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Portuguese shrimps: nutritional added value and contaminants evaluation. Risk-benefit

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Portuguese shrimps: nutritional added value and contaminants evaluation. Risk-benefit.

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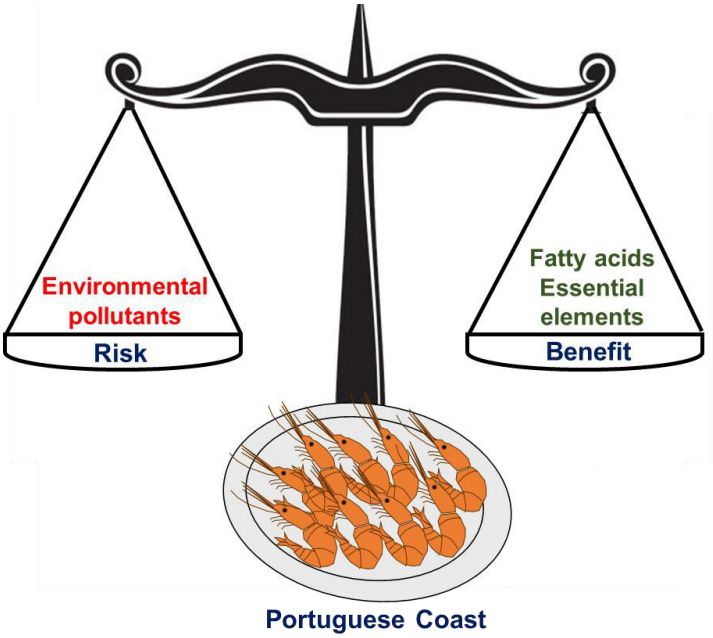
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"It is not the strongest of the species that survives, nor the most intelligent, but the one most responsive to change."

Charles Darwin

Abstract

Environmental pollution has been a growing concern in recent decades as a result of the development and intensification of commerce, agriculture, and industry. In particular, water pollution can generate impacts on species and cause the scarcity of this natural resource. The water quality of coastal and estuarine regions has been affected by the presence of the chemicals that can be found in waters, and that are accumulated in marine organisms that, once consumed, become a potential risk to human health. These chemicals include: organochlorine (OCPs) and organophosphorus (OPPs) pesticides widely used in insect and pest control, brominated flame retardants (BFRs) used to reduce the flammability of various materials, polychlorinated biphenyls (PCBs) used in a wide variety of industrial materials, polycyclic aromatic hydrocarbons (PAHs) formed from the production and use of petroleum and during incomplete combustion of organic materials, synthetic musks (SMs) used as fragrance additives in household and personal care products and heavy metals, comprising metals and metalloids with high density that can be toxic even at low levels.

Most of these pollutants are persistent in the environment and have bioaccumulative properties, being capable of accumulating in aquatic biota such as shrimps. Shrimps are a highly consumed seafood around the world and particularly among the Portuguese population. Despite shrimps potentially accumulating environmental pollutants, these crustaceans can also be a good source of diverse nutrients, for instance, protein, ω-3 fatty acids, minerals, and vitamins.

The present work evaluated the presence of environmental pollutants and the nutritional profile of two shrimp species from Portugal. The sampling was performed between 2017 and 2019, twice a year corresponding to the spring and autumn seasons. *Palaemon serratus* was sampled in Vila do Conde, Matosinhos, Aveiro and Figueira da Foz and *Palaemon varians* was sampled in Sado estuary. Water from shrimp's habitat was also collected and analyzed for the presence of environmental pollutants.

Diverse optimized methodologies were applied to achieve the purposed goals: in shrimp samples, QuEChERS (quick, easy, cheap, effective, rugged, and safe) was the extraction method of all compounds analyzed except for heavy metals, essential elements, and fatty acids. On which, heavy metals and essential elements were extracted by microwave digestion and the fatty acids by Folch method and base-catalyzed derivatization. For water samples, solid phase extraction (SPE) was performed for all environmental pollutants analyzed. The determination was performed using gas chromatography (GC) for OCPs,

OPPs, BFRs, PCBs, SMs and fatty acids, high-performance liquid chromatography (HPLC) for PAHs, inductively coupled plasma (ICP) for heavy metals and micro-elements and flame atomic absorption spectrometry (FAAS) for macro-elements.

The analysis shows the presence of OCPs and BFRs in water samples and OCPs, SMs, PAHs and heavy metals in shrimp samples. Some variations in the concentrations of the pollutants between seasons, species and locations were found. The risk assessment for SMs, PAHs and heavy metals were evaluated, and no significant hazard was associated with the consumption of Portuguese shrimp.

The possible outcomes from environmental pollutants exposure (specifically BFRs) were investigated through *in vitro* studies. The results showed that BFRs could cause a disturbance in lipid metabolism.

Following the risk analysis of shrimp intake, the evaluation of beneficial consumption of shrimp was evaluated through the analysis of minerals and fatty acids content. Portuguese shrimps proved to be a good source of Eicosapentaenoic acid (EPA) + Docosahexaenoic acid (DHA), copper, selenium, iron, manganese, sodium, potassium, calcium, and iodine and can be considered a good nutritional quality food, assessed by the calculation of nutritional indices. Moreover, risk-benefit ratio was assessed regarding heavy metals (risk) and EPA + DHA (benefit), proving once again the benefit of Portuguese shrimp consumption.

This work findings highlight the importance of monitoring Portuguese coastal area and assessing the risks and benefits of eating seafood products widely consumed in Portugal.

Keywords

Portuguese shrimp; Heavy metals; Organochlorine pesticides; Organophosphorus pesticides; Brominated flame retardants; Polychlorinated biphenyls; Polycyclic aromatic hydrocarbons; Synthetic musks; Coastal pollution; Fatty acids; Minerals; Risk assessment; Nutritional quality; Risk-benefit.

Resumo

A poluição ambiental tem sido uma preocupação crescente nas últimas décadas fruto do desenvolvimento e intensificação do comércio, da agricultura e da indústria. Em particular, a poluição aquática pode gerar impactos sobre as espécies e provocar a escassez destes recursos naturais. A qualidade das águas das regiões costeiras e estuarinas tem sido afetada pela presença de contaminantes que podem ser encontrados nas águas, e que são acumulados em organismos marinhos que, uma vez consumidos, tornam-se um potencial risco para a saúde humana. Essas substâncias químicas compreendem: pesticidas organoclorados (OCPs) e organofosforados (OPPs) amplamente utilizados no controlo de insetos e pragas, retardadores de chama bromados (BFRs) utilizados para reduzir a inflamabilidade de diversos materiais, bifenilos policlorados (PCBs) usados numa ampla variedade de materiais industriais, hidrocarbonetos aromáticos policíclicos (PAHs) formados a partir da produção e uso de petróleo e durante a combustão incompleta de materiais orgânicos, almíscares sintéticos (SMs) usados como aditivos de fragrâncias em produtos de limpeza e produtos de higiene pessoal e metais pesados que compreendem metais e metalóides com alta densidade que podem ser tóxicos mesmos em quantidades residuais. Na sua maioria estes poluentes são persistentes no meio ambiente e possuem propriedades bioacumulativas, podendo acumular-se em organismos aquáticos, como os camarões. O camarão é um tipo de marisco muito consumido em todo o mundo, particularmente entre a população portuguesa. Apesar de potencialmente os camarões acumularem poluentes ambientais, estes crustáceos podem ser uma boa fonte de diversos nutrientes, como proteínas, ácidos gordos ω-3, minerais e vitaminas.

O presente trabalho avaliou a presença de poluentes ambientais assim como o perfil nutricional de duas espécies de camarão da costa Portuguesa. A amostragem foi realizada entre 2017 e 2019, duas vezes por ano correspondendo à primavera e outono. O *Palaemon serratus* foi capturado em Vila do Conde, Matosinhos, Aveiro e Figueira da Foz enquanto a espécie *Palaemon varians* foi capturada no estuário do Sado. No local de captura do camarão foi também colhida água e analisada quanto à presença de poluentes ambientais. De forma a atingir os objetivos propostos, diversas metodologias foram otimizadas e aplicadas. O método QuEChERS (rápido, fácil, barato, eficaz, robusto e seguro) foi utilizado para a extração de todos os compostos analisados no camarão, exceto metais pesados, elementos essenciais e ácidos gordos. Os metais pesados e elementos essenciais foram extraídos com recurso a digestão em micro-ondas e os ácidos gordos utilizando o método

de Folch com derivatização catalisada por uma base. No caso das amostras de água, foi utilizada extração em fase sólida (SPE) para análise de todos os poluentes. Na quantificação dos contaminantes usou-se cromatografia gasosa (GC) para OCPs, OPPs, BFRs, PCBs, SMs e ácidos gordos, cromatografia líquida de alta eficiência (HPLC) para PAHs, plasma indutivamente acoplado (ICP) para metais pesados e microelementos e espectrometria de absorção atómica com chama (FAAS) para macroelementos. Nas amostras de água foi detetada a presença de OCPs e BFRs e nas amostras de camarão os OCPs, SMs, PAHs e metais pesados. Foi encontrada alguma variabilidade nas concentrações de poluentes relativamente às estações do ano, espécies e locais de amostragem. Procedeu-se à avaliação de risco para SMs, PAHs e metais pesados atendendo ao consumo de camarão, o resultado mostrou não haver um risco significativo associado ao consumo de camarão português.

Através de estudos *in vitro* foram estudados os possíveis efeitos da exposição a poluentes ambientais (especificamente BFRs). Os resultados mostram que os BFRs podem causar alterações no metabolismo lipídico.

A avaliação do benefício nutritivo do consumo de camarão foi efetuada através da análise do teor de minerais e ácidos gordos. O camarão português avaliado mostrou ser uma boa fonte de ácido eicosapentaenóico (EPA) + ácido docosahexaenóico (DHA), cobre, selénio, ferro, manganês, sódio, potássio, cálcio e iodo. Através do cálculo dos índices nutricionais efetuado, pode ser considerado um alimento de boa qualidade nutricional. Além disto, o risco-benefício foi avaliado tendo em conta metais pesados (risco) e EPA + DHA (benefício), comprovando mais uma vez o benefício do consumo de camarão português.

Os resultados deste trabalho destacam a importância de monitorizar a zona costeira portuguesa, avaliando os riscos e benefícios da ingestão de marisco amplamente consumido em Portugal.

Palavras-Chave

Camarão português; Metais pesados; Pesticidas organoclorados; Pesticidas organofosforados; Retardadores de chama bromados; Bifenilos policlorados; Hidrocarbonetos aromáticos policíclicos; Almíscares sintéticos; Poluição costeira; Ácidos gordos; Minerais; Avaliação de risco; Qualidade nutricional; Risco-benefício.

List of publications

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Publications in international peer reviewed journals

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Communications in national and international scientific meetings

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Poster presentations

Maria Luz Maia, Conceição Calhau, Cristina Delerue-Matos, Valentina F. Domingues. "Biomonitoring of organochlorine pesticides in Portuguese coast shrimp", XIV Encontro de química dos alimentos, 2018.

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Maria Luz Maia, Paula Paíga, Maria João Ramalhosa, Cristina Delerue-Matos, Conceição Calhau, Valentina Fernandes Domingues. "Polycyclic Aromatic Hydrocarbons evaluation in Portuguese shrimp samples", North American Chemical Residue Workshop, 2021.

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List of abbreviations and acronyms

3T3-L1	Pre-adipocyte cell line
Ace	Acenaphthene
Acy	Acenaphthylene
ACN	Acetonitrile
ADI	Acceptable daily intake
AI	Adequate intake
AHTN	Tonalide
Ant	Anthracene
BaA	Benz-a-anthracene
BAF	Bioaccumulation factor
BaP	Benzo-a-pyrene
BbFt	Benzo-b-fluoranthene
BDE 28	2,4,4′-tribromodiphenyl ether
BDE 47	2,2′,4,4′- tetrabromodiphenyl ether
BDE 99	2,2′,4,4′,5-pentabromodiphenyl ether
BDE 100	2,2′,4,4′,6-pentabromodiphenyl ether
BDE 153	2,2′,4,4′,5,5′-hexabromodiphenyl ether
BDE 154	2,2′,4,4′,5,6′-hexabromodiphenyl ether
BDE 183	2,2′,3,4,4′,5′,6-heptabromodiphenyl ether
BF_3	Boron trifluoride-methanol
BFRs	Brominated flame retardants
Bg,h,iP	Benzo-g,h,i-perylene
BHT	Butylated hydroxytoluene
BjFt	Benzo-j-fluoranthene
BkFt	Benzo-k-fluoranthene
BTBPE	1,2-bis(2,4,6-tribromophenoxy) ethane
C13:0	Tridecanoic acid
C16:0	Palmitic acid
C18:0	Stearic acid
C18:1 n-9 c	Oleic acid
Chry	Chrysene
CVD	Cardiovascular disease

DBa,hA	Dibenz-a,h-anthracene
DBa,lP	Dibenzo-a,l-pyrene
DDD	Dichlodiphenyldichloro-ethane
DDE	2,2-bis(p-chlorophenyl)-1,1-dichloroethylene
DDT	1,1,1 trichloro-2,2- bis-(p-chlorophenyl) ethane)
DHA	Docosahexaenoic acid
DI	Daily intake
DMEM	Dulbecco's modifed Eagle's medium (DMEM)
DMSO	Dimethyl sulfoxide
DV	Daily value
ECD	Electron capture detector
EDs	Endocrine disruptors
EDI	Estimated daily intake
EFSA	European food safety authority
EPA	Eicosapentaenoic acid
FAAS	Flame atomic absorption spectrometry
FAME	Fatty acid methyl esters
FAO	Food and agriculture organization
FBS	Fetal bovine serum
FID	Flame ionization detector
FLD	Fluorescence
Fln	Fluoranthene
Flu	Fluorene
FPD	Flame photometric detector
GC	Gas chromatography
HBB	Hexabromobenzene
HBCD	Hexabromocyclododecane
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HepG2	Hepatocyte cell line
HHCB	Galaxolide
HI	Hazard index
HMW-PAHs	High molecular weight - Polycyclic aromatic hydrocarbons

IARC	International Agency for Research on Cancer
ICP	Inductively coupled plasma
InP	Indeno-1,2,3-cd-pyrene
IPCS	International program on chemical safety
IS	Internal standard
JECFA	Joint FAO/WHO expert committee on food additives
LCFA	Long chain fatty acids
LMW-PAHs	Low molecular weight - Polycyclic aromatic hydrocarbons
LOC	Levels of concern
MDL	Method detection limit
ME	Matrix effect
MEME	Minimum essential medium eagle (MEME)
MQL	Method quantification limit
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
MUFA	Monounsaturated fatty acids
NaOMe	Sodium methoxide
Naph	Naphthalene
NBFRs	Novel brominated flame retardants
ND	Not detected
NOAEL	No observed adverse effect level
NW	Northwest
OCPs	Organochlorine pesticides
OPPs	Organophosphorus pesticides
PAHs	Polycyclic aromatic hydrocarbons
PBBs	Polybrominated biphenyls
PBDEs	Polybrominated diphenyl ethers
PBEB	Pentabromoethylbenzene
PBT	Pentabromotoluene
PCB	Polychlorinated biphenyl
PCB 28	2,4,4′-Trichlorobiphenyl
PCB 52	2,2′,5,5′-Tetrachlorobiphenyl

