019; Verachia et al., 2012a), or no significant alterations during 8 days of transport (**Subchapter 4.1**). Changes in turbidity, on the other hand, may be associated with responses to adverse external conditions, as also seen in another study under similar air conditions (Verachia et al., 2012a).

3.2.2 Appearance and odour of live sea urchin

The appearance and odour changes of live sea urchin over the storage at 7 °C were assessed using **Table 4.3-1** (initial QIM scheme) and results are shown in **Figure 4.3-2**.

Table 4.3-1 — Initial Quality Index Method (QIM) scheme for freshness evaluation of commercial size live *P. lividus* refrigerated at 7.1 ± 0.8 °C.

Parameters		Descriptors	Demerit points
Appearance	Spines* activity and wetness	Active, bright, wet	0
		Reactive, still bright	1
		Less reactive/few reactive, slightly dull	2
		Unreactive, dull	3
	Spine's position and firmness	Upright position, very firms	0
		Still in upright position, firms	1
		Withered, disorganized, fragile	2
		Fallen/collapsed, brittle (breaking readily), loss of spines	3
	Oral area	Mouth centered and closed, reactive	0
		Mouth semi-open (still reacts)	1
		Mouth open, decentered	2
Odour - Oral area		Slight marine (ocean-air), clean seaweed, fresh	0
		Marine, with sweet notes, sulfide, slight ammonia-like odour	1
		"Air-exposed seaweed", sulfide/sulphur, slightly yeasty, cloying/sickly sweet	2
		Putrid, rotten, yeasty, sewer	3
Quality Index (QI): 0 – 11 demerit points			

*Typical colour of the spines are purple, black-purple, red-brown, yellow-brown, light brown or olive green (Boudouresque and Verlaque, 2013).

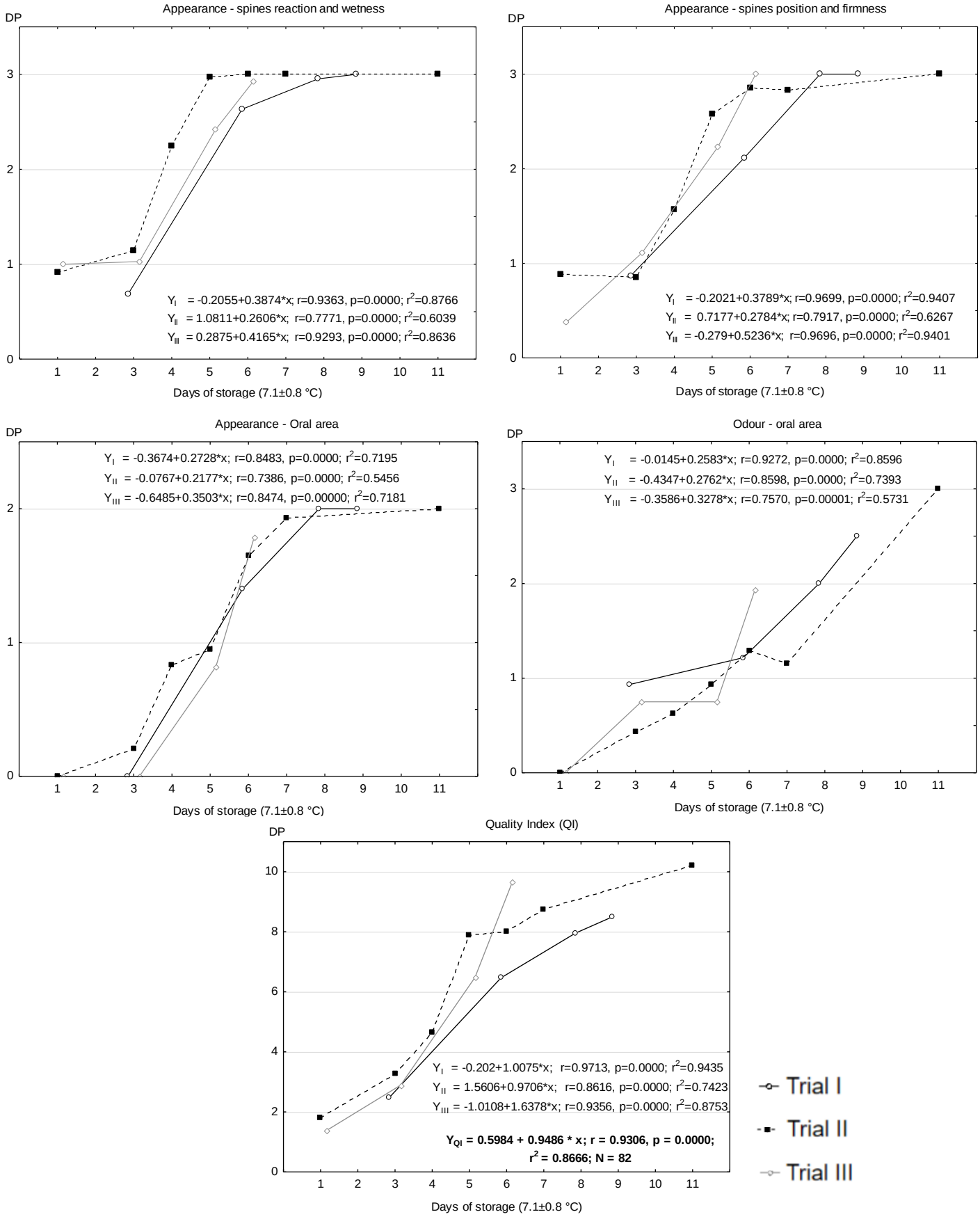


Figure 4.3-2 — Appearance, odour and quality index evolution of commercial size live sea urchin during the refrigerated storage. Each point is the mean value of the urchins assessed (N = 5 to 16; 0.1 DP < standard deviation < 1.3 DP). DP – demerit points; Y_I, Y_{II}, Y_{III} are regression equations, considering all data, of Trial I, Trial II and Trial III, respectively; Y_{QI} is the global regression equation considering all data.

In general, the parameters reflect the quality changes of live sea urchin through storage; positive and significant linear correlations were observed for all parameters evaluated ($r > 0.7386$, $p < 0.0001$). Some variability in the adjustment of the linear model was recorded for the evaluated parameters ($0.5456 \leq r^2 \leq 0.9407$); although the linear model fit is not as strong in trial II, is still significant.

The evolution of sum of all demerit points assigned to the attributes and descriptors, i.e., the quality index (QI) values was also presented in **Figure 4.3-2**. Significant linear correlation between the QI and storage time was observed for each of the trials. The global regression equation of QI is Y_{QI} (**Figure 4.3-2**) that allows the prediction of the storage time, through the equation:

$$\text{Predicted storage time (days)} = (QI - 0.5984) / 0.9486$$

The spines at day 1 and 3 were reactive to touch and still brights (1 DP) and firms (1 DP), as seen in **Figure 4.3-3** (aboral area, day 1).

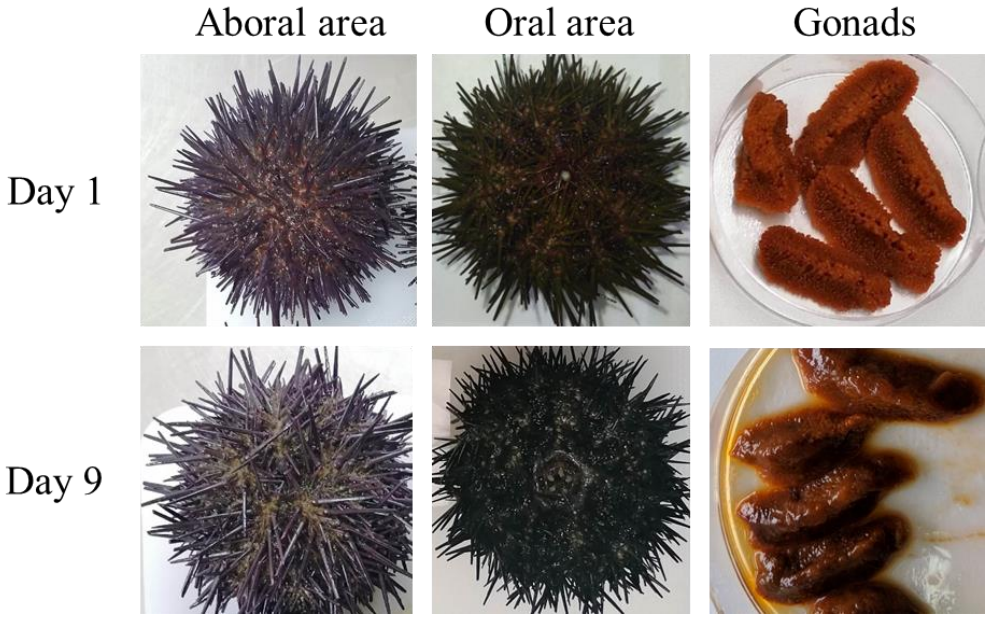


Figure 4.3-3 — Visual changes of commercial size live sea urchin (aboral and oral area perspectives) and respective gonads at days 1 and 9 of storage under refrigeration (7.1 ± 0.8 °C).

The spines become less reactive (2 DP) to without reaction (3 DP) at day 5 (trial II) and 6, respectively. The spines were completely fall off and fragile at day 6 (trials II and III) and day 8 (trial I); were evaluated with 3 DP. The upright position and firmness of the spines is characteristic of a very fresh urchin, which then undergoes after some time of harvesting, as it was verified. This parameter was discussed previously (section **3.1.1**, **pp. 133**). Regarding the oral area, urchins up to 3 days presented centered and closed mouth (0 DP) and a flat to concave peristome (**Figure 4.3-3** oral area, day 1). This area evolved to closed mouth (1 DP, 4-5 days) with

the peristome mostly concave, and after 7-8 days (trials I and II), the mouth was decentered and opened (2 DP) (**Figure 4.3-3**; oral area, day 9).

As living organisms outside their natural habitat, although generally considered to be robust animals, they are subjected to stress from abiotic conditions, leading to physiological changes and consequent loss of quality. A report indicate changes in the oral area of *S. droebachiensis* sea urchins stored at 3-4 °C: mouth closed up to 5 days after capture and mouth open after 13 days (Stefánsson and Ólafsdóttir, 2017).

The odour was initially scored as 0 DP (associated to fresh/marine), but it changed over storage time, reaching an average 2 DP on the 6th (trial III), 8th (trial I) and 9th (trial II) days of storage. The maximum score (3 DP) was reached on day 11 (trial II). The report by Stefánsson and Ólafsdóttir (2017) point out that sea urchins *S. droebachiensis*, kept alive at 3-4 °C, had a fresh odour up to 9 days of storage and after 13 days a strong spoilage odour was detected. The sensory changes in terms of appearance and odour in live *P. lividus* were previously characterised in **Subchapter 4.1**, although in a lesser extent (up to 8 days) and in a transport context.

The parameters evaluated in the live sea urchin correlated positively with mass loss and turbidity of the coelomic fluid ($r > 0.5701$, $p < 0.0004$); negative correlations were recorded between the four parameters and the pH of the coelomic fluid ($r < -0.5158$, $p < 0.0019$). It is interesting to note that the live urchin parameters correlated with the chemical indicators AEC (negative correlation, $r < -0.7817$, $p < 0.0001$) and *K*-value (positive correlation, $r > 0.4594$, $p < 0.0031$), both quantified in the gonads (**Subchapter 4.2**), highlighting the usefulness and complementarity of sensory and chemical parameters in assessing sea urchin freshness.

It has been shown that handling, transport and storage are conditions that induce stress in bivalves due to reduced oxygen availability, leading to quality losses (e.g. Anacleto et al., 2013). Probably, the unavailability of oxygen also induced the changes observed in the urchins of the present study, since the gas exchanges in these organisms is mainly through the buccal podia (tube feet near oral area) and high water flow rate is necessary to maintain adequate oxygen levels (Harris and Eddy, 2015).

3.2.3 Assessment of gonads from live sea urchin

The evaluation of raw gonads from live sea urchin is presented in terms of appearance (**Figure 4.3-4**), odour (**Figure 4.3-5**) and mouth test (**Figure 4.3-6**) over the storage at 7.1 ± 0.8 °C. Most of the parameters reflect the quality changes of gonads from live sea urchin.

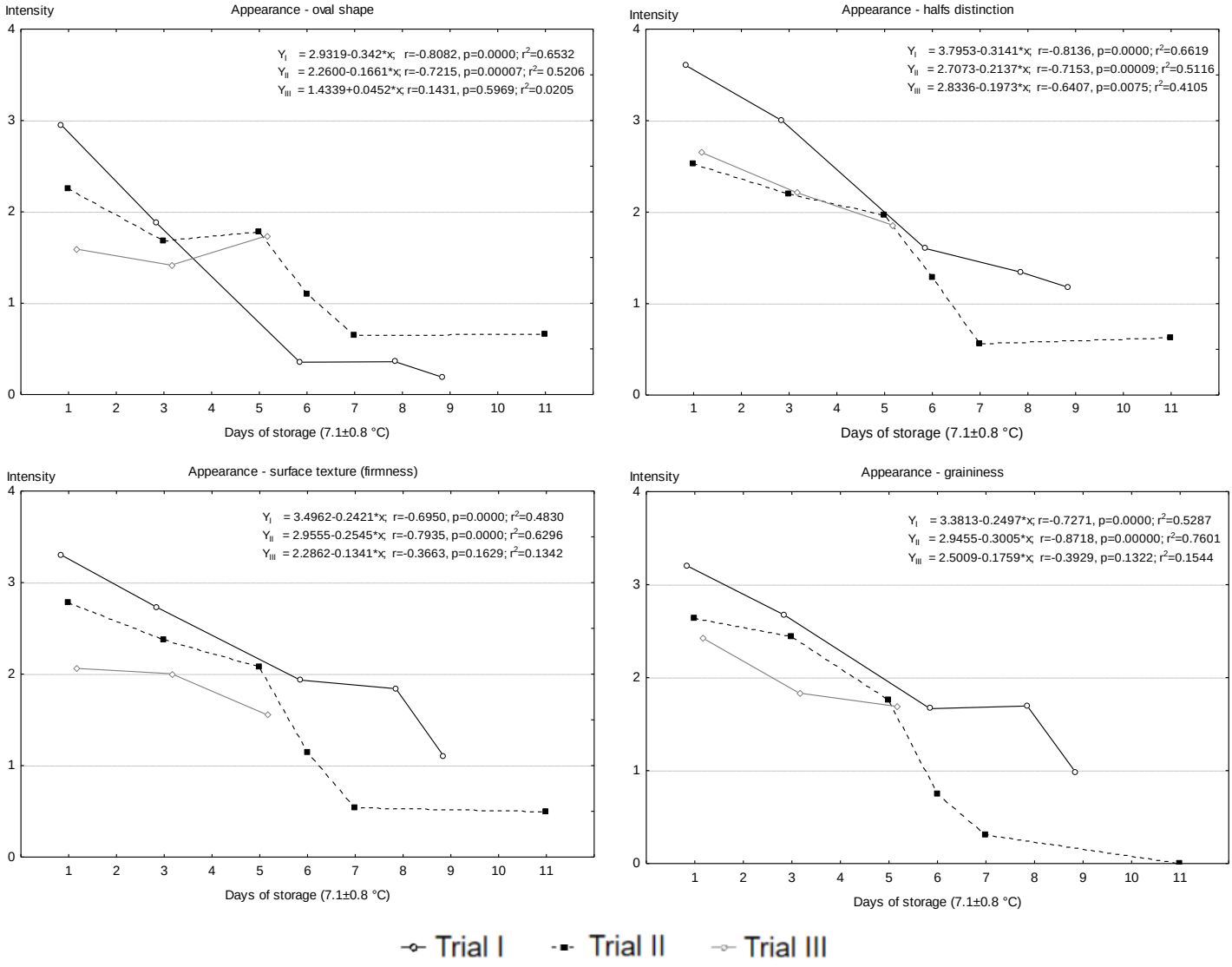


Figure 4.3-4 — Appearance of raw gonads from commercial size live sea urchin throughout refrigerated storage for each final experiment.

Intensity scale: 0-absent; 1-slight; 2-moderate; 3-strong; 4-extreme; Each point is the mean value of the gonads assessed (N= 4 to 12; standard deviation < 1); Y_I, Y_{II}, Y_{III} are regression equations, considering all data, of Trial I, Trial II and Trial III, respectively.

Linear and significant correlations were obtained between most of the parameters evaluated and storage time ($r < 0.6950$, $p < 0.0001$; trials I and II); with some fluctuations in the adjustment of the linear model ($0.4830 \leq r^2 \leq 0.7601$). In trial III the linear model did not fit. Overall, very fresh gonads (mainly those of trial I) showed higher intensity in the appearance parameters (**Figure 4.3-4**). At the beginning of storage (1 or 3 days), very fresh gonads in trial I were visually firm (strong intensity), with a distinct oval shape and graininess (**Figure 4.3-3**, day 1). The gonads in trials II and III were visually less firm at the beginning of storage (moderate to strong intensity), with an oval shape and graininess less evident (**Figure 4.3-4**).

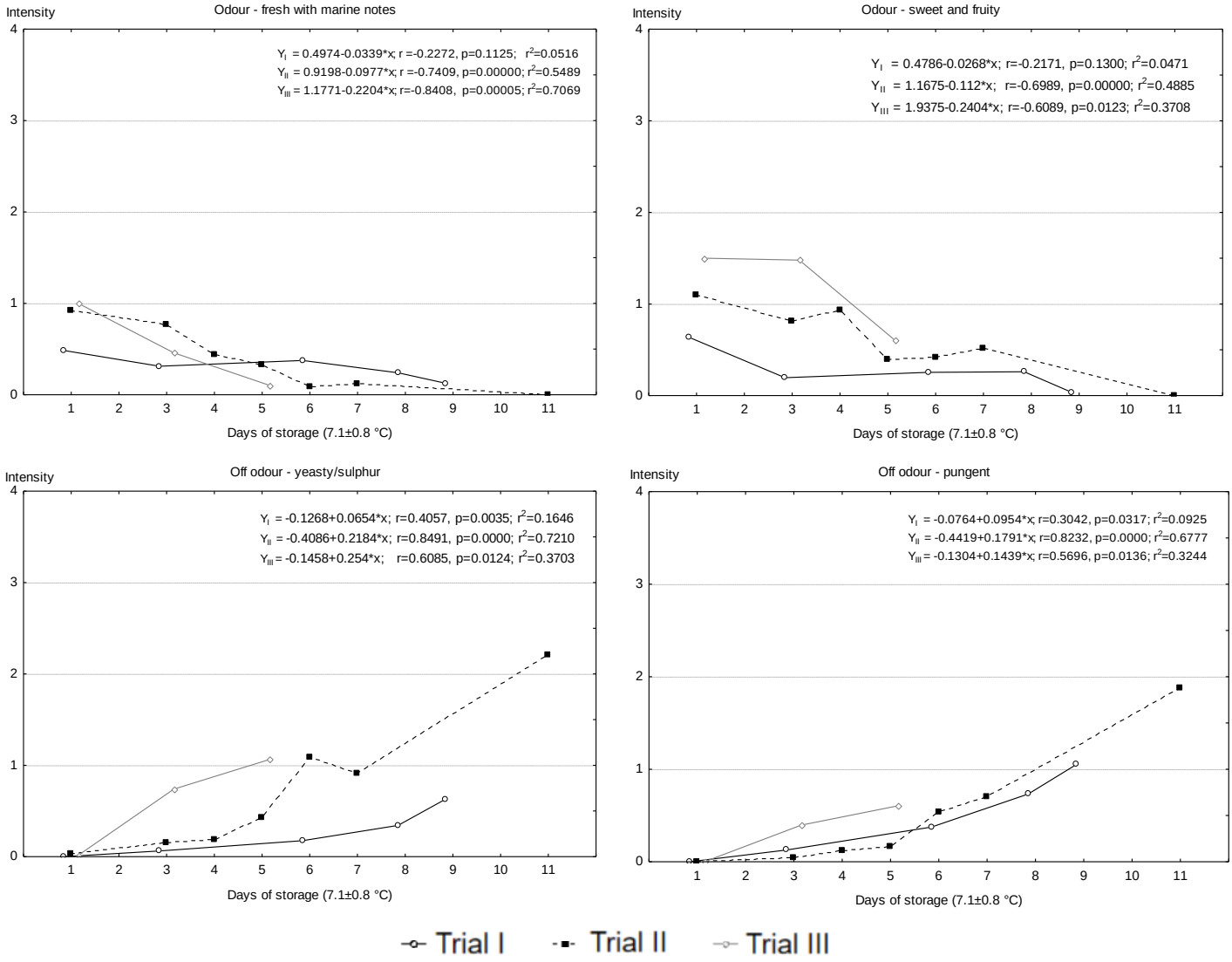


Figure 4.3-5 — Odours of raw gonads from commercial size live sea urchin throughout refrigerated storage for each final experiment.

Intensity scale: 0-absent; 1-slight; 2-moderate; 3-strong; 4-extreme; Each point is the mean value of the gonads assessed (N= 4 to 12; standard deviation < 1); Y_I, Y_{II}, Y_{III} are regression equations, considering all data, of Trial I, Trial II and Trial III, respectively.

Initially the odour was slightly fresh and marine (ocean-air, clean seaweed), as well as slightly sweet and fruity, more intense in the samples of trial III in the latter descriptors (**Figure 4.3-5**); the off-odours were non-existent.

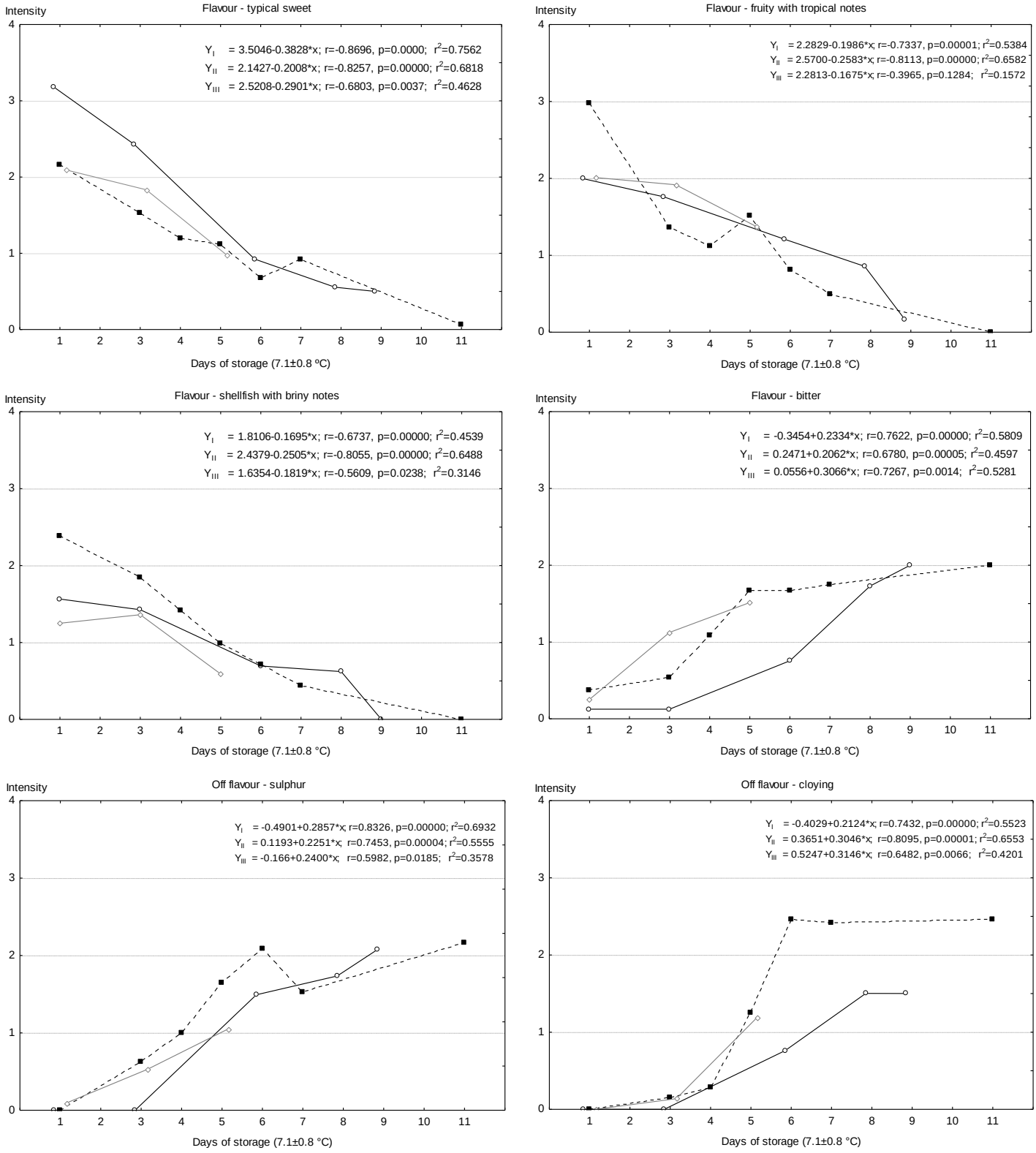


Figure 4.3-6 — Flavour and mouth feeling of raw gonads from commercial size live sea urchin throughout refrigerated storage for each final experiment.

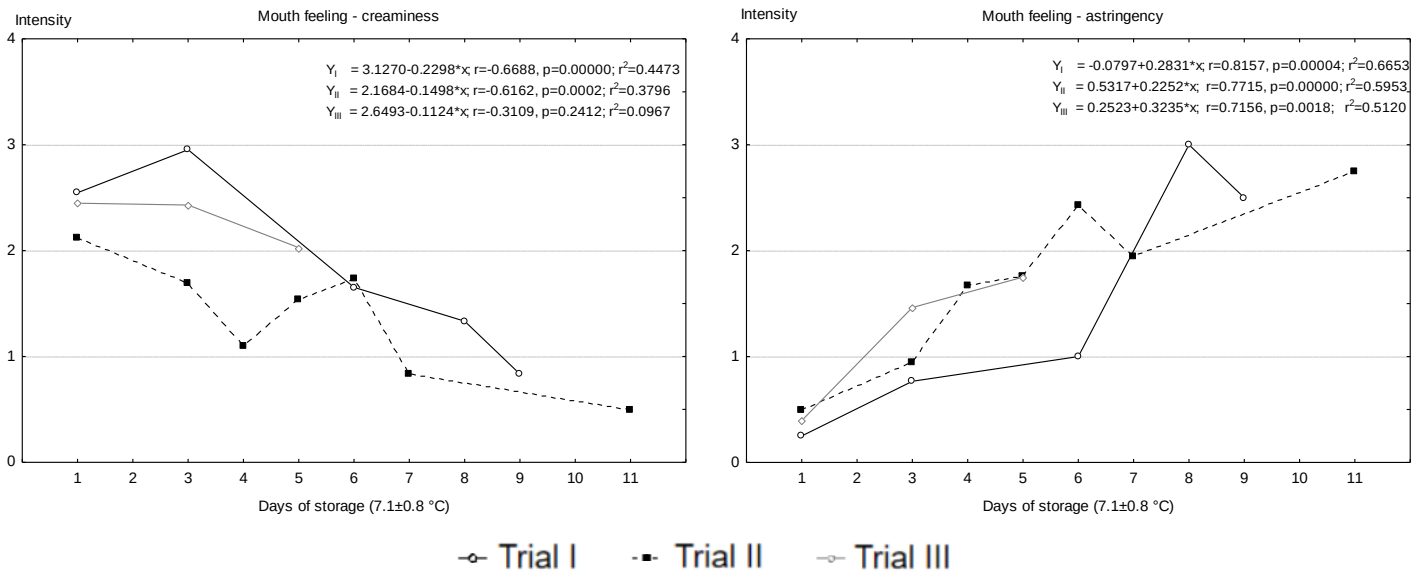


Figure 4.3-6 (*continuation*) — Flavour and mouth feeling of raw gonads from commercial size live sea urchin throughout refrigerated storage for each final experiment.

Intensity scale: 0-absent; 1-slight; 2-moderate; 3-strong; 4-extreme; Each point is the mean value of the gonads assessed (N=4 to 8; standard deviation < 1). Y_I, Y_{II}, Y_{III} are regression equations, considering all data, of Trial I, Trial II and Trial III, respectively.

Regarding mouth test (**Figure 4.3-6**), gonads had moderate to strong typical sweet taste and fruity flavour (days 1 and 3). The shellfish flavour was less intense, and the bitter as well as off-flavours were absent. Regarding the mouth sensations, the raw gonads melted in the mouth and then became very creamy (moderate to strong intensity), with a sweet after-taste. The appearance and odour of fresh raw gonads were like what has been described in other studies. The sweet is one of the most characteristic taste descriptors in raw gonads of *P. lividus* and *E. chloroticus*, although influenced by sex (Baião et al., 2021b; Phillips et al., 2010; Walker et al., 2015). According to the grades that have been used for other species (**Table 2.2-3**, pp. **29**), the gonads evaluated up to day 3 would correspond to a grade A (Californian Uni grade; Proville, 2009), or score 1 according to gonad texture and firmness evaluated subjectively by eye (Pearce et al., 2004).

As storage time go ahead, the gonads lost their shape and the granules were less evident in the gonad surface (**Figure 4.3-3**, day 1; **Figure 4.3-4**); the off-odours appear with descriptors as yeasty and sulphur (**Figure 4.3-5**), with cloying notes, and in a later stage, very offensive/pungent. As regards mouth test, the creaminess decreased as well, a pasty sensation remained in the mouth, which did not keep its shape nor melt (slimy). The bitterness started earlier in trial III. The off-flavours were mentioned as complex, with descriptors mainly associated to sulphur,

cloying (**Figure 4.3-6**), others such as sour, yeasty, and bitter aftertaste were also mentioned by the panel.

Gonads with more days of storage (5 - 6 days) may be equivalent to a grade C (in terms of appearance, excluding colour), a grade 4 (distinction of gonad segments not possible; very soft) or grade 5 if bitter taste is taken into consideration – gonads with no commercial value (Azad et al., 2011; Pearce et al., 2004; Proville, 2009).

There is evidence that the undesirable sulphur off-odours is due to benzothiazole and phenol compounds identified in *P. lividus* (De Quirós et al., 2001a). Indeed, odour plays a central role in any sensory scheme applied to seafood products, in particular in the development of QIM schemes and acceptance determination (Di Natale and Ólafsdóttir, 2009).

The bitter taste has been mentioned by chefs as an intrinsic characteristic that negatively influences the acceptance of gonads of *P. lividus* (Baião et al., 2021a). In this work it was found that bitterness was imperceptible in the fresh product, revealing to be a good indicator to follow changes during storage (significant linear correlation with storage time; see **Figure 4.3-6**).

According to the study of Rocha et al. (2019b), the most favourable period to harvest high quality *P. lividus* from the Portuguese coast is between November and February. For trials I and II, urchins were caught in this period and in the case of trial III harvesting was slightly later. As gonad tissues become fragile as gametogenesis progresses (Unuma and Walker, 2009), some sensory characteristics of sea urchins used in the latter trial could be slightly affected.

Instrumental texture and colour results of gonads from live sea urchin (trial I) are shown in **Table 4.3-2**. The gonads showed significantly higher hardness and adhesiveness after 8 days of storage ($p < 0.0002$, Dunnett test), while resilience and chewiness increases were only perceived at day 9 ($p < 0.0234$, Dunnett test). The values of work of shear and firmness were highest after 6 days ($p < 0.0131$, Dunnett test). Springiness and cohesiveness parameters were significantly lower than day 1, after 3 and 8 days, respectively ($p < 0.0389$, Dunnett test).

Negative correlations were found between instrumental texture and texture evaluated in the mouth. Specifically, between hardness, work of shear and the 4 appearance parameters (oval shape, halves distinction, graininess, and firmness) ($r < -0.6160$, $r^2 > 0.3343$, $p < 0.0002$).

The adhesiveness and cohesiveness results corroborate the visual appearance as positive correlations were recorded between them ($r > 0.5231$, $r^2 > 0.2731$, $p < 0.0001$). Positive correlations were also found between instrumental texture parameters and mouth sensations, namely between adhesiveness and creaminess ($r > 0.4719$, $r^2 > 0.2227$, $p < 0.0107$).

Table 4.3-2 — Texture and colour parameters of gonads from commercial size live sea urchin throughout refrigerated storage - final experiment trial I.