PCB 77	3,3′,4,4′-Tetrachlorobiphenyl
PCB 101	2,2′,4,5,5′-Pentachlorobiphenyl
PCB 114	2,3,4,5,4'-Pentachlorobiphenyl
PCB 118	2,3′,4,4′,5′-Pentachlorobiphenyl
PCB 126	3,3',4,4',5'-Pentachlorobiphenyl
PCB 138	2,2′,3,4,4′,5′-Hexachlorobiphenyl
PCB 153	2,2′,4,4′,5,5′-Hexachlorobiphenyl
PCB 156	2,3,3′,4,4′,5-Hexachlorobiphenyl
PCB 157	2,3,3',4,4',5'-Hexachlorobiphenyl
PCB 169	3,4,5,3',4',5'-Hexachlorobiphenyl
PCB 180	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl
PDA	Photodiode array
Phe	Phenanthrene
Pyr	Pyrene
PUFA	Polyunsaturated fatty acids
QuEChERS	Quick, easy, cheap, effective, rugged, and safe
RDA	Recommended Dietary Allowance
REACH	Registration, Evaluation, Authorization and Restriction of Chemicals
R*f*D	Reference dose for oral exposure
RT-PCR	Real Time - Polymerase Chain Reaction
SCF	Scientific committee on food
SCFA	Short-chain fatty acids
SD	Standard deviation
SEM	Standard error of mean
SFA	Saturated fatty acids
SMs	Synthetic musks
SPE	Solid phase extraction
SRB	Sulforhodamine B
TBB	2-ethylhexyl 2,3,4,5-tetrabromobenzoate
TBBPA	Tetrabromobisphenol A
TBECH	1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane
TCA	Trichloroacetic acid
TDI	Tolerable daily intake

THQ	Target hazard quotient
TLC	Total lipid content
TMAH	Tetramethylammonium hydroxide
TU	Toxic unit
TWI	Tolerable Weekly Intake
UFA	Unsaturated fatty acids
WHO	World Health Organization

Preface

Preface

Relevance and motivation

Environmental pollutants have been an increasing concern over the last decades, due to agricultural, commercial, and industrial rapid developments. The oceans play a crucial role in the transport of these environmental pollutants, affecting the aquatic biota (Bashir et al., 2020). Shrimp is one of the most consumed seafood in the world, and a good source of nutrients, protein, ω-3 fatty acids (Ayas et al., 2013), phosphorus, copper, selenium, zinc, iodine, astaxanthin as well as B-complex vitamins, vitamin A and E (Akonor et al., 2016; Mesko et al., 2016). However, this crustacean can also be a source of environmental pollutants in diet, namely heavy metals (Raissy, 2016), organochlorine pesticides (OCPs) (Ferrante et al., 2007), organophosphorus pesticides (OPPs) (Chang et al., 2019), brominated flame retardants (BFRs) (Van Leeuwen and De Boer, 2008), polychlorinated biphenyls (PCBs) (Ferrante et al., 2007), polycyclic aromatic hydrocarbons (PAHs) (Sun et al., 2016) and synthetic musks (SMs) (Sapozhnikova et al., 2010). Some of these pollutants are endocrine disruptors and persistent in the environment with bioaccumulation properties. Even though the nutritional benefits of shrimp consumption, the potential risk from the environmental pollutants present in shrimp is raising concern. Public awareness about the health effects of foodborne diseases related to pollutants highlights the need of carrying out studies in this field.

Objectives

Concerning the raise of aquatic environmental pollution, the present work aims to assess the environmental contamination of shrimp samples and nutritional elements in order to explore the risk-benefit of shrimp consumption, it was also aimed to assess the environmental contamination of the water from the different locations of the shrimp sampling. For that purpose, specific objectives were established:

- Assess the levels of environmental pollutants, such as heavy metals, OCPs, OPPs, BFRs, PCBs, PAHs and SMs in shrimp samples obtained in different coastal areas of Portugal.

- Assess the levels of environmental pollutants, such as OCPs, OPPs, BFRs, PCBs, and SMs in water samples obtained in the same places where shrimps were collected, in different coastal areas of Portugal.
- Assess the biological risks of environmental pollutants, such as BFRs, through *in vitro* assays in order to evaluate the metabolic impact of these pollutants.
- Assess of fatty acids and micro and macro-elements content in shrimp samples obtained in different coastal areas of Portugal.
- Analysis of the variation of environmental pollutants concentrations between species, seasons, and locations of sampling.
- Analysis of risk assessment and risk-benefit ratios.

Organization and structure of the thesis

The present thesis compiles all the work developed in the scope of the doctoral project in sustainable chemistry. It is divided in five chapters that included a compilation of the scientific articles produced over the last four years. For all the articles, the original content, structure, and formatting were maintained in agreement with where these were published or accepted.

Firstly, the relevance and motivation, main objectives, and thesis structure were provided in this Preface. Chapter 1 comprises the contextualization of the developed work, including a theoretical background. This chapter includes a general overview of environmental pollutants (section 1.1) with a literature review concerning the presence of environmental pollutants in shrimps collected around the world (section 1.1.3), and a nutritional perspective regarding minerals and fatty acids (section 1.2). Chapter 2, 3 and 4 describe the experimental work developed during this doctoral project. Chapter 2 shows the results obtained in the monitoring of environmental pollutants in Portuguese shrimps and a set of pollutants in the waters of the shrimp's habitat. Chapter 3 continues the description of the work with the study of the effects of BFRs on lipid metabolism. In Chapter 4, the evaluation of fatty acids and minerals composition of Portuguese coast shrimps were determined as a contribution to know their nutritional value.

Finally, Chapter 5 includes the final concluding remarks. The main conclusions of the developed work are highlighted as well as future perspectives are pointed out.

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Chapter 1

Introduction

1.1

Environmental pollutants: a general overview

1.1.1 Polychlorinated biphenyls

1.1.2 Organophosphorus pesticides

1.1.3 Organochlorine pesticides, Brominated flame retardants, Polycyclic aromatic hydrocarbons and Synthetic musks

1.1.4 Heavy metals

1.2

Minerals and fatty acids: a nutritional perspective

1.2.1 Essential and probably essential elements

1.2.2 Fatty acids

Introduction

1.1 Environmental pollutants: a general overview

The increase of environmental pollution, with an emphasis on the coastal regions, which suffered rapid development in the last decades, leads humans to be exposed to a wide range of man-made chemicals widespread in the environment through contaminated food and water. Examples of these chemicals are organophosphorus pesticides (OPPs), organochlorine pesticides (OCPs), brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs), synthetic musks (SMs) and heavy metals.

Oceans are known to play an important role in the transport and fate of environmental pollutants, functioning many times as a reservoir for them (Jurado et al., 2004). In these aquatic environments, the environmental pollutants can bioaccumulate through direct uptake from the water or trophic transfer (Al-Malki and Moselhy, 2011). The major exposure routes to them are diet, inhalation, occupational exposure or absorption through the skin and them have been identified in a wide variety of samples and locations of the planet, even in remote zones such as the Arctic Archipelago (Svendsen et al., 2018).

The fact that many of these compounds have been found in human adipose tissue are a proof that humans are exposed to them (Sousa et al., 2022). Exposure to environmental pollutants can lead to several adverse health effects, including diabetes, thyroid dysfunction, cancers and respiratory, neurological, immunological and reproductive disorders (Kim et al., 2014; Mostafalou and Abdollahi, 2017; Pinkas et al., 2017; Saktrakulkla et al., 2020; Xiao et al., 2017).

1.1.1 Polychlorinated biphenyls

PCBs are synthetic aromatic chemicals, not naturally present in the environment (Fillos et al., 2012; WHO, 2000). PCB's structure consists of biphenyl formation with two linked benzene rings in which some or all the hydrogen atoms have been switched by chlorine atoms. This group includes 209 theoretical congeners differing in the positions of chlorine atoms in the benzine ring, however, only around 130 have been found in commercial products (Dai et al., 2016; WHO, 2000). Due to their properties, such as thermal stability, resistance to acids, insulating ability and flame resistance, these chemicals were first synthesized at the beginning of the 1920s as technical mixtures and began to be employed

for industrial applications, like electronic products, particularly in transformers and power capacitors. Additionally, some paint and dye manufacturing methods can produce PCB congeners as by-products (Dai et al., 2016; Fillos et al., 2012; Gandhi et al., 2015). Although PCBs have beneficial properties for industrial usage, their properties also lead to a widespread PCBs contamination in the environment in several parts of the world due to their usage and disposal (Gandhi et al., 2015). The rising of the ecological and human health concerns from PCBs exposure led to the ban of their production in North America and most of Europe in the 70s and 80s (Dai et al., 2016; Gandhi et al., 2015). Although PCBs production have been banned for decades in some regions, these chemicals are hydrophobic, lipophilic and degrade slowly in the environment, consequently, these have been abundantly found in the sediment, soil and biota worldwide (Fillos et al., 2012). Due to these properties, PCBs tend to accumulate in biota, being magnified through the food chain, so diet is a major exposure route to these chemicals in humans (Dai et al., 2016). The presence of these chemicals in foodstuffs has consequences for human health, disrupting endocrine, immune, nervous and reproductive systems (Saktrakulkla et al., 2020). As a result of these findings, in 2001 the Stockholm Convention classified PCBs as persistent organic pollutants (Stockholm Convention, 2001) and the International Agency for Research on Cancer (IARC) classified PCBs as Group I - carcinogenic to humans (IARC, 2016).
Some aquatic food sources, such as fish, mussels, squids, crabs and shrimps have been reported to contain measurable levels of PCBs (Fillos et al., 2012). Shrimp is an aquatic food source of particular interest to study due to the high consumption levels worldwide. PCBs have been detected in shrimps all over the world with a wide range of concentrations, depending on the location of the sampling and the number of PCBs analyzed in each study (Carlsson et al., 2016; Fillos et al., 2012; Saktrakulkla et al., 2020; Van Ael et al., 2012).

1.1.2 Organophosphorus pesticides

Pesticides according to their chemical composition can be divided into classes. OPPs are a class of chemicals widely used as agricultural pesticides in continuous for the last five decades for pest control in crops and the household for a pest repellant and parasitic control in domestic animals. These pesticides contribute to about 38% of the total pesticides used throughout the world (Pundir et al., 2019). These chemicals are constituted by a phosphorus atom double bonded with oxygen or sulfur and single bonded with alkoxy/aryloxy/thioalkoxy group (Pundir et al., 2019). Although OPPs have been conceived to prevent and control

pests and weeds, their high efficiency led to their spread use, which leads to the risk for public health through occupational and environmental exposure (Teodoro et al., 2019).

The vast usage of OPPs in fruits, vegetables and water resources undesirably affects the wellbeing of non-target organisms, for instance, fishes, birds and humans (Pundir et al., 2019). The OPPs exposure caused toxicity through the inhibition of the activity of the acetylcholinesterase enzyme. This enzyme is important for the appropriate functioning of central nervous system. Some of the most used OPPs are chlorpyrifos, malathion, parathion and parathion-methyl (Pundir et al., 2019). In January 2020 the European Union (EU) announced a short period for final storage, disposal and use of chlorpyrifos and chlorpyrifos-methyl**,** after that period these products can no longer be commercialized or used in the EU (European Commission, 2022). Since August 2021, Environmental Protection Agency stopped the usage of the chlorpyrifos pesticide on all food to protect human health, especially of children and farmworkers (EPA, 2021). In addition, malathion was classified as probably carcinogenic to humans (Group 2A) by IARC (IARC, 2015) and dimethoate were also banned in EU (PAN Europe, 2020). OPPs, such as chlorpyrifos, trichlorfon and fenitrothion have been found in shrimps (Chang et al., 2019; Kaonga et al., 2015).

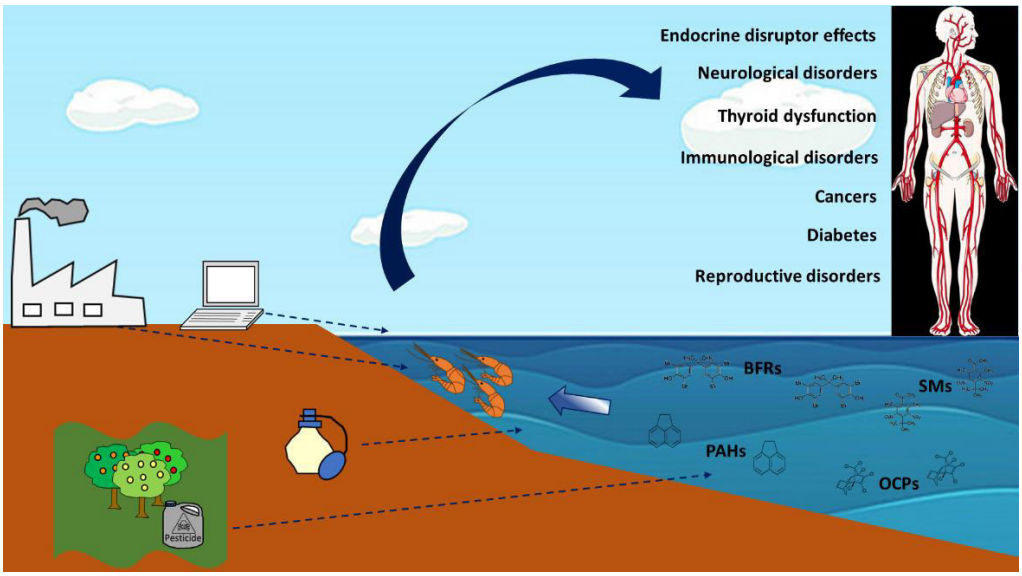
1.1.3 Organochlorine pesticides, Brominated flame retardants, Polycyclic aromatic hydrocarbons and Synthetic musks

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Organochlorine pesticides, brominated flame retardants, synthetic musks and polycyclic aromatic hydrocarbons in shrimps. An overview of occurrence and its implication on human exposure.

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Statement of contribution

The contribution of the candidate, Maria Luz Maia, in this work includes the literature review on environmental pollutants in shrimp samples found around the world in the last years and the written of the original draft.

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Review article

Organochlorine pesticides, brominated flame retardants, synthetic musks and polycyclic aromatic hydrocarbons in shrimps. An overview of occurrence and its implication on human exposure

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ABSTRACT

Shrimps are widely distributed in coastal areas, estuaries and rivers. Although this shellfish is a good source of nutrients, it can also accumulate environmental contaminants, such as organochlorine pesticides (OCPs), brominated flame retardants (BFRs), synthetic musks (SMs) and polycyclic aromatic hydrocarbons (PAHs). Due to their bioaccumulative properties, these pollutants are endocrine disruptors. In this review, an overview of the world's shrimp market, pollutants legislation and values found in shrimp samples will be discussed. Shrimps analysed from all continents showed the presence of contaminants, Asia being the continent with the highest values reported. The concentration values reached a maximum of 26100 ng/g wet weight (ww) for OCPs, of 226.45 ng/g ww for BFRs, of 12.1 ng/g ww for SMs and of 50650 ng/g ww for PAHs. Exposure data and risk, taken from different studies, are very variable and indicate that shrimp's consumption may represent a risk especially in certain geographic areas.

1. Introduction

Seafood is consumed worldwide and considered high quality food. The seafood market has experienced a constant growth in the last century, making it an important source of nutrients and energy worldwide. Shrimps are detritivores contributing to the breakdown of organic matter. They feed on phytoplankton and are a food source for larger animals. To the human population they are considered a delicacy [1, 2]. Shrimp is one of the most popular seafood in the world and they can be a healthy addition to our diet. Shrimps are low in fat and calories. They are composed of a larger amount of water (three-fourths of the edible portion), and primarily made of protein (80% of the remaining portion, dry matter). In fact, the average protein content of fresh shrimp is 19.4 g/100 g and it contributes to 87% of the total energy [3]. Shrimps have low levels of lipids, containing 65–70% phospholipids, 15–20% cholesterol and 10–20% total acyl glycerol. 32 % of the total lipid composition is made up of polyunsaturated fatty acids, a term usually associated with high-quality seafood. These lipids are rich in omega-3 fatty acids, especially eicosapentaenoic acid and docosahexaenoic acid [3, 4]. This shellfish is also a good source of key nutrients such as phosphorus, choline, copper, zinc, iodine, B-complex vitamins, vitamin A and E [3, 5, 6]. While the majority of the antioxidants we ingest originated from vegetables and fruits, shrimp is a good source of compounds with antioxidant properties such as selenium and astaxanthin. Astaxanthin has been found to be a potent natural antioxidant [3].

Nonetheless, seafood can also be a source of environmental contaminants due to the pollution of many coastal areas that affect marine ecosystems, biodiversity and fisheries. Shrimp is widespread and can be found near the seafloor of most coasts and estuaries, as well as in rivers and lakes. Hence, they live in the aquatic environments affected by lipophilic pollutants that have been accumulating over time. This raises a concern about their safety for human consumption. Organochlorine pesticides (OCPs) used in agriculture, brominated flame retardants (BFRs) routinely added to products in order to reduce their flammability, synthetic musks (SMs) used in personal care product fragrances and polycyclic aromatic hydrocarbons (PAHs) generated primarily during the

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incomplete combustion of organic materials are major chemical pollutants. The release of chemical pollutants in aquatic environments raises awareness about the health and environmental impact of these pollutants on seafood.

This study aims to review the world's shrimp market, legislation on OCPs, BFRs, SMs and PAHs, studies published from 2004 to 2020, reporting the occurrence of OCPs, BFRs, SMs and PAHs in shrimps, the possible effects of these on human health and an evaluation of risk assessment.

2. The status of world's shrimp production, importation, exportation and consumption

Regarding shrimp importation, the top five importers in 2017 were the United States (US), the European Union (EU), Vietnam, China and the Republic of Korea [7]. In terms of production and exportation, China remained the largest producer of farmed shrimp in the world. However, most of China's harvest remains in the domestic market. India's shrimp industry, on the other hand, mainly exports and is the world's second largest producer of farmed shrimp. The top two world exporters in the first half of 2017 continue to be India and Ecuador, with market values increasing in supplies 35% and 18%, respectively. Among the other top exporters, Vietnam and China reported higher shipments January–June 2017, whereas exports declined in Thailand [7].

The average annual *per capita* worldwide consumption of shrimp in 2013 was 1.3 kg. According to the FAO database and trace statistics a total of 9,129,021 tons of shrimp was consumed worldwide in 2013. Asia is the largest consumer of shrimp in the world, with China and Japan accounting for 4,035,409 tons and 661,624 tons, respectively. The America continent is in second place, responsible for 19.6% of the world consumption, the USA being the country with the highest shrimp consumption (1,287,094 tons). Although, considering the amount of shrimp consumed *per capita* in 2013 (Figure 1) Australia & New Zeeland surpassed Asia with 2.29 kg/*per capita* consumption versus 1.45 kg/*per capita*. In second place, once more, is the America continent with 1.85 kg/*per capita*. Interestingly, Norway was in 2013 the country with highest *per capita* consumption, achieving 9.38 kg/*per capita*, followed by Japan with 5.20 kg/*per capita* and USA with 4.02 kg/*per capita*. Shrimp consumption *per capita* was rising in the Netherlands, the Republic of Korea and China between 2000 and 2013, it has been declining in Japan since 2005 and in Spain since 2006. In Norway the consumption oscillated until 2008, when it continually increased. In Portugal the consumption in 2013 reached 1.4 kg/*per capita*, values similar to Belgium and Italy. All this reported data emphasizes the importance of shrimp in the global economy [8, 9].

3. Pollutants

The rapid industrialization and urbanization have led to severe pollution of environmental matrices. New products are being developed without any regard to their long-term potential risk to humans and the environment. Coastal regions are developing rapidly due to commercial, agricultural, and industrial activities, leading to an increase in the contamination of the aquatic environment, affecting those locations and the biota living there. Shrimps are wildly consumed all over the world as described above, with the increase in coastal pollution, the impact on human health is an ever-increasing concern.

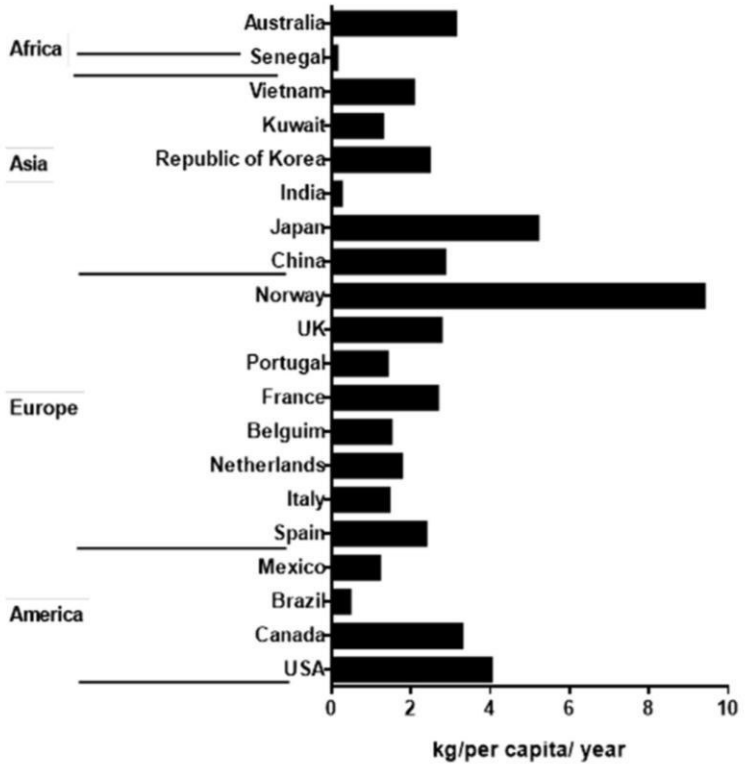


Figure 1. Shrimp estimated consumption in 2013 divided by countries adapted from [8, 9].

3.1. Organochlorine pesticides

OCPs have been used around the world as pest and insect control for more than half a century. The agricultural usage of most OCPs have been banned worldwide. However, these chemicals are hard to degrade, and hence capable of remaining in the environment for decades [10]. OCPs are chemical persistent, lipophilic, and hydrophobic compounds that can accumulate in biota, become biomagnified through the food chain, meaning that the concentrations in biota increase as the trophic level increases [11, 12]. In aquatic environments, hydrophobic compounds such as OCPs can enter shrimps, mainly via two pathways: bioconcentration, directly through the water environment and/or biomagnification, through food web preys. Lipid content, depuration rates, size and time of exposure of the organism, as well as the structure of the food and the environmental chemical concentrations are all potential factors influencing bioaccumulation of these compounds in aquatic organisms [10]. These compounds have clearly demonstrated to induce metabolic alterations in human cell lines and be harmful to humans. In fact, continuous exposure to them can cause several adverse health effects, such as, endocrine disrupting effects, cancers, neurodegenerative disorders, respiratory disorders, reproductive disorders [13], thyroid dysfunction, immunological disorders [14], increase risk for obesity and type 2 diabetes [15].

3.2. Brominated flame retardants

BFRs are applied in high quantities to reduce the flammability of polymers used in indoor applications and products including electronics, vehicles, plastics and textiles. This group of chemicals consists of tetrabromobisphenol A (TBBPA), polybrominated diphenyl ethers (PBDEs) that comprises 209 congeners, polybrominated biphenyls (PBBs), and hexabromocyclododecane (HBCDD). BFRs is a huge and complex chemical family, with molecular weights varying from 249 for MonoBDEs to 959 for decabromodiphenyl (DecaBDE). These compounds are also, lipophilic and persistent in the environment, they have the capacity to accumulate in animal fats including aquatic species. The harmful health effects of these chemicals can be related to their persistency, bioaccumulation and biomagnification potential through the food chain [16, 17]. PBDE is one of the most studied group of BFRs, this group is widely detected in human tissues, such as blood and breast milk [17]. BFRs can act as endocrine disruptors and the continuous human exposure to them is associated with several disorders, including diabetes [16], cancers, neurological effects, thyroid disorders [17] and reproductive disorders [18].

3.3. Synthetic musks

SMs have been used over the years. Nowadays large quantities of SMs are manufactured and used as fragrance additives and fixative in a great variety of personal care products and household products, such as perfumes, shampoos, lotions, deodorants, soaps, and detergents. SMs can be divided into four groups: nitro, polycyclic, macrocyclic and alicyclic, among them polycyclic and nitro musks are dominant in terms of production volume; comprising 61% and 35%, respectively, of the total amount of SMs produced in the world. Due to their highly lipophilic nature, SMs tend to accumulate in sediments, sludge, and biological tissues, such as blood, breast milk and adipose tissue [19, 20, 21]. Little is known about the biological effects of SMs in humans after a prolonged exposure. The health concerns associated with SMs are endocrine disruptor effects, cancers and neurological disorders [22].

3.4. Polycyclic aromatic hydrocarbons

PAHs have at least two fused benzene rings in linear, angular, or cluster arrangements. They are a group of chemicals that usually occur naturally and generally appear as complex mixtures, not as single compounds. PAHs are primarily derived from incomplete combustion or pyrolysis of organic materials, for instance when coal, oil, gas, wood, garbage or tobacco are burned [23]. PAHs enter the marine environment through several mechanisms including atmospheric deposition, discharge of industrial sewage, marine transport, terrestrial runoff, and petroleum spills [24]. PAHs are distributed among different trophic levels through bioaccumulation processes. They start to accumulate in sediment, a contamination source to the surrounding water and aquatic biota. Aquatic organisms such as shrimp, are capable of concentrate pollutants directly from sediments and water and transfer these pollutants through the food web [25]. In terms of human exposure, PAHs have been reported to be carcinogenic, mutagenic and teratogenic [24]. The most significant endpoint of PAH toxicity after long term exposure is cancer, they can lead to proliferation of mutated cells resulting in cancer growth [26]. Furthermore, they have been reported to have endocrine disruptor capacity [27]. PAHs are also linked to adverse neurobehavioral effects, reproductive disorders and the prenatal exposure to them is associated with adverse health effects on children [28, 29]. Recent studies regarding the interaction of PAHs and obesity, reported that obesity may enhance the effect of PAHs exposure contributing to a greater risk for diabetes [30].

4. Legislation

The Stockholm Convention concerning persistent organic pollutants was adopted on the 22nd of May 2001 in Stockholm, Sweden [31]. OCPs such as, aldrin, endrin, chlordane, dichlorodiphenyltrichloroethane (DDT), heptachlor, mirex, toxaphene and hexachlorobenzene (HCB), have been banned for agricultural or domestic usage in many European, North American and South American countries since 1970s–1980s [32]. However, some specific OCPs are still allowed to be used in some countries. One example is DDT that is still used to prevent spreading of malaria and other vector-borne diseases such as dengue, leishmaniosis and Japanese encephalitis through the prevention of mosquito growth. Another example of widely used OCP is lindane (γ-HCH), this OCP had been used to treat head lice in children [33].

As for BFRs, some of them are already restricted in the EU in order to protect the health of the environment and the human population. However, due to their persistence in the environment there are still concerns about the risks that these chemicals pose to public health. The EU has directives to control, reduce or stop the sale and use of some BFRs. Directive 2003/11/EC, amends Directive 76/769/EEC on the marketing and use of certain dangerous substances and preparations, bans the sale of two commercial mixtures of PBDEs, known as PentaBDE and OctaBDE, in concentrations higher than 0.1% by mass. As of July 2006, under Directive 2002/95/EC, all new electrical and electronic equipment can no longer contain PBBs and PBDEs in any concentration. In July 2008, DecaBDE, originally exempted from the restrictions, was also banned by the European Court of Justice [34]. In the US, Washington State's law ESHB 2545 mandates that after July 1, 2017, no children's products may be sold within the state containing more than 1,000 ppm of DecaBDE, HBCDD and TBBPA [35].

In the case of SMs the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation, has classified in 2008, musk xylene as a substance of high concern with a very persistent and very bioaccumulative designation. A restricted use warning was placed on musk ketone. Nitro musk compounds do not degrade easily, being highly stable and ubiquitous in the environment. Therefore, nitro musks have been banned in several countries and replaced by polycyclic musks. Nevertheless, nitro musks are still being produced in China and India and used in non-cosmetic compounds. The US has no restrictions to the use of SMs [21].

Lastly, PAHs have restricted regulations too. In this group more than 200 PAHs are known, in which, 16 PAHs were classified as priority pollutants by the EU and US Environmental Protection Agency (EPA) [36]. The EU has regulations regarding the levels of PAHs in food.

Regulation (EU) No 835/2011 amending Regulation (EC) No 1881/2006 regards maximum levels for PAHs in foodstuffs. Regulation (EU) No 1327/2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of PAHs in traditionally smoked meat and meat products and traditionally smoked fish and fishery products. Regulation (EU) 2015/1125 amending Regulation (EC) No 1881/2006 as regards maximum levels for PAHs in Katsuobushi (dried bonito) and certain smoked Baltic herring. Regulation (EU) 2015/1933 amending Regulation (EC) No 1881/2006 as regards maximum levels for PAHs in cocoa fiber, banana chips, food supplements, dried herbs and dried spices [37].

Several guidelines have been implemented by different countries and continents to protect water of natural resources from pollutants. Water protection has been considered a top priority for some time for human consumption. However, the protection of the environment, the water resources and consequently the biota that lives in aquatic environment have been a priority as well. Table 1 resumes guidelines of quality standards for some pollutants in natural waters from different geographical areas, such as EU [38], US [39], Australia [40], South Africa [41] and Vietnam [42]. Vietnam has guidelines with the highest values of OCPs allowed. This data can explain some of the differences in the pollutant's concentrations found in shrimp from different geographic areas.

5. Occurrence of pollutants in shrimps

For this review article, a thorough search on Web of Science database from 2004 to 2020 was performed. In this search a total of 33 original research articles were found for OCPs, 37 for BFRs, 3 for SMs and 24 for PAHs. When possible, the reported concentrations were converted to the same unit (ng/g ww), allowing fair comparison.

5.1. Occurrence of OCPs

Table S1 (supplementary data) summarizes published studies from 2004 to 2020 regarding shrimp contamination with OCPs [43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76]. The most studied OCPs in these publications are hexachlorocyclohexanes (HCHs), DDT, dichlorodiphenyldichloroethylene (DDE), and dichlorodiphenyldichloroethane (DDD). HCHs reached values as high as 17.84 ng/g wet weight (ww) in shrimp species from Cauvery River, India. In this location the large number of industries and agriculture activities can contribute to the high pollution [46]. An exceptionally high value, 26100 ng/g ww, was found in *Penaeus monodon* from Kolleru Lake, India [71]. For DDE the values ranged from ND to 143 ng/g ww, the maximum value was detected in gei wai shrimp (*Metapenaeus sp.*) from Mai Po (a Ramsar site) in Hong Kong, China. Domestic and industrial effluent discharged in the Pearl River Delta, have been a principal pollution source of this Ramsar site [54]. For DDD values ranged from ND to 14.83 ng/g ww, maximum value was detected in *Exopalaemon modestus* from Qiantang River, China. Upstream of the river, in Lanxi, a pesticide factory still produces OCPs which is a potential pollution source in the region [76]. As for DDT values were between ND and 16 ng/g ww in Chinese prawn from Yellow Sea, China. The Yellow Sea has been suffering with the rapid industrial and agricultural development [48]. An exceptionally high value was found in *P. monodon* (9800 ng/g ww) from Kolleru Lake, India [71]. The Σendosulfan, reached values as high as 7.31 ng/g ww, this value was detected in a *Litopenaeus spp.* sample from Pozo-Rey estuary. Agricultural practices of the region and sanitary campaigns for mosquito control may contribute to the pollution in this estuary [66]. An exceptionally high value was detected in *P. monodon* sample (27800 ng/g ww) from Kolleru Lake, India. As potential sources of pollution in this lake are the direct discharges of industrial effluents, sewage and agricultural wastes. The concentration of OCPs found in shrimps from Kolleru Lake were higher than the allowable limits for human consumption recommended by FAO/WHO [71]. A total of 31 OCPs were analysed in shrimp samples from the studies shown in Table S1 (supplementary data). The number of samples analysed in these studies vary between 2 [74] and 205 [64]. Some studies indicated the individuals collected varying between 9 [63] and 180 [70], and the specimens pooled per analyses ranged from 5 [68, 76] to 30 [67]. Various authors collected the samples by purchasing them at the local markets [47, 48, 50, 52, 59, 61, 65, 75]. Other samples were collected directly from their natural habitat, in some cases collected by

Table 1. Allowed values of quality standards (μg/L) for some pollutants in natural waters in different geographical areas.