	Days of storage (7.1 ± 0.8 °C)				
	1	**3**	**6**	**8**	**9**
Texture					
Hardness (N)	1.20 ± 0.35	1.62 ± 0.38	2.24 ± 0.83	3.69 ± 1.38*	4.56 ± 1.25*
Adhesiveness ($g \cdot cm^{-1}$)	-0.06 ± 0.05	-0.04 ± 0.02	-0.07 ± 0.02	-0.23 ± 0.08*	-0.32 ± 0.16*
Springiness	0.65 ± 0.11	0.66 ± 0.14	0.54 ± 0.09	0.46 ± 0.04#	0.47 ± 0.07#
Cohesiveness	0.35 ± 0.06	0.30 ± 0.03#	0.30 ± 0.04#	0.23 ± 0.02#	0.20 ± 0.03#
Resilience	0.15 ± 0.06	0.15 ± 0.02	0.14 ± 0.02	0.12 ± 0.03	0.09 ± 0.02*
Chewiness	0.26 ± 0.1	0.30 ± 0.09	0.35 ± 0.08	0.29 ± 0.17	0.43 ± 0.08*
Work of shear ($N \cdot mm \cdot s^{-1}$)	0.23 ± 0.05	0.32 ± 0.09	0.40 ± 0.14*	0.44 ± 0.09*	0.46 ± 0.24*
Firmness ($N \cdot mm^{-1}$)	0.05 ± 0.01	0.10 ± 0.04	0.12 ± 0.05*	0.14 ± 0.02*	0.11 ± 0.05*
Colour					
*L** (lightness)	44.01 ± 4.72	38.58 ± 5.01	38.04 ± 5.6	35.77 ± 5.07	38.06 ± 3.63
*a** (green (-) to red (+) colour)	19.02 ± 3.52	18.59 ± 3.07	18.68 ± 2.44	18.39 ± 3.12	16.66 ± 2.68
*b** (blue (-) to yellow (+) colour)	41.57 ± 9.23	36.25 ± 7.08	36.18 ± 9.66	30.24 ± 7.02	32.84 ± 5.69
$C^*_{a,b}$ (Chroma, saturation)	45.74 ± 9.73	40.82 ± 7.2	40.86 ± 9.33	35.47 ± 7.31	36.89 ± 5.83
h° (hue angle)	65.17 ± 2.13	62.6 ± 4.22	61.74 ± 5.45	58.19 ± 3.85	62.87 ± 3.56

Values are mean ± standard deviation (N = 7).

* represent significant higher values in relation to day 1 ($p < 0.0131$; Dunnett one-sided test);

represent significant lower values in relation to day 1 ($p < 0.0389$; Dunnett one-sided test).

The colour parameters range were: *L** from 35.7 to 44.01; *a** from 16.66 to 19.02; *b** from 30.24 to 41.57; C from 35.47 to 45.74; and h° from 58.19 to 65.17 (**Table 4.3-2**); no significant changes ($p > 0.05$; Dunnett test) were found through storage in comparison with very fresh gonads (day 1). Other authors reported absence of colour changes through 10 days of storage at 4 °C for *E. chloroticus* gonads (Phillips et al., 2009). Thus, the colour results seem to indicate that this parameter is not suitable enough to assess gonad freshness and that's why it was not included in the QIM scheme, only as a note regarding the most acceptable colour of this species was added. Colours were classified by the panel from bright orange or yellow, with some intermediate descriptions such as orange-earth, and these were taken as the characteristics of the species.

Similar *L** and *b** colour parameters were reported for wild *P. lividus*, from Portuguese coast (Rocha et al., 2019b), as well as enhanced ones with different diets (Cook and Kelly, 2007). The *a** parameter in the present study was slightly lower than those found in the referred

studies (20.1 - 48.1). The *a** e *b** were identical of those reported for *S. franciscanus* gonads, while *L** was lower (McBride et al., 2004). The carotenoids echinenone, β-carotene, α-carotene and lutein are the main compounds influencing colour perception, however some authors reinforce the hypothesis that there are other factors modulating colour, namely the transformations during gametogenesis (Rocha et al., 2019b), such as the growth of nutritive phagocytes (Unuma and Walker, 2009).

The chemical determinations in the gonads of this study, namely chemical composition, nucleotide content and free amino acids (FAA) are presented in **Subchapter 4.2**, with emphasis on the contribution of these compounds in freshness evaluation and taste of gonads from live urchin. These are the main non-volatile taste active compounds in marine invertebrates, including sea urchins (Fuke and Konosu, 1991), especially if they are present above a certain threshold (Engel et al., 2002).

The moderate to intense sweet taste in fresh gonads (day 1 and 3) is mostly due to the presence of FAA such as Gly and Ala, with taste active value (TAV) of 10 and 2, respectively (**Table 4.2-3** in **Subchapter 4.2**). The intensity of the sweet taste decreased over storage time (**Figure 4.3-6**) and the TAV of the FAA associated with the sweet remained like initial values.

It is interesting to note that the TAV of the compounds associated with bitter taste (mainly Arg, Lys, His) remained stable throughout the storage time, in contrast to the increased bitterness sensory evaluated. This is certainly related to the complex interaction between these low molecular weight compounds and volatile compounds that interfere in the flavour perception, specifically the increase of off-odours/off-flavours with the progression of the storage time.

The descriptor umami was not mentioned by the panel but the descriptors briny, savoury, stocky that characterise the umami flavour of the gonads (Phillips et al., 2009) were stated, mainly when the product had the greatest freshness (days 1 and 3). The TAV of compounds associated to umami showed values with a significant contribution to the overall taste of the gonads (**Table 4.2-3** in **Subchapter 4.2**). The sweet taste was more intense in trial I, corresponding to a higher contribution of umami, assessed through EUC calculation (

Figure 4.2-3 in **Subchapter 4.2**).

Some correlations between sensory data from the gonads of live sea urchins and some of these chemical parameters should be highlighted: overall appearance and AEC ($r > 0.6319$, $p < 0.0029$); overall off-odours/off-flavours and AEC ($r < -0.5815$, $p < 0.00046$).

In summary, the FAA and their respective contributions to flavour together with the sensory results, confirm that these compounds are not good indicators of the sea urchin freshness.

On the other hand, the nucleotides through the AEC index, can follow the changes in the freshness of this product, corroborating the sensory results.

3.2.4 Shelf-life estimation

A gradual increase in the intensity of off-odours and off-flavours was observed in gonads from live urchin over the storage time (final trials). In general, the off-odours were slightly higher than the off flavours, and a positive correlation was found between these two parameters (r = 0.7924, $p < 0.001$). There was a positive and significant linear correlation between the mean intensities of off-odour/off-flavour in the gonads and storage time. An increase in off-odours/off-flavours throughout the storage time was registered, with a slight intensity i.e., close to the defined acceptability limit (section 2.3.2, pp. 131), of 0.87 and 1.20, at day 4 and 5, respectively.

Statistical differences compared to day 1 (control) were found after 4 days of storage ($p = 0.0083$, Dunnett test), where the gonads were classified with slight to moderate typical sweet flavour (on average) and were accepted by the panel. On day 5, about 42 % of the gonads from the different urchins had almost slight off-odours/off-flavours, while on day 6, 93 % were recorded above 1 (slight) and were rejected by the panel on this day. In trial III, the panel rejected the raw gonads on day 5, so they were not sensory evaluated in the following days. The limit of acceptability was identical in all three trials, being reached on day 4-5.

These results agreed with the qualitative evaluation of the odour of urchins after dissection (with internal content: coelomic fluid, viscera, gonads). At the beginning, a slight marine odour, which intensified later at the same time as off-odours began to emerge (although earlier than for the gonads) associated with putrid, faecal, phenolic, with cloying sweet notes.

Considering the sensory results described above (off-odours and off-flavours), the shelf-life of live sea urchin kept under refrigeration (7 °C) is estimated as 4-5 days. The absence or slight intensity of typical sweet flavour and the off-odours were the main attributes that led to the rejection of gonads, however the visual evaluation (appearance) and texture/mouthfeel are also an important role in the assessment of freshness, as significant linear correlations were obtained with storage time. Assuming this shelf-life, the chemical indicators, namely AEC should not be less than 45 % for the product to be considered for consumption (Subchapter 4.2).

There are suggestions that sea urchins of the species *P. lividus* can be kept alive for about 3 or 4 days (no temperature details) (Meloni and Fois, 2013), however this is the first time that a shelf-life study has been carried out in this species. Stefánsson and Ólafsdóttir (2017)

reported a shelf-life of 5-9 days at 3-4 °C for *S. droebachiensis* species, however it was based only in qualitative assessment.

Some studies point out 3-4 days preservation of live mussels (*Mytilus galloprovincialis*) in air at 2-3 °C and an extension of 2-3 additional days in oxygen-rich modified atmosphere (Bernárdez and Pastoriza, 2011; Pastoriza et al., 2004). Other studies with live clams (*Ruditapes decussatus*) reported a benefit of oxygen-rich packaging over air storage, both at 6.1 ± 0.7 °C during 6 days (Gonçalves et al., 2009).

3.2.5 QIM Proposal

Most of the parameters assessed in the live sea urchin and respective gonads were useful in reflecting sensory changes and revealed linear regressions with storage time of the product at 7 °C, therefore included in a proposed QIM scheme.

The study presented in **Subchapter 4.1** (effect of transport on the quality changes of live sea urchin) provided useful information regarding the quality alterations and development of a sensory scheme for the evaluation of the freshness degree of the product. Thus, it was possible to start the final experiments with an initial QIM scheme (**Table 4.3-1**).

A QI value between 6 and 8 was reported for live sea urchin at the 5th day (limit of shelf-life), in a total of 11 DP (**Figure 4.3-2**). The global QI (all data) significantly correlated with off-odours/off-flavours assessed in the gonads (r^2 = 0.6724, r = 0.8200, p < 0.001), evidencing the utility of the QIM scheme. In addition, the remaining shelf-life can be calculated through the equation:

Remaining shelf-life (days) = 5 days (estimated shelf-life) - Predicted storage time (days).

In terms of usefulness and applicability, the QIM scheme of live sea urchin constitutes a valuable non-destructive method for the estimation of gonads quality that can be employed in the commercialisation chain (e.g., auctions, industry).

Still, some modifications were needed to allow a better adjustment of the QI score at the rejection time. Moreover, intermediate descriptors regarding the activity and position of the spines revealed difficult to evaluate and adjustments were made accordingly. In this sense, the demerit points (spines evaluation) and descriptors (mainly oral area and odour) were adjusted, allowing to propose a final QIM scheme, which is presented in **Table 4.3-3**.

Table 4.3-3 — Final Quality Index Method (QIM) scheme for freshness evaluation of commercial sized live *P. lividus* refrigerated at 7 °C.

Parameters		Descriptors	Demerit points
Appearance	Spines reaction and wetness	Active or reactive, bright, wet	0
		Less reactive, still bright	1
		Unreactive/lethargic, dull	2
	Spines position and firmness	Upright position, firms	0
		Withered, disorganized, fragile	1
		Fallen/collapsed, brittle (breaking readily), loss of spines	2
	Oral area	Mouth centered and closed, reactive, peristome taut and flat	0
		Mouth centered, closed to semi-open (still reacts), peristome flat to concave	1
		Mouth decentered, open (without reaction), peristome mostly concave	2
Odour - Oral area		Slight marine (clean seaweed, ocean-air), fresh, iodine-like	0
		Marine with sweet notes, sulfide, slight phenolic	1
		"Air-exposed seaweed", sulfide/sulphur, ammonia-like and moderate phenolic, slight yeasty, cloying/sickly sweet	2
Quality Index (QI): 0 – 8 demerit points			

*Typical colour of the spines are purple, black-purple, red-brown, yellow-brown, light brown or olive green (Boudouresque and Verlaque, 2013).

One new parameter associated to the oral area was the peristome, where panellists commented on the position of this structure in the final trials, which was then included in the QIM scheme. The peristome is a sensitive region in the oral area (lower surface) of the sea urchins and it is from here that the mouth opens (Boudouresque and Verlaque, 2013; Harris and Eddy, 2015). In fresh sea urchin the peristome should be predominantly taut and flat (Meloni and Fois, 2013). At days 7 and 8, the peristome assumed concave as well as an imploded position in the different sea urchins evaluated.

In the case of gonads from live sea urchins, the results obtained from its shelf-life establishment, allow to propose an initial QIM scheme (**Table 4.3-4**). However, it was not possible to test this scheme within the frame of the present work once the harvesting season was already finished.

Table 4.3-4 — Proposal of Quality Index Method (QIM) scheme for freshness evaluation of raw gonads from commercial size live *P. lividus* refrigerated at 7 °C.

Parameters	Descriptors	Demerit points
Appearance Shape, apparent firmness, and colour*	Oval shape, distinct halves, very firm, bright colour	0
	Shape still defined, distinct halves, firm, bright colour	1
	Loss of shape, halves still distinct, soft, slight dull	2
	Amorphous, shapeless, very soft, sticky, dull	3
Appearance Graininess	Grainy, evident granules, perfectly individualised	0
	Less evident granules, partially individualised	1
	Flat and smooth surface	2
Odour	Odourless, fresh, slight marine notes (ocean-air)	0
	Slight off-odours	1
	Cloying sweet notes, fruity (non-citrous) rip notes, mildly yeasty	2
	Sulphur, musty, pungent	3
Taste and mouth feeling	Delicate sweet-briny taste, tropical notes, creamy	0
	Slight sweet, bitter notes, slight creamy	1
	Bitter and cloying, slimy, astringent	2
Quality index (QI): 0 – 10 demerit points		

*typical colour of gonads from *Paracentrotus lividus* are orange-yellow (somewhat dependent on sex). Gonads with brown colour are common but were not considered for this sensory scheme because they are not normally accepted for raw consumption.

Given the specificity of the sea urchin within the seafood products, this scheme includes the sensory test in the mouth (taste and mouth feeling), which is quite uncommon in this type of sensory schemes. The rationale for this broader approach is related to its applicability. In case of processing, it is particularly relevant because the taste may be characterised prior processing (e.g. freezing). Thus, a wider quality assessment can be achieved with both schemes.

4 Conclusion

This work allowed to develop, for the first time, two QIM schemes to assess the freshness of live sea urchin *P. lividus* and its gonads stored in a moist environment at 7 °C. For this purpose, specific descriptors were selected, and the evolution of sensory changes followed. It was found that most of the selected descriptors followed the sensory changes (significant linear correlations *vs* storage time). The shelf-life was determined to be up to 4 - 5 days based on the

scores of typical sweet flavour and off-odours/off-flavours. The chemical indicators, namely AEC should not be lower than 45 % in the gonads, corresponding to the chemical limit for the acceptability of live product. Two QIM schemes were proposed: one for live urchins with a total of 8 DP that includes 3 parameters (appearance (spines, oral area) and odour) and another for gonads with 10 DP that includes appearance, odour, taste and mouth feeling. It is important to refine the definition of parameters and descriptors to make it possible to apply these schemes to sea urchins harvested in different seasons and to those from aquaculture. Finally, it is considered that the proposed schemes can be used as a basis for assessing the freshness of other sea urchin species.

Acknowledgements

This research was supported by the CAVIAR project - Market valorisation of sea urchin gonads through dietary modulation (FA_05_2017_015), financed through Fundo Azul programme and by Portuguese Foundation for Science and Technology (FCT) through the strategic projects IDB/04077/2020, UIDB/04423/2020 and UIDP/04423/2020 (CIIMAR). We acknowledge the sensory panel for their availability and commitment to perform the sensory evaluation during consecutive days and weeks in difficult conditions due to covid-19. Carolina Camacho also acknowledges the FCT for the PhD Grant (SFRH/BD/141315/2018).

5

INNOVATION AND VALORISATION OF SEA URCHIN GONAD

5.1 Canning: a traditional solution to promote the valorisation and quality of sea urchin (*Paracentrotus lividus*) gonads

Abstract

Sea urchin (*Paracentrotus lividus*) gonads have been considered a delicacy in Mediterranean cuisine due to their sensory features. However, the short seasonal availability of this species highlights the challenge of valuing gonads that have lower quality. In this context, the aim of this work was to evaluate the effect of canning process, and the addition of a macroalgae (*Porphyra* spp.) on physical, chemical (nutritional), and sensory quality of sea urchin gonads prepared with a light brine under industrial conditions. Furthermore, the stability (shelf-life) of control canned gonads was also evaluated through an accelerated ageing experiment equivalent to 12 months at room temperature. After processing, the contents of protein, K and most of carotenoids decreased, while total lipids, ash, Na and Se levels increased. The addition of macroalgae led to a darker, less smooth orange product having reduced levels of protein, 16:3 *n*-3 fatty acid, cryptoxanthin and increased content of carbohydrates, Na, Se, carotenoids (echinenone, β-carotene and lutein). Ageing led to lower levels of protein, 16:3 *n*-3 and β-carotene, as well as higher Na and lutein contents (compared to the control formulation). The consumption of a 25 g dose of canned gonads by adults contributes to a significant daily intake of protein (8 – 9 %), EPA+DHA (47 - 53 %), I (35 - 62 %) and Se (30 - 47 %). The products (with and without macroalgae) were well accepted by the sensory panel; however, the formulation of those with macroalgae should be slightly adjusted as they were found to be as the saltiest and with affected appearance. Canning is an adequate solution to promote the valorisation of an important species through its consumption throughout the year.

Keywords: macroalgae, heat processing, ageing, macro and micronutrients, carotenoids, sensory properties

This subchapter is under submission:

Camacho, C., Oliveira, H., Serrano, C., Delgado, I., Coelho, I., Pedro, S., Lourenço, H., Bandarra, N.M., Marques, A., Pessoa, M.F., Gonçalves, A., Nunes, M.L. 2023. Canning: a traditional solution to promote the valorisation and quality of sea urchin (*Paracentrotus lividus*) gonads.

Contribution of Carolina Camacho: Investigation, Data curation, Validation, Formal analysis, Visualization, Writing – original draft, review & editing.

1 Introduction

The sea urchin *Paracentrotus lividus* is seasonally harvested in Atlantic and Mediterranean coastal areas (Boudouresque and Verlaque, 2013). Its gonads (or roe) are considered a luxury product, highly appreciated for their unique sensory characteristics (Baião et al., 2021b). However, since the gonads are only available with high quality during a short period throughout the year (from November to March; Rocha et al., 2019), the application of preservation and processing technologies is of utmost importance to extend their shelf-life and meet demand during off-season. Several thermal (freezing and canning) and non-thermal (brine-salting, fermentation, and high pressure) processes have been tested and accepted to valorise such resource, especially those with less quality (coming from low-yield, non-typical colour, and damaged shape gonads). For instance, freezing conducted at low temperatures (-30/-40 ºC) or more often at -18 ºC, allows increased storage periods before noticeable changes on texture and flavour attributes (Tillocca, 2016). Furthermore, drying, salting, brining and fermenting have been the most popular in Japan market (Sun and Chiang, 2015).

After freezing, the second most popular method of processing seafood intended for direct human consumption is canning (Aubourg, 2001; Hall, 2011) and Portugal has a long tradition in the production of such products. In this sense, canning seems a good solution to valorise sea urchin gonads and contribute for the consumption of this product all year round, even in some Portuguese restaurants, where chefs complain about irregular supply throughout the year (Baião et al., 2021a). Although canned sea urchin gonads are rarely sold in stores, some canned or preserved roe can be found in European special retailers with an indication of about 2 years shelf-life (Monfort, 2002).

The effect of canning on physicochemical quality of sea urchin gonads has been assessed by different authors (Cruz-García et al., 2000; De Quirós et al., 2001a, 2001b; Lee et al., 2012). Some studies analysed the protein, amino acids and fatty acids contents (Cruz-García et al., 2000), volatile components (De Quirós et al., 2001a) and carotenoids and liposoluble vitamins levels (De Quirós et al., 2001b) of raw and canned sea urchin gonads from *P. lividus*. Moreover, the physicochemical properties (e.g., contents of water, ash, carbohydrates, fatty acids and minerals – such as K, Na) of steamed and canned gonads of three popular species in Korea (*Anthocidaris crassispina*, *Pseudocentrotus depressus*, *Hernicentrotus pulcherrimus*) have also been tested by Lee et al. (2012). However, such studies did not encompass the acceptance of the product, and sensory changes due to the addition of other ingredients, nor its stability shelf-life.

Despite the popularity of sea urchin gonads in many markets, to the authors' knowledge there is still little information on the impacts of processing on their quality (Bledsoe et al., 2003). In this context, the aim of this study was to: i) develop high quality canned sea urchin (*P. lividus*) gonads and assess its overall quality, stability and acceptance; and ii) evaluate the effect of adding macroalgae on the nutritional value, physical and sensory quality of canned gonads.

2 Materials and methods

2.1 Raw materials

Live sea urchins (*P. lividus*) of commercial size (i.e., test diameter ≥ 5 cm) were harvested at Praia Norte (Viana do Castelo, Northern Portugal) during the winter, corresponding to the time of year when in this area the somatic index is the highest (Rocha et al., 2019b). The wild specimens were transported in polystyrene boxes with cold accumulators and processed within approximately 14 hours. For raw material characterisation, the gonads of twelve live sea urchins were removed and 3 independent pools were randomly constituted with 20 gonads of different specimens). One red macroalgae (*Porphyra* spp. produced by ALGAMAR) was purchased in a Portuguese supermarket (Lisbon) and used as ingredient in the preparation of canned gonad products.

2.2 Canning of gonads

Processing was performed at a Portuguese seafood canning factory with Marine Stewardship Council certification on sustainable seafood. Upon reception, sea urchins were steamed for 10 min (**Figure 5.1-1 A**) and after cooling, the gonads were carefully removed with a stainless-steel spoon. Then, for batch 1 (30 cans; canned gonads in light brine), about 50 g of gonads were randomly distributed in easy-to-open aluminium containers (cylinder shape; mass = 10 g; volume = 80 cm^3, diameter = 7 cm; height = 3 cm) (**Figure 5.1-1 B**) to which 5 mL of a brine (5 % NaCl) was added (corresponding to 0.25 g of salt), the remaining volume was filled with water (filling medium). The batch 2 (15 cans; canned gonads +Macroalgae in light brine) was prepared similarly but with the previous addition of 500 mg of crushed *Porphyra* spp. to the cans.

A) B)

Figure 5.1-1 — Steamed sea urchins (**A**); Gonads in the cans (**B**).

Cans of both batches were hermetically sealed and properly sterilised by heat treatment (121 °C/30 min) according to the common practices of the industrial unit. After cooling and washing, cans were left during a quarantine period at room temperature and then tested according to NP 4404-1 (2002) to assure stability. Then both batches were sent to IPMA laboratories. Fifteen packages of canned gonads (Control cans), and 15 cans with macroalgae (+Macroalgae group) were maintained at room temperature (≈ 20 °C) for 3 months.

2.3 Accelerated ageing experiment

Stability (shelf-life), corresponding to the time of product with the best sensory and microbial quality, was assessed on control batch (canned gonads without macroalgae). For this purpose, 15 packages were stored under heat stress conditions at 40 °C for 3 months, to mimic a 12-month ageing period at 20 °C. The calculation of accelerated aging was based on the Arrhenius equation, which states that an increase of 10 °C at a given temperature doubles the rate of chemical reactions and/or microbiological growth (Labuza and Fu, 1993). With this last procedure, a third group of cans was obtained (referred as "aged group").

2.4 Analyses

The products were assessed after the 3 months of storage. Nine packages of each canned products (Control, +Macroalgae, Aged) were used in stability and sterility tests. The remaining were utilised for sensory, physical, and chemical analyses.

2.4.1 Stability and sterility testing

The stability test was performed according to the procedure of Portuguese standard NP 4404-1 (2002). Briefly, this test consists in the evaluation of the i) package (external examination), ii) visual and odour of the content, and iii) pH (pH meter 539, WTW, Weilheim, Germany)

after a 7-day period at three different temperatures (≈ 20 °C, 37 °C and 55 °C). Canned products sterility was determined following Portuguese standard NP 2309-2 (1988) for low-acid food, which involves the detection of aerobic and anaerobic mesophiles and thermophiles, at 37 °C and 55 °C, respectively.

2.4.2 Chemical analyses

Homogenized samples (N = 3 pools of raw gonads or packages of canned products) were stored at -80 °C, then freeze-dried (24 h at -45 °C under low pressure ≈ 10132.5 Pa; Power Dry 150 LL3000, Heto, Czech Republic), powdered by means of mortar and pestle and kept at -80 °C until further chemical analysis. Proximate composition, fatty acids profile, macro (Na and K) and micro (I and Se) elements levels and carotenoids content, were determined in both raw materials (sea urchin gonads and macroalgae) and in the canned products (Control, +Macroalgae, Aged). Calculations were made to present data in wet weight.

2.4.2.1 Proximate composition

Moisture content was determined by mass difference through freeze-drying process. The ash content was quantified by incineration of freeze-dried sample in a muffle furnace (Heraeus, Thermo Scientific), for 16 h at 500 ± 25 °C according to standard procedure (AOAC, 2005). The protein level was determined through the combustion of nitrogen (Dumas method; Saint-Denis and Goupy, 2004) using an automatic analyser (FP-528 DSP LECO, St. Joseph, USA). The nitrogen released after combustion (850 °C) was quantified using EDTA calibration curve and converted into protein using the usual factor (6.25) for animal proteins (FAO, 2003). The total lipids was quantified following the Folch method (Folch et al., 1957). This procedure comprised the homogenization of sample in a mixture of chloroform:methanol (2:1), followed by the addition of an acid (HCl 0.1 N) and a saline solution ($MgCl_2$ at 0.5 %), contributing for the protein precipitation and to promote the lipid migration to the organic phase (lower phase after separation). Afterwards, the lower phase was carefully collected. A second step of extraction was conducted and both organic phases were combined and evaporated to dryness. Results given in $g \cdot (100\ g)^{-1}$ ww.

2.4.2.2 Fatty acids

The determination of fatty acid methyl esters (FAME) was performed according to Lepage and Roy (1986) procedure with modifications (Bandarra et al., 1997; Cohen et al., 1988), through acid-catalysed transesterification. Briefly, each sample (approximately 200 mg dw) and 50 µL

of an internal standard solution (C23:0 at 10 mg·mL^{-1}) were homogenised in 5 mL acetyl chloride-methanol solution (1:19 v/v, Merck, Darmstadt, Germany) (in a 15 mL tube) and heated in a water bath for 1 h at 80 °C. After cooling, 1 mL of Milli-Q water and 2 mL of n-heptane (99.5 % Merck, Darmstadt, Germany) were added. Transesterified sample extracts were separated through centrifugation (2300 *g*, 5 min, 5 °C; Sigma 3 K30, Germany) after being shaken. The upper phase was gently collected, and moisture content was removed with anhydrous sodium sulphate (99.0 %, Panreac, Darmstadt, Germany). About 2 µL was injected into a gas chromatograph (Bruker Scicon 456, Livingston, UK) equipped with an auto sampler. The separation of FAME was performed in a polyethylene glycol capillary column (30 m length × 0.25 mm internal diameter, 0.25 µm thickness; Stabilwax-MS) with helium as a carrier gas, programmed to start at 180 °C, held for 5 min, then raised to 220 °C at 4 °C min^{-1}, and maintained at 220 °C for 25 min. Detection was done on a flame ionization detector at 250 °C and the identification were accomplished by retention times comparison using standards: Supelco PUFA No.1 (Marine Source, 99 %-Ref. 47033) and PUFA No.3 (Menhaden oil 99 %-Ref. 47085-U). Data on fatty acid (FA) profile were calculated in mg·(100 g)$^{-1}$ ww of gonads using the peak area ratio (% of total fatty acids).

2.4.2.3 Macro and micro elements

Potassium (K) and sodium (Na) were analysed based on the method described by Jorhem (2000) through flame atomic absorption spectrophotometry (Spectr AA 55B spectrophotometer, Agilent, Santa Clara, USA) with a background deuterium correction. The concentrations were calculated using linear calibration obtained from absorbance measurements of at least, five different concentrations of standard solutions (KNO_3 and $NaNO_3$, both dissolved in 0.5 M HNO_3). Results given in mg·(100 g)$^{-1}$ ww.

Iodine (I) and selenium (Se) were determined by inductively coupled plasma mass spectrometer (ICP-MS) (Thermo X series II, Thermo Fisher Scientific, Waltham, USA), after acid digestion (Se) or alkaline extraction (I). Se content was quantified using the European standard procedure EN 15763:2009 (CEN, 2009). Briefly, about 200 mg of sample (dw) was weighed into 50 mL polypropylene DigiTUBEs (SCP Science, Quebec, Canada) and digested overnight in 7 mL of nitric acid (60 % ultrapure w/w). Then, digestion continued in a 48-well heating block (DigiPREP, SCP Science, Courtaboeuf, France) for 3.5 h at 85 °C after adding 1 mL of hydrogen peroxide (30 % w/w, Merck). After cooling, the digests were diluted up to 25 mL with MilliQ water and kept at 5 ± 3 °C until ICP-MS analysis (Coelho et al., 2019). Quantification of I was performed separately, following the European Standard procedure EN 15111:2007 (CEN, 2007).

For each replicate, about 200 mg of sample (dw) was weighed into 50 mL of polypropylene DigiTUBEs (SCP Science, Quebec, Canada) and extracted with tetramethylammonium hydroxide (TMAH; 25 %, Fluka, St. Gallen, Switzerland) in MilliQ water (1:8 ratio) using a 48-well heating block (DigiPREP, SCP Science, Courtaboeuf, France) for 3 h at 90 °C. After cooling, the extracts were diluted up to 50 mL with MilliQ water and centrifuged at 10 000 rpm (15 min at 20 °C). The supernatant was filtered through a 0.45 µm syringe filter (Millipore) before ICP-MS analysis (Delgado et al., 2019). Quantification was attained by linear calibration using solutions prepared from single-element high-purity ICP stock standards for Se (SCP Science, Marktoberdorf, Germany) and I (Inorganic Ventures, Christiansburg, Virginia), ranging between 0.5 – 5 $\mu g \cdot g^{-1}$ and 1 – 50 $\mu g \cdot g^{-1}$, respectively. Results given in $mg \cdot kg^{-1}$ ww.

2.4.2.4 Carotenoids content

The method to analyse carotenoids was adapted from Abdel-Aal et al. (2007) and Serrano et al. (2017). Briefly, carotenoids (two technical replicates of 200 mg each) were extracted twice with 2 mL of water-saturated *n*-butanol, by means of stirring (30 s at 2236 *g*), followed by 15 min in an orbital shaker and then left to rest for 60 min. Then the mixture was centrifuged (5 min at 10 000 *g*) and the supernatant was collected, after filtration through a 0.22 µm syringe filter (PET-20/25). All the procedures were performed whenever possible in the absence of light. The extracts were kept at -80 °C until further analysis. The extracts (20 µL) were analysed on a HPLC-PDA workstation system (2795, Waters, USA) equipped with a photodiode array detector (2996, Waters, USA) and a degassed inline system. The detector slider and diode width were 2.4 nm and the response time was 1 s. The wavelength was monitored between 250 and 750 nm. The chromatographic separation was achieved on a Carotenoid C30 reversed phase column (4.6 mm × 100 mm, 5 µm particle size; YMC, Kyoto) at 35 °C in an oven (Waters, USA), using a flow rate of 0.6 $mL \cdot min^{-1}$. The mobile phase was methanol/water (75:25, v/v, eluent A) and ethyl acetate (eluent B), the elution gradient was as follows: 0-25 min,70 % A and 30 % B; 25-50 min, 45 % A and 55 % B; 50-60 min 10 % A and 90 % B; 60-70 min, 70 % A and 30 % B. Data acquisition and processing were performed using the Millenium 3.2 software. The identification of the unknown carotenoids was performed by retention times comparison and ultraviolet spectra at 450 nm with the following reference compounds: astaxanthin, lutein, zeaxanthin, cryptoxanthin, echinenone and β-carotene. Quantification of carotenoids was based on external calibration using linear regression analysis. The calibration plots, relating signal (peak area) to concentration, yielded linear equations: astaxanthin ($y = 288135x – 17845$; $R^2 = 0.99$), lutein ($y = 396469x – 73833$; $R^2 = 0.91$), zeaxanthin ($y = 418259x – 232053$;

R^2 = 0.97), cryptoxanthin (y = 380658x – 59301; R^2 = 0.98), echinenone (y = 247681x – 2E6; R^2 = 0.99) and β-carotene (y = 67263x – 6072.9; R^2 = 0.99). Results given in $\mu g \cdot g^{-1}$ ww.

2.4.3 Physical analyses

Instrumental colour and texture were evaluated in canned products, and colour was also determined in raw gonads.

2.4.3.1 Colour

Colour was measured using the model MACBETH COLOUR-EYE 3000 colorimeter (Macbeth, New Windsor, NY, USA) at standard illuminant D65 and 2-degree observer, previously calibrated with a white standard tile (L^* = 98.0; a^* = -0.3; b^* = 2.4). The L^*, a^* and b^* coordinates from CIELAB system were recorded. In this system, L^* values denote lightness on a scale of 0 (black) to 100 (white); the a^* values describe the intensity from green (-) to red (+); and the b^* values from blue (-) to yellow (+). The chroma (saturation; C^*) was determined according to the following equation (Kalinowski et al., 2005): $C^*_{a,b} = (a^{*2} + b^{*2})^{1/2}$. The colour difference was determined through Euclidean distance between two colour stimuli in CIELAB space (Schubring, 2009; Sharma and Bala, 2017): $\Delta E^*_{a,b} = [(L^* - L^*_s)^2 + (a^* - a^*_s)^2 + (b^* - b^*_s)^2]^{1/2}$. This colour distance was calculated after canning (raw gonads as standard product, L^*_s, a^*_s, b^*_s), after the addition of macroalgae and after ageing (control canned gonads as standard product, L^*_s, a^*_s, b^*_s).