Pollutants		European Union	United States		Australia		South Africa*	Vietnam
		Surface waters	Salt water	Fresh water	Salt water	Fresh water	Coastal and marine waters	Fresh Water
OCPs	Aldrin		1.3	3		0.01	0.003	
	Chlordane		0.09	2.4	0.004	0.01		
	DDT		0.13	1.1		0.0015	0.025	42
	Dieldrin		0.71	2.5		0.005	0.003	
	Endosulfan	0.004	0.034	0.22	0.001	0.003	0.0005	
	Endrin		0.037	0.18		0.002		
	HCB	0.05						
	Heptachlor	0.00003	0.053	0.52		0.005		18
	Lindane		0.16	2	0.004	0.01		56
	Methoxychlor					0.03		
BFRs	HBCDD	0.05						
	BDEs	0.014						
PAHs	Acenaphthene						20	
	Anthracene	0.1					0.1	
	Benzo(a)pyrene	0.027					0.00017	
	Benzo(b)fluoranthene	0.017						
	Benzo(k)fluoranthene	0.017						
	Benzo(g,h,i)perylene	0.00082						
	Fluoranthene	0.12					0.0063	
	Naphthalene	130					2	
Ref.		[38]	[39]		[40]		[41]	[42]

* In the case of South Africa, the values presented are chronic values, for the other geographical areas the value presented, represents the maximum allowed value.

professional fishermen [63, 68, 70, 73]. The sample catchment methods applied were trawl fishing [43, 49, 51, 62, 64] or fishing with the use of different nets, such as scoop net [71]. It is worth noting that Asia generally has higher levels of OCPs in shrimps than other continents (Figure 2). Although, we must consider that there are more studies available in this continent, which increase the chances to find contaminants. China is the country with the most studies conducted [48, 54, 57, 58, 60, 61, 64, 67, 68, 70, 74, 75, 76] contributing extensively to the larger number of papers in Asia and making the public aware of the risk of shrimp consumption. The next highest values found for ΣOCPs in shrimp were Egypt [65] and Mexico [66], followed by some European countries [43, 47, 49, 51, 73]. As possible contamination sources of the sampling areas the authors indicated industrial effluents and sewage [43, 45, 57, 71, 75, 76], agricultural activities [44, 45, 56, 60, 66, 71, 72], intensive shipping and fishing [60, 64] and sanitary campaigns for mosquito control [66]. Even in recent studies, shrimps analysed from several continents contain OCPs residues despite the limited usage imposed since the Stockholm Convention.

5.2. Occurrence of BFRs

Table S2 (supplementary data) summarizes published studies from 2004 to 2020 regarding shrimp contamination with BFRs [47, 49, 51, 55, 59, 62, 64, 68, 69, 70, 74, 75, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102]. As shown in Table S2, 30 different PBDEs were analysed. This group has the most number of compounds. BDE209 was the one with the highest values, with concentrations ranging between ND and 226.45 ng/g ww, the highest value reported was in *Neocaridina denticulate* from Qingyuan city, China [81]. For TBBPA the values reached were 0.10 ng/g ww, being the highest value detected in *Neomysis integer* from Scheldt estuary (Schaar van Waarde), Netherlands [100]. Regarding ΣHBCDD values ranged from ND to 8 ng/g ww, with the highest value found in *Neomysis integer* from Scheldt estuary (Bath), Netherlands [100], the same estuary where the highest value of TBBPA were reported however at a different site. Regarding the number of samples analysed per study, the number varies between 2 [74,99] and 205 [64]. Being the number of individuals collected between 2 [96] and 180 [70], and the number of specimens per pool analysed between 7 [84–86] and 50 [97]. Concerning the sample collection, some authors reported that they purchased the samples at local markets [47, 59, 75, 78, 79, 82, 89, 91, 93, 95, 96] or directly from local fishermen [68, 70, 94]. The sample catchment methods applied were trawl fishing [49, 51, 62, 64, 77, 97, 101] and electric pulse fishing [68, 84, 85, 86]. As possible pollution sources near the sampling locations the authors indicated highly urbanized and industrialized areas [49, 51, 68, 70, 75, 78, 83, 87, 88, 89, 91, 93, 94, 97, 101], e-waste recycling areas [59, 70, 75, 81, 84, 85, 86] and intensive shipping and fishing [64]. Concerning ΣBFRs as we can see in Figure 3, the location with the highest value detected was in Qingyuan city, China [81]. Similar to what happened with OCPs, Asia still is the continent with the greatest amounts of studies performed in this field. In Europe some studies were performed [47, 49, 51, 95, 96, 97, 98, 99, 100, 101, 102] and the highest value was detected in the Netherlands [100]. These findings reveal that BFRs are already widespread and the legislators must be aware of them.

5.3. Occurrence of SMs in shrimp

Table S3 (supplementary data) summarizes published studies from 2004-2020 regarding shrimp contamination with SMs [20, 103, 104]. These are emergent pollutants that have been a cause for concern. Regardless of the few papers available in the last years, we can see that some SMs, such as Galaxolide (HHCB) and Tonalide (AHTN) are present in the shrimp samples in the three studies published. Emphasising the need for more studies on the existence and concentrations of SMs in shrimps worldwide. Regarding the number of samples collected the authors indicated 25 [104] and 33 specimens purchased at fish markets [20]. Sapozhnikova et al. suggested wastewater and sewage as possible contamination sources of SMs in the aquatic environment.

5.4. Occurrence of PAHs in shrimp

Table S4 (supplementary data) summarizes published studies from 2004-2020 regarding shrimp contamination with PAHs [24, 25, 36, 52, 54, 60, 69, 71, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120]. As demonstrated in Table S4 the 16 PAHs that EPA listed as priority pollutants are the most studied ones. Among these

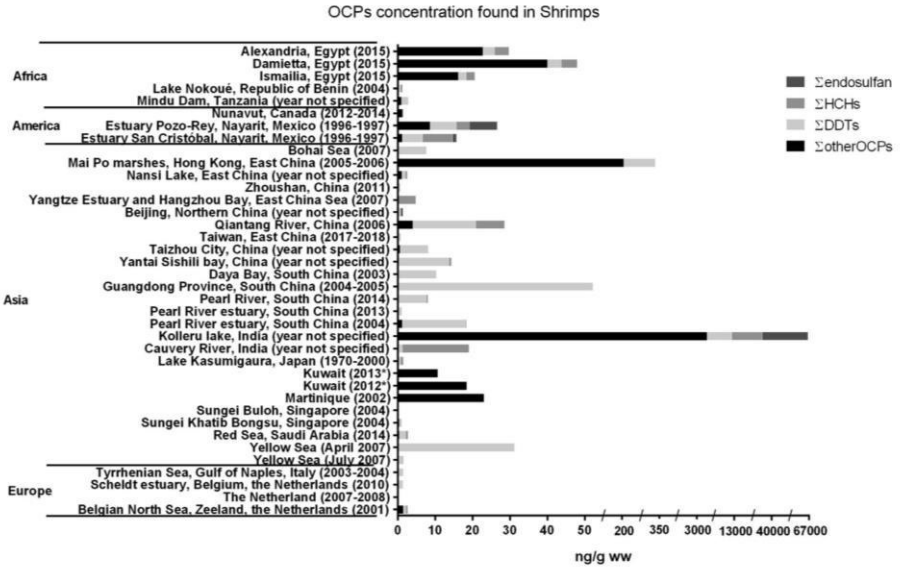


Figure 2. Reported concentrations of ΣOCPs found in shrimp samples, from 2004 to 2020. * studies performed by the same author. Year of sampling not specified. Presented year of publication.

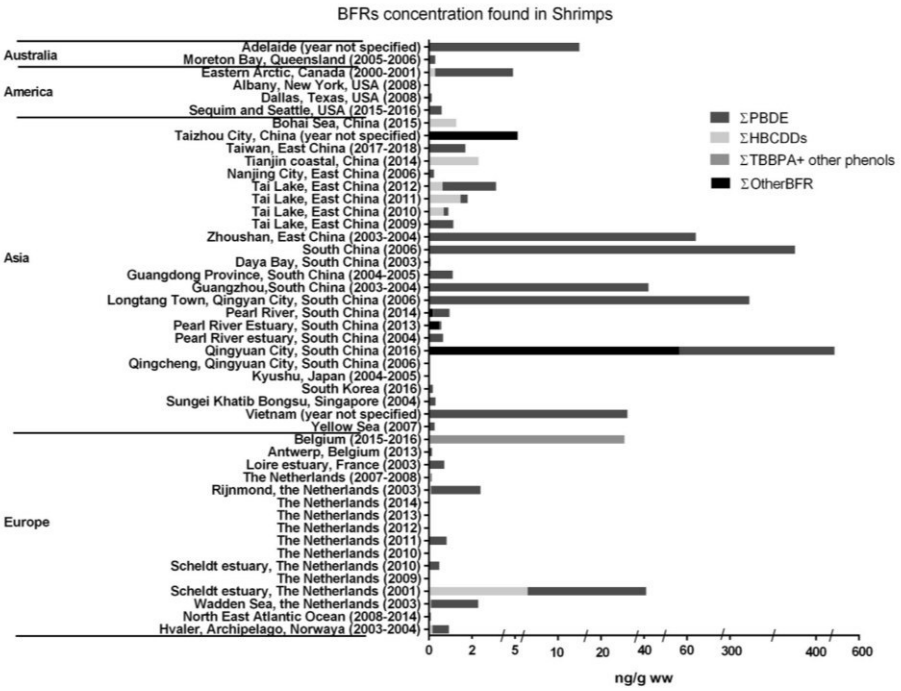


Figure 3. Reported concentrations of ΣBFRs found in shrimp samples, from 2004 to 2020.

PAHs, with a maximum value of (50650 ± 21880 ng/g ww) were found in *Penaeus notialis,* for naphthalene, in Lagos, Nigeria [115]. The most detected PAHs were from petrogenic origin, indicating that anthropogenic activities were influencing PAH concentrations in this area. With the exception of the values reported in Lagos, Nigeria [115], Asia is the continent with more studies and the highest values reported, as shown in Figure 4. The comparison between the concentrations found from two different places of the same species (*Penaeus monodon*), India [71] and Africa [117] shows that the habitat seems to be more important to PAHs accumulation in shrimp then the species itself. Moreover, the shrimps

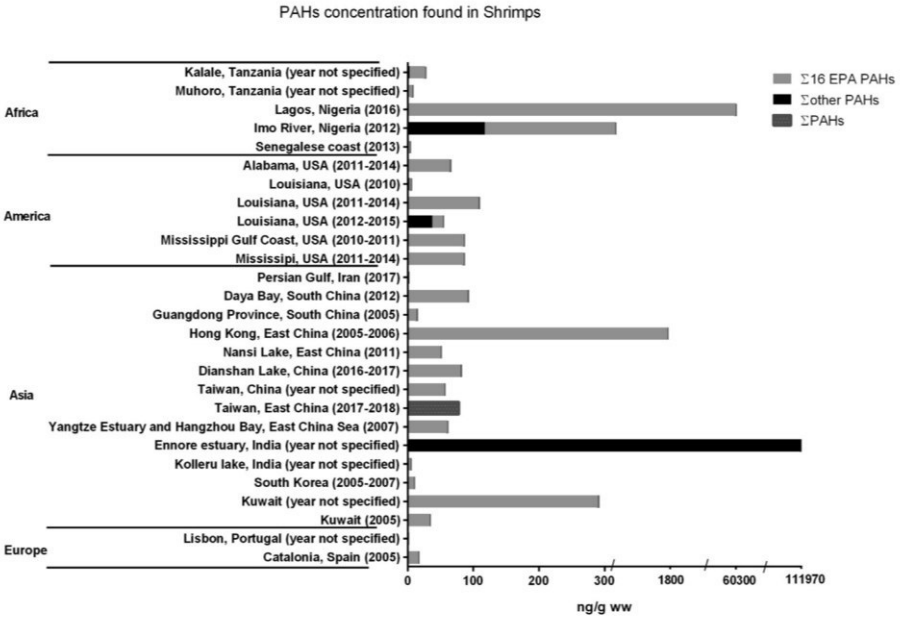


Figure 4. Reported concentrations of Σ16 priority PAHs found in shrimp samples, from 2004 to 2020.

sampled in estuary regions seem to be the mo contaminated ones [24, 36, 54, 60, 106, 109, 117]. For the PAHs evaluation the number of samples analysed varied between 3 [54,116,117,119,120] and 10 [24], the number of individuals collected ranged between 10 [117] and 300 [24]. The number of specimens pooled per sample ranged between 7 [109] and 200 [120]. Regarding the sample collection, some authors reported that the samples were purchased at local markets or directly from local fishermen [52, 105, 107, 108, 111, 114, 115, 119, 120]. The most applied sample catchment method was trawl fishing [24, 113, 116, 118] and one author reported the use of a scoop net [71]. As possible pollution sources of PAHs near the sampling locations the authors indicated highly urbanized and industrialized areas [54, 60, 71, 110, 117, 118], more specifically industry, such as petrochemical refineries, nuclear power stations and thermal power stations [24, 106, 109, 113], oil spills [105, 112, 115, 120], and specifically the deepwater horizon oil spill [36, 114, 116], another possible source of PAHs formation can be the grilling of food [108, 111].

6. Risk assessment

Until now only a few studies that reported levels of these lipophilic pollutants in shrimps included a risk assessment [56, 58, 59, 75, 82, 104, 107, 109].

Concerning the OCPs, a study conducted in Africa (Republic of Bénin) reported levels in shrimps and risk assessment data [56]. Calculated daily intakes (from shrimp) for ∑DDT and for α-endosulfan were of 0.65 and 0.15 ng/kg bw/day respectively. A toxic unit (TU) was also calculated by dividing the estimated daily intake of each pesticide by its tolerable daily intake. The sum of TU for the analysed pesticides was 0.00009, thus far below the value of 1 that would indicate possible health risks. The authors concluded that the risk for humans consuming shrimp from the lake or lagoon, was low [56]. Labunska et al. estimated adult and child exposure to selected organohalogen contaminants via different types of food at e-waste recycling sites in Taizhou, China. The daily intake of ∑DDTs for children and adults was 0.81 and 3.16 ng/kg bw/day, respectively. For HCB the calculated DIs were 0.06 and 0.22 ng/kg bw/day for adults and children. These exposure values were well below the tolerable daily intakes [59]. Some studies also presented a carcinogenic risk assessment for OCPs [58, 75]. To assess the potential health risk related to dietary exposure to DDTs, authors applied a method based on the benchmark concentration. In this method the hazard ratios (HRs) from the 50th and the 95th percentile assessed concentrations were considered. For the calculation of Benchmark concentration for carcinogenic effect it was used the USEPA cancer slope factor by setting cancer risk to one in one million due to lifetime exposure. The results showed that the 50th percentile cancer HRs for all age groups were significantly higher than one without significant differences amongst the different age groups. Therefore, dietary intake of DDTs via fish consumption were considered to cause a possible serious concern with a lifetime cancer risk. However, for shrimps, authors concluded that the risk was lower than one [75]. In another study, that applied the same calculation method to assess cancer risk, the HRs of DDTs and HCHs were 0.43 and 1.55, respectively. For the HCHs, results pointed that daily exposure to OCPs had a lifetime cancer risk of greater than one in one million [58].