2.4.3.2 Texture

Texture profile analysis (TPA, double compression test) was carried out on a TA.XTplus analyser and software (Stable Micro Systems, Surrey, UK) using a 5 kg load cell. The individualized gonads were compressed twice up to 50 % of the original height with a cylindrical probe of 50 mm diameter (P50) and applying a constant speed of 1 $mm \cdot s^{-1}$. The time between compressions was 5 s. The primary characteristics (i.e., hardness, N; springiness, %; and adhesiveness, $g \cdot cm^{-1}$) were obtained.

2.4.4 Sensory evaluation

The sensory analysis was performed after 3 months of storage, which corresponds to the time necessary to obtain the typical palatability of most canned seafood products (Aubourg, 2001), and after verifying the results of stability and sterility tests. The three canned products (Control, +Macroalgae and Aged) were assessed in a sensory laboratory equipped according to international standard ISO 8589 (2007) through a quantitative descriptive method used by

five trained panellists. The cans were open 15 min before testing and one or two gonads were presented to each panellist in white coded plates.

The panellists were asked to use the line scale (12 cm; with marks at the ends and in the middle: 0 – absent; 6 – moderate; 12 – extreme) provided in the evaluation forms to evaluate their perceived intensity of the selected attribute/descriptor: odour (typical of canned seafood, marine, algae, fresh, uncharacteristic/off-odour), appearance (colour, firmness, shape, graininess), flavour (typical of canned seafood, sweet, salty, acid, bitter, tropical), texture (firmness, succulence, adhesivity) and mouth sensations (astringency, sweetness, saltiness). The panel was also encouraged to make additional comments.

An acceptance test of the three canned products (Control, +Macroalgae and Aged) was also performed. This test was attended by 32 frequent consumers of canned seafood products. The assessment was focused on appearance, odour, taste, texture and overall acceptance using a 5-point hedonic scale (0 – dislike very much, 1 – dislike slightly; 2 – neither like nor dislike; 3 – like slightly; 4 – like very much).

2.5 Nutritional contribution

The nutritional contribution (NC) of canned sea urchin gonads was calculated based on protein, EPA + DHA, macro (Na and K) and micro (I and Se) elements, considering a portion of 25 g since this product is usually consumed as a side dish, appetizer or even used as an addition to give flavour and texture to several recipes (Baião et al., 2021a; Sun and Chiang, 2015). The dietary reference values (DRVs) recommended by European Food Safety Authority (EFSA, 2016, 2017, 2019) were considered and the following formula was used:

NC (%) = 100 × (C × M)/AI, where C = mean concentration of the nutrient ($mg \cdot kg^{-1}$); M = weight of the defined portion (kg); and AI = adequate intake ($mg \cdot day^{-1}$).

2.6 Statistical analyses

Data were tested for normality of distribution and homogeneity of variances using Kolmogorov–Smirnov and Levene's tests, respectively. The effect of canning (i.e., the comparison between raw and control canned gonads) on the analysed parameters (except texture) was tested by the Student's *t*-test. The impact of the macroalgae addition and aging on the assessed parameters was tested by analysis of variance (one-way ANOVA), followed by post-hoc one-sided Dunnett test (+Macroalgae and Aged groups: lower or higher compared to Control group). Statistical analysis was performed using the STATISTICA™ software, version 7.0 (StatSoft Inc., USA) with significance set at $\alpha = 0.05$ (p-value < 0.05) for all tests (Zar, 2014).

3 Results and discussion

3.1 Stability and sterility testing

The results of stability and sterility tests are shown in **Table 5.1-1**. External visual examination of the package did not reveal any physical damage, nor leakage of the filling media in both canned products stored for 3 months at room temperature or aged ones (those that mimics a 12-month storage at room temperature). The appearance and odour of the sea urchin gonads were characteristic of a canned product. The differences in pH between cans maintained at the three different temperatures (≈ 20, 37 and 55 °C) were less than 0.5. Additionally, no microbial growth was observed under the specified conditions of the tests (**Table 5.1-1**).

Table 5.1-1 — Summary of stability and sterility tests performed after 3 months of storage in all canned sea urchin gonads (in light brine).

Test	Storage temperature (°C)	Range/limit[1,2]	Results
Stability[1]			
Package examination[3]	≈ 20*	-	Conforming/Normal (at ≈ 20, 37 and 55 °C)
	40#	-	Conforming/Normal (at ≈ 20, 37 and 55 °C)
Content examination[4]	≈ 20*	-	Conforming/Normal (at ≈ 20, 37 and 55 °C)
	40#	-	Conforming/Normal (at ≈ 20, 37 and 55 °C)
pH	≈ 20*	5.5 – 6.5	6.36 - 6.37 (at 37 and 55 °C)
	40#	5.5 – 6.5	6.36 - 6.37 (at 37 and 55 °C)
Sterility[2]	≈ 20*	Negative	Negative, sterile products (at ≈ 20, 37 and 55 °C)
	40#	Negative	Negative, sterile products (at ≈ 20, 37 and 55 °C)

*Groups with and without macroalgae; #Group without macroalgae; [1]According to NP 4401-1 (2002); [2]According to NP 2309-2 (1988); [3]Physical damages and leakages; [4]Appearance (colour and texture) and odour examination.

The results of the microbiological analyses were negative for thermophilic and mesophilic aerobes and anaerobes. Based on these results, it was considered that the products had commercial stability and sterility for at least 3 (canned gonads with macroalgae) and 12 months (canned gonads without macroalgae) after processing, so it was decided to proceed with the remaining analyses.

3.2 Proximate composition

Thermal processing techniques or culinary processes can affect the composition of seafood products, leading to the losses of nutrients, breakdown of some constituents with further

interactions, depending on the intrinsic characteristics of the product, the methods used, as well as the exposure time and temperature (Aubourg, 2001; Barbosa et al., 2021; Oliveira et al., 2019).

The chemical composition of raw materials and canned products can be found in **Table 5.1-2**. In general, the values obtained for raw gonads are in agreement with those reported by other studies with wild *P. lividus* (Dincer and Cakli, 2007; Rocha et al., 2019b).

Table 5.1-2 — Proximate composition of raw materials (sea urchin gonads and macroalgae) and canned gonads (in light brine).

Parameter, g·(100 g)$^{-1}$ ww	Raw materials		Canned sea urchin gonads		
	Gonads	Macroalgae[1]	Control[2]	+ Macroalgae[2]	Aged[3]
Moisture	73.8 ± 0.5	9.5 ± 0.3	73.1 ± 0.9	72.8 ± 0.3	72.3 ± 0.3
Protein	17.0 ± 0.1*	36.7 ± 0.6	15.7 ± 0.6* $^{a; A}$	13.1 ± 0.1^{b}	14.7 ± 0.4^{B}
Total lipids	4.1 ± 0.2*	1.4 ± 0.2	5.1 ± 0.3*	4.7 ± 0.2	5.0 ± 0.4
Ash	2.5 ± 0.1*	10.2 ± 0.6	3.6 ± 0.1* ^{a}	5.5 ± 0.0^{b}	3.7 ± 0.2
Carbohydrates[4]	2.6 ± 0.5	42.3 ± 1.0	3.1 ± 0.5^{a}	4.2 ± 0.3^{b}	3.8 ± 0.4

Results are given as mean values ± standard deviation (n = 3). For each parameter, values assigned with asterisk (*) indicate significant differences (*t*-Student test, $p \leq 0.018$); values assigned with different small (a-b) and capital letters (A-B) indicate significant lower or higher values than the control (Dunnett one-side test, $p \leq 0.040$).
[1]Dried macroalgae; [2]Three months of storage at ≈ 20 °C and at [3]40 °C; [4]Calculated by difference.

The moisture content in fresh gonads was ≈ 73 g·(100 g)$^{-1}$, without significant differences ($p = 0.334$, *t*-Student test; **Table 5.1-2**) after canning. Indeed, like in the present study, the moisture content of canned tuna using water as a filling medium was equivalent to the raw material (Mol et al., 2008a). With oil as filling medium the water losses can vary in the range of 9 – 28 % (Aubourg, 2001).

On the other hand, total lipids, protein, and ash contents changed significantly with the processing ($p < 0.019$, *t*-Student test; **Table 5.1-2**). The increase of total lipids may be due to the water loss stimulated by pre-cooking and heat processing. Moreover, the ethereal extract presented an orange colour, suggesting that other compounds (pigments such as carotenoids which are fat soluble; Pither, 2003), besides crude fat, were co-extracted and consequently the total lipid content observed in the gonad samples may have been overquantified. The protein decrease can be due to three possible reasons: solubilisation during pre-cooking, diffusion into

filling medium and heat denaturation during thermal processing (Sampels, 2015). Regarding ash, the increase can be ascribed to the NaCl added in the light brine (Estevez et al., 2021).

Other works mentioned slight alterations after canning of gonads: higher moisture in *P. lividus* (Cruz-García et al., 2000), *A. crassispina* and *P. depressus* (Lee et al., 2012); and lower protein and lipid levels in the three mentioned species (Cruz-García et al., 2000; Lee et al., 2012). With steaming, the slight alterations observed in gonads are generally the opposite of canning (Lee et al., 2012).

The aged canned gonads differ significantly from the control canned gonads in terms of protein (lower content, $p = 0.024$, Dunnett one-side test). The lower amounts of protein found may be due to an increased solubilisation during the ageing step (Sampels, 2015).

The incorporation of macroalgae induced changes in protein, ash, and carbohydrates levels ($p < 0.024$; **Table 5.1-2**). The increment of ash and carbohydrates content may be related precisely to the addition of this ingredient rich in such components (**Table 5.1-2**; Circuncisão et al., 2018). The lower protein value may be ascribed to the higher solubilisation of this macronutrient due to the high ionic strength in the gonads and in the filling medium (brine).

3.3 Fatty acids profile

A total of 38 FAs were identified in the samples. The main FA of the raw and canned sea urchin gonads are displayed in **Table 5.1-3**. The polyunsaturated FA (PUFA) were the most abundant in the gonads (46 – 50 %), followed by monounsaturated FA (MUFA, 27 - 29 %) and saturated FA (SFA, 14 - 17 %). Identical profiles of FA groups were found in the three canned gonads.

Table 5.1-3 — Main fatty acids (FA) composition of raw materials (sea urchin gonads and macroalgae) and canned gonads (in light brine).

Fatty acid,	Raw materials		Canned sea urchin gonads		
mg·(100 g)$^{-1}$ ww	Gonads	Macroalgae[1]	Control[2]	+ Macroalgae[2]	Aged[3]
14:0	40.26 ± 10.46	4.46 ± 0.26	60.40 ± 19.66	63.46 ± 12.57	88.41 ± 11.48
16:0	176.65 ± 34.97	179.52 ± 12.03	256.01 ± 77.39	263.29 ± 39.25	334.91 ± 40.60
18:0	50.71 ± 4.19*	5.57 ± 0.58	71.75 ± 9.77*	63.83 ± 6.37	82.34 ± 6.06
20:0	19.01 ± 1.02	0.79 ± 0.08	25.11 ± 5.17	23.31 ± 4.03	28.68 ± 2.43
Σ SFA	311.42 ± 52.68	258.97 ± 15.90	449.32 ± 116.76	428.60 ± 62.30	578 ± 64.12
18:1 *n*-9	30.60 ± 5.62	14.97 ± 0.96	50.76 ± 14.78	55.75 ± 8.18	65.48 ± 9.97
18:1 *n*-7	56.28 ± 8.75	13.07 ± 0.89	76.03 ± 21.85	79.24 ± 8.69	91.53 ± 12.90
20:1 *n*-11	132.64 ± 21.78*	---	191.51 ± 23.75*	169.82 ± 12.22	200.86 ± 20.93
20:1 *n*-9	170.27 ± 12.75	82.82 ± 3.80	219.28 ± 41.01	213.91 ± 12.03	238.85 ± 26.39
20:1 *n*-4	101.00 ± 26.34	---	155.89 ± 33.30	149.61 ± 13.27	165.34 ± 16.35
22:1 *n*-9	82.81 ± 6.97*	13.46 ± 0.42	110.06 ± 9.55*	105.49 ± 6.51	114.06 ± 10.18
Σ MUFA	608.50 ± 81.70	125.23 ± 6.05	852.50 ± 160.18	824.49 ± 66.48	937.45 ± 103.18
16:3 *n*-3	68.17 ± 9.64	---	62.13 ± 5.17$^{a; A}$	37.05 ± 4.17^{b}	45.36 ± 3.27^{B}
18:4 *n*-3	79.80 ± 11.28	3.31 ± 0.26	109.30 ± 31.78	111.77 ± 11.00	136.69 ± 21.72
20:3 *n*-3	64.19 ± 6.50	3.71 ± 0.21	78.63 ± 11.04	72.24 ± 2.05	84.33 ± 9.29
20:4 *n*-3	55.06 ± 6.85	16.43 ± 0.62	65.45 ± 13.17	57.55 ± 4.07	75.98 ± 8.24
20:5 *n*-3 (EPA)	354.62 ± 26.47	884.54 ± 38.07	460.22 ± 56.77	450.44 ± 24.68	504.81 ± 48.35
22:6 *n*-3 (DHA)	15.65 ± 1.33*	---	23.66 ± 2.15*	21.45 ± 1.91	21.62 ± 3.21
Σ n-3 PUFA	697.31 ± 59.02	910.34 ± 39.21	877.79 ± 126.67	824.89 ± 50.85	959.07 ± 98.78
20:2 *n*-6	49.89 ± 4.34*	47.22 ± 2.45	66.34 ± 4.51*	58.45 ± 1.85	68.33 ± 5.92
20:4 *n*-6 (ARA)	314.42 ± 23.24*	27.44 ± 1.05	411.86 ± 29.24*	364.80 ± 21.24	442.03 ± 40.29
Σ *n*-6 PUFA	398.81 ± 30.02*	109.37 ± 1.81	531.39 ± 42.09*	474.64 ± 27.47	570.73 ± 50.28
Σ PUFA	1101.56 ± 88.92	1020.14 ± 38.46	1418.96 ± 165.16	1308.02 ± 78.02	1539.94 ± 148.74
Σ *n*-3 PUFA/Σ *n*-6 PUFA	1.75 ± 0.05	8.33 ± 0.43	1.65 ± 0.16	1.74 ± 0.04	1.68 ± 0.05

Results are given as mean values ± standard deviation (n = 3); For each parameter, values assigned with asterisk (*) indicate significant differences (*t*-Student test, $p \leq 0.049$); values assigned with different small (a-b) and capital letters (A-B) indicate significant lower or higher values than the control (Dunnett one-side test, $p \leq 0.048$).

[1]Dried macroalgae; [2]Three months of storage at ≈ 20 °C and at [3]40 °C; Σ SFA – sum of saturated FA; Σ MUFA – sum of monounsaturated FA; Σ *n*-3 PUFA – sum of omega-3 polyunsaturated FA; Σ *n*-6 PUFA - Sum of omega-6 polyunsaturated FA; Σ PUFA - sum of all polyunsaturated FA; EPA – eicosapentaenoic acid; DHA – docosahexaenoic acid; ARA – arachidonic acid.

In raw gonads, EPA was the most abundant (355 mg·(100 g)$^{-1}$) within PUFA, followed by ARA (314 mg·(100 g)$^{-1}$). The FAs 16:3 *n*-3, 18:4 *n*-3, 20:3 *n*-3 and 20:4 *n*-3 were found in levels between 55 and 80 mg·(100 g)$^{-1}$ and the remaining below 29 mg·(100 g)$^{-1}$ (**Table 5.1-3**). The most abundant MUFA were 20:1 *n*-9 (170 mg·(100 g)$^{-1}$), followed by 20:1 *n*-11 (133 mg·(100 g)$^{-1}$). All other MUFA were found in levels equal to or less than 101 mg·(100 g)$^{-1}$. Concerning SFA, 16:0 was the one with the highest concentration (177 mg·(100 g)$^{-1}$) in raw gonads. Such profile is in line with what has been reported by other authors, although in different proportions for some FA groups (Angioni and Addis, 2014; Arafa et al., 2012; Volpe et al., 2018).

In general, the individual FA levels were not significantly influenced by sterilisation and storage. However, some exceptions were found in the values of SFA (18:0), MUFA (20:1 *n*-11 and 22:1 *n*-9), PUFA (22:6 *n*-3, 20:2 *n*-6, 20:4 *n*-6) and Σ *n*-6 PUFA, after processing ($p \leq 0.049$, *t*-Student test). In other studies with canned sea urchin gonads, a general absence of changes or a significant decrease in some FA, specially SFA, were reported (Cruz-García et al., 2000). In studies conducted on canned sardine, herring, mackerel (Hale and Brown, 1983), tuna (Aubourg et al., 1990) and crab (Giddings and Hill, 1975), changes in in FA concentrations due to heat processing were also not found.

Ageing did not induce significant changes ($p > 0.05$) in the concentrations of most FA, which indicates good stability up to 12-month storage period at 20 °C. Similarly, no significant differences ($p > 0.05$) were found between the +Macroalgae formulation and the control, which can be attributed to the reduced amount of macroalgae added, which in turn contained very low levels of total lipids (**Table 5.1-2**), but high PUFA concentrations (**Table 5.1-3**). It is also noteworthy, that the ratio Σ *n*-3/Σ *n*-6 (around 1.7) was not affected ($p > 0.05$) by canning, ageing and macroalgae addition (**Table 5.1-3**).

3.4 Macro and micro elements

Concentrations of macro and micronutrients are presented in **Table 5.1-4**. The mean Na and K contents of the raw gonads were 270 and 430 mg·(100 g)$^{-1}$, respectively. The concentration of the macro element Na significantly increased 3.9-fold after canning ($p < 0.0001$, *t*-Student test), due to the salt (i.e., NaCl) addition in the product preparation; while K significantly decreased ($p < 0.0001$, *t*-Student test) (**Table 5.1-4**).

Table 5.1-4 — Concentrations of macro and micro elements of raw materials (sea urchin gonads and macroalgae) and canned gonads (in light brine).

Elements	Raw materials		Canned sea urchin gonads		
	Gonads	Macroalgae[1]	Control[2]	+ Macroalgae[2]	Aged[3]
Macro, mg·(100 g)$^{-1}$ ww					
K[4]	434.3 ± 25.8*	3899.4 ± 69.4	266.8 ± 21.6*	244.3 ± 34.7	292.6 ± 5.1
Na[5]	267.3 ± 22.6*	1262.0 ± 148.1	1050.7 ± 26.8* [a; A]	1427.5 ± 27.9[b]	1134.7 ± 20.0[B]
Micro, mg·kg^{-1} ww					
I[6]	2.1 ± 0.4	12.5 ± 0.4	2.9 ± 0.5	3.7 ± 0.6	2.1 ± 0.3
Se[7]	0.29 ± 0.03*	1.74 ± 0.25	0.84 ± 0.05* [a]	1.32 ± 0.10[b]	0.84 ± 0.18

Results are given as mean values ± standard deviation (n = 3); For each parameter, values assigned with asterisk (*) indicate significant differences (*t*-Student test, $p < 0.001$); values assigned with different small (a-b) and capital letters (A-B) indicate significant lower or higher values than the control (Dunnett one-side test, $p \leq 0.005$).
[1]Dried macroalgae; [2]Three months of storage at ≈ 20 °C and at [3]40 °C; [4]Detection limit = 0.01 mg·kg^{-1}; Certified reference material = Dorm-4, fish protein (National Research Council of Canada, Canada); Certified value (average ± uncertainty) = 15500 ± 1000 mg·kg^{-1}; Present work (average ± standard deviation) = 14500 ± 495 mg·kg^{-1}; Certified reference material = LUTS-1, non-defatted lobster hepatopancreas (National Research Council of Canada, Canada); Certified value (average ± uncertainty) = 948 ± 72 mg·kg^{-1}; Present work (average ± standard deviation) = 791 ± 0 mg·kg^{-1}. [5]Detection limit = 0.09 mg·kg^{-1}; Proficiency Test = FAPAS Test 01120, Nutritional Components in Canned Meat, January–March 2018 (Fera Science Ltd., York, UK); Certified value (average ± uncertainty) = 0.60 ± 0.03 mg·kg^{-1}; Present work (average ± standard deviation) = 0.55 ± 0.02 mg·kg^{-1}. [6]Detection limit = 0.07 μg·g^{-1}; Certified reference material = ERM®-BB422, fish muscle (Joint Research Centre (JRC), Brussels); Certified value (average ± uncertainty) = 1.40 ± 0.40 μg·g^{-1}; Present work (average ± standard deviation) = 1.10 ± 0.04 μg·g^{-1} (79 % recovery). [7]Detection limit = 0.03 μg·g^{-1}; Certified reference material = ERM®-BB422, Fish muscle (Joint Research Centre (JRC), Brussels); Certified value (average ± uncertainty) = 1.33 ± 0.13 μg·g^{-1}; Present work (average ± standard deviation) = 1.33 ± 0.18 μg·g^{-1} (105 % recovery).

The fluctuations in the macro elements with canning are generally caused by interactions between the foodstuff and the filling medium (Pither, 2003). In the case of Na, there was probably an uptake from the light brine, and in the case of K, a leaching of about 38 % to the filling medium. This last element is particularly susceptible to leaching in canned products with recorded losses between 15 and 50 % in the case of canned vegetables (Pither, 2003). Both increases and decreases in some macro elements (Na, K, Ca, and Cu) contents were also reported by other authors after seasoning and canning of gonads from three different sea urchin species (Lee et al., 2012). The addition of macroalgae led to a significant increase of the Na content ($p < 0.0001$, Dunnett one-sided test), while K level remained like control canned gonads. A similar trend on the content of these elements was registered with ageing (**Table 5.1-4**). Thus,

it is difficult to point out a general trend on the effect of canning on these elements' levels. It is important to mention that the Na/K ratio in canned products was above the reference threshold recommended by WHO (< 1) for maintaining cardiovascular health (Whelton, 2014). Given that the consumption of this product is not frequent, this figure does not compromise its consumption.

The concentrations of the micronutrients I and Se in the raw gonads were 2.1 and 0.29 $mg \cdot kg^{-1}$, respectively (**Table 5.1-4**). and processing induced significant increases in Se content ($p < 0.0001$, *t*-Student test). For I, the values found in the control group were higher than those observed in the raw gonads, although this difference was not significant ($p > 0.05$). Previous studies suggest a primary binding of Se and I to proteins (Hou, 2009; Vicente-Zurdo et al., 2019), which consequently can make these elements less susceptible to be leached during processing (including the use of the heat). In addition, higher I and Se contents after steaming and boiling were reported by other authors, who linked such trends to a moisture loss (Barbosa et al., 2021; Oliveira et al., 2019), a change not observed in the present study.

The obtained results show that canning and ageing did not significantly ($p > 0.05$) influence the levels of micronutrients I and Se (≈ 2.1 $mg \cdot kg^{-1}$ and 0.84 $mg \cdot kg^{-1}$ for I and Se, respectively) naturally present in the gonads.

The addition of the macroalgae (+Macroalgae group) led to a significantly higher concentration of Se in comparison to control canned gonads (about 50 %) ($p = 0.0024$; Dunnett one-sided test). The I content found in the +Macroalgae group was higher, but not significantly different ($p = 0.0829$ Dunnett one-sided test) from that observed in the control formulation.

3.5 Nutritional contribution

One side effect of canning can be a shift in the nutritional value of seafood depending on the filling medium (Sampels, 2015). In the case of canned gonads in light brine, the protein contribution could be almost 10 % of the daily intake for adults with a portion of 25 g (**Table 5.1-5**), which constitutes a valuable contribution for tissue maintenance (EFSA, 2017). The level of *n*-3 FA in canned products is quite relevant, providing between 47 (+Macroalgae group) to 53 % (Aged group) of EPA+DHA (**Table 5.1-5**), an extremely important contribution in the prevention of cardiovascular diseases (Sheppard and Cheatham, 2018). Thus, products prepared with sea urchin roe can be recommended as an additional and interesting source of polyunsaturated FA.

Table 5.1-5 — Nutritional contribution (%) of canned sea urchin gonads (in light brine), considering a portion of 25 g.

	DRVs[1] (mg·day^{-1})	Canned sea urchin gonads		
		Control[2]	+ Macroalgae[2]	Aged[3]
Proximate composition				
Protein	41778[4]* (AR)	9.4 ± 0.4$^{a, A}$	7.8 ± 0.1^{b}	8.8 ± 0.3^{B}
***n*-3 fatty acids**				
EPA+DHA	250[4] (AI)	48.4 ± 5.7	47.2 ± 2.7	52.6 ± 4.9
Macro elements				
K	3500[4] (AI)	1.9 ± 0.2	1.7 ± 0.3	2.1 ± 0.1
Na	2000[4,5] (AI)	13.1 ± 0.3$^{a, A}$	17.8 ± 0.3^{b}	14.2 ± 0.2^{B}
Micro elements				
I	0.15[4] (AI)	49.0 ± 8.4	61.6 ± 9.6	34.7 ± 4.8
Se	0.07[4] (AI)	30.1 ± 1.7^{a}	47.1 ± 3.4^{b}	30.1 ± 6.2

Values are means ± standard deviation (n = 3). For each parameter, values assigned with different small (a-b) and capital letters (A-B) indicate significant lower or higher values than the control (Dunnett one-side test, $p \leq 0.024$). [1]The Dietary Reference Values (DRVs) given as average requirements (AR) or adequate intakes (AI) are presented for adults (women/men); [2]Three months of storage at ≈ 20 °C and at [3]40 °C; [4]EFSA (2017); [5]EFSA (2019); *Reference body weight for adults (63.3 kg, mean of the values for males and females; EFSA, 2017) was considered to calculate DRV.

Regarding the macro elements, the contribution of canned products is less pronounced, ≈ 2 % of K and ≈ 15 % of Na of daily intake for adults (**Table 5.1-5**), corresponding to a very low amount in relation to the limit of 5 g NaCl (equivalent to a 2 g of Na) recommended by EFSA (2019).

A higher NC was detected for microelements, specifically for I (between 35 and 62 % in Aged and +Macroalgae groups, respectively). The consumption of 25 g-dose of control and Aged canned gonads contributes around 30 % of daily Se intake for adults. The formulation with macroalgae can provide up to 47 % of this micronutrient (**Table 5.1-5**). The health effects associated with the consumption of foods rich in these elements (such as canned gonads) are related to the structural and functional tasks on the thyroid hormones in the case of iodine. Selenium is essential in the protection against organs degeneration (EFSA, 2017).

All selenium and iodine values obtained in canned products reached > 30 % of the nutrient reference value (NRV) (70 and 150 µg)·day^{-1} defined by EFSA (2017). Hence, these data support the claim that all canned gonads are "high in selenium" and "high in iodine" (European Parliament, 2011, 2006). Thus, these products can be valuable options for target groups that have special Se and I needs (e.g., for pregnant women that have special I needs for increased

thyroid hormone production to cover maternal and foetal needs; EFSA, 2017). Additionally, the consumption of a dose of 25 g of all canned gonads had I values far below the Upper Limit (600 μg·day^{-1}) defined for adults including pregnant and lactating women (EFSA, 2006).

3.6 Carotenoids

Carotenoids are pigments with great importance in foods since colour is one of the first quality criteria (e.g. Baião et al., 2021b; Rocha et al., 2019). Their levels in raw materials and canned products can be found in **Table 5.1-6**. Four carotenoid pigments were identified in the gonads: echinenone, β-carotene, lutein and cryptoxanthin.

Table 5.1-6 — Carotenoids concentrations of raw materials (sea urchin gonads and macroalgae) and canned gonads (in light brine).

Carotenoids	Raw materials		Canned sea urchin gonads		
(μg·g^{-1} ww)	Gonads	Macroalgae[1]	Control[2]	+ Macroalgae[2]	Aged[3]
Echinenone[4]	51.3 ± 2.9*	n.d.	27.3 ± 3.0* ^{a}	41.8 ± 0.9^{b}	26.4 ± 8.0
β-carotene[5]	7.8 ± 1.1*	15.1 ± 3.4	4.5 ± 0.4* $^{a; A}$	8.8 ± 0.9^{b}	< 3.3 (LQ)^{B}
Lutein[6]	1.6 ± 0.7	n.d.	0.5 ± 0.1$^{a; A}$	2.4 ± 0.2^{b}	1.2 ± 0.4^{B}
Cryptoxanthin[7]	2.3 ± 0.3*	n.d.	1.1 ± 0.0* ^{a}	0.9 ± 0.1^{b}	1.0 ± 0.0
Zeaxanthin[8]	n.d.	11.6 ± 1.9	n.d.	n.d.	n.d.

Results are given as mean values ± standard deviation (n = 3). For each parameter, values assigned with asterisk (*) indicate significant differences (*t*-Student test, $p \leq 0.008$); values assigned with different small (a-b) and capital letters (A-B) indicate significant lower or higher values than the control (Dunnett one-side test, $p \leq 0.043$).
[1]Dried macroalgae; [2]Three months of storage at ≈ 20 °C and at [3]40 °C; [4]Detection and quantification limits = 1.9 and 5.9 μg·g^{-1}, respectively; [5]Detection and quantification limits = 1.1 and 3.3 μg·g^{-1}, respectively; [6]Detection and quantification limits = 3×10^{-3} and 9×10^{-3} μg·g^{-1}, respectively; [7]Detection and quantification limits = 0.1 and 0.3 μg·g^{-1}, respectively; [8]Detection and quantification limits = 0.2 and 1.1 μg·g^{-1}, respectively; Abbreviations: n.d. – not detected; LQ – quantification limit.

In the raw gonads, echinenone was the predominant carotenoid (51.3 μg·g^{-1}), followed by β-carotene (7.8 μg·g^{-1}), cryptoxanthin (2.3 μg·g^{-1}) and lutein (1.6 μg·g^{-1}). The zeaxanthin was only found in the macroalgae. The dominance of the echinenone is in accordance with what was previously reported for the *P. lividus* species (Rocha et al., 2019b; Symonds et al., 2007). Other pigments such as echinochrome A (EchA), a quinone with pharmaceutical properties, can also be found in sea urchins (Rubilar et al., 2021).

Canned gonads (Control group) exhibited lower carotenoid's levels (echinenone, β-carotene and cryptoxanthin; $p < 0.008$, *t*-Student test) than the raw gonads, except in the case of

lutein (p = 0.061, t-Student test). Similarly, De Quirós et al. (2001b) registered a 20 – 30 % decrease in the carotenoids levels (except for echinenone) after canning. The reduction of the carotenoids levels was quite expectable since these compounds are typically damaged, oxidised or even susceptible to isomerisation through heat processing and low pH, as is the case of canning (Pither, 2003).

Significant changes with ageing were observed in the case of β-carotene (decrease) and lutein (increase) (p < 0.008, Dunnett one-sided test). In the case of lutein, changes can be attributed to the intrinsic variability of the raw materials. The decrease in the β-carotene may be due to the displacement of the equilibrium between the cis and trans forms of β-carotene, that can be easily isomerized into cis-isomers, the less stable form (Khoo et al., 2011).

The addition of macroalgae seems to contribute to the slowdown of carotenoids degradation induced by heat treatment. The carotenoids concentrations (except in the case of cryptoxanthin) were significantly higher in the +Macroalgae group in relation to control (p < 0.044, Dunnett one-sided test). This result can be attributed to the great antioxidant activity of the macroalgae *Porphyra* spp. (Supawong et al., 2022).

3.7 Colour and texture

The colour and texture parameters of raw and canned products are shown in **Table 5.1-7**.

Table 5.1-7 — Colour and texture of raw and canned sea urchin gonads (in light brine).