For the BFRs, Miyake et al. [82] published results for two Chinese coastal cities, Guangzhou and Zhoushan. In their study, the authors determined daily intakes of 132 and 80.9 pg/kg bw/day for ∑PBDEs, of 1.20 or 0.483 9 pg/kg bw/day for ∑polybrominated dibenzo-p-dioxins (PBDDs) and of 0.934 or 0.619 for 9 pg/kg bw/day ∑polybrominated dibenzofurans (PBDFs), depending on the sampling location. The authors concluded that the results of the performed risk assessment indicated that exposure to lower brominated PBDEs and BDE 209 was unlikely to result in significant health risks. However, for the PBDDs/DFs it was considered that the determined levels could pose some health risks to the local population [82].

A study concerning SMs fragrances in seafood commercialized in several countries from EU was undertaken [104]. For the selected contaminants, no established health-based guidance values were available, therefore the authors calculated a Tolerable Weekly Intake (TWI_cal) value based on the available no-observed-adverse-effect level values. In these calculations, it was applied an uncertaint factor of 100 (to account for species differences and human variability). Considering all the studied countries, a mean value of 0.0024 μg/kg bw/week for HHCB and of 0.013 μg/kg bw/week for AHTN was estimated. These values were below the TWI_cal, which was also true even under a worst-case scenario (percentile 99). However, authors highlighted that southern European countries such as Spain and Portugal, due to a higher seafood consumption, presented higher exposure levels.

Hong-Gang Ni et al. reported risk assessment data for parent PAHs and halogenated polycyclic aromatic hydrocarbons (HPAHs) [107]. Shrimp was one of the major contributors for the total calculated intakes. Considering that the toxic equivalency quotient (TEQ) can be regarded as a better index for the potent toxicity than the concentration, authors also calculated the TEQ of PAHs and HPAHs. The excess cancer risk (ECR) induced by dietary exposure to PAHs and HPAHs via seafood consumption was also determined. The calculated mean TEQ of Σ_{16}PAHs was of 69.2 pg TEQ/g ww for shrimp. DahA (dibenzo[a,h]anthrancene) and B[a]P were the major contributors to the total TEQ of 16 PAHs. The median values of ECRs induced by 16PAHs for all subgroups (according to age and gender) were lower than the acceptable risk level. Thus, authors concluded that the results showed no significant cancer risk related to seafood consumption for people in South China [107]. Also, for PAHs cancer risk associated with consumption of shrimps in Nigeria was assessed through comparison of estimated daily intakes (EDI) and references values from US EPA and EFSA. The EDI for naphthalene and BaP were of 3 and 62.53, and of 4 and 84.25 ng/kg bw/day for adults and children, respectively. Therefore, the determined values were lower than the US EPA benchmarks and EFSA levels of concern values for adults and children population, suggesting a low probability of cancer development [109].

Even though, the results of the available studies pointed to levels of exposure that were below benchmark levels (or calculated), more studies are needed to better explain associated risks from pollutants exposure through shrimp consumption. This being particularly relevant for countries that have higher seafood consumption and consequently probable higher exposure levels for the respective population.

Considering the maximum values found in shrimps (Tables S1 from to and Table S4 supplementary data), respective average world shrimp consumption and average weight from an adult, a conservative risk assessment was performed. Consequently, results are presented by each group of compounds in Tables 2, 3, and 4.

For OCPs EFSA acceptable daily intakes (ADI) [121] were considered for risk assessment. Following our procedure, applying the maximum reported concentration values (worst scenario) the HQ values for a few pesticides exceeded the value of 1 (Tables 2, 3, and 4). Particularly, considering the extremely high concentration reported by Amaraneni et al [71].

To assess potential public health risks concerning PBDEs, maximum exposure concentrations were compared to minimal risk derived by Agency for Toxic Substances and Disease Registry following the same procedure as applied by Miyake et al [82]. A value of 0.5 was obtained, thus lower than 1. However, in one particular study exceptional high exposure values were reported in South China [84, 85] and if considered those values for risk assessment evaluation a value of 3.12 (Table 3) would be obtained. This value would indicate reasons for concern.

For SMs two compounds were considered HCBB and AHTN. Even considering the maximum reported values in the literature, risk assessment revealed no reasons for concern at the present moment. According to EPA a no-observed-effect level for oral dose of 10 000 μg/kg bw/day, with an uncertainty factor of 100, for HCBB was established. Considering the maximum reported values, a maximum of 0.00041 μg/kg bw/day

Table 2. Organochlorine pesticides hazard quotient.

Compound	Max. Value (ng/g ww)	Daily consumption (in 2013)	Average weight (kg) [123]	Calculated daily intake	Hazard risk#	References	EU ADI (μg/kgbw/day)
Chlordane	182*	3.58	62	10.509	**21.018**	[54]	0.5
Chlordane	1.05	3.58	62	0.061	0.121	[74]	
DDT/DDE	143	3.58	62	8.257	0.826	[54]	10
	9800*	3.58	62	565.871	**56.587**	[71]	
Heptachlor	4.53*	3.58	62	0.262	**2.616**	[66]	0.1
	2.97	3.58	62	0.171	**1.715**	[76]	0.1
Aldrin	2.03	3.58	62	0.117	**1.172**	[76]	0.1
Dieldrin	0.86	3.58	62	0.050	0.497	[76]	0.1
	3100*	3.58	62	179	**1790**	[71]	0.1
Endrin	2.94	3.58	62	0.170	0.849	[66]	0.2
	3.22*	3.58	62	0.186	0.930	[76]	0.2
Endosulfan	5.15	3.58	62	0.297	0.005	[66]	60
Endosulfan	27800*	3.58	62	1605.226	**26.754**	[71]	60

* Extemporaneous value, # bold values: Hazard risk ≥ 1.

Table 3. PBDEs hazard quotient.

	Compound	Max. Value (ng/g ww)	Daily consumption (in 2013)	Average weight (kg) [123]	Calculated daily intake	Hazard risk#	Reference
World	PBDEs	61.15	3.58	62	3.531	0.50	[82]
		378*	3.58	62	21.827	**3.12**	[84]

* Extemporaneous value, # bold values: Hazard risk ≥ 1.

Table 4. PAHs hazard quotient.

Compound	Max. Value (ng/g ww)	Daily consumption (in 2013)	Average weight (kg) [123]	Calculated daily intake	Hazard risk#	Reference	EPA RfD
acenaphthene	11.52	3.58	62	0.665		[24]	nd
naphthalene	55.5	3.58	62	3.205	0.160	[108]	20
	650*	3.58	62	37.532	**1.877**	[54]	20
fluorene	15.29	3.58	62	0.883	0.022	[25]	40
	63.7*	3.58	62	3.678	0.092	[54]	40
phenanthrene	37.92	3.58	62	2.190		[25]	n.a
	318.5*	3.58	62	18.391		[54]	nd
anthracene	9.84	3.58	62	0.568	0.019	[24]	30
fluoranthene	8.15	3.58	62	0.471	0.012	[36]	40
	188.5*	3.58	62	10.884	0.272	[54]	40
pyrene	17.38	3.58	62	1.004	0.033	[120]	30
	448.5*	3.58	62	25.897	0.863	[54]	30
benzo[α]anthracene	0.76	3.58	62	0.044		[25]	n.a
	39*	3.58	62	2.252		[54]	n.a
chrysene	2.08	3.58	62	0.120		[36]	n.a
	71.5*	3.58	62	4.129		[54]	n.a
benzo[*b*]fluoranthene	2.65	3.58	62	0.153		[36]	n.a
benzo[α]pyrene	6.35	3.58	62	0.367	**1.222**	[36]	0.3
benzo[*k*]fluoranthene	3.6	3.58	62	0.208		[36]	n.a
dibenz[α,*h*]anthracene	8.06	3.58	62	0.465		[36]	n.a
benzo[*g,h,i*]perylene	1.1	3.58	62	0.064		[25]	n.a
indeno[1,2,3-*c,d*]pyrene	3.71	3.58	62	0.214		[36]	n.a

* Extemporaneous value, # bold values: Hazard risk ≥ 1.

was calculated considering the average world daily consumption. The hazard quotient (HQ), obtained dividing the calculated DI by the NOAEL of 10 000 μg/kg bw/day was well below 1, which would indicate reasons for concern.

The non-cancer risk for PAHs associated with consumption of shrimps was assessed through comparison of estimated daily intakes based on maximum reported values and reference values from US EPA [122]. For the majority 16 EPA PAHs the calculated hazard risk values were below

1, thus not indicating reason for concern. However, for B[a]P Xia et al. reported a value of 6.35 ng/g in shrimp wet weight (6.35 μg/kg) [36] which resulted in a HQ of 1.22 (Table 4).

The risk assessment reported in this section, as mentioned, was based upon the highest reported values which can be regarded as the worst-case scenario. If median values are considered, generally no HQ values of ≥1 are achieved, which is in accordance with what has been reported by other authors [56, 59, 82, 104, 107, 109]. Average weight of 62 kg were considered for the general population [123].

For SMs no reference values are available thus values from [104] were taken into account. For PAHs risk assessment was performed as described by Dosunmu et al. [109] applying EPA reference values. For OCPs EU ADI reference values were taken into account. Hazard risk was calculated dividing the calculated daily intake by reference daily intake values; values equal or higher than 1 can indicate possible associated health risks.

7. Conclusion

This work is an overview on reported contamination levels of some lipophilic pollutants in shrimp samples from all over the world. A vast amount of published data on these pollutants' presence in shrimp species has been reported in recent years. Asia is the continent with more publications in this field and perhaps that's why this is the continent with the highest values of contamination in shrimp reported, in conjunction with the fact that this seems to be where the legislation regarding natural waters is not as restrictive. Guidelines of quality standards for natural waters have different values depending on the geographical area and government policies. These differences in guidelines and restriction laws can reflect different pollution values in waters and consequently in biota from different geographical areas of the planet. Pollutants were found in concentrations ranging from ND to 26100 ng/g ww (exceptional value in *Penaeus monodon* from India) for HCHs, OCP; from ND to 226.45 ng/g ww (found in *Neocaridina denticulate* from Qingyuan city, China) for BDE209, BFR; from ND to 12.1 ng/g ww (found in Asia) for HHCB-Lactone, SM; and from ND to 50650 ng/g ww (exceptional value found in *Penaeus notialis* in Lagos, Nigeria) for naphthalene, PAH. The pollutants deposited in the environment can enter the food chain and ultimately affect the human population. Although contamination levels were generally relatively low, the risk for accumulation of pollutants could pose risks to human health through the consumption of seafood such as shrimp. Public concern about the health effects of foodborne diseases related to pollutants, highlight the need of carrying out studies in this field. These pollutants have been associated with endocrine disruptive effects, cancer growth, neurobehavioral and reproductive disorders and diabetes. This review highlights some significant concerns regarding human health associated with hazard risks. Future studies on shrimp are needed in order to detect levels that could represent a risk for human health and to fully understand the contamination of biota such as, shrimps.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

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Supplementary data

Organochlorine Pesticides, Brominated Flame Retardants, Synthetic Musks and Polycyclic Aromatic Hydrocarbons in shrimps. An overview of occurrence and its implication on human exposure

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Table S1. Summary of reported studies on assessment of OCPs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected.

Species	Sampling location	Sampling period	Concentrations OCPs ng/g ww																				Ref.
			oxychlordane	trans-nonachlor	cis-nonachlor	cis-chlordane	trans-chlordane	Heptachlor epoxide	Heptachlor	HCHs	DDE	DDD	DDT	Aldrin	Dieldrin	Endrin	metoxychlor	Endrin aldehyde	Endrin Ketone	Endosulphan I	Endosulphan II	Endosulphan sulphate	HCB	Pentachlorobenzene	Others	
Penaeus setiferus	Ismailia, Egypt	March to June 2015					ND - 0.56	ND - 4.80	ND - 0.33	ND - 2.32	ND - 1.50	ND - 0.22	ND - 0.48	ND - 0.70		ND - 9.44							ND - 0.25			[65]
	Damietta, Egypt						ND - 0.78	ND - 6.00	ND - 4.00	ND - 4.15	ND - 2.70	ND - 0.53	ND - 0.60	ND - 0.85		ND - 28.00						ND - 0.36				
	Alexandria, Egypt						ND - 0.72	ND - 5.34	ND - 0.71	ND - 3.76	ND - 2.26	ND - 0.46	ND - 0.54	ND		ND - 14.87				0.22						[56]
Penaeus notialis	Lake Nokoué, Republic of Bénin	2004								ND	0.94															
Macrobrachium rude	Mindu Dam, Tanzania				0 - 0.6						0 - 1.9											0.3				[45]
Pandalus borealis	Clyde River, Nunavut, Canada	From 2012 to 2014								0.04 - 0.16		0.02 - 0.09			0.09 - 0.36										0.01 - 0.03 (Mirex)	[62]
Macrobrachium rosenbergii	Fish farm located on polluted grounds in Martinique	January and February 2002			0.24 - 0.84																				23 (Chlordecone)	[72]
Litopenaeus spp.	Estuary Pozo-Rey Mexico	October 1996, February, May and August 1997						4.53	ND	0.24 - 3.67	0.24	0.24 - 6.11	0.72	0.22	0.27	0.59				2.29 - 3.20	1.95	0.41 - 2.16					[66]
	Estuary San Cristóbal Mexico							ND	ND	ND - 8.04	ND	0.20 - 5.46	ND	0.97	ND	0.14				0.72	ND	0.37					

Table S1. Summary of reported studies on assessment of OCPs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling period	Concentrations OCPs ng/g ww																		Ref.
			oxychlordane	trans-nonachlor	cis-nonachlor	cis-chlordane	trans-chlordane	HCHs	DDE	DDD	DDT	Heptachlor epoxide	Heptachlor	Aldrin	Dieldrin	Endrin	metoxychlor	Endrin aldehyde	Endrin Ketone	Endosulphan I	Endosulphan II	Endosulphan sulphate	HCB	Pentachlorobenzene	Others	
Not identified	Yangtze Estuary and Hangzhou Bay, East China Sea	Autumn of 2007									Σ ≈ 4.80															[60]
Penaeus orientalis, P. merguiensis, Metapenaeus ensis and *Oratosquilla oratória*	Pearl River estuary China	February 2004				1.09		0.23		17																[74]
	Daya Bay China	November 2003					0.24	0.07		10																
Not identified	Bohai Sea China	April 2007							0.28 - 1.61	ND - 1.35	ND - 4.47															[48]
	Yellow Sea China - Korea								8.21 - 10.6	ND - 4.47	2.74 - 16												ND - 0.127			
Metapenaeus sp.	Ramsar site at Mai Po marshes, Hong Kong China	August–September 2005 and 2006		ND - 19.5	ND	ND	91 - 182		84.5 - 143														ND			[54]
Penaeus chinensis	Local markets or supermarkets in Beijing, China								0.364 - 0.595	0.014 - 0.036	ND - 0.109	ND	0.007				ND			0.015	0.398	0.026				[61]

Table S1. Summary of reported studies on assessment of OCPs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling period	Concentrations OCPs ng/g ww																							Ref.
			oxychlordane	trans-nonachlor	cis-nonachlor	cis-chlordane	trans-chlordane	HCHs	DDE	DDD	DDT	Heptachlor epoxide	Heptachlor	Aldrin	Dieldrin	Endrin	metoxychlor	Endrin aldehyde	Endrin Ketone	Endosulphan I	Endosulphan II	Endosulphan sulphate	HCB	Pentachlorobenzene	Others	
Macrobrachium nipponense	Nansi Lake, China S1	May 2011						ND - 0.76	1.09	ND	ND	0.65	0.43	ND	0.45	ND										[58]
	Nansi Lake, China S4							0.22	0.86	ND	ND	0.15	0.25	ND	0.11	ND										
	Nansi Lake, China S6							ND - 0.38	1.26	0.23	ND	0.30	0.33	ND	0.24	0.03										
Oratosquilla aratoria and Alpheus distinguendus	Yantai Sishili bay China Sewage outfall area							0.02 - 0.51	0.07 - 4.81	0.05 - 3.03	0.07 - 3.81															[57]
	Yantai Sishili bay China Port area							0.02 - 0.52	0.06 - 9.00	0.10 - 5.42	0.21 - 6.61															
	Yantai Sishili bay China Contrast area							0.02 - 0.44	0.06 - 3.42	0.04 - 2.32	0.06 - 3.03															
P. monodon, P. japonicus, Metapenaeus ensis, Macrobrachium rosenbergii, Proambarus clarkia, and *Squilla oraloria*	Local markets of Guangdong Province, South China	November 2004 and October 2005						ND - 52.2																		[75]
Not identified	Taizhou City (e-waste recycling area), China							7.65															0.52			[59]