Parameter	Raw material (gonads)	Canned sea urchin gonads		
		Control[1]	+ Macroalgae[1]	Aged[2]
Colour				
L^* (lightness)	64.3 ± 1.5	64.5 ± 0.2[a]	62.8 ± 0.2[b]	64.6 ± 0.4
a^* (green (-) to red (+) colour)	5.4 ± 0.6	6.7 ± 0.7[a]	5.1 ± 0.6[b]	7.3 ± 0.4
b^* (blue (-) to yellow (+) colour)	9.2 ± 0.7	9.6 ± 0.5[a]	8.3 ± 0.8[b]	9.6 ± 0.2
$C^*_{a,b}$ (Chroma, saturation)	10.7 ± 0.9	11.7 ± 0.8[a]	9.7 ± 1.0[b]	12.1 ± 0.2
$\Delta E^*_{a,b}$ (colour difference)	---	1.9 ± 0.4[#]	3.2 ± 0.6[$]	1.0 ± 0.4[¥]
Texture[3]				
Hardness (N)	---	0.78 ± 0.19[a]	1.12 ± 0.37[b]	0.56 ± 0.18
Adhesiveness (g·cm^{-1})	---	-0.004 ± 0.001	-0.010 ± 0.004	-0.006 ± 0.003
Springiness (%)	---	72.6 ± 6.4	75.3 ± 3.1	69.2 ± 5.0

Results are given as mean values ± standard deviation (n = 3 for colour; n = 9 for texture). For each parameter, values assigned with different small letters (a-b) indicate significant lower or higher values than the control (Dunnett one-side test, $p \leq 0.023$). For colour parameters, no significant differences were found between raw and control canned gonads (*t*-Student test, $p \geq 0.085$).

[1]Three months of storage at ≈ 20 °C and at [2]40 °C; [3]It was not possible to evaluate the texture parameters in the raw material. [#]small difference between raw and control canned gonads (effect of canning); [$]just noticeable colour difference between control and +Macroalgae groups (effect of macroalgae addition); [¥]small difference between control and aged groups (effect of ageing).

The colour parameters (L^* - lightness; a^* - redness; b^* - yellowness; and $C^*_{a,b}$) were not significantly affected by processing ($p > 0.085$, *t*-Student test), which is quite unexpected since the compounds strictly linked with colour i.e., the carotenoid pigments, were deteriorated due to heat processing (section 3.6), a quality change usually associated with product discoloration (Pither, 2003). Still, the Euclidean distance showed a value of 1.9 (**Table 5.1-7**) between the raw material and canned gonads, which is a small difference, associated to heat. Additionally, it is known that the colour depends on samples' dimension and shape which can influence the colour determination (Schubring, 2009).

The colour of the canned gonads was not influenced by ageing ($p > 0.085$ for L^*, a^*, b^* and C* parameters, Dunnett one-sided test), with a small colour difference of 1.0 (**Table 5.1-7**). On the other hand, the macroalgae addition significantly changed all the colour parameters analysed ($p < 0.022$, Dunnett one-sided test). Specifically, the L^*, a^* and b^* values decreased, turning the gonads less bright and with intense orange colour. Consequently, the incorporation of macroalgae to the cans promoted a reduction in the colour saturation. The colour difference

was 3.2 which falls within the criteria of a noticeable colour difference between control and +Macroalgae groups (**Table 5.1-7**).

It was not possible to evaluate the texture properties of raw gonads, but in general it was observed that canned gonads presented higher firmness and lower springiness than raw ones from the same area, harvested during five seasons (Rocha et al., 2019b). Indeed, it seems that the texture of seafood is susceptible to be affected by sterilisation process (Pither, 2003; Sampels, 2015). For instance, Hui et al. (2006) mentioned that the oxidation of proteins that may occur after a thermal process can cause protein cross-linking and thus tissue toughening. However, only few studies reported colour and texture changes due to processing in roe products (Farag et al., 2021).

The texture parameters were not affected by ageing ($p > 0.08$). In turn, the hardness was affected by macroalgae addition ($p = 0.023$). Such change may be due to the salt addition (higher concentrations of Na were found in this group of canned gonads; section 3.4), which is considered a technological ingredient, capable to solubilize proteins and to form a cohesive network. Moreover, the compounds presented in the macroalgae may interact with the surface of the gonads during the heat treatment. Indeed, a macroalgae of the genus *Porphyra* spp. presents high swelling and water holding capacity when in flaked form (Supawong et al., 2022), whose may affect the textural properties of processed foods when added as ingredient.

3.6 Sensory analysis

The main results of the descriptive test performed with the three canned products (in light brine) are presented in **Figure 5.1-2**.

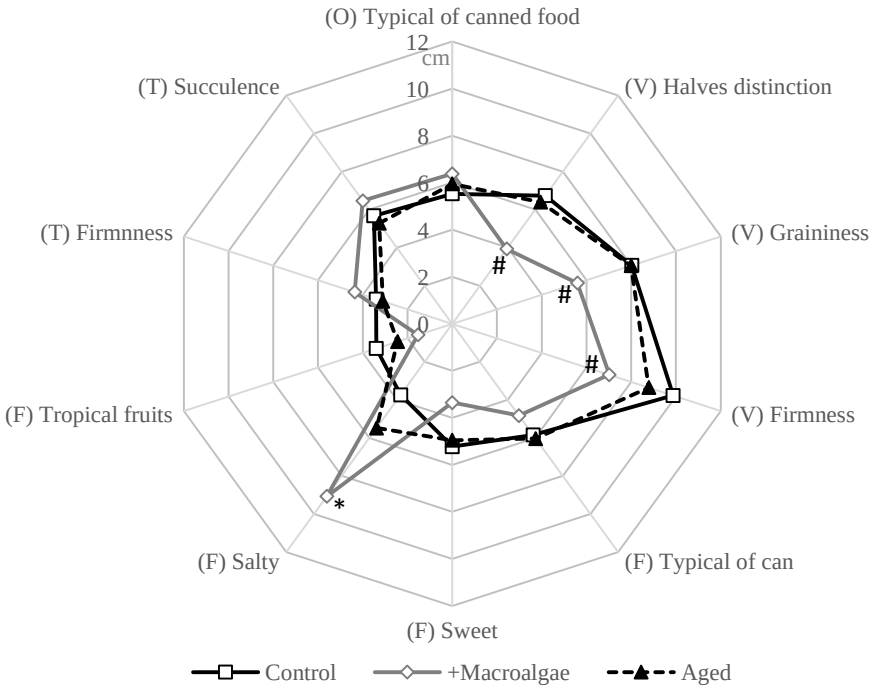


Figure 5.1-2 — Sensory profile of canned sea urchin gonads (in light brine).

Tests performed after 3 months of storage at ≈ 20 °C (Control and +Macroalgae groups) and 40 °C (Aged group); Results correspond to mean values (0.1 ≤ SD ≤ 2.0; N = 5 assessors);
12-cm scale was used: 0-absent, 6-moderate, 12-extreme; (O) – odour;
(V) - visual assessment; (F) – flavour/taste; (T) – texture in the mouth;
*indicates significant higher value in relation to Control (p = 0.0002; Dunnett one-sided test);
#indicates significant lower value in comparison to Control (p < 0.0218; Dunnett one-sided test).

The panel identified the products as having a moderate odour typical of canned food odour (5.5 – 6.4 cm; **Figure 5.1-2**); but almost absent in what regards sea and algae odours (≈ 1 cm). In terms of visual evaluation, the addition of macroalgae affected the appearance of canned gonads, making the halves distinction, graininess, and firmness significantly lower in relation to control formulation (p < 0.0218 Dunnett one-sided test). The control canned gonads were scored as having moderate intensity (≈ 5 cm) of sweet taste, which remained with ageing. The addition of macroalgae slightly decreased (but not significantly) the intensity of sweet taste. Concerning the salty taste, the classification given ranged between 3.7 and 5.5 cm in the control and aged canned gonads, respectively. In turn, the macroalgae addition induced a significant increase of the salty taste (p = 0.0002; Dunnett one-sided test), being classified as strong by the panel (≈ 9 cm; **Figure 5.1-2**). Indeed, the Na content in the formulation with macroalgae was highest in comparison to control canned gonads (**Table 5.1-4**).

Both formulations (with and without macroalgae addition) and ageing did not influence the tropical fruit flavour nor the texture in the mouth (measured through firmness and

succulence; Figure 5.1-2). The astringency was absent in all canned gonads. The off-odours/off-flavours were negligible (only one panellist mentioned a detectable oxidised odour and an acid/sour taste in all tested products).

Consumers also gave it a significantly lower hedonic rate to the appearance of gonads with macroalgae compared to the control formulation (p = 0.0160, Dunnett one-sided test) (Table 5.1-8).

Table 5.1-8 — Sensory evaluation (hedonic test) of canned sea urchin gonads (in light brine).

Attributes	Canned sea urchin gonads		
	Control[1]	+ Macroalgae[1]	Aged[2]
Appearance	3.7 ± 0.8	3.2 ± 1.0*	3.5 ± 0.8
Odour	3.7 ± 0.7	3.3 ± 1.0	3.6 ± 0.6
Flavour	3.7 ± 1.0	3.3 ± 1.0	3.7 ± 1.0
Texture	3.7 ± 0.9	3.6 ± 1.0	3.5 ± 0.9
Overall acceptance	3.6 ± 0.9	3.3 ± 1.0	3.7 ± 1.0

Results are given as mean values ± standard deviation (N = 32 consumers of canned seafood);
*indicates significant lower value in relation to Control (p = 0.0160, Dunnett one-sided test).
[1]Three months of storage at ≈ 20 °C and at [2]40 °C.

Some consumers reported a high salt content in the formulation with macroalgae, as verified by the descriptive test (Figure 5.1-2). Thus, in general, the products were well accepted by consumers (Table 5.1-8), although the formulation with macroalgae should be refined to improve some attributes and descriptors namely the appearance and salty taste. Other authors examined that pasteurisation of several seafood roes (sturgeon caviar and roes-producing species) is associated to sensory changes (Bledsoe et al., 2003; Farag et al., 2021).

4 Conclusion

The results obtained allow to conclude that canning did not significantly affect the contents of moisture, carbohydrates, most of the fatty acids, iodine and lutein, nor the colour of gonads. However, the process caused a reduction in protein, potassium and carotenoids (echinenone, β-carotene and cryptoxanthin) contents and the concentration of total lipids, sodium and selenium levels. The canned gonads were commercially stable up to 12 months (evaluated by accelerated ageing) with slight changes on the nutritional profile, such as lower protein, 16:3 *n*-3 fatty acid and β-carotene contents, and higher sodium and lutein levels.

The incorporation of macroalgae led to a decrease in levels of 16:3 *n*-3 fatty acid and cryptoxanthin and, on the other hand, to a rise in carbohydrate, sodium, selenium and carotenoids (echinenone, β-carotene and lutein) contents. Additionally, the +Macroalgae group presented less smooth texture (although not perceived by the sensory panel) and darker orange colour compared to the control formulation.

It is also important to highlight that the consumption of a 25 g-portion of any of the canned sea urchin gonads contributes to meet the daily requirement of nutrients beneficial to adult health, such as protein (8 – 9 %), EPA+DHA (47 – 53 %), iodine (35 – 62 %; highest contribution in +Macroalgae formulation) and selenium (30 – 47 %; highest percentage in +Macroalgae group). For sodium, the contribution is 13 – 18 %.

Sensory evaluation indicated good acceptance for all canned gonads (with and without macroalgae); suggesting that they could be interesting alternatives for consumers who do not appreciate raw gonads and for off-season consumption and culinary applications. The +Macroalgae formulation should be adjusted slightly to improve appearance and reduce sodium content and perceived saltiness.

Acknowledgements

This study was carried out in the scope of "BLUE BIOECONOMY PACT" (Project Nº. C644915664-00000026), co-funded by Next Generation EU European Fund, under the incentive line "Agendas for Business Innovation" within Component 5 - Capitalization and Business Innovation of the Portuguese of the Recovery and Resilience Plan (RRP) and CAVIAR project - Market valorisation of sea urchin gonads through dietary modulation (FA_05_2017_015) (financed through Fundo Azul programme) also contributed with financial support. The authors acknowledge the Portuguese Foundation for Science and Technology (FCT) through the strategic projects IDB/04077/2020, UIDB/04423/2020 and UIDP/04423/2020 (CIIMAR). Carolina Camacho also acknowledges the FCT for the PhD Grant (SFRH/BD/141315/2018). Finally, the authors are grateful for the collaboration of the industrial unit.

6

Concluding remarks and future perspectives

The current chapter aims to summarise and combine the main findings of Chapters 3 to 5, present the answers to each of the questions proposed in Chapter 1, linking the general and specific objectives previously defined.

The studies carried out within the framework of this thesis have made a valuable contribution to the knowledge of wild *Paracentrotus lividus* of the Portuguese coast from the quality and valorisation point of view. Important topics were covered with special emphasis on chemical and emerging contaminants; freshness evaluation, sensory and physical-chemical traits of the gonads, the only edible part of this product. This thesis constitutes a valuable contribution in the scientific field and for more informed decision making by the different stakeholders. The most relevant outcomes are: i) better knowledge of the risks associated with the consumption of wild sea urchins (**Chapter 3**); ii) new insights on the effect of transport and preservation on sea urchin quality (**Chapter 4**); iii) potential of canning to valorise urchins caught out of season, obtaining stable and appealing products from a sensory and nutritional point of view (**Chapter 5**).

In the field of biology, the sea urchin *P. lividus* is considered a very annoying species, bending or debunking several theories (Boudouresque and Verlaque, 2013). This perspective is corroborated by food technologists and quality managers since the processing and quality assessment of the species are challenging tasks, as partially demonstrated by the present work.

To date, few studies have reported quality changes in this important species. This study is of major interest since the sea urchin is a niche product, highly valued for the unique sensory features of its gonads.

6.1 Answers to the research questions

1. Is the sea urchin from Portuguese coast safe for consumption?

In terms of chemical contamination, the answer is not a complete yes due to Cd levels, especially of urchins coming from Carreço area. The levels of toxic elements (e.g., As, Cd, Pb, Hg, iAs) were always below the maximum levels allowed in foodstuffs, with minimal risk to consumer health. Overall, it can be concluded that an occasional consumption or a regular serving of sea urchin gonads (up 50 g) does not pose a chemical risk to consumers.

A total of 99 emerging and persistent organic contaminants (butyltins, BTs; polycyclic aromatic hydrocarbons, PAHs; pesticides including pyrethroids; pharmaceuticals and personal

care products, PCPs; and flame retardants; **Subchapter 3.1**) and macro and trace elements (including non-nutritive; **Subchapter 3.2**) were assessed in the gonads of *P. lividus* of highly exploited areas along the NW Atlantic coast of Portugal (Carreço, Praia Norte and Vila Chã). The non-detected or measurable in low levels of persistent and emerging contaminants allow to verify the chemical quality of gonads. Nonetheless, a body of evidence was achieved on the link between anthropogenic pressures of the studied areas and the presence of some contaminants in the edible part of urchins. This is in line with previous studies that showed that urchin effectively bioaccumulate and is considered a biomonitor species (e.g., Bouiba et al., 2023; Cunha et al., 2005; El Idrissi et al., 2020).

Regarding microbiological contaminations, despite not have been an output of the present work, it can be considered that the urchins from Portuguese coastal areas were safe for consumption. According to the IPMA, I.P., the *E. coli* counts were irrelevant, in general less than 18 MPN·(100 g)$^{-1}$, in sea urchin gonads during the last 5 years of a monitoring program (IPMA, 2023).

In conclusion, sea urchin caught off the Northwest Portuguese coast is safe for human consumption and a weekly consumption of 50 g of gonads (usually as a starter) provides an important contribution of iodine (70 %), phosphorus (34 %), selenium (20 %) and iron (19 %). In this scenario the risk for consumers (see **Table 3.2-3** in **Subchapter 3.2**) is very low.

2. Does transport conditioning affect the quality of sea urchins?

Yes, it does. The transport conditioning affects the quality of live sea urchins at 7 °C (in a moist environment) and should not exceed 4 - 5 days. Both bulk and individual compartments led to a change in its appearance and odour. Nonetheless, the bulk should be avoided to minimise mass loss (more 3 % at day 6) (**Subchapter 4.1**). The study pioneered the use of biomarkers tools in the gonads to complement the quality assessment in a transport simulation trial. The antioxidant system of sea urchins indicates some oxidative stress with cell damage (and ultimately affected animal welfare), with special emphasis on day 6, which followed the degradation of external appearance and odour. The freshness of sea urchin gonads is significantly influenced by lipid oxidation by degrading flavour, colour, odour, texture, and presence of hazardous chemicals (Ning et al., 2022).

3. Which quality changes occurred during refrigerated storage of the live sea urchin *P. lividus*?

In terms of chemical alterations, the nucleotides change through 11 days of storage at 7 °C were clear in the gonads from commercial size live sea urchin trials. The adenylate energy charge (AEC) was the most adequate indicator to assess the vitality and freshness of live sea urchin preserved at 7 °C, presented a linear and negative trend throughout the storage period. The *K*-value, an index that is usually used to evaluate the extension of nucleotides degradation, was generally low, not allowing a distinction of freshness grades. No noticeable changes in FAA concentrations were recorded through storage. However, the effect of maturation stage was verified with higher contribution of the FAA related to sweet (glycine, alanine) taste and lower expected contributions for bitterness (arginine, lysine, histidine and valine) and umami (glutamic acid).

Regarding sensory changes, the upright position of spines as well as the gonads odour (sweet and marine less developed), the specific sweet taste as well as slight off-odours/off-flavours were the most relevant attributes to discriminate the freshness of sea urchin.

4. How can the freshness of sea urchins be characterised and assessed?

The freshness of live sea urchin *P. lividus* can be characterised through its appearance: active or reactive and bright spines which are very firms and upright positioned. The peristome in the oral area mostly flat; mouth centered and closed (or reactive). The overall odour and specially on the oral area is slightly marine (ocean-air), clean seaweed, iodine-like.

Regarding the gonads, very fresh ones are oval-shaped, allowing to distinguish the halves, firms in appearance with typical orange-yellow.

The freshness of sea urchin is a key attribute to ensure greater economic value and facilitate commercialisation. To date, few studies reported quality changes in this species. In the present work, a quality index method (QIM) was developed for commercial size live sea urchin *P. lividus* and respective gonads for the first time, allowing to assess its freshness. One QIM was devoted to live urchins with a total of 8 demerit points (DP) that includes 3 parameters (spines, oral area and odour; **Table 4.3-3**, **pp. 150**) and another for gonads with 10 DP that includes appearance, odour, taste and mouth feeling (**Table 4.3-4**, **pp. 151**).

5. Which is the shelf-life of live sea urchins?

The shelf-life of commercial size live *P. lividus* stored at 7 °C (in a moist environment) was estimated to be 4 - 5 days based on sensory criteria. The sensory quality was compromised on day 6 when the sensory panel rejected the gonads.

6. Which changes occur in canned sea urchin gonads? Are the canned gonads accepted by consumers?

In this work, canning process was applied to the gonads from *P. lividus*. The processing caused a reduction in protein, potassium and carotenoids contents and an increase of total lipids, sodium (due to the added light brine) and selenium levels. The canned gonads were commercially stable up to 12 months (evaluated by accelerated ageing) with slight changes on the nutritional profile. One batch with incorporation of macroalgae (*Porphyra* spp.) led to a rise in carbohydrate, sodium, selenium and carotenoids contents, a less smooth texture and darker orange colour.

The canned products (with and without macroalgae) were highly accepted (58 % in the category "like very much") by consumers, being the marine flavour the most appreciated attribute.

6.2 Major outcomes and recommendations

The results of the present thesis highlight important considerations of sea urchin quality dimensions and as such will provide useful information for the future aquaculture and processing of the species. A summary of the main valuable findings to valorise the fishery are, as follows:

- The sea urchins from Portuguese coast are chemical safe for consumption;
- The elements profile of the sea urchin gonads from the highly explored South West Atlantic areas was Cl> K>P >Ca >S>Zn>Br>Fe>Sr>I>Rb>Cu>Se> Cr>Ni;
- Bulk transport and storage should be avoided to minimise animal stress, mass losses, overall quality losses, and consequent decrease of commercial value;
- Transport and commercialisation of live sea urchin should not exceed 4 - 5 days at 7 °C;
- A single dose of 25 g of the canned product contribute to significant daily intake of protein (up 9 %), EPA+DHA (53 %), iodine (62 %), and selenium (47 %);

- Canning allows the consumption of sea urchin gonads off season. It is a viable solution mainly for niche markets and induce a major interest in echinoculture development, being an alternative route when there is no flow of fresh product. However, it should be noted that this is a different product from fresh one, although with similar sensory attributes and descriptors;
- Canning is a good solution to valorise gonads that are less appealing (size and colour) for fresh consumption (raw). The quality of the product is generally maintained, being sterile and stable after 12 months;
- QIM schemes to assess freshness of live sea urchin (**Table 4.3-3**, **pp. 150**) and respective gonads (**Table 4.3-4**, **pp. 151**).

6.3 Future perspectives

The results of this work allow to highlight areas that require further research, particularly in:

- Studies on transport and commercialisation techniques (such as immersion in aerated salt water), in order to avoid stress and delay quality changes in live sea urchin;
- Adjustment of the QIM devoted to gonads from live sea urchin; refinement and application of both QIM schemes, particularly with regards to the establishment of parameters and descriptors, as well as the potential adaptation of the developed schemes to sea urchins from the aquaculture sector or even to other species;
- Impact of adding ingredients (e.g., with health promoting properties) or adjusting (reducing) sodium content on stability, sensory and nutritional characteristics of canned gonads;
- Factors affecting the stability and quality properties of sea urchin gonads during frozen storage;
- Development of sea urchin products (e.g., sauces, salad portions);
- Understanding consumer motivation and preferences towards sea urchin gonads;
- Development of solutions to valorise the whole resource, including waste (cellular tissues, tests and spines) for sustainability reasons and "zero waste" trends.

References

Abdel-Aal, E.-S.M., Young, J.C., Rabalski, I., Hucl, P., Fregeau-Reid, J., 2007. Identification and quantification of seed carotenoids in selected wheat species. Journal of Agricultural and Food Chemistry 55, 787–794. https://doi.org/10.1021/jf062764p

Abdel-Shafy, H.I., Mansour, M.S.M., 2016. A review on polycyclic aromatic hydrocarbons: source, environmental impact, effect on human health and remediation. Egyptian Journal of Petroleum 25, 107–123. https://doi.org/10.1016/j.ejpe.2015.03.011

Abe, H., Yoshikawa, N., Sarower, M.G., Okada, S., 2005. Physiological function and metabolism of free D-alanine in aquatic animals. Biological and Pharmaceutical Bulletin 28, 1571–1577. https://doi.org/10.1248/bpb.28.1571

Ablanedo, N., González, H., Ramírez, M., Torres, I., 1990. Evaluacion del erizo de mar *Echinometra lucunter* como indicador de contaminacion por metales pesados, Cuba. Aquatic living resources 3, 113–120.

Agnello, M., 2017. Introductory Chapter: Sea Urchin-Knowledge and Perspectives, in: Agnello, M. (Ed.), Sea Urchin - From Environment to Aquaculture and Biomedicine. pp. 1–16. https://doi.org/10.5772/intechopen.70415

Ahn, I.-Y., Ji, J.Y., Park, H., 2009. Metal accumulation in sea urchins and their kelp diet in an Arctic fjord (Kongsfjorden, Svalbard). Marine Pollution Bulletin 58, 1571–1577. https://doi.org/10.1016/j.marpolbul.2009.07.013

Alamdari, D.H., Kostidou, E., Paletas, K., Sarigianni, M., Konstas, A.G.P., Karapiperidou, A., Koliakos, G., 2005. High sensitivity enzyme-linked immunosorbent assay (ELISA) method for measuring protein carbonyl in samples with low amounts of protein. Free Radical Biology and Medicine 39, 1362–1367. https://doi.org/10.1016/j.freeradbiomed.2005.06.023

Alasalvar, C., Grigor, J., Ali, Z., 2011a. Practical evaluation of fish quality by objective, subjective, and statistical testing, in: Alasalvar, C., Shahidi, F., Miyashita, K., Wanasundara, U. (Eds.), Handbook of Seafood Quality, Safety and Health Applications. Wiley-Blackwell, Oxford, pp. 13–28.

Alasalvar, C., Shahidi, F., Miyashita, K., Wanasundara, U., 2011b. Seafood quality, safety, and health applications: an overview, in: Alasalvar, C., Shahidi, F., Miyashita, K., Wanasundara, U. (Eds.), Handbook of Seafood Quality, Safety and Health Applications. Wiley-Blackwell, Oxford, pp. 1–10.

Ali, M.M., Ali, M.L., Islam, M.S., Rahman, M.Z., 2016. Preliminary assessment of heavy metals in water and sediment of Karnaphuli River, Bangladesh. Environmental Nanotechnology, Monitoring & Management 5, 27–35.

Alishahi, A., Aïder, M., 2012. Applications of Chitosan in the Seafood Industry and Aquaculture:

A Review. Food and Bioprocess Technology 5, 817–830. https://doi.org/10.1007/s11947-011-0664-x

Aminot, Y., Belles, A., Alary, C., Readman, J.W., 2017. Near-surface distribution of pollutants in coastal waters as assessed by novel polyethylene passive samplers. Marine Pollution Bulletin 119, 92–101. https://doi.org/10.1016/j.marpolbul.2017.03.022

Amri, S., Samar, M.F., Sellem, F., Ouali, K., 2017. Seasonal antioxidant responses in the sea urchin *Paracentrotus lividus* (Lamarck 1816) used as a bioindicator of the environmental contamination in the South-East Mediterranean. Marine Pollution Bulletin 122, 392–402. https://doi.org/10.1016/j.marpolbul.2017.06.079

Anacleto, P., Maulvault, A.L., Barrento, S., Mendes, R., Nunes, M.L., Rosa, R., Marques, A., 2013. Physiological responses to depuration and transport of native and exotic clams at different temperatures. Aquaculture 408–409, 136–146. https://doi.org/10.1016/j.aquaculture.2013.05.035

Andrew, N.L., Agatsuma, Y., Ballesteros, E., Bazhin, A.G., Creaser, E.P., Barnes, D.K.A., Botsford, L.W., Bradbury, A., Campbell, A., Dixon, J.D., 2002. Status and management of world sea urchin fisheries, in: Gibson, R.N., Barnes, M., Atkinson, R.J.A. (Eds.), Oceanography and Marine Biology: An Annual Review. CRC Press, pp. 343–425.

Angioni, A., Addis, P., 2014. Characterization of the lipid fraction of wild sea urchin from the Sardinian Sea (western Mediterranean). Journal of Food Science 79, C155–C162. https://doi.org/10.1111/1750-3841.12330

Angioni, A., Cau, A., Secci, M., Addis, P., 2014. GC–ITMS analysis of PAH contamination levels in the marine sea urchin *Paracentrotus lividus* in Sardinia. Marine Pollution Bulletin 82, 201–207. https://doi.org/10.1016/j.marpolbul.2014.02.031

Angioni, A., Porcu, L., Secci, M., Addis, P., 2012. QuEChERS method for the determination of PAH compounds in Sardinia Sea Urchin (*Paracentrotus lividus*) Roe, using gas chromatography ITMS-MS analysis. Food Analytical Methods 5, 1131–1136. https://doi.org/10.1007/s12161-011-9353-7

Antwi, F.B., Reddy, G.V.P., 2015. Toxicological effects of pyrethroids on non-target aquatic insects. Environmental Toxicology and Pharmacology 40, 915–923. https://doi.org/10.1016/j.etap.2015.09.023

AOAC, 2007. Pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulphate. Journal of AOAC International 90, 17–26.

AOAC, 2005. Official Methods of Analysis of the Association of Official Analytical Communities International, 18th ed. Washington DC.

APA, 2015. Plano de Gestão de Região Hidrográfica: parte 2 – Caracterização e Diagnóstico. Região Hidrográfica do Minho e Lima (RH1).

APA, 2012a. Plano de gestão da região hidrográfica do Minho e Lima: Relatório Técnico – Comissão Europeia.

APA, 2012b. Plano de Gestão da Região hidrográfica do Cávado, Ave e Leça: Relatório Técnico – Comissão Europeia.

Arafa, S., Chouaibi, M., Sadok, S., El Abed, A., 2012. The influence of season on the gonad index and biochemical composition of the sea urchin *Paracentrotus lividus* from the Golf of Tunis. The Scientific World Journal 2012. https://doi.org/10.1100/2012/815935

Archana, A., Babu, K.R., 2016. Nutrient composition and antioxidant activity of gonads of sea urchin *Stomopneustes variolaris*. Food Chemistry 197, 597–602.

https://doi.org/10.1016/J.FOODCHEM.2015.11.003

Archer, E., Petrie, B., Kasprzyk-Hordern, B., Wolfaardt, G.M., 2017. The fate of pharmaceuticals and personal care products (PPCPs), endocrine disrupting contaminants (EDCs), metabolites and illicit drugs in a WWTW and environmental waters. Chemosphere 174, 437–446. https://doi.org/10.1016/j.chemosphere.2017.01.101

Atkinson, D.E., 1968. Energy charge of the adenylate pool as a regulatory parameter. Interaction with feedback modifiers. Biochemistry 7, 4030–4034. https://doi.org/10.1021/bi00851a033

Aubourg, S.P., 2001. Loss of quality during the manufacture of canned fish products. Food Science and Technology International 7, 199–215. https://doi.org/10.1106/4H8U-9GAD-VMG0-3GLR

Aubourg, S.P., Sotelo, C.G., Gallardo, J.M., 1990. Changes in flesh lipids and fill oils of albacore (*Thunnus alalunga*) during canning and storage. Journal of Agricultural and Food Chemistry 38, 809–812. https://doi.org/10.1021/jf00093a047

Augier, H., Ramonda, G., Rolland, J., Santimone, M., 1989. Teneurs en metaux lourds des oursins comestibles *Paracentrotus lividus* (Lamarck) preleves dans quatre secteurs tests du littoral de Marseille (Mediterranee, France). Vie Marine 10, 226–239.