Table S1. Summary of reported studies on assessment of OCPs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling period	Concentrations OCPs ng/g ww																		Ref.
			oxychlordane	trans-nonachlor	cis-nonachlor	cis-chlordane	trans-chlordane	HCHs	DDE	DDD	DDT	Heptachlor epoxide	Heptachlor	Aldrin	Dieldrin	Endrin	metoxychlor	Endrin aldehyde	Endrin Ketone	Endosulphan I	Endosulphan II	Endosulphan sulphate	HCB	Pentachlorobenzene	Others	
Exopalaemon modestus	Qiantang River China Jinghua	July 2006						ND - 7.07	7.12	ND	ND	2.03	0.88	ND	0	ND										[76]
	Qiantang River China Fuyang							ND - 1.61	1.16	ND	ND - 2.45	ND	ND	ND	ND	ND										
	Qiantang River China Hangzhou							2.02 - 14.45	22.19	14.83	0.64 - 2.76	1.30	1.67	2.03	0.86	3.22										
Oratosquilla oratoria, Palaemon gravieri, Crangon hakodatei and *Solenocera melantho*	Yellow Sea China - Korea	July 2007			0.0068 - 0.087			0.03 - 0.15	0.12 - 1.17														0.02 - 0.07			[64]
Metapenaeus joyneri and *Parapenaeopsis hardwickii*	Pearl River Estuary, South China	October 2013							0.50 - 1.01																	[70]
Macrobrachium nipponense	Pearl River and Dongjiang River, China	August 2014						0.26 - 0.46	6.58 - 7.57																	[68]
Trachypenaeus curvirostris	Zhoushan Fishing Ground, China	November 2011					0.01–0.04	0.06–0.16	0.24–0.40																	[67]
Penaeus monodon	Prawn ponds near Kolleru lake, India							6100 - 26100			9800				3100						27800					[71]

Table S1. Summary of reported studies on assessment of OCPs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected; BLD-below limit of detection (cont.)

Species	Sampling location	Sampling period	Concentrations OCPs ng/g ww																							Ref.
			oxychlordane	trans-nonachlor	cis-nonachlor	Heptachlor epoxide	Heptachlor	cis-chlordane	trans-chlordane	HCHs	DDE	DDD	DDT	Aldrin	Dieldrin	Endrin	metoxychlor	Endrin aldehyde	Endrin Ketone	Endosulphan I	Endosulphan II	Endosulphan sulphate	HCB	Pentachlorobenzene	Others	
Caridina spp., *Litopenaeu* spp., *Macrobrachium* spp. and *Neocaridina* spp.	Cauvery River India							ND - 17.84	0.11 - 0.79	ND - 0.15	ND - 0.24															[46]
Macrobrachium nipponense	Lake Kasumigaura Japan	1970 - 2000								0.09 - 0.62	<0.04 - 0.8												0.0022 - 0.025			[44]
Not identified	Local market in Kuwait			∑OCPs 18.39																						[50]
				∑OCPs 10.61																						[52]
Litopenaeus vannamei	Aquacultures farming areas in Malaysia	February to November 2011							Negligible levels of OCPs were detected in the 60 samples																	[53]
Austinogebia edulis	Station A Industrial Park (mud shrimp conservation area), Taiwan	September 2017 to January 2018									0.4															[69]
	Station E Industrial Park, Taiwan										1.1															
Pleoticus muelleri	Red Sea in Saudi Arabia	June and December 2014		0.14	0.03		0.01	0.02		0.20 - 0.52	0.14 - 1.12	0.20 - 0.56	0.17 - 0.26										0.09			[63]

Table S1. Summary of reported studies on assessment of OCPs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling period	Concentrations OCPs ng/g ww																			Ref.
			oxychlordane	trans-nonachlor	cis-nonachlor	Heptachlor epoxide	Heptachlor	cis-chlordane	trans-chlordane	HCHs	DDE	DDD	DDT	Aldrin	Dieldrin	Endrin	metoxychlor	Endrin aldehyde	Endrin Ketone	Endosulphan I	Endosulphan II	Endosulphan sulphate	HCB	Pentachlorobenzene	Others	
Alpheus microrhynchus and *Peneaus* spp.	Sungei Buloh, Singapore	April 2004				0.06 - 0.29	0.10			BLD	BLD	BLD	BLD							BLD	BLD					[55]
	Sungei Khatib Bongsu, Singapore									BLD - 0.09	BLD - 0.57															
Crangon crangon	Scheldt Estuary (Belgian North Sea Western)	October and November 2001	ND - 0.19	ND		ND	ND			ND - 0.42	ND - 0.81	ND	ND									0.08 - 0.50	ND			[43]
Crangon crangon	Scheldt estuary (Belgium–Netherlands)	March, July and September 2010	ND	ND	ND	ND	ND			ND	ND - 1.03	ND - 0.20	ND - 0.14										ND			[49,51]
Aristeus antennatus	Tyrrhenian Sea in the Gulf of Naples	October 2003 to December 2004									ND - 1.02	ND - 0.12	ND - 0.17										ND - 0.12			[73]
P. monoden, P. vannamei and *Litopenaeus vannamе*	Suppliers from different places in Netherlands	October 2007 and January 2008								ND	ND	ND		ND	ND	ND				ND				0.000072 - 0.000132	ND (trans-heptachlor epoxide, cis-heptachlor epoxide, telodrin and isodrin)	[47]

Table S2. Summary of reported studies on assessment of BFRs in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected; MDL-method detection limit.

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																																					Ref.
			BDE75	BDE71	BDE77	BDE49	BDE17	BDE119	BDE126	BDE191	BDE156	BDE184	BDE196	BDE206	BDE66	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE209	BDE138	BDE85	BDE197	BDE207	BDE3	BDE15	BDE60	BDE105	BDE190	BTBPE	DBDPE	TBBPA	α-HBCDD	β-HBCDD	γ-HBCDD	Others	
Pandalus borealis and *Hymenodora glacialis*	Eastern Canadian Arctic	October of 2000 and 2001															0.12 - 0.77	0.06 - 1.19	0.04 - 0.79	<MDL - 0.29	<MDL - 0.22		0.08 - 1.32		<MDL - 0.02											0.07 - 0.20	<MDL	0.02 - 0.09		[77]
Not identified	Los Angeles, California, USA	May–June 2008				ND										ND	ND	ND	ND		ND	ND	ND																	[78]
	Dallas, Texas, USA					ND										ND	0.010	ND	ND		ND	ND	0.120																	
	Albany, New York, USA					ND										ND	0.010	ND	ND		ND	ND	ND																	
Pandalus borealis	Clyde River, Nunavut, Canada	From 2012 to 2014			ND											ND	ND	ND	ND		ND				ND														ND (PBEB)	[62]
Litopenaeus setiferus	Purchased from local grocery stores in Sequim and Seattle, USA	From October 2015 to January 2016															0.27	0.1656	0.0416	0.0088																			0.087 (OH-PBDEs); 0.024 (MeO-PBDEs)	[79]
Sand shrimp	Bohai Sea, China	2012																																					ND- (OH-PBDEs, MeO-PBDEs)	[80]
Oratosquilla oratoria, *Palaemon gravieri*, *Crangon hakodatei* and *Solenocera melantho*	Yellow Sea China - Korea	July 2007		ΣPBDE 0.09 - 0.27																																				[64]

Table S2. Summary of reported studies on assessment of BFRs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected; LOQ-limit of quantification (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																																			Ref.
			BDE75	BDE71	BDE77	BDE49	BDE17	BDE119	BDE126	BDE191	BDE156	BDE184	BDE196	BDE206	BDE66	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE209	BDE138	BDE85	BDE197	BDE207	BDE3	BDE15	BDE60	BDE105	BDE190	BTBPE	DBDPE	TBBPA	α-HBCDD	β-HBCDD	γ-HBCDD	Others	
Neocaridina denticulata	Qingyuan City, China	2016											0.776 ± 0.19	7.135 ± 2.95	4.803 ± 0.39	19.284 ± 2.31	159.24 ± 20.06	13.752 ± 1.09	22.283 ± 2.30	12.822 ± 1.38	19.129 ± 3.25	2.730 ± 0.44	226.45 ± 13.65	6.824 ± 0.51		1.577 ± 0.31	4.482 ± 1.84						8.375 ± 3.11	42.549 ± 13.86					2.161 ± 0.43 (BDE202); 0.987 ± 0.23 (BDE203); 3.304 ± 1.08 (BDE208); 6.101 ± 3.38 (HBB); 0.941 ± 0.08 (PBEB); 0.225 ± 0.07 (PBT)	[81]
Macrobrachium nipponense	Pearl River and Dongjiang River, China	August 2014													0.456 - 0.756																		0.0024 - 0.0038	0.136 - 0.193					0.0024 - 0.0031(pTBX); 0.0013 - 0.002(PBT)	[68]
Oratosquilla oratoria and *Metapenaeus ensis*	Guangzhou, China	Between 2003 and 2004			<LOQ - 1.60				0.26 - 0.37								12.10 - 28.20	2.66 - 4.99	0.44 - 1.23	0.93 - 3.64		<LOQ - 0.56	<LOQ - 0.27								<LOQ	<LOQ								[82]
	Zhoushan, China				0.97				<LOQ								28.31	4.52	2.87	1.82		0.54	22.93								<LOQ	0.16								
Penaeus orientalis, P. merguiensis, Metapenaeus ensis and *Oratosquilla oratoria*	Pearl River estuary, China	February 2004														∑PBDE ≈ 0.22 - 0.66																			ND				ND (MoBrPCDD/DFs); ND (PBDD/DFs)	[74]
	Daya Bay, China	November 2003														∑PBDE ≈ 0 - 0.075																			0.0001 - 0.0003					

Table S2. Summary of reported studies on assessment of BFRs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																																			Ref.
			BDE75	BDE71	BDE77	BDE49	BDE17	BDE119	BDE126	BDE191	BDE156	BDE184	BDE196	BDE206	BDE66	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE209	BDE138	BDE85	BDE197	BDE207	BDE3	BDE15	BDE60	BDE105	BDE190	BTBPE	DBDPE	TBBPA	α-HBCDD	β-HBCDD	γ-HBCDD	Others	
Metapenaeus joyner and *Parapenaeopsis hardwickii*	Pearl River Estuary, South China	October 2013													∑PBDE 0.009 - 0.08																			0.007 - 0.5						[70]
Elminius modestus	Tai Lake, China	2009		ND			0.059 - 0.062								ND	0.06 - 0.07	0.63 - 0.95	0.006 - 0.007	0.007 - 0.009	0.012 - 0.017	0.011 - 0.014				0.0006 - 0.003											ND	ND	ND		[83]
		2010		ND			0.03 - 0.10								ND	0.01 - 0.03	0.07 - 0.10	ND	ND	ND	ND				ND											ND - 0.68	ND	ND		
		2011		0.01 - 0.01			0.007 - 0.018								ND	0.007 - 0.021	0.08 - 0.10	ND	ND	ND	0.004 - 0.18				ND											0.51 - 1.20	ND	ND - 0.27		
		2012		ND			0.37 - 0.41								ND	0.10 - 0.15	1.46 - 1.89	ND	ND	0.009 - 0.02	0.02 - 0.04				ND											0.32 - 0.52	ND	ND - 0.10		
Macrobrachium nipponense	E-waste recycling site in South China	2006														6.01 - 17.9	61.6 - 127	12.6 - 34.8	11.6 - 37.1	10.1 - 18.7	6.94 - 21.6		2.81 - 47.7	9.36 - 14.6																[85]
																5.02 - 20.08	50.19 - 158	10.28 - 34.42	9.56 - 48.76	10.04 - 18.40	5.74 - 21.99		2.39 - 62.62	8.60 - 14.34																[84]
																																	0.001 ± 0.0002	0.002 ± 0.002		0.006 ± 0.002	0.0002 ± 0.000065	0.003 ± 0.0007	0.005 ± 0.001 (HBB); 0.0002 ± 0.00006 (PBEB); 0.000037 ± 0.000015 (PBT)	[86]

Table S2. Summary of reported studies on assessment of BFRs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																																			Ref.
			BDE75	BDE71	BDE77	BDE49	BDE17	BDE119	BDE126	BDE191	BDE156	BDE184	BDE196	BDE206	BDE66	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE209	BDE138	BDE85	BDE197	BDE207	BDE3	BDE15	BDE60	BDE105	BDE190	BTBPE	DBDPE	TBBPA	α-HBCDD	β-HBCDD	γ-HBCDD	Others	
Fenneropenaeus chinensis and *Oratosquilla oratoria*	Estuary Mihe River, Bohai Sea, China	November 2015																																		0.19 - 0.77	0.17 - 0.19	0.10 - 0.31		[87]
Oratosquilla oratoria	Tianjin coastal sites, China	September 2014																																		0.205 - 1.21	0.058-0.288	0.111-0.811		[88]
Not identified	Taizhou City (e-waste recycling area), China																															0.41	2.04		<0.0009	<0.0009	<0.0009	<0.007 (HBB); 0.31 (PBEB); 1.66 (EH-TBB); 0.73 (BEH-TEBP)	[59]
Macrobrachium nipponensis	Markets in Nanjing City, China	July 2006		ND - 0.013			ND								ND - 0.022	ND - 0.021	ND - 0.120	ND - 0.013	ND - 0.019	ND - 0.011	ND - 0.022																		[89]
Penaeus monodon, *P. japonicus*, *Metapenaeus ensis*, *Macrobrachium rosenbergii*, *Proambarus clarkia*, and *Squilla oraloria*	Local fishery markets of Guangdong Province, South China	November 2004 and October 2005													ND - 1.11																								[75]
Not identified	India	April-June 2014 and September-January 2015														ND							ND										ND					ND (HBB); ND (PBEB)	[90]

Table S2. Summary of reported studies on assessment of BFRs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected; BLD-below limit of detection (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																																				Ref.
			BDE75	BDE71	BDE77	BDE49	BDE17	BDE119	BDE126	BDE191	BDE156	BDE184	BDE196	BDE206	BDE66	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE209	BDE138	BDE85	BDE197	BDE207	BDE3	BDE15	BDE60	BDE105	BDE190	BTBPE	DBDPE	TBBPA	α-HBCDD	β-HBCDD	γ-HBCDD	Others	
Not identified	An industrialized area, Japan	October 2004 to February 2005																																	ND				ND (MoBrPCDD/DFs); ND (PBDD/DFs)	[91]
	A less industrialized area, Japan																																		0.01 - 0.02					
Solenocera melantho	South Korea	January to February 2016	0.074																																ND		ND		0.007(MeO-BDEs); 0.102(OH-BDEs)	[92]
Austinogebia edulis	Station A Industrial Park (mud shrimp conservation area), Taiwan	September 2017 to January 2018		*			*							1.2 *															*			*							* BDE 2, 203 and 205	[69]
	Station E Industrial Park, Taiwan			*			*							2.2 *															*			*							* BDE 2, 203 and 205	
Alpheus microrhynchus and *Peneaus spp.*	Sungei Buloh, Singapore	April 2004															BLD																							[55]
	Sungei Khatib Bongsu, Singapore																BLD - 0.31																							
Penaeus laspisulcate	Adelaide fish market (Vietnam)													0.79			0.70	0.58	0.77	2.27	0.88	2.51	11.39	2.01			1.19													[93]
Melicertus latisulcatus	Adelaide fish market (Australia)													ND			0.78	0.83	0.98	1.70	1.09	1.49	3.63	1.18			ND													
Penaeus merguiensis	Moreton Bay, Queensland, Australia	July 2005 and August 2006				0.004										0.003	0.16	0.09	0.02	0.007	0.006																			[94]

*∑BDE 2, 15, 17, 28, 47, 66, 71, 85, 99, 100, 138, 153, 154, 183, 190, 203, 205, 206 and 209 for each station.