Azad, A.K., Pearce, C.M., McKinley, R.S., 2011. Effects of diet and temperature on ingestion, absorption, assimilation, gonad yield, and gonad quality of the purple sea urchin (*Strongylocentrotus purpuratus*). Aquaculture 317, 187–196. https://doi.org/10.1016/j.aquaculture.2011.03.019

Aznar-Alemany, Ò., Trabalón, L., Jacobs, S., Barbosa, V.L., Tejedor, M.F., Granby, K., Kwadijk, C., Cunha, S.C., Ferrari, F., Vandermeersch, G., 2017. Occurrence of halogenated flame retardants in commercial seafood species available in European markets. Food and Chemical Toxicology 104, 35–47. https://doi.org/10.1016/j.fct.2016.12.034

Baião, L.F., Moura, A.P., Rocha, C., Valente, L.M.P., Cunha, L.M., 2021a. Dimensions for the valorisation of sea urchin (*Paracentrotus lividus*) gonads production through the eyes of experienced chefs. International Journal of Gastronomy and Food Science 26, 100438. https://doi.org/10.1016/j.ijgfs.2021.100438

Baião, L.F., Oliveira, A.S., Gonçalves, A., de Pinho, P.G., Valente, L.M.P., Cunha, L.M., 2020. Analysis of volatile compounds in *Paracentrotus lividus* by HS-SPME/GS-MS and relation to its sensorial properties. LWT 130, 109629. https://doi.org/10.1016/j.lwt.2020.109629

Baião, L.F., Rocha, C., Lima, R.C., Marques, A., Valente, L.M.P., Cunha, L.M., 2021b. Sensory profiling, liking and acceptance of sea urchin gonads from the North Atlantic coast of Portugal, aiming future aquaculture applications. Food Research International 140, 109873. https://doi.org/10.1016/j.foodres.2020.109873

Baião, L.F., Rocha, F., Costa, M., Sá, T., Oliveira, A., Maia, M.R.G., Fonseca, A.J.M., Pintado, M., Valente, L.M.P., 2019. Effect of protein and lipid levels in diets for adult sea urchin *Paracentrotus lividus* (Lamarck, 1816). Aquaculture 506, 127–138. https://doi.org/10.1016/j.aquaculture.2019.03.005

Baião, L.F., Rocha, F., Sá, T., Oliveira, A., Pintado, M., Lima, R.C., Cunha, L.M., Valente, L.M.P., 2022. Sensory profiling, liking and gonad composition of sea urchin gonads fed synthetic or natural sources of β-carotene enriched diets. Aquaculture 549, 737778. https://doi.org/10.1016/j.aquaculture.2021.737778

Baião, L.F.L., 2021. Characterization of the sensory profile of sea urchin aiming at the selection

of premium gonads. Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto.
Bandarra, N.M., Batista, I., Nunes, M.L., Empis, J.M., Christie, W.W., 1997. Seasonal changes in lipid composition of sardine (*Sardina pilchardus*). Journal of Food Science 62, 40–42. https://doi.org/10.1111/j.1365-2621.1997.tb04364.x
Banks, E.R. (Ed.), 2014. Sea Urchins: Habitat, Embryonic Development and Importance in the Environment, Marine Biology. Nova Science Publishers, Inc., New York.
Barbosa, V., Maulvault, A.L., Alves, R.N., Anacleto, P., Pousão-Ferreira, P., Carvalho, M.L., Nunes, M.L., Rosa, R., Marques, A., 2017. Will seabass (*Dicentrarchus labrax*) quality change in a warmer ocean? Food Research International 97, 27–36. https://doi.org/10.1016/j.foodres.2017.03.024
Barbosa, V., Maulvault, A.L., Anacleto, P., Santos, M., Mai, M., Oliveira, H., Delgado, I., Coelho, I., Barata, M., Araújo-Luna, R., 2021. Effects of steaming on health-valuable nutrients from fortified farmed fish: Gilthead seabream (*Sparus aurata*) and common carp (*Cyprinus carpio*) as case studies. Food and Chemical Toxicology 152, 112218. https://doi.org/10.1016/j.fct.2021.112218
Barón, E., Eljarrat, E., Barceló, D., 2014. Gas chromatography/tandem mass spectrometry method for the simultaneous analysis of 19 brominated compounds in environmental and biological samples. Analytical and Bioanalytical Chemistry 406, 7667–7676. https://doi.org/10.1007/s00216-014-8196-7
Barón, E., Eljarrat, E., Barceló, D., 2012. Analytical method for the determination of halogenated norbornene flame retardants in environmental and biota matrices by gas chromatography coupled to tandem mass spectrometry. Journal of Chromatography A 1248, 154–160. https://doi.org/10.1016/j.chroma.2012.05.079
Barrento, S., Lupatsch, I., Keay, I., Shields, S., 2013. Protocol on Best Practice and handling Transportation of Live Mussels, Technical Report Supported by Project No. 243452, FP7-SME.
Barroco, A., Costa, A., Miranda, D., Portugal, J., Freitas, C., Oliveira, M., Almendra, R., 2007. Plano Estratégico do Sítio da Rede Natura do Litoral Norte - Relatório Final. Viana do Castelo.
Barsh, R., Bell, J., Blaine, E., Daniel, C., Reeve, J., 2009. Pyrethroid Pesticides and PCBs in Bivalves from East Sound, San Juan County, WA, KWIAHT (Center for the Historical Ecology of the Salish Sea). Lopez, WA.
Bayed, A., Quiniou, F., Benrha, A., Guillou, M., 2005. The *Paracentrotus lividus* populations from the northern Moroccan Atlantic coast: growth, reproduction and health condition. Journal of the Marine Biological Association of the United Kingdom 85, 999–1007. https://doi.org/10.1017/S0025315405012026
Bayne, B.L., Brown, D.A., Burns, K., Dixon, D.R., Ivanovici, A., Livingstone, D.R., Lowe, D.M., Moore, M.N., Stebbing, A.R.D., Widdows, J., 1985. The effects of stress and pollution on marine animals. New York (USA) Praeger, New York.
Bendimerad, M., 2014. Impact of the heavy metals (Cd, Fe, Cu, Pb, Zn, Ni) concentration on the edible sea urchin *Paracentrotus lividus* (Lamarck, 1816) at the level of the bay of Honaïne (W. de Tlemcen–North West Algerian). Int. J. of Multidisciplinary and Current Research 2, 14–17.
Berdanier, C.D., 1998. Advanced Nutrition: Micronutrients. CRC Press, Washington DC.
Bernárdez, M., Pastoriza, L., 2011. Quality of live packaged mussels during storage as a function of size and oxygen concentration. Food Control 22, 257–265.

https://doi.org/10.1016/j.foodcont.2010.07.007
Bernardi, D.C., Márcico, E.T., Freitas, M.Q. de, 2013. Quality Index Method (QIM) to assess the freshness and shelf life of fish. Brazilian Archives of Biology and Technology 56, 587–598. https://doi.org/10.1590/S1516-89132013000400009
Bernardo, Y.A.A., Rosario, D.K.A., Delgado, I.F., Conte-Junior, C.A., 2020. Fish Quality Index Method: Principles, weaknesses, validation, and alternatives—A review. Comprehensive Reviews in Food Science and Food Safety 19, 2657–2676. https://doi.org/10.1111/1541-4337.12600
Bertocci, I., Dominguez, R., Freitas, C., Sousa-Pinto, I., 2012. Patterns of variation of intertidal species of commercial interest in the Parque Litoral Norte (north Portugal) MPA: Comparison with three reference shores. Marine Environmental Research 77, 60–70. https://doi.org/10.1016/j.marenvres.2012.02.003
Bertocci, I., Dominguez, R., Machado, I., Freitas, C., Godino, J.D., Sousa-Pinto, I., Gonçalves, M., Gaspar, M.B., 2014. Multiple effects of harvesting on populations of the purple sea urchin *Paracentrotus lividus* in north Portugal. Fisheries Research 150, 60–65. https://doi.org/10.1016/j.fishres.2013.10.010
Bi, S., Chen, L., Sun, Z., Wen, Y., Xue, Q., Xue, C., Li, Z., Sun, C., Wei, Z., Liu, H., 2021. Investigating influence of aquaculture seawater with different salinities on non-volatile taste-active compounds in Pacific oyster (*Crassostrea gigas*). Journal of Food Measurement and Characterization 15, 2078–2087. https://doi.org/10.1007/s11694-020-00807-4
Bielmyer, G.K., Jarvis, T.A., Harper, B.T., Butler, B., Rice, L., Ryan, S., McLoughlin, P., 2012. Metal accumulation from dietary exposure in the sea urchin, *Strongylocentrotus droebachiensis*. Archives of Environmental Contamination and Toxicology 63, 86–94. https://doi.org/10.1007/s00244-012-9755-6
Bledsoe, G.E., Bledsoe, C.D., Rasco, B., 2003. Caviars and Fish Roe Products. Critical Reviews in Food Science and Nutrition 43, 317–356. https://doi.org/10.1080/10408690390826545
Borisovets, E.E., Zadorozhny, P.A., Kalinina, M. V, Lepskaya, N. V, Yakush, E. V, 2002. Changes of major carotenoids in gonads of sea urchins (*Strongylocentrotus intermedius* and *S. nudus*) at maturation. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 132, 779–790. https://doi.org/10.1016/S1096-4959(02)00099-4
Bouchoucha, M., Piquet, J.-C., Chavanon, F., Dufresne, C., Le Guyader, F.S., 2015. Faecal contamination of echinoderms: first report of heavy *Escherichia coli* loading of sea urchins from a natural growing area. Letters in Applied Microbiology 62, 105–110. https://doi.org/10.1111/lam.12524
Boudouresque, C.F., Verlaque, M., 2013. *Paracentrotus lividus*, in: Lawrence, J.M. (Ed.), Sea Urchins: Biology and Ecology. Tampa, Florida, pp. 297–327. https://doi.org/10.1016/B978-0-12-396491-5.00021-6
Bouiba, S., Bendimerad, M.E.A., Rouane-Hacene, O., Boualga, A., Richir, J., 2023. Metallic trace element dynamics in *Paracentrotus lividus* from Algeria: Environmental and human health risk assessment. Marine Pollution Bulletin 187, 114485. https://doi.org/10.1016/j.marpolbul.2022.114485
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Analytical Biochemistry 72, 248–254. https://doi.org/10.1016/0003-2697(76)90527-3
Bremner, H.A., 2002. Understanding the concepts of quality and freshness in fish, in: Bremner,

H. (Ed.), Safety and Quality Issues in Fish Processing. Woodhead Publishing, Cambridge, pp. 163–172.

Brewster, J., Garama, D., Lamare, M., Carne, A., 2018. Carotenoid composition of a New Zealand (*Evechinus chloroticus*) and an Australian (*Heliocidaris erythrogramma*) sea urchin in relation to gonad colour. Aquaculture Research 49, 2751–2762. https://doi.org/10.1111/are.13736

Bulleri, F., Benedetti-Cecchi, L., Cinelli, F., 1999. Grazing by the sea urchins *Arbacia lixula* L. and *Paracentrotus lividus* Lam. in the Northwest Mediterranean. Journal of Experimental Marine Biology and Ecology 241, 81–95. https://doi.org/10.1016/S0022-0981(99)00073-8

Burnett, L., Terwilliger, N., Carroll, A., Jorgensen, D., Scholnick, D., 2002. Respiratory and acid-base physiology of the purple sea urchin, *Strongylocentrotus purpuratus*, during air exposure: presence and function of a facultative lung. The Biological Bulletin 203, 42–50.

Bustamante, P., Lahaye, V., Durnez, C., Churlaud, C., Caurant, F., 2006. Total and organic Hg concentrations in cephalopods from the North Eastern Atlantic waters: influence of geographical origin and feeding ecology. Science of the total environment 368, 585–596. https://doi.org/10.1016/j.scitotenv.2006.01.038

Byrne, M., 1990. Annual reproductive cycles of the commercial sea urchin *Paracentrotus lividus* from an exposed intertidal and a sheltered subtidal habitat on the west coast of Ireland. Marine Biology 104, 275–289.

Cairrão, E., Couderchet, M., Soares, A., Guilhermino, L., 2004. Glutathione-S-transferase activity of *Fucus* spp. as a biomarker of environmental contamination. Aquatic Toxicology 70, 277–286. https://doi.org/10.1016/j.aquatox.2004.09.005

Campbell, L.M., Fisk, A.T., Wang, X., Köck, G., Muir, D.C.G., 2005. Evidence for biomagnification of rubidium in freshwater and marine food webs. Canadian Journal of Fisheries and Aquatic Sciences 62, 1161–1167. https://doi.org/10.1139/f05-027

Carboni, S., 2013. Research and development of hatchery techniques to optimise juvenile production of the edible Sea Urchin, *Paracentrotus lividus*. University of Stirling, Institute of Aquaculture.

Carvalho, M.L., Santiago, S., Nunes, M.L., 2005. Assessment of the essential element and heavy metal content of edible fish muscle. Analytical and Bioanalytical Chemistry 382, 426–432. https://doi.org/10.1007/s00216-004-3005-3

Carvalho, P.N., Pinto, L.F., Basto, M.C.P., Vasconcelos, M.T.S.D., 2007. Headspace solid-phase micro-extraction and gas chromatography-ion trap tandem mass spectrometry method for butyltin analysis in sediments: Optimization and validation. Microchemical Journal 87, 147–153. https://doi.org/10.1016/j.microc.2007.07.003

Carvalho, P.N., Rodrigues, P.N.R., Basto, M., Vasconcelos, M., 2009. Butyltin levels in several Portuguese coastal areas. Environmental Monitoring and Assessment 159, 183–190. https://doi.org/10.1007/s10661-008-0621-y

CEN, 2016. European Standard EN 16802 - Foodstuffs - Determination of Elements and Their Chemical Species - Determination of Inorganic Arsenic in Foodstuffs of Marine and Plant Origin by Anion-exchange HPLC-ICP-MS.

CEN, 2009. European Standard EN 15763 - Foodstuffs - Determination of trace elements - Determination of arsenic, cadmium, mercury and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion.

CEN, 2007. European Standard EN 15111 - Foodstuffs - Determination of trace elements -

Determination of iodine by ICP-MS (inductively coupled plasma mass spectrometry).
Chamika, W.A.S., Ho, T.C., Roy, V.C., Kiddane, A.T., Park, J.-S., Kim, G.-D., Chun, B.-S., 2021. In vitro characterization of bioactive compounds extracted from sea urchin (*Stomopneustes variolaris*) using green and conventional techniques. Food Chemistry 361, 129866. https://doi.org/10.1016/j.foodchem.2021.129866
Chen, Y.-C., Chen, T.-Y., Chiou, T.-K., Hwang, D.-F., 2013. Seasonal variation on general composition, free amino acids and fatty acids in the gonad of taiwan's sea urchin Tripneustes gratilla. Journal of Marine Science and Technology 21, 14. https://doi.org/10.6119/JMST-013-0429-1
Chinprahast, N., Shirai, T., Hoamuangkaew, K., 2013. Comparative study on extractive components of fresh and boiled gonads of three species of sea urchins from the Gulf of Thailand. Fisheries science 79, 707–714. https://doi.org/10.1007/s12562-013-0641-6
Choi, B., Surh, J., 2015. Effect of Salt Fermentation on the Physicochemical Properties and Antioxidant Activities of Sea Urchin Roe from *Anthocidaris crassispina* and *Pseudocentrotus depressus*. Korean Journal of Food Science and Technology 47, 460–467. https://doi.org/10.9721/KJFST.2015.47.4.460
Circuncisão, A.R., Catarino, M.D., Cardoso, S.M., Silva, A.M.S., 2018. Minerals from macroalgae origin: Health benefits and risks for consumers. Marine Drugs 16, 400. https://doi.org/10.3390/md16110400
Cirino, P., Brunet, C., Ciaravolo, M., Galasso, C., Musco, L., Vega Fernández, T., Sansone, C., Toscano, A., 2017. The sea urchin *Arbacia lixula*: A novel natural source of astaxanthin. Marine Drugs 15, 187. https://doi.org/10.3390/md15060187
CNADS, 2002. Reflexão do CNADS sobre o desenvolvimento sustentável da zona costeira. Conselho Nacional do Ambiente e Desenvolvimento Sustentável, Ministério Do Ambiente, do Ordenamento do Território e do Desenvolvimento Regional: 53p.
Coelho, I., Matos, A.S., Teixeira, R., Nascimento, A., Bordado, J., Donard, O., Castanheira, I., 2019. Combining multielement analysis and chemometrics to trace the geographical origin of Rocha pear. Journal of Food Composition and Analysis 77, 1–8. https://doi.org/10.1016/j.jfca.2018.12.005
Cohen, Z., Vonshak, A., Richmond, A., 1988. Effect of environmental conditions on fatty acid composition of the red alga *Porphyridium cruentum*: Correlation to growth rate 1. Journal of Phycology 24, 328–332. https://doi.org/10.1111/j.1529-8817.1988.tb04474.x
Collard, M., Laitat, K., Moulin, L., Catarino, A.I., Grosjean, P., Dubois, P., 2013. Buffer capacity of the coelomic fluid in echinoderms. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 166, 199–206. https://doi.org/10.1016/j.cbpa.2013.06.002
Cook, E.J., Kelly, M.S., 2007. Enhanced production of the sea urchin *Paracentrotus lividus* in integrated open-water cultivation with Atlantic salmon *Salmo salar*. Aquaculture 273, 573–585. https://doi.org/10.1016/j.aquaculture.2007.10.038
Corcellas, C., Eljarrat, E., Barceló, D., 2015. First report of pyrethroid bioaccumulation in wild river fish: a case study in Iberian river basins (Spain). Environment International 75, 110–116. https://doi.org/10.1016/j.envint.2014.11.007
Coroneo, V., Corrias, F., Brutti, A., Addis, P., Scano, E., Angioni, A., 2022. Effect of High-Pressure Processing on Fresh Sea Urchin Gonads in Terms of Shelf Life, Chemical Composition, and Microbiological Properties. Foods 11, 260. https://doi.org/10.3390/foods11030260

Costa, S., Moreira, S., Almeida, M., Antunes, C., Bastos, L., Henriques, M.A., Mucha, A.P., Pereira, R., Salgado, M.A., Santos, M., Veloso Gomes, F., Vieira, M.N., Guilhermino, L., 2015. ECORISK Project Final report – Ecological Risk Assessment of Oils and Noxious Substances in the NW Portuguese Coast.

Covaci, A., Harrad, S., Abdallah, M.A.-E., Ali, N., Law, R.J., Herzke, D., de Wit, C.A., 2011. Novel brominated flame retardants: a review of their analysis, environmental fate and behaviour. Environment International 37, 532–556. https://doi.org/10.1016/j.envint.2010.11.007

Cruz-García, C., López-Hernández, J., González-Castro, M.J., Rodríguez-Bernaldo De Quirós, A.I., Simal-Lozano, J., 2000. Protein, amino acid and fatty acid contents in raw and canned sea urchin (*Paracentrotus lividus*) harvested in Galicia (NW Spain). Journal of the Science of Food and Agriculture 80, 1189–1192. https://doi.org/10.1002/1097-0010(200006)80:8<1189::AID-JSFA618>3.0.CO;2-7

Cuevas-Acuña, D.A., Valenzuela, M.H.G., Santacruz-Ortega, H.C., Melchor, R.G.V., Arias-Moscoso, J.L., 2019. Composición química, contenido de proteína, aminoácidos y morfología de gónadas de erizo de mar (*Strongylocentrotus franciscanus*). Biotecnia 21, 86–91. https://doi.org/10.18633/biotecnia.v21i3.1015

Cunha, I., García, L.M., Guilhermino, L., 2005. Sea-urchin (*Paracentrotus lividus*) glutathione S-transferases and cholinesterase activities as biomarkers of environmental contamination. Journal of Environmental Monitoring 7, 288–294. https://doi.org/10.1039/B414773A

Custódio, P.J., Carvalho, M.L., Nunes, F., 2003. Trace elements determination by energy dispersive X-ray fluorescence (EDXRF) in human placenta and membrane: a comparative study. Analytical and Bioanalytical Chemistry 375, 1101–1106. https://doi.org/10.1007/s00216-003-1765-9

Dale, T., Siikavuopio, S., Aas, K., 2005. Roe enhancement in sea urchin: effects of handling during harvest and transport on mortality and gonad growth in *Strongylocentrotus droebachiensis*. Journal of Shellfish Research 24, 1235–1239. https://doi.org/10.2983/0730-8000(2005)24[1235:REISUE]2.0.CO;2

Danis, B., Cotret, O., Teyssié, J.-L., Bustamante, P., Fowler, S.W., Warnau, M., 2005. Bioaccumulation of PCBs in the sea urchin *Paracentrotus lividus*: seawater and food exposures to a 14C-radiolabelled congener (PCB# 153). Environmental Pollution 135, 11–16. https://doi.org/10.1016/j.envpol.2004.10.011

De Quirós, A.R.-B., López-Hernández, J., González-Castro, M., De la Cruz-Garcia, C., Simal-Lozano, J., 2001a. Comparison of volatile components in fresh and canned sea urchin (*Paracentrotus lividus*, Lamarck) gonads by GC–MS using dynamic headspace sampling and microwave desorption. European Food Research and Technology 212, 643–647. https://doi.org/10.1007/s002170100315

De Quirós, A.R.-B., López-Hernández, J., Simal-Lozano, J., 2001b. Determination of carotenoids and liposoluble vitamins in sea urchin (*Paracentrotus lividus*) by high performance liquid chromatography. European Food Research and Technology 212, 687–690. https://doi.org/10.1007/s002170100316

de Solla, S.R., Gilroy, È.A.M., Klinck, J.S., King, L.E., McInnis, R., Struger, J., Backus, S.M., Gillis, P.L., 2016. Bioaccumulation of pharmaceuticals and personal care products in the unionid mussel *Lasmigona costata* in a river receiving wastewater effluent. Chemosphere 146, 486–496. https://doi.org/10.1016/j.chemosphere.2015.12.022

De Zoysa, H.K.S., 2014. Nutritional Composition and Trace Metals Accumulation of Edible Sea

Urchin (*Stomopneustes variolaris*) in Selected Areas of Sri Lanka. Rajarata University of Sri Lanka.

Delgado, I., Ventura, M., Gueifão, S., Coelho, I., Nascimento, A.C., Silva, J.A.L., Castanheira, I., 2019. 12th IFDC 2017 special issue–Iodine, selenium and iron contents in Portuguese key foods as consumed. Journal of Food Composition and Analysis 79, 39–46. https://doi.org/10.1016/j.jfca.2019.03.004

Di Natale, C., Ólafsdóttir, G., 2009. Electronic nose and electronic tongue, in: Rehbein, H., Oehlenschläger, J. (Eds.), Fishery Products Quality, Safety and Authenticity. Wiley-Blackwell, Oxford, pp. 105–126.

Díaz-Enrich, M.J., Ramos-Martínez, J.I., Ibarguren, I., 2002. Implication of guanosine 3′, 5′-cyclic monophosphate, adenosine 3′, 5′-cyclic monophosphate, adenosine 5′-mono-, di-and triphosphate and fructose-2, 6-bisphosphate in the regulation of the glycolytic pathway in hypoxic/anoxic mussel, *Mytilus galloprovincialis*. Molecular and cellular biochemistry 240, 111–118. https://doi.org/10.1023/a:1020666623094

Dincer, T., Cakli, S., 2007. Chemical composition and biometrical measurements of the Turkish sea urchin (*Paracentrotus lividus*, Lamarck, 1816). Critical Reviews in Food Science and Nutrition 47, 21–26. https://doi.org/10.1080/10408390600550265

Duan, W., Huang, Y., Xiao, J., Zhang, Y., Tang, Y., 2020. Determination of free amino acids, organic acids, and nucleotides in 29 elegant spices. Food Science & Nutrition 8, 3777–3792. https://doi.org/10.1002/fsn3.1667

Dunkel, A., Hofmann, T., 2009. Sensory-directed identification of β-alanyl dipeptides as contributors to the thick-sour and white-meaty orosensation induced by chicken broth. Journal of Agricultural and Food Chemistry 57, 9867–9877. https://doi.org/10.1021/JF900948R

Ebele, A.J., Abdallah, M.A.-E., Harrad, S., 2017. Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. Emerging Contaminants 3, 1–16. https://doi.org/10.1016/j.emcon.2016.12.004

Ebert, T.A., 2013. Growth and Survival of Postsettlement Sea Urchins, in: Lawrence, J.M. (Ed.), Sea Urchins: Biology and Ecology. Tampa, Florida, pp. 83–117.

EC, 2021. SANTE/10252/2021 Magnitude of pesticides residues in fish [WWW Document]. URL https://food.ec.europa.eu/system/files/2021-10/pesticides_mrl_guidelines_app-j-10252-2021_en.pdf (accessed 1.26.23).

EC, 2015a. Regulation No 2015/2285 of 8 December 2015 amending annex II to Regulation (EC) No 854/2004 of the European Parliament and of the Council laying down specific rules for the organisation of official controls on products of animal origin intended for human . Official Journal of the European Union 323, 2–4.

EC, 2015b. Regulamento (UE) 2015/2285 da Comissão de 8 de dezembro de 2015 que altera o anexo II do Regulamento (CE) n.º 854/2004 do Parlamento Europeu e do Conselho, que estabelece regras específicas de organização dos controlos oficiais de produtos de origem anima. Jornal Oficial da União Europeia 2015–2017.

EC, 2011a. Commission regulation (EC) No 420/2011 of 29 April 2011 amending Regulation (EC) No 1881/2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L111/3.

EC, 2011b. Commission Regulation (EU) No 1129/2011 of 11 November 2011 amending Annex II to Regulation (EC) No 1333/2008 of the European Parliament and of the Council by

establishing a Union list of food additives. Official Journal of the European Union L 295/1.
EC, 2011c. Commission Regulation (EU) No 836/2011 of 19 August 2011 amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo (a) pyrene in. Official Journal of the European Union 215, 1–8.
EC, 2008. Directive 2008/56/EC of the European Parliament and of the Council of 17 June 2008 establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive). Official Journal of the European Union L 164/19.
EC, 2006. Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. Official Journal of the European Union L364/5.
EC, 2005. Commission Regulation (EC) No 2073/2005 of 15 November 2005 on microbiological criteria for foodstuffs. Official Journal of the European Union 50, 1–26.
EC, 2004a. Regulation (EC) No 854/2004 of the European Parliament and of the Council of 29 April 2004 laying down specific rules for the organisation of official controls on products of animal origin intended for human consumption. Official Journal of the European Communities 24, 83–127.
EC, 2004b. Regulation EC/853/2004 of the European Parliament and of the Council of 29th April 2004 laying down specific hygiene rules for food of animal origin. Official Journal of the European Union 139.
EC, 2000. Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 Establishing a Framework for Community Action in the Field of Water Policy. Official Journal of the European Union 327, 1–73.
EFSA, 2017. Dietary Reference Values for nutrients. Summary report. EFSA supporting publication e15121. https://doi.org/10.2903/sp.efsa.2017.e15121
EFSA, 2014. Scientific opinion on dietary reference values for iodine. EFSA Journal 12, 3660.
EFSA, 2012a. Scientific opinion on emerging and novel brominated flame retardants (BFRs) in food. EFSA Journal 10, 2908.
EFSA, 2012b. Scientific opinion on the tolerable upper intake level of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) and docosapentaenoic acid (DPA). EFSA Journal 10, 2815.
EFSA, 2012c. Scientific Opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA Journal 10, 2985. https://doi.org/10.2903/j.efsa.2012.2985
EFSA, 2011. Statement on tolerable weekly intake for cadmium. EFSA Journal 9, 1975.
EFSA, 2010. Scientific Opinion on Lead in food. EFSA Journal 8(4), 1570.
EFSA, 2009a. Scientific Opinion of the Panel on Contaminants in the Food Chain on a request from the European Commission on cadmium in food. EFSA Journal 7, 1–139.
EFSA, 2009b. Scientific Opinion on Arsenic in food. EFSA Journal 7(10), 1351. https://doi.org/10.2903/j.efsa.2009.1351
EFSA, 2006. Tolerable upper intake levels for vitamins and minerals, Scientific Panel on Dietetic Products, Nutrition and Allergies. Parma, Italy.
EFSA Panel on NDA, Turck, D., Bresson, J.-L., Burlingame, B., Dean, T., Fairweather-Tait, S., Heinonen, M., Hirsch-Ernst, K.-I., Mangelsdorf, I., McArdle, H.J., Neuhäuser-Berthold, M., Nowicka, G., Pentieva, K., Sanz, Y., Siani, A., Sjödin, A., Stern, M., Tomé, D., Van Loveren, H.,

Vincenti, M., Willatts, P., Aggett, P., Martin, A., Przyrembel, H., Brönstrup, A., Ciok, J., Gómez Ruiz, J.A., Sesmaisons-Lecarré, A., Naska, A., 2016. Dietary reference values for potassium. EFSA Journal 14(10), 4592. https://doi.org/10.2903/j.efsa.2016.4592

EFSA Panel on NDA, Turck, D., Castenmiller, J., Henauw, S., Hirsch-Ernst, K.-I., Kearney, J., Knutsen, H.K., Maciuk, A., Mangelsdorf, I., McArdle, H.J., Pelaez, C., Pentieva, K., Siani, A., Thies, F., Tsabouri, S., Vincenti, M., Aggett, P., Fairweather-Tait, S., Martin, A., Przyrembel, H., Ciccolallo, L., Sesmaisons-Lecarré, A., Martinez, S. V, Martino, L., Naska, A., 2019. Dietary reference values for sodium. EFSA Journal 17(9), 5778. https://doi.org/10.2903/j.efsa.2019.5778

El Idrissi, O., Marengo, M., Aiello, A., Gobert, S., Pasqualini, V., Ternengo, S., 2020. Seasonal change in trace element concentrations of *Paracentrotus lividus*: Its use as a bioindicator. Ecological Indicators 112, 106063. https://doi.org/10.1016/j.ecolind.2019.106063

Engel, E., Nicklaus, S., Salles, C., Le Quéré, J.-L., 2002. Relevance of omission tests to determine flavour-active compounds in food: application to cheese taste. Food quality and preference 13, 505–513. https://doi.org/10.1016/S0950-3293(02)00136-2

Erkmen, B., 2015. Spermiotoxicity and embryotoxicity of permethrin in the sea urchin *Paracentrotus lividus*. Bulletin of Environmental Contamination and Toxicology 94, 419–424. https://doi.org/10.1007/s00128-015-1482-z

Estevez, A., Camacho, C., Correia, T., Barbosa, V., Marques, A., Lourenco, H., Serrano, C., Sapata, M., Duarte, M.P., Pires, C., 2021. Strategies to reduce sodium levels in European seabass sausages. Food and Chemical Toxicology 153, 112262. https://doi.org/10.1016/j.fct.2021.112262

European Parliament, 2011. Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, amending Regulations (EC) No 1924/2006 and (EC) No 1925/2006 of the European Parliament and of the Council, an. Off. J. Eur. Union 20, 168–213.

European Parliament, 2006. Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on nutrition and health claims made on foods. Off. J. Eur. Union 404/9, 9–25.

FAO/WHO, 2011. Exposure to Arsenic: A Major Public Health Concern [WWW Document]. URL http://www.who.int/ipcs/features/arsenic.pdf (accessed 7.14.17).

FAO, 2022. The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation. Rome, FAO. https://doi.org/10.4060/cc0461en

FAO, 2004. Data and Statistics Unit (FAO-FIDI) - Collation, Analysis and Dissemination of Global and Regional Fishery Statistics. FAO, Rome.

FAO, 2003. Food energy - methods of analysis and conversion factors, FAO Food and Nutrition Paper. Rome.

Farag, M.A., Abib, B., Tawfik, S., Shafik, N., Khattab, A.R., 2021. Caviar and fish roe substitutes: Current status of their nutritive value, bio-chemical diversity, authenticity and quality control methods with future perspectives. Trends in Food Science & Technology 110, 405–417. https://doi.org/10.1016/j.tifs.2021.02.015

Feo, M.L., Eljarrat, E., Barceló, D., 2011. Performance of gas chromatography/tandem mass spectrometry in the analysis of pyrethroid insecticides in environmental and food samples. Rapid Communications in Mass Spectrometry 25, 869–876. https://doi.org/10.1002/rcm.4936

Fernández-Boán, M., Fernández, L., Freire, J., 2012. History and management strategies of the sea urchin *Paracentrotus lividus* fishery in Galicia (NW Spain). Ocean & Coastal Management 69, 265–272. https://doi.org/10.1016/j.ocecoaman.2012.07.032

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226, 497–509. https://doi.org/10.1016/S0021-9258(18)64849-5

Freitas, J., Vaz-Pires, P., Câmara, J.S., 2021. Quality Index Method for fish quality control: Understanding the applications, the appointed limits and the upcoming trends. Trends in Food Science & Technology 111, 333–345. https://doi.org/10.1016/j.tifs.2021.03.011

Fuke, S., Konosu, S., 1991. Taste-active components in some foods: a review of Japanese research. Physiology & Behavior 49, 863–868. https://doi.org/10.1016/0031-9384(91)90195-T

Gagné, F., 2014. Biochemical Ecotoxicology: Principles and Methods. Elsevier, Oxford.

Gago-Ferrero, P., Alonso, M.B., Bertozzi, C.P., Marigo, J., Barbosa, L., Cremer, M., Secchi, E.R., Azevedo, A., Lailson-Brito Jr, J., Torres, J.P.M., 2013. First determination of UV filters in marine mammals. Octocrylene levels in Franciscana dolphins. Environmental Science & Technology 47, 5619–5625. https://doi.org/10.1021/es400675y

Gao, Y., Tian, Y., Shen, X., 2008. Current techniques for assessing developmental neurotoxicity of pesticides. Frontiers of Medicine in China 2, 337–343. https://doi.org/10.1007/s11684-008-0064-4

Garmendia, J.M., Menchaca, I., Belzunce, M.J., Franco, J., Revilla, M., 2010. Seasonal variability in gonad development in the sea urchin (*Paracentrotus lividus*) on the Basque coast (Southeastern Bay of Biscay). Marine Pollution Bulletin 61, 259–266. https://doi.org/10.1016/j.marpolbul.2010.02.023

Gianguzza, P., Chiantore, M., Bonaviri, C., Cattaneo-Vietti, R., Vielmini, I., Riggio, S., 2006. The effects of recreational *Paracentrotus lividus* fishing on distribution patterns of sea urchins at Ustica Island MPA (Western Mediterranean, Italy). Fisheries Research 81, 37–44. https://doi.org/10.1016/j.fishres.2006.06.002

Giddings, G.G., Hill, L.H., 1975. Processing effects on the lipid fractions and principal fatty acids of blue crab (*Callinectes sapidus*) muscle. Journal of Food Science 40, 1127–1129. https://doi.org/10.1111/j.1365-2621.1975.tb01033.x

Giménez, A., Ares, F., Ares, G., 2012. Sensory shelf-life estimation: A review of current methodological approaches. Food Research International 49, 311–325. https://doi.org/10.1016/j.foodres.2012.07.008

Giraldo, A., Montes, R., Rodil, R., Quintana, J.B., Vidal-Liñán, L., Beiras, R., 2017. Ecotoxicological evaluation of the UV filters ethylhexyl dimethyl p-aminobenzoic acid and octocrylene using marine organisms *Isochrysis galbana*, *Mytilus galloprovincialis* and *Paracentrotus lividus*. Archives of Environmental Contamination and Toxicology 72, 606–611. https://doi.org/10.1007/s00244-017-0399-4

Giri, A., Bharti, V.K., Angmo, K., Kalia, S., Vivek, P., Kumar, B., 2016. Arsenic and it's adjuvantic role on oxidative stress in animals: a brief overview. Int. J. Curr. Biosci. Plant Biol. 3(12), 105–114.

Giulivo, M., Capri, E., Eljarrat, E., Barceló, D., 2016. Analysis of organophosphorus flame retardants in environmental and biotic matrices using on-line turbulent flow chromatography-liquid chromatography-tandem mass spectrometry. Journal of

Chromatography A 1474, 71–78. https://doi.org/10.1016/j.chroma.2016.10.042
Gómez, J.L.C., 1992. La pêche des oursins en Galice, Espagne, pendant la campagne 1990–1991, in: Echinoderm Research 1991. CRC Press, pp. 199–200.
Gonçalves, A., Pedro, S., Duarte, A., Nunes, M.L., 2009. Effect of enriched oxygen atmosphere storage on the quality of live clams (*Ruditapes decussatus*). International Journal of Food Science & Technology 44, 2598–2605. https://doi.org/10.1111/j.1365-2621.2009.02090.x
Gonçalves, A.M., Alpuim, T., 2011. Water quality monitoring using cluster analysis and linear models. Environmetrics 22, 933–945. https://doi.org/10.1002/env.1112
Gonçalves, E.P.R., Boaventura, R.A.R., Mouvet, C., 1992. Sediments and aquatic mosses as pollution indicators for heavy metals in the Ave river basin (Portugal). Science of the Total Environment 114, 7–24. https://doi.org/10.1016/0048-9697(92)90410-T
González-Irusta, J.M., De Cerio, F.G., Canteras, J.C., 2010. Reproductive cycle of the sea urchin *Paracentrotus lividus* in the Cantabrian Sea (northern Spain): environmental effects. Journal of the Marine Biological Association of the United Kingdom 90, 699–709. https://doi.org/10.1017/S002531540999110X
Goutte, A., Chevreuil, M., Alliot, F., Chastel, O., Cherel, Y., Eléaume, M., Massé, G., 2013. Persistent organic pollutants in benthic and pelagic organisms off Adélie Land, Antarctica. Marine pollution bulletin 77, 82–89. https://doi.org/10.1016/j.marpolbul.2013.10.027
Gray, J.S., 2002. Biomagnification in marine systems: the perspective of an ecologist. Marine Pollution Bulletin 45, 46–52. https://doi.org/10.1016/S0025-326X(01)00323-X
Grosjean, P., 2001. Growth model of the reared sea urchin *Paracentrotus lividus* (Lamarck, 1816). Université de Mons.
Groz, M.P., Bueno, M.J.M., Rosain, D., Fenet, H., Casellas, C., Pereira, C., Maria, V., Bebianno, M.J., Gomez, E., 2014. Detection of emerging contaminants (UV filters, UV stabilizers and musks) in marine mussels from Portuguese coast by QuEChERS extraction and GC–MS/MS. Science of the Total Environment 493, 162–169. https://doi.org/10.1016/j.scitotenv.2014.05.062
Gu, S.-Y., Ekpeghere, K.I., Kim, H.-Y., Lee, I.-S., Kim, D.-H., Choo, G., Oh, J.-E., 2017. Brominated flame retardants in marine environment focused on aquaculture area: Occurrence, source and bioaccumulation. Science of the Total Environment 601, 1182–1191. https://doi.org/10.1016/j.scitotenv.2017.05.209
Guérin, T., Chekri, R., Vastel, C., Sirot, V., Volatier, J.-L., Leblanc, J.-C., Noël, L., 2011. Determination of 20 trace elements in fish and other seafood from the French market. Food Chemistry 127, 934–942. https://doi.org/10.1016/j.foodchem.2011.01.061
Guidetti, P., Fraschetti, S., Terlizzi, A., Boero, F., 2003. Distribution patterns of sea urchins and barrens in shallow Mediterranean rocky reefs impacted by the illegal fishery of the rock-boring mollusc *Lithophaga lithophaga*. Marine Biology 143, 1135–1142. https://doi.org/10.1007/s00227-003-1163-z
Guidetti, P., Mori, M., 2005. Morpho-functional defences of Mediterranean sea urchins, *Paracentrotus lividus* and *Arbacia lixula*, against fish predators. Marine Biology 147, 797–802. https://doi.org/10.1007/s00227-005-1611-z
Guo, J., Venier, M., Salamova, A., Hites, R.A., 2017. Bioaccumulation of Dechloranes, organophosphate esters, and other flame retardants in Great Lakes fish. Science of the Total Environment 583, 1–9. https://doi.org/10.1016/j.scitotenv.2016.11.063
Habig, W.H., Pabst, M.J., Jakoby, W.B., 1974. Glutathione S-transferases the first enzymatic step

in mercapturic acid formation. Journal of Biological Chemistry 249, 7130–7139. https://doi.org/10.1016/S0021-9258(19)42083-8

Hagen, N.T., Jørgensen, I., Egeland, E.S., 2008. Sex-specific seasonal variation in the carotenoid content of sea urchin gonads. Aquatic Biology 3, 227–235. https://doi.org/10.3354/ab00084

Hale, M.B., Brown, T., 1983. Fatty acids and lipid classes of three underutilized species and changes due to canning. Marine Fisheries Review 45, 45–48.

Hall, G.M., 2011. Canning Fish and Fish Products, in: Hall, G.M. (Ed.), Fish Processing - Sustainability and New Opportunities. Wiley Online Library, pp. 30–50.

Hammer, H.S., Powell, M.L., Watts, S.A., 2013. Species Profile: Sea Urchins of the Southern Region. SRAC Publication 1–6.

Harris, L.G., Eddy, S.D., 2015. Sea urchin ecology and biology, in: Brown, N., Eddy, S. (Eds.), Echinoderm Aquaculture. Wiley-Blackwell, New Jersey, pp. 3–24.

Henderson, J.W., Ricker, R.D., Bidlingmeyer, B.A., Woodward, C., 2000. Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids.

Hladik, M.L., Smalling, K.L., Kuivila, K.M., 2009. Methods of Analysis - Determination of Pyrethroid Insecticides in Water and Sediment Using Gas Chromatography/mass Spectrometry. US Geological Survey Reston, Virginia, USA.

Hong, H., Regenstein, J.M., Luo, Y., 2017. The importance of ATP-related compounds for the freshness and flavor of post-mortem fish and shellfish muscle: A review. Critical Reviews in Food Science and Nutrition 57, 1787–1798. https://doi.org/10.1080/10408398.2014.1001489

Horrocks, A.R., Davies, P.J., Kandola, B.K., Alderson, A., 2007. The Potential for Volatile Phosphorus-containing Flame Retardants in Textile Back-coatings. Journal of Fire Sciences 25, 523–540. https://doi.org/10.1177/0734904107083553

Hou, X., 2009. Iodine speciation in foodstuffs, tissues, and environmental samples: iodine species and analytical method, in: Preedy, V.R., Burrow, G.N., Watson, R. (Eds.), Comprehensive Handbook of Iodine. Academic Press, pp. 139–150.

Huerta, B., Rodríguez-Mozaz, S., Barceló, D., 2012. Pharmaceuticals in biota in the aquatic environment: analytical methods and environmental implications. Analytical and bioanalytical chemistry 404, 2611–2624. https://doi.org/10.1007/s00216-012-6144-y

Hui, Y.H., Cross, N., Kristinsson, H.G., Lim, M.H., Nip, W.K., Siow, L.F., Stanfield, P.S., 2006. Biochemistry of Seafood Processing, in: Hui, Y.H. (Ed.), Food Biochemistry and Food Processing. Blackwell Publishing, pp. 350–378.

Hyldig, G., Bremner, A., Martinsdóttir, E., Schelvis, R., 2012. Quality Index Methods, in: Nollet, L.M.L. (Ed.), Handbook of Meat, Poultry and Seafood Quality. Wiley-Blackwell, Oxford, UK, pp. 437–458.

Hyldig, G., Green-Petersen, D.M.B., 2005. Quality Index Method — an objective tool for determination of sensory quality. Journal of Aquatic Food Product Technology 13, 71–80. https://doi.org/10.1300/J030v13n04_06

IARC, 1999. Some chemicals that cause tumours of the kidney or urinary bladder in rodents and some other substances. IARC monographs on the evaluation of carcinogenic risks to humans 73, 131–182.

IoM, 2001. Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc.,

National A. ed. Washington DC.
IPMA, 2023. SNMB - Sistema Nacional de Monitorização de Moluscos Bivalves [WWW Document]. URL https://www.ipma.pt/pt/bivalves/ (accessed 1.19.23).
ISO, 2008. International Standard 5492. Sensory analysis — Vocabulary, International Standard ISO. International Organisation for Standardisation, Genéve.
ISO, 2007. International Standard 8589. Sensory Analysis - General guidance for the design of test rooms, International Standard ISO. International Organization for Standardization, Genève.
ISO, 1994. International Standard 11035. Sensory analysis - Identification and selection of descriptors for establishing a sensory profile by multidimensional approach, International Standard ISO. International Organization for Standardization, Genève.
Jabeen, F., Chaudhry, A.S., Manzoor, S., Shaheen, T., 2015. Examining pyrethroids, carbamates and neonicotenoids in fish, water and sediments from the Indus River for potential health risks. Environmental Monitoring and Assessment 187, 1–11. https://doi.org/10.1007/s10661-015-4273-4
James, P., Evensen, T., 2018. Sea urchin transportation systems for land, sea and air (Activity A6.4.1 of the NPA URCHIN project), Nofima rapportserie. Nofima AS, Tromsø.
James, P., Evensen, T., Samuelsen, A., 2017. Commercial scale sea urchin roe enhancement in Norway: Enhancement, transport and market assessment. Tromsø.
James, P., Noble, C., Siikavuopio, S., Sloan, R., Hannon, C., Þórarinsdóttir, G., Ziemer, N., Lochead, J., 2016. Sea urchin fishing techniques report (Activity A3.1.1 of the NPA URCHIN project), Nofima rapportserie. Nofima AS, Tromsø.
James, P., Siikavuopio, S.I., 2014. Alternative Low-Cost Methods of Fishing Sea Urchins. Nofima Internal Report No. 38B/2014, Nofima rapportserie. Tromsø.
Johansson, L.H., Borg, L. a, 1988. A spectrophotometric method for determination of catalase activity in small tissue samples. Analytical biochemistry 174, 331–336. https://doi.org/10.1016/0003-2697(88)90554-4
Johnstone, J., Nash, S., Hernandez, E., Rahman, M.S., 2019. Effects of elevated temperature on gonadal functions, cellular apoptosis, and oxidative stress in Atlantic sea urchin *Arbacia punculata*. Marine environmental research 149, 40–49. https://doi.org/10.1016/j.marenvres.2019.05.017
Jonze, T., 2018. Sea urchin is in demand. It's the ocean's foie gras: delicious, but not entirely ethical [WWW Document]. The Guardian. URL https://www.theguardian.com/food/2018/oct/17/taste-of-uni-fresh-salty-opulent-edible-sea-urchin-demand-rising-uk-restaurants (accessed 6.10.20).
Jorhem, L., 2000. Determination of metals in foods by atomic absorption spectrometry after dry ashing: NMKL1 collaborative study. Journal of AOAC International 83, 1204–1211. https://doi.org/10.1093/jaoac/83.5.1204
Kaharudin, H.A.F., 2020. Prehistoric fast food: Sea urchin exploitation on Alor island.
Kalinowski, C.T., Robaina, L.E., Fernandez-Palacios, H., Schuchardt, D., Izquierdo, M.S., 2005. Effect of different carotenoid sources and their dietary levels on red porgy (*Pagrus pagrus*) growth and skin colour. Aquaculture 244, 223–231. https://doi.org/10.1016/j.aquaculture.2004.11.001
Kaneko, K., Matsumoto, H., Shirai, T., Kamei, M., Okazaki, E., Osako, K., 2012. Seasonal variations in free amino acid composition and taste aspects of black sea urchin, *Diadema setosum*,

gonad. Food Science and Technology Research 18, 835–842. https://doi.org/10.3136/fstr.18.835

Karouna-Renier, N.K., Snyder, R.A., Allison, J.G., Wagner, M.G., Rao, K.R., 2007. Accumulation of organic and inorganic contaminants in shellfish collected in estuarine waters near Pensacola, Florida: contamination profiles and risks to human consumers. Environmental Pollution 145, 474–488. https://doi.org/10.1016/j.envpol.2006.04.035

Kato, H., Rhue, M.R., Nishimura, T., 1989. Role of Free Amino Acids and Peptides in Food Taste, in: Teranishi, R., Buttery, R.G., Shahidi, F. (Eds.), Flavor Chemistry. American Chemical Society, pp. 158–174.

Kato, S., Schroeter, S.C., 1985. Biology of the red sea urchin, *Strongylocentrotus franciscanus*, and its fishery in California. Marine fisheries review 47, 1–20.

Kawabe, S., Takada, M., Shibuya, R., Yokoyama, Y., 2010. Biochemical changes in oyster tissues and hemolymph during long-term air exposure. Fisheries Science 76, 841–855. https://doi.org/10.1007/s12562-010-0263-1

Kelly, M., Carboni, S., Cook, E., Hughes, A., 2015. Sea urchin aquaculture in Scotland, in: Brown, N., Eddy, S. (Eds.), Echinoderm Aquaculture. John Wiley and Sons, Inc., New Jersey, pp. 211–224.

Kelly, M.S., 2005. Echinoderms: Their Culture and Bioactive Compounds, in: Matranga, V. (Ed.), Echinodermata. Springer, Berlin, pp. 139–165.

Kelly, M.S., Symonds, R.C., 2013. Carotenoids in sea urchins, in: Lawrence, J.M. (Ed.), Sea Urchins: Biology and Ecology. Elsevier, Tam, pp. 171–177.

Khoo, H.-E., Prasad, K.N., Kong, K.-W., Jiang, Y., Ismail, A., 2011. Carotenoids and their isomers: color pigments in fruits and vegetables. Molecules 16, 1710–1738. https://doi.org/10.3390/molecules16021710

Kim, J.-W., Ramaswamy, B.R., Chang, K.-H., Isobe, T., Tanabe, S., 2011. Multiresidue analytical method for the determination of antimicrobials, preservatives, benzotriazole UV stabilizers, flame retardants and plasticizers in fish using ultra high performance liquid chromatography coupled with tandem mass spectrometry. Journal of Chromatography A 1218, 3511–3520. https://doi.org/10.1016/j.chroma.2011.04.006

Kinoshita, Y., Yoshioka, T., Miyazaki, S., Kato, S., Konno, K., 2009. Storage of sea urchin gonad in oxygenated sea water. Nippon Suisan Gakkaishi 75, 237–243. https://doi.org/10.2331/suisan.75.237

Kolbe, E., Kramer, D.E., 2007. Planning for seafood freezing, Alaska Sea. ed, Mab. Fairbanks.

Komata, Y., 1964. Studies on the extractives of" Uni"-IV. Taste of each component in the extractives. Bull. Japan Soc. Sci. Fish. 30, 749–756.

Kosma, C.I., Lambropoulou, D.A., Albanis, T.A., 2014. Investigation of PPCPs in wastewater treatment plants in Greece: occurrence, removal and environmental risk assessment. Science of the Total Environment 466, 421–438. https://doi.org/10.1016/j.scitotenv.2013.07.044

Kruzynski, G.M., 2004. Cadmium in oysters and scallops: the BC experience. Toxicology Letters 148, 159–169. https://doi.org/10.1016/j.toxlet.2003.10.030

Labuza, T.P., Fu, B., 1993. Growth kinetics for shelf-life prediction: theory and practice. Journal of Industrial Microbiology 12, 309–323.

Lall, S.P., 2003. The minerals, in: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press. San Diego, California, pp. 259–308.

Lawrence, J.L. (Ed.), 2013. Sea Urchins: Biology and Ecology, Third. ed, Developments in Aquaculture and Fisheries Science Volume 38. Elsevier, Tampa, Florida.

Lebrun, J.D., Leroy, D., Giusti, A., Gourlay-Francé, C., Thomé, J.-P., 2014. Bioaccumulation of polybrominated diphenyl ethers (PBDEs) in Gammarus pulex: relative importance of different exposure routes and multipathway modeling. Aquatic toxicology 154, 107–113.

Lee, S.-U., Lee, H.-Y., Kim, S.-H., Kim, D.-J., 2012. Changes in physicochemical composition of sea urchin roe by steaming treatment. Journal of the Korean Society of Food Science and Nutrition 41, 550–560. https://doi.org/10.3746/jkfn.2012.41.4.550

Lepage, G., Roy, C.C., 1986. Direct transesterification of all classes of lipids in a one-step reaction. Journal of lipid research 27, 114–120. https://doi.org/10.1016/S0022-2275(20)38861-1

Lesser, M.P., 2006. Oxidative Stress In Marine Environments: Biochemistry and Physiological Ecology. Annual Review of Physiology 68, 253–278. https://doi.org/10.1146/annurev.physiol.68.040104.110001

Liang, L., Duan, W., Zhang, J., Huang, Y., Zhang, Y., Sun, B., 2022. Characterization and molecular docking study of taste peptides from chicken soup by sensory analysis combined with nano-LC-Q-TOF-MS/MS. Food Chemistry 383, 132455. https://doi.org/10.1016/j.foodchem.2022.132455

Liu, Y., Qiu, C., 2016. Calculated Taste Activity Values and Umami Equivalences Explain Why Dried Sha-chong (*Sipunculus nudus*) Is a Valuable Condiment. Journal of Aquatic Food Product Technology 25, 177–184. https://doi.org/10.1080/10498850.2013.839591

Lopes, A.R., Sampaio, E., Santos, C., Couto, A., Pegado, M.R., Diniz, M., Munday, P.L., Rummer, J.L., Rosa, R., 2018. Absence of cellular damage in tropical newly hatched sharks (*Chiloscyllium plagiosum*) under ocean acidification conditions. Cell Stress and Chaperones 23, 837–846. https://doi.org/10.1007/s12192-018-0892-3

Lourenço, S., José, R., Neves, P., Góis, A., Cordeiro, N., Andrade, C., Ribeiro, C., 2022a. Population density, reproduction cycle and nutritional value of *Sphaerechinus granularis* (Echinodermata: Echinoidea) in an oceanic insular ecosystem. Frontiers in Marine Science 8, 2040. https://doi.org/10.3389/fmars.2021.699942

Lourenço, S., Mendes, S., Raposo, A., Santos, P.M., Gomes, A.S., Ganhão, R., Costa, J.L., Gil, M.M., Pombo, A., 2021. Motivation and preferences of Portuguese consumers' towards sea urchin roe. International Journal of Gastronomy and Food Science 24, 100312. https://doi.org/10.1016/j.ijgfs.2021.100312

Lourenço, S., Raposo, A., Cunha, B., Pinheiro, J., Santos, P.M., Gomes, A.S., Ferreira, S., Gil, M.M., Costa, J.L., Pombo, A., 2022b. Temporal changes in sex-specific color attributes and carotenoid concentration in the gonads (roe) of the purple sea urchin (*Paracentrotus lividus*) provided dry feeds supplemented with β-carotene. Aquaculture 560, 738608. https://doi.org/10.1016/j.aquaculture.2022.738608

Lourenço, S., Valente, L.M.P., Andrade, C., 2019. Meta-analysis on nutrition studies modulating sea urchin roe growth, colour and taste. Reviews in Aquaculture 11, 766–781. https://doi.org/10.1111/raq.12256

Lozano, J., Galera, J., López, S., Turon, X., Palacin, C., 1995. Biological cycles and recruitment of *Paracentrotus lividus* (Echinodermata: Echinoidea) in two contrasting habitats. Marine Ecology Progress Series 122, 179–191. https://doi.org/10.3354/meps122179

Madeira, C., Madeira, D., Diniz, M.S., Cabral, H.N., Vinagre, C., 2017. Comparing biomarker

responses during thermal acclimation: A lethal vs non-lethal approach in a tropical reef clownfish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 204, 104–112. https://doi.org/10.1016/J.CBPA.2016.11.018

Madeira, D., Vinagre, C., Diniz, M.S., 2016. Are fish in hot water? Effects of warming on oxidative stress metabolism in the commercial species *Sparus aurata*. Ecological Indicators 63, 324–331. https://doi.org/10.1016/j.ecolind.2015.12.008

MADRP, 2011. Ministério da Agricultura, do Desenvolvimento Rural e das Pescas - Portaria n.º 82/2011 de 22 de Fevereiro. Diário da República 1.ª série, 886–887.

Mamede, R., Duarte, I.A., Caçador, I., Tanner, S.E., Silva, M., Jacinto, D., Fonseca, V.F., Duarte, B., 2022. Elemental fingerprinting of sea urchin (*Paracentrotus lividus*) gonads to assess food safety and trace its geographic origin. Journal of Food Composition and Analysis 114, 104764. https://doi.org/10.1016/j.jfca.2022.104764

Marsh, A.G., Powell, M.L., Watts, S.A., 2013. Biochemical and energy requirements of gonad development, in: Lawrence, J.M. (Ed.), Sea Urchins: Biology and Ecology. Elsevier, Tampa, Florida, pp. 45–57.

Martínez-Pita, I., García, F.J., Pita, M.-L., 2010. Males and females gonad fatty acids of the sea urchins *Paracentrotus lividus* and *Arbacia lixula* (Echinodermata). Helgoland Marine Research 64, 135–142. https://doi.org/10.1007/s10152-009-0174-7

Martinsdóttir, E., Schelvis, R., Hyldig, G., Sveinsdóttir, K., 2009a. Sensory evaluation of seafood: methods, in: Rehbein, H., Oehlenschläger, J. (Eds.), Fishery Products: Quality, Safety and Authenticity. Wiley-Blackwell, Oxford, pp. 425–443.

Martinsdóttir, E., Schelvis, R., Hylding, G., Sveinsdóttir, K., 2009b. Sensory evaluation of seafood: general principles and guidelines, in: Rehbein, H., Oehlenschläger, J. (Eds.), Fishery Products: Quality, Safety and Authenticity. Wiley-Blackwell, Oxford, pp. 411–424.

Maulvault, A.L., Anacleto, P., Barbosa, V., Sloth, J.J., Rasmussen, R.R., Tediosi, A., Fernandez-Tejedor, M., van den Heuvel, F.H.M., Kotterman, M., Marques, A., 2015. Toxic elements and speciation in seafood samples from different contaminated sites in Europe. Environmental Research 143, 72–81. https://doi.org/10.1016/j.envres.2015.09.016

Maulvault, A.L., Barbosa, V., Alves, R., Anacleto, P., Camacho, C., Cunha, S., Fernandes, J.O., Ferreira, P.P., Rosa, R., Marques, A., Diniz, M., 2018. Integrated multi-biomarker responses of juvenile seabass to diclofenac, warming and acidification co-exposure. Aquatic Toxicology 202, 65–79. https://doi.org/10.1016/j.aquatox.2018.06.016

McBride, S.C., 2005. Sea urchin aquaculture, in: American Fisheries Society Symposium. American Fisheries Society, pp. 179–208.

McBride, S.C., Price, R.J., Tom, P.D., Lawrence, J.M., Lawrence, A.L., 2004. Comparison of gonad quality factors: color, hardness and resilience, of *Strongylocentrotus franciscanus* between sea urchins fed prepared feed or algal diets and sea urchins harvested from the Northern California fishery. Aquaculture 233, 405–422. https://doi.org/10.1016/j.aquaculture.2003.10.014

McCord, J.M., Fridovich, I., 1969. Superoxide dismutase an enzymic function for erythrocuprein (hemocuprein). Journal of Biological Chemistry 244, 6049–6055. https://doi.org/10.1016/S0021-9258(18)63504-5

Meilgaard, M.C., Civille, G.V., Carr, B.T., 2016. Sensory Evaluation Techniques, 5th ed. CRC press, Boca Raton.

Meloni, D., Fois, F., 2013. Aspetti biologici, note igienico-sanitarie e commerciali del riccio di

mare nella costa nord-occidentale della Sardegna [WWW Document]. La pagina scientifica. URL https://www.pubblicitaitalia.com/pesce/prodotti/il-pesce/2013/5/12750 (accessed 7.13.22).

Mendes, A., Araújo, J., Soares, F., Bandarra, N., Pousão-Ferreira, P., 2019. Production of Purple Sea Urchin *Paracentrotus lividus* in Portugal. The World Aquaculture Society 50, 46–50.

Mendes, R., Quinta, R., Nunes, M.L., 2001. Changes in baseline levels of nucleotides during ice storage of fish and crustaceans from the Portuguese coast. European Food Research and Technology 212, 141–146. https://doi.org/10.1007/s002170000222

Merino, M., Vicente, E., Gonzales, K.N., Torres, F.G., 2017. Ageing and degradation determines failure mode on sea urchin spines. Materials Science and Engineering: C 78, 1086–1092. https://doi.org/10.1016/j.msec.2017.04.155

Mizukawa, K., Takada, H., Takeuchi, I., Ikemoto, T., Omori, K., Tsuchiya, K., 2009. Bioconcentration and biomagnification of polybrominated diphenyl ethers (PBDEs) through lower-trophic-level coastal marine food web. Marine Pollution Bulletin 58, 1217–1224. https://doi.org/10.1016/j.marpolbul.2009.03.008

Mol, S., Alakavuk, D., Tosun, Ş.Y., 2008a. Effects of different processing technologies on the chemical composition of seafoods. Food Science and Technology Research 14, 467. https://doi.org/10.3136/fstr.14.467

Mol, S., Baygar, T., Varlik, C., Tosun, Ş.Y., 2008b. Seasonal variations in yield, fatty acids, amino acids and proximate compositions of sea urchin (*Paracentrotus lividus*) roe. Journal of Food and Drug Analysis 16, 68–74. https://doi.org/10.38212/2224-6614.2363

Monfort, M.C., 2002. Fish roe in Europe: Supply and demand conditions. Food and Agriculture Organization of the United Nations, GLOBEFISH, Fishery …, Rome, FAO.

Morillo, J., Usero, J., El Bakouri, H., 2008. Biomonitoring of heavy metals in the coastal waters of two industrialised bays in southern Spain using the barnacle *Balanus amphitrite*. Chemical Speciation & Bioavailability 20, 227–237. https://doi.org/10.3184/095422908X380992

Moskovitz, J., Oien, D.B., 2010. Protein carbonyl and the methionine sulfoxide reductase system. Antioxidants & Redox Signaling 12, 405–415. https://doi.org/10.1089/ars.2009.2809

Mottaleb, M.A., Usenko, S., O'Donnell, J.G., Ramirez, A.J., Brooks, B.W., Chambliss, C.K., 2009. Gas chromatography–mass spectrometry screening methods for select UV filters, synthetic musks, alkylphenols, an antimicrobial agent, and an insect repellent in fish. Journal of Chromatography A 1216, 815–823. https://doi.org/10.1016/j.chroma.2008.11.072

Moureaux, C., Pérez-Huerta, A., Compère, P., Zhu, W., Leloup, T., Cusack, M., Dubois, P., 2010. Structure, composition and mechanical relations to function in sea urchin spine. Journal of Structural Biology 170, 41–49. https://doi.org/10.1016/J.JSB.2010.01.003

Murata, Y., Yoshimura, H., Unuma, T., 2020. Compositions of extractive components in the testes and ovaries of various sea urchins: comparisons among species, sexes, and maturational status. Fisheries Science 86, 203–213. https://doi.org/10.1007/s12562-019-01388-y

Murphy, C.A., Rose, K.A., del Carmen Alvarez, M., Fuiman, L.A., 2008. Modeling larval fish behavior: scaling the sublethal effects of methylmercury to population-relevant endpoints. Aquatic Toxicology 86, 470–484. https://doi.org/10.1016/j.aquatox.2007.12.009

Mykles, D.L., 1998. Intracellular proteinases of invertebrates: calcium-dependent and proteasome/ubiquitin-dependent systems. International Review of Cytology 184, 157–289. https://doi.org/10.1016/S0074-7696(08)62181-6

Nancharaiah, Y. V, Reddy, G.K.K., Mohan, T.V.K., Venugopalan, V.P., 2015. Biodegradation of tributyl phosphate, an organosphate triester, by aerobic granular biofilms. Journal of Hazardous Materials 283, 705–711. https://doi.org/10.1016/j.jhazmat.2014.09.065

Nielsen, S.P., Bärenholdt, O., Bärenholdt-Schiøler, C., Mauras, Y., Allain, P., 2004. Noninvasive measurement of bone strontium. Journal of Clinical Densitometry 7, 262–268. https://doi.org/10.1385/JCD:7:3:262

Ning, Y., Zhang, F., Tang, L., Song, J., Ding, J., Chang, Y., Zuo, R., 2022. Effects of dietary lipid sources on the growth, gonad development, nutritional and organoleptic quality, transcription of fatty acid synthesis related genes and antioxidant capacity during cold storage in adult sea urchin (*Strongylocentrotus intermedius*). Aquaculture 548, 737688. https://doi.org/10.1016/j.aquaculture.2021.737688

Nishimura, T., Kato, H., 1988. Taste of free amino acids and peptides. Food Reviews International 4, 175–194. https://doi.org/10.1080/87559128809540828

Nordin, D.M.A., Kramer, D.E., 1979. Studies on the Handling and Processing of Sea Urchin Roe: Fresh Product. I. Technology Services Branch, Fisheries Management, Pacific Region, Department ..., Vancouver.

NP 2309-2, 1988. Microbiologia Alimentar: Conservas. Parte 2: Apreciação da esterilidade.

NP 4404-1, 2002. Microbiologia Alimentar: Conservas. Parte 1: Apreciação da estabilidade, provas de estufa (método corrente).

Nunes, M.L., Bandarra, N.M., Batista, I., 2011. Health benefits associated with seafood consumption, in: Alasalvar, C., Shahidi, F., Miyashita, K., Wanasundara, U. (Eds.), Handbook of Seafood Quality, Safety and Health Applications. Wiley-Blackwell, Chichester, pp. 369–379.

Oceatec, 2022. Oceatec - Services in seafood processing equipment design [WWW Document]. URL http://oceatec.com/urchin.html (accessed 12.30.22).

Oehlenschläger, J., 1997. Marine Fish - A source for essential elements?!, in: Luten, J.B., Borresen, T., Oehlenschläger, J. (Eds.), Seafood from Producer to Consumer, Integrated Approach to Quality. Amsterdam, pp. 641–652.

Ólafsdóttir, G., Martinsdóttir, E., Oehlenschläger, J., Dalgaard, P., Jensen, B., Undeland, I., Mackie, I.M., Henehan, G., Nielsen, J., Nilsen, H., 1997. Methods to evaluate fish freshness in research and industry. Trends in Food Science & Technology 8, 258–265. https://doi.org/10.1016/S0924-2244(97)01049-2

Oliveira, H., Muniz, J.A., Bandarra, N.M., Castanheira, I., Coelho, I.R., Delgado, I., Gonçalves, S., Lourenço, H.M., Motta, C., Duarte, M.P., 2019. Effects of industrial boiling on the nutritional profile of common octopus (*Octopus vulgaris*). Foods 8, 411. https://doi.org/10.3390/foods8090411

Olmedo, P., Pla, A., Hernández, A.F., Barbier, F., Ayouni, L., Gil, F., 2013. Determination of toxic elements (mercury, cadmium, lead, tin and arsenic) in fish and shellfish samples. Risk assessment for the consumers. Environment International 59, 63–72. https://doi.org/10.1016/j.envint.2013.05.005

Osako, K., Fujii, A., Ruttanapornvareesakul, Y., Nagano, N., Kuwahara, K., Okamoto, A., 2007. Differences in free amino acid composition between testis and ovary of sea urchin

Anthocidaris crassispina during gonadal development. Fisheries Science 73, 660–667. https://doi.org/10.1111/j.1444-2906.2007.01379.x

Pagano, G., Meriç, S., De Biase, A., Petruzzelli, D., Tünay, O., Warnau, M., 2002. Toxicity of bauxite manufacturing by-products in sea urchin embryos. Ecotoxicology and Environmental Safety 51, 28–34. https://doi.org/10.1006/eesa.2001.2114

Paglia, D.E., Valentine, W.N., 1967. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. The Journal of Laboratory and Clinical Medicine 70, 158–169. https://doi.org/10.5555/uri:pii:0022214367900765

Pais, A., Saba, S., Rubattu, R., Meloni, G., Montisci, S., 2011. Proximate composition of edible sea urchin *Paracentrotus lividus* roe commercialised in Sardinia. Biologia Marina Mediterranea 18, 390–391.

Palmquist, K., Salatas, J., Fairbrother, A., 2012. Pyrethroid insecticides: use, environmental fate, and ecotoxicology. Insecticides-advances in integrated pest management 251–278.