Table S2. Summary of reported studies on assessment of BFRs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																										Ref.
			BDE75	BDE71	BDE77	BDE49	BDE17	BDE119	BDE126	BDE191	BDE156	BDE184	BDE196	BDE206	BDE66	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE209	BDE138	BDE85	BDE197	BDE207	BDE3	BDE15	BDE60	BDE105	BDE190	BTBPE	DBDPE	TBBPA	α-HBCDD	β-HBCDD	γ-HBCDD	Others	
Not identified	Supermarkets from Antwerp (Belgium)	2013													<0.003	<0.001	0.003	<0.003	<0.003	<0.003	<0.003	<0.003	<0.06		<0.003								<0.006					<0.034 (EH-TBB), <0.006 (BEH-TEBP) and <0.013 (HCDBCO)	[95]
Pink shrimp, Grey shrimp	Belgian supermarkets	2015 - 2016																																				0.786-21.042 (4-BP); 0.165-0.751 (2,4-DBP); 0.093-0.946 (2,4,6-TBP)	[96]
Crangon crangon, *Palaemon elegans* and *P. serratus*	Loire estuary France	Spring and summer														0.01 - 0.19	0.08 - 0.30	0.01 - 0.12	0.01 - 0.09		0.01 - 0.02																		[97]
Not identified	Netherlands	2009						0.011-0.013*																	*						*							[98]
		2010						0.015 ± 0.0073*											*						*						*							
		2011						0.823*											*						*						*							
		2012						0.0033 ± 0.0024*											*						*						*							
		2013						0.0032*											*						*						*							
		2014						0.03*											*						*						*							
Crangon crangon	Wadden Sea, Netherlands	September and December 2003													<0.1	<1.0	0.7	0.8	<0.1	0.2	<0.1	<0.1	<0.3												<0.1	<0.1		[99]
	Rijnmond, Netherlands															<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.7												<0.1	<0.1		
Penaeus monoden, *P. vannamei*, *Litopenaeus vannamei*	Suppliers from different places in Netherlands	October 2007 and January 2008														∑PBDE ≈ 0.010 - 0.050																				0.008 - 0.080		[47]

*∑BDE 75, 71, 77, 49, 17, 119, 66, 28, 47, 99, 100, 153, 154, 183, 138, 85 and 190 each year

Table S2. Summary of reported studies on assessment of BFRs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																													Ref.
			BDE75	BDE71	BDE77	BDE49	BDE17	BDE119	BDE126	BDE191	BDE156	BDE184	BDE196	BDE206	BDE66	BDE28	BDE47	BDE99	BDE100	BDE153	BDE154	BDE183	BDE209	BDE138	BDE85	BDE197	BDE207	BDE3	BDE15	BDE60	BDE105	BDE190	BTBPE	DBDPE	TBBPA	α-HBCDD	β-HBCDD	γ-HBCDD	Others	
Crangon crangon	Scheldt estuary (SE, the Netherlands–Belgium)	March, July and September 2010														ND	0.02 - 0.23	0.02 - 0.20	0.01 - 0.05	ND	ND	ND																	[49,51]
Neomysis integer	Schaar van Waarde Netherlands	November 2001		<0.10				0.01							0.40	0.40	9.61	8.40	2.80	0.70	0.60		4.30	0.04	0.20						<0.10			<0.10					[100]
	Overloop van Valkenisse Netherlands			<0.10				0.01							0.40	0.60	12.00	8.80	3.41	0.90	0.80		3.50	0.06	0.30						<0.10			0.01	7.31				
	Bath Netherlands		<0.10					<0.10							0.30	0.50	13.00	12.00	4.00	1.20	1.10		6.60	0.08	0.40						0.01			0.01	8.00				
Palaemon adspersus, P. elegans and *Pandalus borealis*	Hvaler, Archipelago, Norway	Summer of 2003 or late spring of 2004														<0.003 - 0.007	0.16 - 0.28	0.02 - 0.18	0.02 - 0.06	<0.023 - 0.155	0.005 - 0.026		<0.006 - 0.053													<0.02 - 0.17				[101]
P. borealis	North East Atlantic Ocean	2008–2014																				ND													ND				[102]

Table S3. Summary of reported studies on assessment of SMs, in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected.

Species	Sampling location	Sampling date	HHCB	AHTN	DPMI	ADBI	AHMI	ATTI	MIX	MIM	MIK	HHCB-Lactone	Unit	Ref.
Not identified	Wild and farmed shrimp from the USA and other countries (Mexico, India, Equador, Thailand, China and others)	2006	48 - 762	1 - 384									ng/g lipid w	[20]
Not identified	Asia	April-June 2014, September-January 2015	5.3	1.5	ND	ND	ND	2.3	ND	ND	ND	12.1	ng/g ww	[104]
Aristeus antennatus	From three establishments (supermarket, local market and fish store) in Tarragona, Spain		3.8	2.9	<MQL	ND	ND	ND	ND	ND	ND	4.8	ng/g ww	[103]

Table S4. Summary of reported studies on assessment of PAHs in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected.

Species	Sampling location	Sampling date	Concentrations (ng/g ww)														Ref.
			acenaphthylene	acenaphthene	naphthalene	fluorene	phenanthrene	anthracene	fluoranthene	pyrene	benzo[a]anthracene	chrysene	benzo[b]fluoranthene	benzo[a]pyrene	benzo[k]fluoranthene	dibenz[a,h]anthracene	benzo[g,h,i]perylene	indeno[1,2,3-c,d]pyrene	Others	
Macrobrachium felicinum	Estuarine mangrove area, Imo River, Nigeria	12 August 2012	19.84 - 213.6	23.15 - 344.31	15.31 - 398.27	14.63 - 181.62	26.69 - 57.51	12.26 - 20.52	5.11 - 9.09	4.81 - 15.41	9.15 - 9.86	9.15 - 9.85	7.58 - 8.99	2.83 - 2.93	7.77 - 8.75	1.09 - 11.16	2.91 - 3.12	9.26 - 9.6	3.05 - 56.23 (1-Methyl-naphthalene); 2.78 - 11.34 (2-Methyl-naphthalene); 7.44 - 14.48 (1-Methyl-phenanthrene); 9.42 - 27.06 (dibenzothiophene); 3.48 - 6.62 (Perylene); 1.65 - 1.78 (Retene)	[109]
Penaeus notialis	Lagos, Nigeria	June to August 2016	80 ± 130	1310 ± 1720	50650 ± 21880	1780 ± 3070	1650 ± 2040	3340 ± 2100	910 ± 1200	570 ± 590	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL		[115]
Penaeus kerathurus	Senegalese coast	2013									1.92 - 2.88									[110]
Panaeus monodon	Muhoro, Rufiji estuary, Tanzania	June and July 2014	0.53 ± 0.07	ND	0.17 ± 0.02	1.66 ± 0.19	2.18 ± 0.07	0.46 ± 0.98	ND	0.07 ± 0.02	ND	ND	ND	0.19 ± 0.07	0.50 ± 0.34	0.07 ± 0.02	ND	ND	ND (benzo[e]pyrene); 0.14 ± 0.02 (benzo[j]fluoranthene); 0.50 ± 0.02 (Perylene)	[117]
	Kalale, Rufiji estuary, Tanzania		0.46 ± 0.31		3.58 ± 1.03	1.39 ± 0.55	11.81 ± 1.94	2.83 ± 0.67	0.50 ± 0.07	1.18 ± 0.46	0.26 ± 0.05	0.84 ± 0.07	0.31 ± 0.17	ND	ND	0.70 ± 0.02	ND	ND	ND (benzo[e]pyrene); 0.46 ± 0.22 (benzo[j]fluoranthene); 0.97 ± 0.50 (Perylene)	
Not identified	Southeast Louisiana, USA	From 2012 to 2015			8.2 ± 7.8		2.2 ± 0.6			4.0 ± 1.7		1.8 ± 0.2							37.4 ± 14.0 (dibenzothiophene)	[112]

Table S4. Summary of reported studies on assessment of PAHs in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected; BDL-below detection limit; RL-reporting limit (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																	Ref.
			acenaphthylene	acenaphthene	naphthalene	fluorene	phenanthrene	anthracene	fluoranthene	pyrene	benzo[a]anthracene	chrysene	benzo[b]fluoranthene	benzo[a]pyrene	benzo[k]fluoranthene	dibenz[a,h]anthracene	benzo[g,h,i]perylene	indeno[1,2,3-c,d]pyrene	Others	
Litopenaeus setiferus	Bayou Bienvenue (inshore area), USA	11 November 2010			≈ 1.9		≈ 0.4												± 0.9 (2-Methyl-naphthalene); ± 0.9 (Biphenyl); ± 0.6 (Dibenzofuran)	[116]
	Chandeleur Sound (offshore area), USA	17 November 2010			≈ 1.7		≈ 0.4			≈ 0.6									± 0.1 (1-Methyl-phenanthrene); ± 0.8 (Biphenyl); ± 0.5 (Dibenzofuran); ± 0.7 (Perylene)	
Farfantepenaeus aztecus and *F. setiferus*	Mississippi Gulf Coast, USA	May 2010 to August 2011			12.5	2.82	31.0		8.15	3.66	0.647	2.08	2.65	6.35	3.60	8.06		3.71		[36]
Farfantepenaeus aztecus and Litopenaeus setiferus	Louisiana, USA	December 2011 to February 2014	19.8 ± 6.1 - 107.9 ± 24.3																	[114]
	Mississippi, USA		13.1 ± 2.2 - 85.2 ± 15.8																	
	Alabama, USA		14.4 ± 2.0 - 64.4 ± 46.5																	
Oratosquilla nepa, O. interrupta and *O. oratoria*	Daya Bay, South China	April 2012	ND - 0.48	2.88 - 11.52	10.56 - 36	0.38 - 7.92	1.90 - 9.60	1.87 - 9.84	0.43 - 1.15	3.12 - 9.60	ND - 0.43	ND - 0.31	ND - 1.54	ND - 0.96	ND - 0.60	ND - 0.62	ND - 0.53	ND - 0.04		[24]
Not identified	Yangtze Estuary and Hangzhou Bay, East China Sea	Autumn of 2007			∑PAH ≈ 60															[60]

Table S4. Summary of reported studies on assessment of PAHs in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected; DL-detection limit (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																Ref.
			acenaphthylene	acenaphthene	naphthalene	fluorene	phenanthrene	anthracene	fluoranthene	pyrene	benzo[a]anthracene	chrysene	benzo[b]fluoranthene	benzo[a]pyrene	benzo[k]fluoranthene	dibenz[a,h]anthracene	benzo[g,h,i]perylene	indeno[1,2,3-c,d]pyrene	Others	
Metapenaeus sp	Ramsar site at Mai Po marshes, Hong Kong, China	Aug–Sept 2005 and 2006	BDL	BDL	383.5 - 650	40.3 - 63.7	188.5 - 318.5	BDL	104 - 188.5	182 - 448.5	BDL - 39	BDL - 71.5								[54]
Squilla oratoria, P. monodon, P. japonicus, Macrobrachium rosenbergii, Proambarus clarkii and *Metapenaeus ensis*	Fishery in 11 coastal cities of Guangdong Province, South China	June to October 2005	<RL - 0.54	<RL - 0.47	<RL	<RL - 1.52	<RL - 4.37	<RL - 3.67	<RL - 1.33	<RL - 0.89	<RL	<RL - 0.14	<RL - 0.22	<RL	<RL - 0.21	<RL	<RL	<RL		[107]
Macrobrachium nipponense	Dianshan Lake, China	22 December 2016 to 5 January 2017	∑PAH 80.52																	[118]
Not identified	Shops in New Taipei City, Taiwan, China		ND	ND	55.2 ± 0.3	0.4 ± 0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		[108]
Macrobrachium nipponense	Nansi Lake, China S1	May 2011	1.19	5.47		11.88	14.66	4.70	5.95	10.97	0.72	1.37	1.43	0.70	0.90	0.24	1.10	1.23		[25]
	Nansi Lake, China S2		1.15	4.15		15.29	37.92	2.69	6.34	12.84	0.76	0.86	0.67	0.28	0.31	0.01	0.29	0.28		
	Nansi Lake, China S4		0.33	1.22		3.94	13.13	ND	2.27	6.55	0.16	0.35	ND	0.007	ND	0.005	0.01	0.005		
	Nansi Lake, China S6		0.35	1.40		3.74	11.21	0.80	2.08	5.81	0.22	0.36	0.10	0.03	ND	0.01	0.09	0.03		
Penaeus indicus	Ennore estuary, India																		95004 (Biphenyl); 16965 (1,1'-biphenyl,3,4'-dimethyl)	[106]

Table S4. Summary of reported studies on assessment of PAHs in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected; DL-detection limit (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																Ref.
			acenaphthylene	acenaphthene	naphthalene	fluorene	phenanthrene	anthracene	fluoranthene	pyrene	benzo[*a*]anthracene	chrysene	benzo[*b*]fluoranthene	benzo[*a*]pyrene	benzo[*k*]fluoranthene	dibenz[*a,h*]anthracene	benzo[*g,h,i*]perylene	indeno[1,2,3-*c,d*]pyrene	Others	
Metapenaeus sp	Ramsar site at Mai Po marshes, Hong Kong, China	Aug–Sept 2005 and 2006	BDL	BDL	383.5 - 650	40.3 - 63.7	188.5 - 318.5	BDL	104 - 188.5	182 - 448.5	BDL - 39	BDL - 71.5								[54]
Squilla oratoria, P. monodon, P. japonicus, Macrobrachium rosenbergii, Proambarus clarkii and *Metapenaeus ensis*	Fishery in 11 coastal cities of Guangdong Province, South China	June to October 2005	<RL - 0.54	<RL - 0.47	<RL	<RL - 1.52	<RL - 4.37	<RL - 3.67	<RL - 1.33	<RL - 0.89	<RL	<RL - 0.14	<RL - 0.22	<RL	<RL - 0.21	<RL	<RL	<RL		[107]
Macrobrachium nipponense	Dianshan Lake, China	22 December 2016 to 5 January 2017	∑PAH 80.52																	[118]
Not identified	Shops in New Taipei City, Taiwan, China		ND	ND	55.2 ± 0.3	0.4 ± 0.0	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND		[108]
Macrobrachium nipponense	Nansi Lake, China S1	May 2011	1.19	5.47		11.88	14.66	4.70	5.95	10.97	0.72	1.37	1.43	0.70	0.90	0.24	1.10	1.23		[25]
	Nansi Lake, China S2		1.15	4.15		15.29	37.92	2.69	6.34	12.84	0.76	0.86	0.67	0.28	0.31	0.01	0.29	0.28		
	Nansi Lake, China S4		0.33	1.22		3.94	13.13	ND	2.27	6.55	0.16	0.35	ND	0.007	ND	0.005	0.01	0.005		
	Nansi Lake, China S6		0.35	1.40		3.74	11.21	0.80	2.08	5.81	0.22	0.36	0.10	0.03	ND	0.01	0.09	0.03		
Penaeus indicus	Ennore estuary, India																		95004 (Biphenyl); 16965 (1,1'-biphenyl,3,4'-dimethyl)	[106]

Table S4. Summary of reported studies on assessment of PAHs in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																	Ref.
			acenaphthylene	acenaphthene	naphthalene	fluorene	phenanthrene	anthracene	fluoranthene	pyrene	benzo[*a*]anthracene	chrysene	benzo[*b*]fluoranthene	benzo[*a*]pyrene	benzo[*k*]fluoranthene	dibenz[*a,h*]anthracene	benzo[*g,h,i*]perylene	indeno[1,2,3-*c,d*]pyrene	Others	
Penaeus monodon	Prawn ponds near Kolleru lake, India							0.125	0.112	0.18		0.086		0.84			2.8			[71]
Metapenaeus afnis and *Penaeus semisulcatus*	Persian Gulf, Iran	January and February 2017	<DL	<DL	0.32 - 0.39	<DL	0.07 - 0.12	<DL - 0.028	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL		[113]
Solenocera melantho	Busan cooperative fish market, in South Korea	From 2005 to 2007	∑PAH 9.17 ± 4.61																	[105]
Not identified	Local fish market in Kuwait		∑PAH 290																	[52]
Penaeus semisulcatus and *Metapenaeus affinis*	Fish market in Kuwait	August 2005			0.53 - 1.12		6.58 - 13.42		0.94 - 1.30	13.54 - 17.38	ND	ND								[120]
Austinogebia edulis	Station A Industrial Park (mud shrimp conservation area), Taiwan	September 2017 to January 2018	61.9 *																Dibenzothiophene, Retene, Benzo[e]pyrene, Perylene, Coronene, Benzo[a]fluorine, Benzo[b]fluorine.*	[69]
	Station E, Industrial Park, Taiwan		94. 1*																	
Litopenaeus vannamei	Purchased at Portuguese markets in Lisbon, Portugal			ND	ND	0.23 ± 0.05	ND			ND			ND		ND					[111]

*∑PAHs for each station

Table S4. Summary of reported studies on assessment of PAHs in shrimp samples, from 2004 to 2020 (source: web of knowledge); ND-not detected (cont.).