Parolini, M., Magni, S., Traversi, I., Villa, S., Finizio, A., Binelli, A., 2015. Environmentally relevant concentrations of galaxolide (HHCB) and tonalide (AHTN) induced oxidative and genetic damage in *Dreissena polymorpha*. Journal of Hazardous Materials 285, 1–10. https://doi.org/10.1016/j.jhazmat.2014.11.037

Pastoriza, L., Bernárdez, M., Sampedro, G., Cabo, M.L., Herrera, J.J.R., 2004. Elevated concentrations of oxygen on the stability of live mussel stored refrigerated. European Food Research and Technology 218, 415–419. https://doi.org/10.1007/s00217-004-0873-x

Pearce, C.M., Daggett, T.L., Robinson, S.M.C., 2004. Effect of urchin size and diet on gonad yield and quality in the green sea urchin (*Strongylocentrotus droebachiensis*). Aquaculture 233, 337–367. https://doi.org/10.1016/j.aquaculture.2003.09.027

Pearce, C.M., Daggett, T.L., Robinson, S.M.C., 2002. Effect of binder type and concentration on prepared feed stability and gonad yield and quality of the green sea urchin, *Strongylocentrotus droebachiensis*. Aquaculture 205, 301–323. https://doi.org/10.1016/S0044-8486(01)00685-8

Pecorino, D., Lamare, M.D., Barker, M.F., 2013. Reproduction of the Diadematidae sea urchin *Centrostephanus rodgersii* in a recently colonized area of northern New Zealand. Marine Biology Research 9, 157–168. https://doi.org/10.1080/17451000.2012.708046

Peng, J., Yuan, J.-P., Wang, J.-H., 2012. Effect of diets supplemented with different sources of astaxanthin on the gonad of the sea urchin *Anthocidaris crassispina*. Nutrients 4, 922–934. https://doi.org/10.3390/nu4080922

Pert, C.G., Swearer, S.E., Dworjanyn, S., Kriegisch, N., Turchini, G.M., Francis, D.S., Dempster, T., 2018a. Barrens of gold: gonad conditioning of an overabundant sea urchin. Aquaculture Environment Interactions 10, 345–361.

Pert, C.G., Swearer, S.E., Dworjanyn, S., Kriegisch, N., Turchini, G.M., Francis, D.S., Dempster, T., 2018b. Barrens of gold: gonad conditioning of an overabundant sea urchin. Aquaculture Environment Interactions 10, 345–361. https://doi.org/10.3354/aei00274

Phillips, K., Bremer, P., Silcock, P., Hamid, N., Delahunty, C., Barker, M., Kissick, J., 2009. Effect of gender, diet and storage time on the physical properties and sensory quality of sea urchin (*Evechinus chloroticus*) gonads. Aquaculture 288, 205–215. https://doi.org/10.1016/j.aquaculture.2008.11.026

Phillips, K., Hamid, N., Silcock, P., Delahunty, C., Barker, M., Bremer, P., 2010. Effect of season on

the sensory quality of sea urchin (*Evechinus chloroticus*) roe. Journal of Food Science 75, S20–S30. https://doi.org/10.1111/j.1750-3841.2009.01388.x

Pinna, M.G., 2016. The Edible sea urchin *Paracentrotus lividus* (Lamarck, 1816): a valuable resource for fisheries and aquaculture purposes in Sardinia (Central-western Mediterranean). Universita'degli studi di Sassari.

Pinsino, A., Della Torre, C., Sammarini, V., Bonaventura, R., Amato, E., Matranga, V., 2008. Sea urchin coelomocytes as a novel cellular biosensor of environmental stress: a field study in the Tremiti Island Marine Protected Area, Southern Adriatic Sea, Italy. Cell Biology and Toxicology 24, 541–552. https://doi.org/10.1007/s10565-008-9055-0

Pinsino, A., Matranga, V., 2015. Sea urchin immune cells as sentinels of environmental stress. Developmental & Comparative Immunology 49, 198–205. https://doi.org/10.1016/j.dci.2014.11.013

Pither, R.J., 2003. Canning| Quality changes during canning. Elsevier, pp. 845–851.

Po, B.H.K., Ho, K.-L., Lam, M.H.W., Giesy, J.P., Chiu, J.M.Y., 2017. Uptake and biotransformation of 2, 2′, 4, 4′-tetrabromodiphenyl ether (BDE-47) in four marine microalgae species. Scientific Reports 7, 1–11. https://doi.org/10.1038/srep44263

Pozharitskaya, O.N., Shikov, A.N., Laakso, I., Seppänen-Laakso, T., Makarenko, I.E., Faustova, N.M., Makarova, M.N., Makarov, V.G., 2015. Bioactivity and chemical characterization of gonads of green sea urchin *Strongylocentrotus droebachiensis* from Barents Sea. Journal of Functional Foodsoods 17, 227–234. https://doi.org/10.1016/j.jff.2015.05.030

Prabhakar, P.K., Vatsa, S., Srivastav, P.P., Pathak, S.S., 2020. A comprehensive review on freshness of fish and assessment: Analytical methods and recent innovations. Food Research International 133, 109157. https://doi.org/10.1016/j.foodres.2020.109157

Prato, E., Fanelli, G., Angioni, A., Biandolino, F., Parlapiano, I., Papa, L., Denti, G., Secci, M., Chiantore, M., Kelly, M.S., Paola, M., Addis, P., 2018. Influence of a prepared diet and a macroalga (Ulva sp.) on the growth , nutritional and sensory qualities of gonads of the sea urchin *Paracentrotus lividus*. Aquaculture 493, 240–250. https://doi.org/10.1016/j.aquaculture.2018.05.010

Price, A.R.G., Readman, J.W., 2013. 12 Booster biocide antifoulants: is history repeating itself?, in: Late Lessons from Early Warnings: Science, Precaution, Innovation.

Proville, J.J., 2009. Uni: The Taste of the Sea, On the Plate [WWW Document]. Starchefs - moving the industry forward. URL https://www.starchefs.com/features/uni/html/ (accessed 3.1.21).

Qin, D., Jiang, H., Bai, S., Tang, S., Mou, Z., 2015. Determination of 28 trace elements in three farmed cyprinid fish species from Northeast China. Food Control 50, 1–8. https://doi.org/10.1016/j.foodcont.2014.08.016

Radenac, G., Fichet, D., Miramand, P., 2001. Bioaccumulation and toxicity of four dissolved metals in *Paracentrotus lividus* sea-urchin embryo. Marine Environmental Research 51, 151–166. https://doi.org/10.1016/S0141-1136(00)00092-1

Rasmussen, R.R., Hedegaard, R. V, Larsen, E.H., Sloth, J.J., 2012. Development and validation of an SPE HG-AAS method for determination of inorganic arsenic in samples of marine origin. Analytical and Bioanalytical Chemistry 403, 2825–2834. https://doi.org/10.1007/s00216-012-6006-7

Reis, P.A., 2013. Cirripides Chathamalus montagui e pollicipes-Pollicipes como biomonitores de contaminação por metais e hidrocarbonetos aromáticos policíclicos ao longo da costa

noroeste de Portugal. Universidade do Porto.

Reis, P.A., Antunes, J.C., Almeida, C.M.R., 2009. Metal levels in sediments from the Minho estuary salt marsh: a metal clean area? Environmental Monitoring and Assessment 159, 191–205. https://doi.org/10.1007/s10661-008-0622-x

Reis, P.A., Salgado, M.A., Vasconcelos, V., 2012. *Chthamalus montagui* as biomonitor of metal contamination in the northwest coast of Portugal. Environmental Monitoring and Assessment 184, 5421–5437. https://doi.org/10.1007/s10661-011-2350-x

Repolho, T.F.B.R., 2012. Evaluation of *Paracentrotus lividus* (Lamarck, 1816) exotrophic larvae as live feed for marine decapod crustacean larvae. Universidade de Lisboa.

Reuver, M., Barbosa, V., Marques, A., Calis, T., Tediosi, A., Cunha, S.C., Fernandes, J.O., 2017. Priority environmental contamination in seafood: safety assessment, impact and public perception, within ECsafeSEAFOOD project.

Reynolds, J.A., Wilen, J.E., 2000. The sea urchin fishery: harvesting, processing and the market. Marine Resource Economics 15, 115–126. https://doi.org/10.1086/mre.15.2.42629295

Ricklund, N., Kierkegaard, A., McLachlan, M.S., 2010. Levels and potential sources of decabromodiphenyl ethane (DBDPE) and decabromodiphenyl ether (DecaBDE) in lake and marine sediments in Sweden. Environmental Science & Technology 44, 1987–1991. https://doi.org/10.1021/es903701q

Rocha, F., Baião, L.F., Moutinho, S., Reis, B., Oliveira, A., Arenas, F., Maia, M.R.G., Fonseca, A.J.M., Pintado, M., Valente, L.M.P., 2019a. The effect of sex, season and gametogenic cycle on gonad yield, biochemical composition and quality traits of Paracentrotus lividus along the North Atlantic coast of Portugal. Scientific Reports 9, 2994. https://doi.org/10.1038/s41598-019-39912-w

Rocha, F., Rocha, A.C., Baião, L.F., Gadelha, J., Camacho, C., Carvalho, M.L., Arenas, F., Oliveira, A., Maia, M.R.G., Cabrita, A.R., Pintado, M., Nunes, M.L., Almeida, C.M.R., Valente, L.M.P., 2019b. Seasonal effect in nutritional quality and safety of the wild sea urchin *Paracentrotus lividus* harvested in the European Atlantic shores. Food Chemistry 282, 84–94. https://doi.org/10.1016/j.foodchem.2018.12.097

Rodrigues, A.P., Lehtonen, K.K., Guilhermino, L., Guimarães, L., 2013. Exposure of *Carcinus maenas* to waterborne fluoranthene: accumulation and multibiomarker responses. Science of the Total Environment 443, 454–463. https://doi.org/10.1016/j.scitotenv.2012.10.077

Rosal, R., Rodríguez, A., Perdigón-Melón, J.A., Petre, A., García-Calvo, E., Gómez, M.J., Agüera, A., Fernández-Alba, A.R., 2010. Occurrence of emerging pollutants in urban wastewater and their removal through biological treatment followed by ozonation. Water Research 44, 578–588. https://doi.org/10.1016/j.watres.2009.07.004

Rotzoll, N., Dunkel, A., Hofmann, T., 2006. Quantitative Studies, Taste Reconstitution, and Omission Experiments on the Key Taste Compounds in Morel Mushrooms (*Morchella deliciosa* Fr.). Journal of Agricultural and Food Chemistry 54, 2705–2711. https://doi.org/10.1021/jf053131y

Rouane-Hacene, O., Boutiba, Z., Benaissa, M., Belhaouari, B., Francour, P., Guibbolini-Sabatier, M.E., Faverney, C.R.-D., 2018. Seasonal assessment of biological indices, bioaccumulation, and bioavailability of heavy metals in sea urchins *Paracentrotus lividus* from Algerian west coast, applied to environmental monitoring. Environmental Science and Pollution Research 25, 11238–11251. https://doi.org/10.1007/s11356-017-8946-0

Rubilar, T., Barbieri, E.S., Gazquez, A., Avaro, M., 2021. Sea urchin pigments: echinochrome a and its potential implication in the cytokine storm syndrome. Marine Drugs 19, 267. https://doi.org/10.3390/md19050267

Rubilar, T., Crespi-Abril, A., 2017. Does Echinoderm research deserve an ethical consideration? Revista de Biología Tropical 65 (1), S11–S22. https://doi.org/10.15517/rbt.v65i1-1.31662

Saint-Denis, T., Goupy, J., 2004. Optimization of a nitrogen analyser based on the Dumas method. Analytica Chimica Acta 515, 191–198. https://doi.org/10.1016/j.aca.2003.10.090

Salgado, R., Noronha, J.P., Oehmen, A., Carvalho, G., Reis, M.A.M., 2010. Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. Water Science and Technology 62, 2862–2871. https://doi.org/10.2166/wst.2010.985

Salvo, A., Cicero, N., Vadalà, R., Mottese, A.F., Bua, D., Mallamace, D., Giannetto, C., Dugo, G., 2016. Toxic and essential metals determination in commercial seafood: *Paracentrotus lividus* by ICP-MS. Natural Product Research 30, 657–664. https://doi.org/10.1080/14786419.2015.1038261

Salvo, A., Potortì, A.G., Cicero, N., Bruno, M., Turco, V. Lo, Bella, G. Di, Dugo, G., 2014. Statistical characterisation of heavy metal contents in *Paracentrotus lividus* from Mediterranean Sea. Natural Product Research 28, 718–726. https://doi.org/10.1080/14786419.2013.878937

Sampels, S., 2015. The effects of processing technologies and preparation on the final quality of fish products. Trends in Food Science & Technology 44, 131–146. https://doi.org/10.1016/j.tifs.2015.04.003

Sant'Anna, V., Gurak, P.D., Marczak, L.D.F., Tessaro, I.C., 2013. Tracking bioactive compounds with colour changes in foods–A review. Dyes and Pigments 98, 601–608. https://doi.org/10.1016/j.dyepig.2013.04.011

Santos-Ferreira, N., Mesquita, J.R., Rivadulla, E., Inácio, Â.S., Nascimento, M.S.J., Romalde, J., da Costa, P.M., 2020. Norovirus contamination of sea urchins (*Paracentrotus lividus*): Potential food risk for consumers. Food Control 111, 107041. https://doi.org/10.1016/j.foodcont.2019.107041

Sarower, G., Farah, A., Hasanuzzaman, M., Abe, H., 2012. Taste producing components in fish and fisheries products: A review. International Journal of Food and Fermentation Technology 2, 113–121.

Schäfer, S., Abele, D., Weihe, E., Köhler, A., 2011. Sex-specific biochemical and histological differences in gonads of sea urchins (*Psammechinus miliaris*) and their response to phenanthrene exposure. Marine Environmental Research 71, 70–78. https://doi.org/10.1016/j.marenvres.2010.10.004

Schubring, R., 2009. Colour measurement, in: Rehbein, H., Oehlenschläger, J. (Eds.), Fishery Products: Quality, Safety and Authenticity. Wiley Blackwell, pp. 127–172.

Senaratna, M., Evans, L.H., Southam, L., Tsvetnenko, E., 2005. Effect of different feed formulations on feed efficiency, gonad yield and gonad quality in the purple sea urchin *Heliocidaris erythrogramma*. Aquaculture Nutrition 11, 199–207. https://doi.org/10.1111/j.1365-2095.2005.00340.x

Serra-Compte, A., Maulvault, A.L., Camacho, C., Álvarez-Muñoz, D., Barceló, D., Rodríguez-Mozaz, S., Marques, A., 2018. Effects of water warming and acidification on bioconcentration, metabolization and depuration of pharmaceuticals and endocrine disrupting compounds in marine mussels (*Mytilus galloprovincialis*). Environmental

Pollution 236, 824–834. https://doi.org/10.1016/j.envpol.2018.02.018

Serrano, C., Carbas, B., Castanho, A., Soares, A., Patto, M.C.V., Brites, C., 2017. Characterisation of nutritional quality traits of a chickpea (*Cicer arietinum*) germplasm collection exploited in chickpea breeding in Europe. Crop and Pasture Science 68, 1031–1040. https://doi.org/10.1071/CP17129

SFT, 2007. Pollution Control Authorities SFT T-2229/2007 - Guidelines for classification of environmental quality in fjords and coastal waters. Revision of Metals and Organic Contaminants in Water and Sediment. Norwegian Pollution Control Authority.

Sharaf, H.M., Shehata, A.M., 2015. Heavy metals and hydrocarbon concentrations in water, sediments and tissue of *Cyclope neritea* from two sites in Suez Canal, Egypt and histopathological effects. Journal of Environmental Health Science and Engineering 13, 1–8. https://doi.org/10.1186/s40201-015-0171-5

Sharma, G., Bala, R., 2017. Digital Color Imaging Handbook. CRC press, Webster, New York.

Sheppard, K.W., Cheatham, C.L., 2018. Omega-6/omega-3 fatty acid intake of children and older adults in the US: dietary intake in comparison to current dietary recommendations and the Healthy Eating Index. Lipids in health and disease 17, 1–12. https://doi.org/10.1186/s12944-018-0693-9

Shimada, K., Nakagawa, S., Hotta, K., Shibata, K., Yagi, T., 1993. Effect of processing and storage on the fate of vitamins B1, B2, and B6 and nicotinamide of sea urchin gonads. Journal of Agricultural and Food Chemistry 41, 1021–1025. https://doi.org/10.1021/jf00031a001

Shimada, K., Ogura, N., 1990. Lipid changes in sea urchin gonads during storage. Journal of Food Science 55, 967–971. https://doi.org/10.1111/j.1365-2621.1990.tb01576.x

Shpigel, M., McBride, S.C., Marciano, S., Ron, S., Ben-Amotz, A., 2005. Improving gonad colour and somatic index in the European sea urchin *Paracentrotus lividus*. Aquaculture 245, 101–109. https://doi.org/10.1016/j.aquaculture.2004.11.043

Shpigel, M., Schlosser, S.C., Ben-Amotz, A., Lawrence, A.L., Lawrence, J.M., 2006. Effects of dietary carotenoid on the gut and the gonad of the sea urchin *Paracentrotus lividus*. Aquaculture 261, 1269–1280. https://doi.org/10.1016/j.aquaculture.2006.08.029

Sibiya, A., Jeyavani, J., Sivakamavalli, J., Ravi, C., Divya, M., Vaseeharan, B., 2021. Bioactive compounds from various types of sea urchin and their therapeutic effects — a review. Regional Studies in Marine Science 44, 101760. https://doi.org/10.1016/j.rsma.2021.101760

Siikavuopio, S.I., Dale, T., Carlehög, M., 2007. Sensory quality of gonads from the green sea urchin, *Strongylocentrotus droebachiensis*, fed different diets. Journal of Shellfish Research 26, 637–643. https://doi.org/10.2983/0730-8000(2007)26[637:SQOGFT]2.0.CO;2

Sikorski, Z.E., Kołakowska, A., Pan, B.S., 1990. The nutritive composition of the major groups of marine food organisms, in: Sikorski, Z.E. (Ed.), Seafood: Resources, Nutritional Composition, and Preservation. CRC Press, Florida, pp. 30–54.

Siliani, S., Melis, R., Loi, B., Guala, I., Baroli, M., Sanna, R., Uzzau, S., Roggio, T., Addis, M.F., Anedda, R., 2016. Influence of seasonal and environmental patterns on the lipid content and fatty acid profiles in gonads of the edible sea urchin *Paracentrotus lividus* from Sardinia. Marine Environmental Research 113, 124–133. https://doi.org/10.1016/j.marenvres.2015.12.001

Silva, L.J.G., Pereira, A.M.P.T., Meisel, L.M., Lino, C.M., Pena, A., 2014. A one-year follow-up analysis of antidepressants in Portuguese wastewaters: occurrence and fate, seasonal

influence, and risk assessment. Science of the Total Environment 490, 279–287. https://doi.org/10.1016/j.scitotenv.2014.04.131

Sloth, J.J., 2017. Report on collaborative trial: Animal feedingstuffs–Determination of iodine in animal feed by ICPMS. National Food Institute, Technical University of Denmark.

Smith, L.C., Ghosh, J., Buckley, K.M., Clow, L.A., Dheilly, N.M., Haug, T., Henson, J.H., Li, C., Lun, C.M., Majeske, A.J., 2010. Echinoderm immunity, in: Söderhäll, K. (Ed.), Invertebrate Immunity. Springer, New York, pp. 260–301.

Smyth, S.A., Lishman, L.A., McBean, E.A., Kleywegt, S., Yang, J.-J., Svoboda, M.L., Lee, H.-B., Seto, P., 2008. Seasonal occurrence and removal of polycyclic and nitro musks from wastewater treatment plants in Ontario, Canada. Journal of Environmental Engineering and Science 7, 299–317. https://doi.org/10.1139/S08-002

Soares, H., Boaventura, R.A.R., Machado, A., Da Silva, J.C.G.E., 1999. Sediments as monitors of heavy metal contamination in the Ave river basin (Portugal): multivariate analysis of data. Environmental Pollution 105, 311–323. https://doi.org/10.1016/S0269-7491(99)00048-2

Soliman, M.M., Fahmy, A.A., Elsawy, A.A., Osman, F., 1983. Effect of salting treatment on the flavor of oyster, donax and sea- urchin. Agricultural And Biological Chemistry 47, 1655–1659. https://doi.org/10.1080/00021369.1983.10865837

Soualili, D., Dubois, P., Gosselin, P., Pernet, P., Guillou, M., 2008. Assessment of seawater pollution by heavy metals in the neighbourhood of Algiers: use of the sea urchin, *Paracentrotus lividus*, as a bioindicator. ICES Journal of Marine Science 65, 132–139. https://doi.org/10.1093/icesjms/fsm183

Spirlet, C., Grosjean, P., Jangoux, M., 2000. Optimization of gonad growth by manipulation of temperature and photoperiod in cultivated sea urchins, *Paracentrotus lividus* (Lamarck) (Echinodermata). Aquaculture 185, 85–99. https://doi.org/10.1016/S0044-8486(99)00340-3

Stamatis, N., Vafidis, D., 2009. Effect of marinating and vacuum storage at 6 °C on the fate of chemical, microbial and sensory quality indices of echinoid gonads *Paracentrotus lividus* Lamark, 1816. International Journal of Food Science & Technology 44, 1626–1633. https://doi.org/10.1111/j.1365-2621.2009.01940.x

Stefánsson, G., Kristinsson, H., Ziemer, N., Hannon, C., James, P., 2017. Markets for sea urchins: A review of global supply and markets, Skýrsla Matís. Reykjavík.

Stefánsson, G., Ólafsdóttir, A., 2019. Holding of Sea Urchins and Scallops in a RAS Transport System. Tromsø. https://doi.org/10.5281/zenodo.3598458

Stefánsson, G., Ólafsdóttir, A., 2017. The keeping quality of chilled sea urchin roe and whole urchins.

Sukhn, C., 2013. Bioaccumulation and depuration in sea urchins *Paracentrotus lividus* (Lebanon) and *Heliocidaris erythrogramma* (Australia). UNSW Sydney.

Sun, J., Chiang, F., 2015. Use and exploitation of sea urchins, in: Brown, N., Eddy, S. (Eds.), Echinoderm Aquaculture. John Wiley and Sons, Inc., New Jersey, pp. 25–45.

Supawong, S., Park, J.W., Park, J., 2022. Functional and Chemical Properties of Gim (*Porphyra yezoensis*) as Affected by the Product Form. Journal of Aquatic Food Product Technology 31, 418–429. https://doi.org/10.1080/10498850.2022.2060053

Symonds, R.C., Kelly, M.S., Caris-Veyrat, C., Young, A.J., 2007. Carotenoids in the sea urchin *Paracentrotus lividus*: Occurrence of 9′-cis-echinenone as the dominant carotenoid in gonad colour determination. Comparative Biochemistry and Physiology Part B:

Biochemistry and Molecular Biology 148, 432–444. https://doi.org/10.1016/j.cbpb.2007.07.012

Takagi, S., Murata, Y., Inomata, E., Endo, H., Aoki, M.N., Agatsuma, Y., 2017. Improvement of gonad quality of the sea urchin *Mesocentrotus nudus* fed the kelp *Saccharina japonica* during offshore cage culture. Aquaculture 477, 50–61. https://doi.org/10.1016/j.aquaculture.2017.04.033

Taylor, A.M., Heflin, L.E., Powell, M.L., Lawrence, A.L., Watts, S.A., 2017. Effects of dietary carbohydrate on weight gain and gonad production in small sea urchins, *Lytechinus variegatus*. Aquaculture Nutrition 23, 375–386. https://doi.org/10.1111/anu.12403

Tejada, M., 2009. ATP-derived products and K-value determination, in: Fishery Products: Quality, Safety and Authenticity. Wiley Online Library, pp. 68–88.

Tejada, S., Deudero, S., Box, A., Sureda, A., 2013. Physiological response of the sea urchin *Paracentrotus lividus* fed with the seagrass *Posidonia oceanica* and the alien algae *Caulerpa racemosa* and *Lophocladia lallemandii*. Marine Environmental Research 83, 48–53. https://doi.org/10.1016/j.marenvres.2012.10.008

Terrosu, G., Tedde, T., Scarano, C., Congiu, S., Caria, G., Uda, M.T., Chessa, G., Lorenzoni, G., Virgilio, S., 2011. Proposal of sanitary management of edible echinoderms in Sardinia. Italian Journal of Food Safety 1, 25–29. https://doi.org/10.4081/ijfs.2011.832

Tillocca, M.G., 2016. Tecnologia di produzione della Polpa di Riccio di mare della specie *Paracentrotus lividus* e controlli sanitari. Università di Pisa.

Tomanek, L., 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. Journal of Experimental Biology 213, 971–979. https://doi.org/10.1242/jeb.038034

Tomšić, S., Conides, A., Radić, I.D., Glamuzina, B., 2010. Growth, size class frequency and reproduction of purple sea urchin, *Paracentrotus lividus* (Lamarck, 1816) in Bistrina Bay (Adriatic Sea, Croatia). Acta Adriatica 51, 67.

Torres, T., Cunha, I., Martins, R., Santos, M.M., 2016. Screening the toxicity of selected personal care products using embryo bioassays: 4-MBC, propylparaben and triclocarban. International Journal of Molecular Sciences 17, 1762. https://doi.org/10.3390/ijms17101762

Tsafnat, N., Fitz Gerald, J.D., Le, H.N., Stachurski, Z.H., 2012. Micromechanics of Sea Urchin Spines. PLoS ONE 7, e44140. https://doi.org/10.1371/journal.pone.0044140

Uchiyama, M., Mihara, M., 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Analytical Biochemistry 86, 271–278. https://doi.org/10.1016/0003-2697(78)90342-1

UNEP, 2005. Ridding the World of POPs: a Guide to the Stockholm Convention on Persistent Organic Pollutants. United Nation Environment Program, Geneva. Geneva.

Unuma, T., Walker, C.W., 2009. Relationship between gametogenesis and food quality in sea urchin gonads, in: Aquaculture Technologies for Invertebrates: Proceedings of the 36th US-Japan Aquaculture Panel Symposium. pp. 45–53.

USDA, 2015. Dietary Reference Intakes (DRIs): Tolerable Upper Intake levels, Elements [WWW Document]. URL http://www.nal.usda.gov/fnic/DRDRI_Tables/DRI_Elements.pdf (accessed 7.14.17).

Vahter, M., 2002. Mechanisms of arsenic biotransformation. Toxicology 181, 211–217.

https://doi.org/10.1016/S0300-483X(02)00285-8
Van Scoy, A., Pennell, A., Zhang, X., 2016. Environmental fate and toxicology of dimethoate, in: Voogt, W.P. de (Ed.), Reviews of Environmental Contamination and Toxicology Volume 237. Springer International Publishing, pp. 53–70.
Vasconcelos, A.S.B., 2015. Estudo da qualidade da água do Rio Ave: relevância da relação entre indicadores microbiológicos, macroinvertebrados e parâmetros físico-químicos. Faculdade de Ciências da Universidade do Porto.
Verachia, W., Lazzarino, G., Niven, B., Bremer, P.J., 2013. The effect of holding live sea urchins (*Evechinus chloroticus*) in air prior to gonad removal on gonad adenine nucleotide profiles during storage at 4 °C. Food Chemistry 141, 841–846. https://doi.org/10.1016/j.foodchem.2013.03.057
Verachia, W., Niven, B., Bremer, P.J., 2012a. The effect of postharvest handling and processing on sea urchin (*Evechinus chloroticus*) gonad quality. International Journal of Food Science and Technology 47, 2545–2553. https://doi.org/10.1111/j.1365-2621.2012.03133.x
Verachia, W., Sewell, M.A., Niven, B., Leus, M., Barker, M.F., Bremer, P.J., 2012b. Seasonal changes in the biochemical composition of *Evechinus chloroticus* gonads (Echinodermata: Echinoidea). New Zealand Journal of Marine and Freshwater Research 46, 399–410. https://doi.org/10.1080/00288330.2012.697070
Verlecar, X.N., Jena, K.B., Chainy, G.B.N., 2008. Seasonal variation of oxidative biomarkers in gills and digestive gland of green-lipped mussel *Perna viridis* from Arabian Sea. Estuarine, Coastal and Shelf Science 76, 745–752. https://doi.org/10.1016/j.ecss.2007.08.002
Verling, E., Crook, A., Barnes, D., 2002. Covering behaviour in *Paracentrotus lividus*: is light important? Marine Biology 140, 391–396. https://doi.org/10.1007/s002270100689
Vicente-Zurdo, D., Gómez-Gómez, B., Pérez-Corona, M.T., Madrid, Y., 2019. Impact of fish growing conditions and cooking methods on selenium species in swordfish and salmon fillets. Journal of Food Composition and Analysis 83, 103275. https://doi.org/10.1016/j.jfca.2019.103275
Viñas, L., Franco, M.A., Soriano, J.A., González, J.J., Ortiz, L., Bayona, J.M., Albaigés, J., 2009. Accumulation trends of petroleum hydrocarbons in commercial shellfish from the Galician coast (NW Spain) affected by the *Prestige* oil spill. Chemosphere 75, 534–541. https://doi.org/10.1016/j.chemosphere.2008.12.003
Volpe, M.G., Fabbrocini, A., Siano, F., Coccia, E., Scordella, G., Licchelli, C., De Sio, F., Paolucci, M., 2018. Gonad quality of sea urchin *Paracentrotus lividus* cultured in an offshore pilot-scale trial on the south-east Italian coast. Aquaculture Nutrition 24, 1444–1455. https://doi.org/10.1111/anu.12681
Walker, C.W., Böttger, S.A., Unuma, T., Watts, S.A., Harris, L.G., Lawrence, A.L., Eddy, S.D., 2015. Enhancing the Commercial Quality of Edible Sea Urchin Gonads – Technologies Emphasizing Nutritive Phagocytes, in: Brown, N.P., Eddy, S.D. (Eds.), Echinoderm Aquaculture. Wiley Online Library, New Jersey, pp. 263–286.
Walker, C.W., Lesser, M.P., Unuma, T., 2013. Sea Urchin Gametogenesis – Structural, Functional and Molecular/Genomic Biology, in: Lawrence, J.M. (Ed.), Sea Urchins: Biology and Ecology. Elsevier, Tampa, Florida, pp. 25–43.
Wang, C., Xue, C., Xue, Y., Li, Z., Lv, Y., Zhang, H., 2012. Quality changes in sea urchin (*Strongylocentrotus nudus*) during storage in artificial seawater saturated with oxygen, nitrogen and air. Journal of the Science of Food and Agriculture 92, 191–196.

https://doi.org/10.1002/jsfa.4564

Wang, Z., de Jager, L.S., Begley, T., Genualdi, S., 2022. Large volume headspace GC/MS analysis for the identification of volatile compounds relating to seafood decomposition. Food Science & Nutrition 10, 1195–1210. https://doi.org/10.1002/fsn3.2751

Warnau, M., Biondo, R., Temara, A., Bouquegneau, J.-M., Jangoux, M., Dubois, P., 1998. Distribution of heavy metals in the echinoid *Paracentrotus lividus* from the Mediterranean Posidonia oceanica ecosystem: seasonal and geographical variations. Journal of Sea Research 39, 267–280. https://doi.org/10.1016/S1385-1101(97)00064-6

Warnau, M., Ledent, G., Temara, A., Alva, V., Jangoux, M., Dubois, P., 1995. Allometry of heavy metal bioconcentration in the echinoid *Paracentrotus lividus*. Archives of Environmental Contamination and Toxicology 29, 393–399.

Warnau, M., Teyssié, J.-L., Fowler, S.W., 1997. Cadmium bioconcentration in the echinoid *Paracentrotus lividus*: influence of the cadmium concentration in seawater. Marine Environmental Research 43, 303–314. https://doi.org/10.1016/S0141-1136(96)00093-1

Weisler, M.I., Mihaljević, M., Rogers, A.J., 2020. Sea urchins: Improving understanding of prehistoric subsistence, diet, foraging behavior, tool use, and ritual practices in Polynesia. The Journal of Island and Coastal Archaeology 15, 547–575. https://doi.org/10.1080/15564894.2019.1679293

Whalen, K., 2008. Sea Urchin Dissection Protocol [WWW Document]. URL https://www.whoi.edu/science/B/students/kwhalen/Sea Urchin Dissection Protocol.pdf (accessed 1.20.23).

Whelton, P.K., 2014. Sodium, Potassium, Blood Pressure, and Cardiovascular Disease in Humans. Current Hypertension Reports 16, 1–8. https://doi.org/10.1007/s11906-014-0465-5

Woods, C., James, P.J., Moss, G.A., Wright, J., Siikavuopio, S., 2008. A comparison of the effect of urchin size and diet on gonad yield and quality in the sea urchin *Evechinus chloroticus* Valenciennes. Aquaculture International 16, 49–68. https://doi.org/10.1007/s10499-007-9124-z

Wu, T., Wang, M., Wang, P., Tian, H., Zhan, P., 2022. Advances in the Formation and Control Methods of Undesirable Flavors in Fish. Foods 11, 2504. https://doi.org/10.3390/foods11162504

Wypych, A., Wypych, G., 2015. Databook of biocides, ChemTech P. ed. Elsevier, Toronto.

Yamaguchi, S., Yoshikawa, T., Ikeda, S., Ninomiya, T., 1971. Measurement of the relative taste intensity of some l-α-amino acids and 5′-nucleotides. Journal of Food Science 36, 846–849. https://doi.org/10.1111/j.1365-2621.1971.tb15541.x

Yamamoto, R., Minami, H., Matsusaki, H., Sakashita, M., Morita, N., Nishimiya, O., Tsutsumi, N., Hosokawa, M., Itabashi, Y., Matsui, T., 2018. Consumption of the edible sea urchin *Mesocentrotus nudus* attenuates body weight gain and hepatic lipid accumulation in mice. Journal of Functional Foods 47, 40–47. https://doi.org/10.1016/j.jff.2018.04.063

Yeruham, E., Rilov, G., Shpigel, M., Abelson, A., 2015. Collapse of the echinoid *Paracentrotus lividus* populations in the Eastern Mediterranean—result of climate change? Scientific Reports 5, 13479. https://doi.org/10.1038/srep13479

Yokoyama, Y., Sakaguchi, M., Kawai, F., Kanamori, M., 1992. Changes in concentration of ATP-related compounds in various tissues of oyster during ice storage. Nippon Suisan Gakkaishi 58, 2125–2136. https://doi.org/10.2331/suisan.58.2125

Zar, J.H., 2014. Biostatistical Analysis: Pearson New International Edition, Fifth Edit. ed. Pearson Higher Ed, New Jersey.

Zhu, B.W., Qin, L., Zhou, D.Y., Wu, H.T., Wu, J., Yang, J.F., Li, D.M., Dong, X.P., Murata, Y., 2010. Extraction of lipid from sea urchin (*Strongylocentrotus nudus*) gonad by enzyme-assisted aqueous and supercritical carbon dioxide methods. European Food Research and Technology 230, 737–743. https://doi.org/10.1007/s00217-010-1216-8

Zlatanos, S., Laskaridis, K., Sagredos, A., 2009. Determination of proximate composition, fatty acid content and amino acid profile of five lesser-common sea organisms from the Mediterranean Sea. International Journal of Food Science & Technology 44, 1590–1594. https://doi.org/10.1111/j.1365-2621.2008.01870.x

Zupo, V., Glaviano, F., Paolucci, M., Ruocco, N., Polese, G., Di Cosmo, A., Costantini, M., Mutalipassi, M., 2019. Roe enhancement of *Paracentrotus lividus*: Nutritional effects of fresh and formulated diets. Aquaculture Nutrition 25, 26–38. https://doi.org/10.1111/anu.12826

A

Appendix

Supplementary material of Subchapter 3.2

Table S1 — Elemental concentration (mg·kg^{-1} dw) and detection limits of certified reference material (CRM) analysed by EDXRF and AAS.

CRM		P	S	Cl	K	Ca	Fe	As	Br	Sr	Cr*	Ni*
	Technique	EDXRF	EDXRF	EDXRF	EDXRF	EDXRF	EDXRF	EDXRF	EDXRF	EDXRF	AAS	AAS
	LOD (mg·kg^{-1} dw)	100	100	100	10	20	3	0.70	0.8	0.6	0.050	0.044
SRM-1571	Present work	2000 ± 500 (95 %)	2200 ± 300 (96 %)	600 ± 100 (86 %)	13500 ± 1300 (92 %)	19500 ± 200 (93 %)	295 ± 10 (98 %)	15 ± 2 (107 %)	11 ± 1 (110 %)	36 ± 2 (97 %)	-	-
	Certified values	2100 ± 100	2300	700	14700 ± 300	20900 ± 300	300 ± 20	14 ± 2	10	37	-	-
DORM-2	Present work	-	-	-	-	-	145 ± 10 (102 %)	19 ± 2 (105 %)	-	-	35.3 ± 0.6 (101 %)	17.1 ± 0.2 (90 %)
	Certified value	-	-	-	-	-	142 ± 10	18.0 ± 1.1	-	-	35 ± 6	19 ± 3
TORT-2	Present work	-	-	-	-	-	102 (97 %)	21 ± 2 (97 %)	-	42 ± 4 (93 %)	0.907 ± 0.05 (113 %)	2.77 ± 0.05 (111 %)
	Certified value	-	-	-	-	-	105 ± 13	21.6 ± 1.8	-	45.2 ± 1.9	0.8 ± 0.2	2.5 ± 0.2

Certified reference materials: SRM-1571 – Orchard leaves reference material; DORM-2 – Fish protein reference material; TORT-2 – Lobster hepatopancreas reference material;

LOD - limit of detection; Recovery percentages is given in parenthesis;

*calibration range for Cr and Ni: 2.5 to 10 µg·L^{-1} with $R^2 > 0.99$.

Table S2 — Accuracy and precision for reference materials and their targets used in ICP-MS and method details.

Sample	**n**	**Cu**	**Zn**	**Se**	**Cd**	**Hg**	**Pb**	**I**	**iAs**
m/z		63	66	82	111	202	208	127	75
ISTD, m/z		Rh^{103}	Rh^{103}	Rh^{103}	In^{115}	Bi^{209}	Bi^{209}	Te^{125}	none
mode		He	He	no gas	no gas	no gas	no gas	no gas	no gas
Cal range ($ng \cdot ml^{-1}$)		2 - 12	4 - 240	0.16 - 9.6	0.02 - 1.2	0.08 - 4.8	0.030 - 0.9	0.15 - 100	0.2 - 20
LOD ($mg \cdot kg^{-1}$)	4	0.76	0.90	0.078	0.0014	0.016	0.0036	nd	nd
LOQ ($mg \cdot kg^{-1}$)		1.67	3.33	0.133	0.017	0.067	0.0025	0.03	0.01
TORT-2 ($mg \cdot kg^{-1}$)	2	106 ± 10	180 ± 6	5.63 ± 0.67	26.7 ± 0.6	0.27 ± 0.06	0.35 ± 0.13	-	-
rec±RSD		92 ± 11%	109 ± 1%	118 ± 3%	89 ± 8%	*143 ± 8%	85 ± 9%		
SRM 2976 ($mg \cdot kg^{-1}$)	2	4.02 ± 0.33	137 ± 13	1.08 ± 0.15	0.82 ± 0.16	0.0610 ± 0.0036	1.19 ± 0.18	-	-
rec±RSD		93 ± 14%	106 ± 7%	128 ± 18%	105 ± 2%	96 ± 9%	100 ± 5%		
BC211 ($mg \cdot kg^{-1}$)	2#	-	-	-	-	-	-	-	0.124 ± 0.011
rec±RSD									104 ± 15%
7405-a ($mg \cdot kg^{-1}$)	6#	-	-	-	-	-	-	-	10.1 ± 0.5
rec±RSD									93 ± 6%
Standard (2 $ng \cdot ml^{-1}$)	5#	-	-	-	-	-	-	-	2.02
rec±RSD									101 ± 3%
CD200 ($mg \cdot kg^{-1}$)	1	-	-	-	-	-	-	631 ± 47	
rec±RSD								93 ± nd %	

Accuracy and precision (rec, recovery ± RSD, relative standard derivation); targets ± ranges (in dw); LOD - limit of detection; LOQ - limit of quantification;

Certified reference materials: TORT-2 – Lobster hepatopancreas; SRM 2976 – Mussel tissue; BC211 – rice; 7405-a – seaweed (Hijiki); CD200 – powdered bladderwrack (*Fucus vesiculosus*);

* Recovery outside target range also when in house method uncertainty is included; # Instrument (injection) replicates; nd - Not determined; n - number of replicates.

Supplementary material of Subchapter 4.2

Table S3 — Nucleotides content (μmol·g^{-1}) of gonads from the live sea urchin previous experiments (trials A, B and C).

		Days of storage (~7 °C)							
		Trial A				Trial B		Trial C[a)]	
μmol·g^{-1}	repli-cate	**2**	**4**	**6**	**9**	**1**[a)]	**5**	**1**	**7**
IMP/GMP	**a**	3.54	3.38	4.28	3.98	3.90	6.54	3.22	3.40
	b	3.38	1.94	4.02	3.02	4.65	5.27	2.58	3.69
ATP	**a**	0.92	0.29 *	0.29 *	< LQ *	0.15	0.32	0.20	0.17
	b	0.71	0.13	0.21	0.13	0.17	0.22	0.17	< LQ
ADP	**a**	0.69	0.53	0.50	0.17 *	0.33	0.45	0.48	0.42
	b	0.59	0.24	0.36	0.17	0.28	0.36	0.43	0.33
AMP	**a**	0.50	1.35 #	1.30 #	0.56	1.29	1.41	1.40	1.13
	b	0.64	1.10	1.58	0.50	1.28	1.55	1.17	1.27
Hx	**a**	ND	< LQ	< LQ	0.20 #	< LQ	< LQ	< LQ	0.14
	b	ND	< LQ	< LQ	0.19	< LQ	0.12	< LQ	0.17
HxR	**a**	< LQ	0.20	0.24 #	0.79 #	0.16	0.33	< LQ	0.32
	b	< LQ	0.16	0.30	0.72	0.24	0.28	< LQ	0.41
Total amount	**a**	5.65	5.75	6.61	5.71	5.84	9.05	5.30	5.59
	b	5.32	3.57	6.47	4.73	6.62	7.80	4.34	5.86
K-value (%)	**a**	<1.48	<4.89 #	<4.86 #	17.21 #	<4.19	<4.50	<3.11	8.29
	b	<1.57	<6.77	<5.85	19.19	<4.91	5.14	<3.77	9.74
AEC (%)	**a**	59.80	25.56 *	25.79 *	<20.93 *	17.98	25.04	21.20	22.00
	b	51.90	16.92	18.19	26.97	17.91	18.72	21.87	14.82

[a)] gonads were handled at room temperature for about 2 hours before quick frozen; ND: not detected; <LQ: above limit of quantification (0.085 μmol·g^{-1}).

*indicate significant lower value in relation to day 2 (p<0.0052; Dunnett one-sided test)

#indicate significant higher value in relation to day 2 (p<0.0114; Dunnett one-sided test)

Table S4 — Nucleotides content (μmol·g^{-1}) of gonads from the live sea urchin final experiments (trials I, II and III) during storage (7.1 ± 0.8 °C).

		Days of storage																
		Trial I					Trial II								Trial III				
μmol·g^{-1}	Pool	1	3	6	8*	9*	1	3	4	5	5*	6*	7*	11*	1	3	5	6	6*
IMP/GMP	1	4.68	6.26	5.28	4.29	4.16	2.72	2.67	2.68	2.47	3.17	3.40	4.04	5.46	4.16	2.90	3.95	3.88	3.47
	2	3.31	5.69	4.53	5.32	3.90	3.54	3.21	-	3.26	2.62	-	-	-	-	-	-	2.84	-
ATP	1	0.99	0.88	0.47	0.26	0.16	1.09	0.60	0.52	0.46	0.50	0.38	0.52	ND	0.79	1.34	0.88	1.11	0.50
	2	0.97	0.50	0.36	0.21	0.19	1.12	0.75	-	0.40	0.32	-	-	-	-	-	-	0.76	-
ADP	1	0.68	0.66	0.57	0.61	0.30	0.51	0.59	0.63	0.79	0.78	0.56	0.72	0.24	0.61	0.66	0.70	0.69	0.80
	2	0.49	0.69	0.63	0.42	0.45	0.49	0.55	-	0.67	0.62	-	-	-	-	-	-	0.79	-
AMP	1	0.29	0.39	0.87	1.05	0.83	ND	0.48	0.50	0.81	0.74	0.67	0.76	0.72	0.46	0.20	0.40	0.34	0.81
	2	0.83	0.65	0.96	1.11	0.98	ND	0.44	-	0.92	0.72	-	-	-	-	-	-	0.58	-
CMP+UMP	1	2.03	2.57	3.04	2.34	2.45	2.84	2.13	2.04	1.62	3.01	3.07	3.27	4.24	3.85	1.98	3.21	3.52	2.07
	2	1.41	2.37	2.50	2.74	2.00	3.32	2.66	-	2.20	2.43	-	-	-	-	-	-	2.33	-
Hx	1	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	< LQ	0.10	0.15	0.55	< LQ	< LQ	< LQ	< LQ	0.15
	2	< LQ	< LQ	< LQ	0.13	< LQ	< LQ	< LQ	-	< LQ	< LQ	-	-	-	-	-	-	< LQ	-
HxR	1	< LQ	< LQ	< LQ	0.23	0.32	< LQ	< LQ	< LQ	< LQ	< LQ	0.11	< LQ	0.59	< LQ	< LQ	< LQ	< LQ	0.15
	2	< LQ	< LQ	< LQ	0.20	0.17	< LQ	< LQ	-	< LQ	< LQ	-	-	-	-	-	-	< LQ	-
Total amount	1	8.67	10.76	10.23	8.78	8.22	7.16	6.47	6.37	6.15	8.20	8.28	9.46	11.80	9.87	7.09	9.15	9.54	7.94
	2	7.00	9.90	8.97	10.13	7.69	8.47	7.61	-	7.45	6.71	-	-	-	-	-	-	7.30	-

ND: not detected; <LQ: above limit of quantification (0.085 μmol·g^{-1}). *pools constituted of sea urchin less reactive.

Table S5 — Concentration of free amino acids, in mg·(100 g)$^{-1}$, of gonads from live sea urchin experiments (trials I, II and III; 7.1 ± 0.8 °C) and from packaged gonads (trial PI; 2.1 ± 0.4 °C) throughout the storage.

		Days of storage (7.1 ± 0.8 °C)														Days of storage (2.1 ± 0.4 °C)		
		Trial I					Trial II						Trial III			Packed gonads (PI)		
mg·(100 g)$^{-1}$	pool	**1**	**3**	**6**	**8***	**9***	**1**	**3**	**4**	**5****	**7***	**11***	**1**	**3**	**6****	**2**	**6**	**9**
Gly	1	1362.71	1278.06	1347.02	1289.76	1193.72	1298.81	1148.46	1329.73	1354.42	1464.66	2015.18	1434.23	1554.41	1534.43	1436.18	1303.30	1398.05
	2	1158.03	1339.53	1317.18	1437.97	1357.80	-	-	-	1330.53	-	-	-	-	1542.04	1372.81	1388.66	1346.35
Ala	1	159.07	102.92	153.72	201.10	140.29	89.35	86.31	106.63	188.60	157.30	159.08	92.32	90.29	150.80	167.85	121.88	164.23
	2	114.75	123.72	153.74	199.55	192.76	-	-	-	179.03	-	-	-	-	186.20	155.29	80.00	184.10
Ser	1	71.70	38.85	52.82	68.07	37.37	44.59	37.50	42.90	59.01	48.53	41.92	76.62	65.59	38.29	61.48	32.92	72.43
	2	63.68	51.51	57.07	77.50	77.61	-	-	-	62.06	-	-	-	-	34.69	63.69	73.43	87.09
Thr	1	73.04	49.89	62.16	75.62	33.58	16.01	29.45	29.61	37.51	17.79	27.91	3.39	4.68	8.26	50.61	53.52	64.70
	2	59.21	61.45	48.56	58.57	65.90	-	-	-	33.26	-	-	-	-	5.96	34.27	58.33	59.80
Pro	1	26.96	21.18	18.08	28.18	22.83	22.66	28.02	18.59	61.52	48.40	46.88	17.12	12.14	36.52	23.38	20.58	30.76
	2	29.00	16.29	26.93	29.77	26.51	-	-	-	58.42	-	-	-	-	41.37	20.80	20.78	25.48
His	1	64.46	85.38	100.43	57.36	45.11	42.24	45.49	38.52	74.79	33.46	31.85	20.94	18.70	27.20	85.15	75.73	81.99
	2	110.65	58.65	81.09	65.33	76.50	-	-	-	49.61	-	-	-	-	25.75	48.39	74.90	62.61
Ile	1	20.14	51.61	39.51	21.20	15.08	8.48	18.66	9.91	31.91	10.21	10.30	6.38	6.22	9.75	30.49	32.34	33.55
	2	42.85	23.90	37.19	19.84	30.31	-	-	-	12.54	-	-	-	-	10.76	8.10	36.55	8.22
Tyr	1	68.35	171.33	183.30	78.28	64.68	57.78	116.49	44.23	117.59	24.77	22.78	20.09	21.20	24.75	124.68	149.92	142.90
	2	229.63	116.23	139.82	120.74	114.23	-	-	-	38.83	-	-	-	-	28.20	60.70	189.52	47.77
Tau	1	38.72	24.27	33.25	51.52	38.01	38.60	43.48	44.95	80.79	75.02	73.41	67.42	45.87	63.43	44.43	25.21	53.26
	2	34.33	29.99	39.42	40.91	71.51	-	-	-	69.17	-	-	-	-	68.35	36.10	33.68	36.16
Val	1	39.61	105.70	86.75	33.21	26.70	12.85	32.33	15.33	51.41	13.21	16.77	13.15	11.36	15.03	67.11	64.30	55.47
	2	94.58	39.95	64.93	44.12	55.00	-	-	-	17.27	-	-	-	-	16.10	15.81	67.55	15.18
Met	1	17.87	34.83	28.54	18.74	11.52	5.56	9.99	6.59	21.71	8.96	8.99	3.85	4.39	7.27	23.99	18.27	21.74
	2	34.43	18.94	26.53	15.81	24.50	-	-	-	8.71	-	-	-	-	7.71	7.62	23.67	7.38
Leu	1	34.74	84.19	61.19	42.28	27.59	12.78	28.23	15.95	58.36	18.82	18.95	9.67	10.15	17.46	51.05	54.21	64.16
	2	73.44	51.13	64.43	34.60	58.64	-	-	-	21.24	-	-	-	-	17.23	16.87	69.01	18.71
Lys	1	293.48	244.75	298.78	309.76	259.69	173.39	158.12	153.93	226.24	157.07	94.58	94.40	85.37	106.33	311.83	263.58	367.39
	2	342.24	261.52	276.34	276.09	354.80	-	-	-	262.36	-	-	-	-	125.70	270.25	346.67	372.25

*pools constituted of sea urchins less reactive; **pool 2 is relative to sea urchins less reactive.

Table S5 (*continuation*) — Concentration of free amino acids, in mg·(100 g)$^{-1}$, of gonads from live sea urchin experiments (trials I, II and III; 7.1 ± 0.8 °C) and from packaged gonads (trial PI; 2.1 ± 0.4 °C) throughout the storage.

		Days of storage (7.1 ± 0.8 °C)													**Days of storage (2.1 ± 0.4 °C)**		
		Trial I					Trial II						Trial III			Packed gonads (PI)		
mg·(100 g)$^{-1}$	pool	**1**	**3**	**6**	**8***	**9***	**1**	**3**	**4**	**5****	**7***	**11***	**1**	**3**	**6****	**2**	**6**	**9**
Arg	1	513.11	603.43	513.42	520.17	476.55	406.93	375.60	335.08	518.96	373.00	243.09	301.52	285.09	289.11	575.78	528.14	677.80
	2	630.53	542.39	527.73	542.45	617.54	-	-	-	553.98	-	-	-	-	347.91	524.31	590.54	575.72
Phe	1	16.43	43.31	28.79	21.09	13.42	10.99	19.20	11.02	28.16	13.10	14.84	9.35	8.93	10.47	28.77	27.41	26.75
	2	39.12	16.80	22.97	18.28	20.60	-	-	-	13.81	-	-	-	-	11.80	5.79	23.32	7.09
Trp	1	17.14	28.92	29.66	20.23	15.14	12.75	19.16	10.86	24.18	9.00	6.45	11.34	10.14	7.94	24.95	29.40	26.32
	2	32.96	15.92	27.06	18.42	17.06	-	-	-	11.57	-	-	-	-	10.10	10.10	34.59	7.91
Glu	1	179.11	175.95	239.57	216.91	173.24	69.65	93.94	97.75	125.31	108.24	69.50	67.33	56.43	97.83	212.69	213.15	134.66
	2	239.54	194.24	222.15	218.11	213.24	-	-	-	132.87	-	-	-	-	103.52	194.63	172.46	194.46
Asp	1	9.18	4.73	3.84	6.15	11.74	2.81	5.86	6.97	5.08	8.13	3.71	3.29	2.41	9.24	8.95	11.08	8.32
	2	9.78	6.26	10.79	5.94	11.46	-	-	-	12.07	-	-	-	-	8.26	11.31	11.54	9.24
Cys	1	9.89	11.31	17.89	19.56	10.76	18.67	10.15	14.66	11.51	13.63	11.86	18.48	21.15	9.80	12.16	13.29	12.17
	2	10.60	17.47	19.87	18.32	9.65	-	-	-	9.03	-	-	-	-	6.26	14.20	14.36	14.64
Asn	1	15.27	9.65	12.31	12.97	8.96	12.56	10.28	8.08	10.21	7.64	7.26	16.87	14.87	10.74	14.53	11.28	16.98
	2	12.50	12.62	10.82	15.85	13.94	-	-	-	13.46	-	-	-	-	10.02	15.14	13.67	12.37
Gln	1	162.94	58.78	82.51	185.68	92.02	65.41	42.55	39.64	48.11	75.70	25.65	47.85	50.07	69.52	102.07	81.03	115.56
	2	101.27	120.33	188.10	103.37	203.19	-	-	-	92.44	-	-	-	-	44.80	224.89	93.17	233.51
Total,	1	3.19	3.23	3.39	3.28	2.72	2.42	2.36	2.38	3.14	2.69	2.95	2.34	2.38	2.54	3.46	3.13	3.57
g·(100 g)$^{-1}$	2	3.46	3.12	3.36	3.36	3.61	-	-	-	2.98	-	-	-	-	2.65	3.11	3.42	3.33

*pools constituted of sea urchins less reactive; **pool 2 is relative to sea urchins less reactive.

Table S6 — Taste active values (TAV, adimentional) of free amino acids and nucleotides of packaged gonads through days of storage (2.0 ± 0.4 °C).

Main taste descriptor	Taste threshold, mg·(100 mL)$^{-1}$	Packed gonads - Days of storage				
		1	2	6	9	11
Sweet						
Gly	130 (1)	9.70±1.11	10.8±0.34	10.35±0.46	10.56±0.28	-
Ala	60 (1)	2.28±0.52	2.69±0.15	1.68±0.49	2.90±0.23	-
Ser	150 (1)	0.45±0.04	0.42±0.01	0.35±0.19	0.53±0.07	-
Thr	260 (1)	0.25±0.04	0.16±0.04	0.22±0.01	0.24±0.01	-
Pro	300 (1)	0.09±0.00	0.07±0.01	0.07±0.00	0.09±0.01	-
Bitter						
Arg+	50 (1)	11.44±1.66	11.00±0.73	11.19±0.88	12.54±1.44	-
Lys+	50 (1)	6.36±0.69	5.82±0.59	6.10±1.18	7.40±0.07	-
His	20 (1)	4.38±1.63	3.34±1.30	3.77±0.03	3.62±0.69	-
Val+	40 (1)	1.68±0.97	1.04±0.91	1.65±0.06	0.88±0.71	-
Tyr	96.6 (2,3)	1.64±1.26	1.02±0.50	1.87±0.31	1.05±0.74	-
Met+	30 (1)	0.87±0.39	0.53±0.39	0.70±0.13	0.49±0.34	-
Ile	90 (1)	0.35±0.18	0.21±0.18	0.38±0.03	0.23±0.2	-
Phe+	90 (1)	0.31±0.18	0.19±0.18	0.28±0.03	0.19±0.15	-
Leu+	190 (1)	0.28±0.14	0.18±0.13	0.32±0.06	0.22±0.17	-
Trp+	90 (1)	0.28±0.12	0.19±0.12	0.36±0.04	0.19±0.14	-
Tau	1877 (3)	0.02±0.00	0.02±0.00	0.02±0.00	0.02±0.01	-
HxR	2575 (3)	<0.0009	<0.0009	<0.0009	<0.0018	0.0026
Hx	122.5 (2)	<0.0009	<0.0009	<0.0009	<0.0009	0.0015
Umami						
Glu	30 (1)	6.98±1.42	6.79±0.43	6.43±0.96	5.49±1.41	-
IMP+GMP a)	25 (4)	5.56±1.35	6.68±1.25	7.22±0.48	4.85±0.40	4.55
AMP	12.5 (5)	1.55±1.06	0.55±0.78	0.43±0.61	1.41±0.27	1.17
Asp	53 (1)	0.18±0.01	0.19±0.03	0.21±0.01	0.17±0.01	-
CMP+UMP a)	9.22 (3)	0.06±0.02	0.09±0.02	0.10±0.01	0.07±0.01	-
Other						
Cys	23.4 (2)	0.44±0.02	0.56±0.06	0.59±0.03	0.57±0.07	-
Asn	100 (1)	0.14±0.02	0.15±0.00	0.12±0.02	0.15±0.03	-
Gln	730 (3)	0.18±0.06	0.22±0.12	0.12±0.01	0.24±0.11	-

No significant differences were found for each compound ($p > 0.05$).

Compounds in bold had TAV > 1; Numbers between parentheses are the reference on taste thresholds: (1) Kato et al. (1989); (2) Rotzoll et al. (2006); (3) Dunkel & Hoffman (2009); (4) Liu & Qiu (2016) and (5) Duan et al. (2020); Threshold values from reference (2) and (3) were transformed according to molar mass of each compound; a) taste threshold is the average value of both compounds; +may elicit sweet taste depending on compound configuration (Nishimura & Kato, 1988).

Supplementary material of Subchapter 4.3

Table S7. Relative frequencies (%) of descriptors in commercial size live sea urchin (previous experiments).

		Days of storage (~7 °C)							
		Trial A				Trial B		Trial C	
	Frequencies (%)	2	4	6	9	0	5	1	7
Spines	Active	0.0	0.0	0.0	0.0	86.7	0.0	0.0	0.0
	Upright position	67.2	20.3	12.5	6.3	13.3	0.0	100.0	0.0
	Firms	65.6	43.8	43.8	0.0	93.3	33.3	87.5	0.0
	Partially firms	28.1	73.4	53.1	93.8	0.0	50.0	0.0	0.0
	Without firmness	4.7	6.3	34.4	0.0	0.0	50.0	0.0	100.0
	Bright	75.0	43.0	0.0	0.0	100.0	0.0	100.0	0.0
Mouth	Closed	83.3	37.5	12.5	12.5	100.0	0.0	100.0	0.0
	Open with reaction	4.2	37.5	12.5	0.0	0.0	0.0	0.0	0.0
	Open	16.7	50.0	87.5	87.5	0.0	100.0	0.0	100.0
Odour	Underdeveloped	33.3	12.5	0.0	0.0	0.0	0.0	0.0	0.0
	Fresh	20.3	6.3	0.0	0.0	0.0	0.0	0.0	0.0
	Marine (ocean-air)	79.7	93.8	59.4	0.0	86.7	0.0	87.5	5.0
	Clean seaweed	0.0	0.0	0.0	0.0	86.7	0.0	87.5	0.0
	"Air-exposed seaweed"	0.0	68.8	66.7	0.0	0.0	100.0	0.0	10.0
	Sweet	0.0	0.0	10.9	0.0	0.0	0.0	0.0	0.0
	Pungent	0.0	3.1	29.7	0.0	0.0	0.0	0.0	0.0
	Ammonia-like	0.0	0.0	0.0	100.0	0.0	0.0	0.0	25.0
	Cloying/sickly sweet	3.1	0.0	9.4	100.0	0.0	0.0	0.0	0.0
	Yeasty	3.1	0.0	0.0	100.0	0.0	0.0	0.0	25.0
	Putrid/rotten	0.0	0.0	9.4	100.0	0.0	0.0	0.0	0.0
	Sewer	0.0	0.0	25.0	0.0	0.0	0.0	0.0	63.0
Sea urchins evaluated (N)		8	8	8	8	15	6	8	5
Assessors (N)		4	4	4	2	1	1	1	4

Table S8 — Relative frequencies (%) of descriptors in gonads from commercial size live sea urchin related to appearance and odour (previous experiments).

		Days of storage (~7 °C)							
		Trial A				Trial B		Trial C	
	Frequencies (%)	2	4	6	9	1	5	1	7
Appearance	Surface texture (firmness)	83.3	36.7	46.7	6.7	85.0	0.0	50.0	16.7
	Distinction of segments	93.3	60.0	30.0	23.3	70.0	83.3	60.0	27.8
	Granule definition	53.3	40.0	30.0	3.3	65.0	83.3	50.0	22.2
Sea urchins evaluated (N)		8	8	8	8	7	6	5	5
Assessors (N)		6	6	6	6	6	1	6	6
Odour	Poorly developed	75.0	12.5	0.0	0.0	13.0	-	33.3	0.0
	Marine	87.5	100.0	62.5	12.5	43.5	-	10.0	20.0
	Fresh seaweed	75.0	62.5	12.5	0.0	0.0	-	0.0	10.0
	Fresh	12.5	37.5	25.0	12.5	78.3	-	10.0	0.0
	Tropical	0.0	62.5	37.5	0.0	52.2	-	3.3	0.0
	Fruity	25.0	50.0	37.5	0.0	8.7	-	0.0	0.0
	Sweet	12.5	25.0	87.5	100.0	43.5	-	0.0	5.0
	Mango	37.5	0.0	0.0	0.0	34.8	-	0.0	0.0
	Citric	0.0	12.5	37.5	0.0	0.0	-	0.0	0.0
	Acid	12.5	25.0	12.5	50.0	0.0	-	0.0	0.0
	Sour	0.0	0.0	12.5	25.0	0.0	-	0.0	25.0
	Earthy	75.0	50.0	12.5	12.5	0.0	-	0.0	5.0
	Ammoniacal	0.0	25.0	12.5	12.5	4.3	-	0.0	15.0
	Woody	25.0	25.0	0.0	0.0	0.0	-	3.3	0.0
	Floral	12.5	37.5	37.5	0.0	0.0	-	0.0	0.0
	Grass	12.5	0.0	0.0	0.0	0.0	-	0.0	0.0
	Pungent	0.0	12.5	62.5	100.0	0.0	-	0.0	40.0
	Alcohol	0.0	12.5	37.5	100.0	0.0	-	0.0	5.0
	Sulphur	0.0	25.0	100.0	25.0	0.0	-	0.0	20.0
	Sewage	0.0	0.0	37.5	75.0	4.3	-	0.0	20.0
Sea urchins evaluated (N)		8	8	8	8	7	0	5	5
Assessors (N)		6	6	4	6	6	0	6	5

Table S9. Relative frequencies (%) of descriptors in gonads from commercial size live sea urchin related to flavour and mouth feeling (previous experiments)

		Days of storage (~7 °C)							
	Frequencies (%)	Trial A				Trial B		Trial C	
		2	4	6	9	1	5	1	7
Flavours	Sweet	62.5	37.5	37.5	37.5	57.1	-	62.5	60.0
	Salty	12.5	12.5	25.0	25.0	71.4	-	37.5	0.0
	Acid	12.5	12.5	50.0	12.5	28.6	-	25.0	0.0
	Bitter	50.0	37.5	62.5	37.5	57.1	-	50.0	40.0
	Fruity	75.0	37.5	37.5	25.0	14.3	-	62.5	40.0
	Tropical	50.0	25.0	37.5	25.0	57.1	-	62.5	20.0
	Marine	62.5	62.5	25.0	0.0	42.9	-	62.5	40.0
	Shellfish	0.0	25.0	37.5	12.5	28.6	-	37.5	20.0
	Seaweed	12.5	25.0	0.0	0.0	0.0	-	0.0	0.0
	Earthy	25.0	25.0	12.5	0.0	14.3	-	50.0	20.0
	Rancid	0.0	0.0	0.0	37.5	0.0	-	0.0	20.0
	Sulphur	0.0	0.0	25.0	0.0	0.0	-	0.0	0.0
	Sewage	0.0	0.0	25.0	0.0	0.0	-	0.0	63.0
Mouth feeling	Creaminess	75.0	62.5	62.5	12.5	71.4	-	62.5	60.0
	Firmness	62.5	37.5	37.5	12.5	71.4	-	50.0	0.0
	Succulency	37.5	12.5	37.5	0.0	71.4	-	50.0	0.0
	Graininess	25.0	25.0	0.0	0.0	28.6	-	12.5	0.0
	Sliminess	37.5	25.0	25.0	75.0	14.3	-	37.5	0.0
	Soft texture	12.5	25.0	50.0	37.5	28.6	-	25.0	40.0
	Spicy	0.0	0.0	37.5	62.5	0.0	-	0.0	60.0
	Astringency	37.5	25.0	37.5	37.5	28.6	-	62.5	40.0
	Easy melting	37.5	25.0	37.5	0.0	85.7	-	62.5	60.0
Sea urchins evaluated (N)		8	8	8	8	8	0	5	4
Assessors (N)		6	6	6	5	6	0	6	6

This work has been developed within the scope of "BLUE BIOECONOMY INNOVATION PACT" (Project Nº. C644915664-00000026) financed by NextGenerationEU, under the incentive line "Agendas for Business Innovation" of the Recovery and Resilience Plan (PRR). This work was also supported by Portuguese Foundation for Science and Technology (projects UIDB/04077/2020, UIDB/04423/2020, UIDP/04423/2020 and Doctoral grant No. SFRH/BD/141315/2018).

Sea Urchin (*Paracentrotus lividus*) Gonads — Quality and Innovative Food Products

CAROLINA RAQUEL LOPES CAMACHO

2023