Species	Sampling location	Sampling date	Concentrations (ng/g ww)																	Ref.
			acenaphthylene	acenaphthene	naphthalene	fluorene	phenanthrene	anthracene	fluoranthene	pyrene	benzo[*a*]anthracene	chrysene	benzo[*b*]fluoranthene	benzo[*a*]pyrene	benzo[*k*]fluoranthene	dibenz[*a,h*]anthracene	benzo[*g,h,i*]perylene	indeno[1,2,3-*c,d*]pyrene	Others	
Penaeus setiferus	Markets from six large cities of Catalonia, Spain	March and April 2005	∑PAH 15.9																	[119]

1.1.4 Heavy metals

Heavy metals are described as metallic elements having a relatively high density when compared to water. This group also comprises metalloids, such as arsenic, that at low levels of exposure are capable to induce toxicity (Tchounwou et al., 2012). These are frequently cataloged between the most toxic chemicals and are considered trace elements due to their presence in residual concentrations in several environmental matrices. Heavy metals are persistent in the environment, toxic and can bioaccumulate through the food web (Lahijanzadeh et al., 2019; Tchounwou et al., 2012). Examples of heavy metals with no recognized biological function, and as such considered non-essential, are cadmium, lead, aluminum and arsenic (Tchounwou et al., 2012). Even though these chemicals are naturally occurring elements observed through the earth's crust, the increase in environmental contamination and consequently higher human exposure results largely from human actions (Tchounwou et al., 2012). These actions include mining, metal industry, burning of fossil fuels and the usage of metals and metal-containing compounds for domestic and agricultural purposes, which causes the alteration of metal distribution leading to the concentration of metals in terrestrial and aquatic ecosystems (Tchounwou et al., 2012; Van Ael et al., 2017). The rise of heavy metal environmental contamination can additionally occur through atmospheric deposition, metal corrosion, soil erosion, weathering, and volcanic eruptions (Tchounwou et al., 2012). Conditions, for example, organic matter, pH and salinity influence metal distribution and bioavailability (Van Ael et al., 2017). In the marine environment, metal bioaccumulation can be influenced by local contamination, specific habitat and behavior of each specie (Van Ael et al., 2017). Aquatic biota, such as crustaceans are sensitive to metals and are described to be significant bioindicators of heavy metal contamination (Gokoglu et al., 2008).

Several studies have described heavy metal contamination in the food chain, which represents a risk to the human population. Over time heavy metals can accumulate in several organs causing numerous adverse health effects, affecting respiratory, cardiovascular, digestive and nervous systems (Gheysari and Rahimi, 2015; Tchounwou et al., 2012). Consequently, aiming to limit the potential impact of heavy metals, the European Union established maximum levels in foodstuffs. In the case of crustaceans, 0.5 mg/kg wet weight was the maximum level established for cadmium and lead (European Commission, 2001). In addition, IARC classified cadmium and arsenic as Group 1 - carcinogenic to humans (IARC, 2012, 2011) and lead as Group 2A - probably carcinogenic to humans

(IARC, 2006). Trace elements, including heavy metals, have been detected in shrimp samples all over the world (Costa et al., 2022; Giri and Singh, 2015; Olgunoglu, 2015; Raissy, 2016; Van Ael et al., 2017).

1.2. Minerals and fatty acids: a nutritional perspective

In recent years, the concern about good quality nutrition has grown. Nutrition is a vital part of development and health. Improved nutrition is associated with a prolonged lifespan, stronger immune systems, lower risks of cardiovascular disease and diabetes (WHO, 2022). Therefore, a healthy, diversified, and a balanced diet are beneficial. Essential minerals and fatty acids are vital for several health associated aspects and the optimized function of the diverse organisms. Their adequate intake is important for the proper function of the human body.

1.2.1 Essential and probably essential elements

The essential elements can be divided in two groups, macro and micro/trace elements depending on larger or minor requirements to the living organisms, respectively (FAO, 1987).

Macro-elements include phosphorus, magnesium, calcium, potassium, and sodium. These essential macro-elements are critical in several biological functions such as maintenance of normal cellular functions, cofactor or activator of enzymes, growth and are crucial to the immune system (Razzaque, 2018).

Essential micro/trace elements comprise iodine, zinc, selenium, cobalt, copper, iron, molybdenum and chromium and probably essential elements group contains manganese, nickel and vanadium (FAO; WHO, 1996; Prashanth, Lingamaneni;Teja Chitturi, Ravi; Kumar Kattapagari et al., 2015). In general, essential elements play a crucial role in biological functions, acting as catalysts or cofactors for several enzymes (Prashanth, Lingamaneni;Teja Chitturi, Ravi; Kumar Kattapagari et al., 2015). Particularly, iodine is a crucial element of the thyroid hormones, thyroxine 3,5,3,5'-tetraiodothyronine (T4) and 3,5,3'-triiodothyronine (T3) and allows brain myelination (Savarino et al., 2021). Zinc is an element essential for the regulation of the immune system, synthesis and degradation of nucleic acids, proteins, lipids and carbohydrates (Prashanth, Lingamaneni;Teja Chitturi, Ravi; Kumar Kattapagari et al., 2015; Savarino et al., 2021). Selenium is a component of the

enzyme glutathione peroxidase acting in the antioxidant defense system (Kieliszek, 2019). Iron is essential for the synthesis of red blood cells and new tissues (Prashanth, Lingamaneni;Teja Chitturi, Ravi; Kumar Kattapagari et al., 2015; Savarino et al., 2021). Cobalt plays a role in methionine metabolism, controlling the transfer of enzymes, such as homocysteine methyltransferase (Prashanth, Lingamaneni;Teja Chitturi, Ravi; Kumar Kattapagari et al., 2015). Copper and molybdenum established physiological functions are linked directly with their role in several copper-containing or molybdenum-containing metalloenzymes, respectively. Such as superoxide dismutase, cytochrome oxidase and dopamine B-monooxygenase for cooper and aldehyde oxidase, xanthine dehydrogenase/oxidase and sulfite oxidase for molybdenum. Chromium is a crucial element that potentiates insulin action, influencing protein, lipid and carbohydrate metabolisms (FAO; WHO, 1996).

Shrimps are a good source of some of these essential elements, such as phosphorous, magnesium, calcium, and sodium, selenium, copper, zinc and iron (Bernard and Bolatito, 2016; Dayal et al., 2013).

1.2.2. Fatty acids

Fatty acids are carboxylic acids present in all organisms, and a key component of cell membrane structure with a variety of biological functions (Glick and Fischer, 2013). Fatty acids diverge from each other, in the number of double bonds and the number of carbon atoms in the chain. Regarding these differences, fatty acids can be divided in saturated fatty acids (SFA) which are single bonded fatty acids and unsaturated fatty acids (UFA) that have at least one double bond. UFA can be further divided into monounsaturated fatty acids (MUFA) with one double bond and polyunsaturated fatty acids (PUFA) when present more than one double bond. PUFA comprises in addition to other omegas, omega-3 (ω-3) and omega-6 (ω-6) fatty acids, for these the first double bond is present in the 3rd and 6th carbon from the methyl end, respectively (Billingsley et al., 2018; Glick and Fischer, 2013). These last two groups are essential fatty acids because are necessary for health maintenance, although must be obtained from dietary sources as the human body is not capable of synthetizing them (Kaur et al., 2014).

The ω-6 fatty acids are precursors for pro-inflammatory eicosanoids, so when ω-6 FAs are consumed in excess induce inflammation, that can lead to coronary chronic diseases, arthritis, diabetes and certain types of cancers (Pires et al., 2018). Alternatively, ω-3 fatty acids are recognized for anti-atherogenic and anti-thrombotic functions, helping the

prevention of cardiovascular diseases and maintaining the health of the nervous system (Soultani et al., 2019). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are ω-3 fatty acids recognized for their beneficial health effects, such as preventing dyslipidemia, thrombosis, hypertension, inflammation, cardiovascular disease and autoimmune disorders (Ayas et al., 2013; Glick and Fischer, 2013). DHA is also known to be crucial for the functional development of the brain in children and the maintenance of normal brain function in adults (Ayas et al., 2013).

Phytoplankton and macroalgae are the principal suppliers of ω-3 fatty acids in the marine ecosystem and these are a food source for other aquatic organisms, such as shrimps (Puccinelli et al., 2021). Shrimps have been recognized as high quality seafood, and a valuable source of fatty acids such as EPA and DHA to the human population (Ayas et al., 2013; Dayal et al., 2013).

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Chapter 2

Environmental pollutants in Portuguese coast shrimps

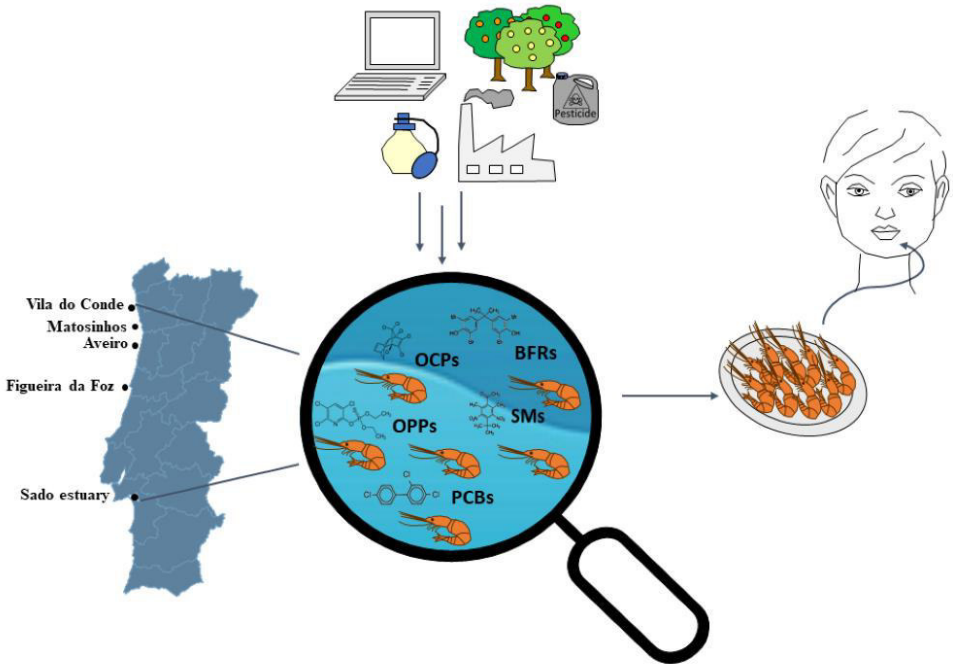
2.1. Validation and Evaluation of Selected Organic Pollutants in Shrimp and Seawater Samples from the NW Portuguese Coast

2.2. Seasonal and spatial comparison of polycyclic aromatic hydrocarbons among decapod shrimp from coastal Portugal

2.1. Validation and Evaluation of Selected Organic Pollutants in Shrimp and Seawater Samples from the NW Portuguese Coast

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Validation and Evaluation of Selected Organic Pollutants in
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Statement of contribution

The contribution of the candidate, Maria Luz Maia, in this work includes the accomplishment of all the experimental work, that is, sample preparation, gas chromatography analysis, statistical analysis and written of the original draft.

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Article

Validation and Evaluation of Selected Organic Pollutants in Shrimp and Seawater Samples from the NW Portuguese Coast

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Abstract: The development of coastal regions has contributed to the intensification of environmental contamination, which can accumulate in aquatic biota, such as shrimps. These crustaceans, besides being delicious and being a good source of nutrients, can also accumulate environmental pollutants. Amongst others, these include organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs) and synthetic musks (SMs). These pollutants, classified as endocrine disruptors, are related to adverse effects in humans and since one of the major routes of exposition is ingestion, this is a cause for concern regarding their presence in food. The aim of the present study was to quantify the presence of environmental pollutants in shrimp samples and in the water from their habitat along the northwest Portuguese coast. In seawater samples, only two OCPs (lindane and DDD) and one BFR (BTBPE) were detected, and in shrimp samples, one OCP (DDD) and three SMs (HHCB, AHTN and ketone) were found. Bioaccumulation and the risk assessment of dietary exposure of SMs in shrimp samples were investigated. It was observed that all shrimp samples analyzed significantly presented bioaccumulation of the three SMs found. Concentrations of SMs detected in shrimp samples do not present a health risk for the adult Portuguese population.

Keywords: endocrine disruptors; aquatic environment; *Palaemon serratus*; *Palaemon varians*; bioaccumulation; biomonitoring

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1. Introduction

In the last several decades, rapid commercial, agricultural and industrial development has contributed to increasing environmental pollution. Oceans are a reservoir of pollutants and play an important role in the transport and fate of environmental pollutants. The large seawater volume in oceans makes this matrix an important inventory for pollutants, particularly in coastal regions [1]. Environmental pollution of surface waters poses a threat to the aquatic environment, through the toxic effects on aquatic organisms. The accumulation of pollutants in the ecosystem is also a potential hazard to human health. As a result, protection of water resources and biota is a priority. The European Union (EU) released maximum allowable concentration levels for some pollutants in surface waters [2]. In aquatic environments, hydrophobic pollutants can bioaccumulate though direct uptake from the water or trophic transfer [3]. This creates safety concerns for human consumption of aquatic organisms. Shrimp is one of the most popular crustaceans consumed worldwide and can be a healthy addition to our diet. Shrimp is low in fat and calories, rich in omega-3 fatty acids and a good source of key nutrients, such as iodine, phosphorus, choline, copper, zinc, B-complex vitamins, vitamin A and E and antioxidants, especially astaxanthin [4,5].

Shrimps can accumulate pollutants from their surrounding environment. These pollutants include organochlorine pesticides (OCPs), organophosphorus pesticides (OPPs), brominated flame retardants (BFRs), polychlorinated biphenyls (PCBs) and synthetic musks (SMs). These chemicals are lipophilic endocrine disruptors that are persistent in the environment and can be transferred through the food chain [6–8]. These pollutants are a global problem for the environment and human health.

OCPs have been used extensively in agriculture as pest and insect control. The use of OCPs, such as dichlorodiphenyltrichloroethane (DDT), hexachlorobenzene (HCB), aldrin, endrin, chlordane, heptachlor, mirex and toxaphene, have been prohibited in many European, North American and South American countries from the 1970s and 1980s [9,10]. Despite this, DDT and lindane (γ-HCH) are still used in some countries. DDT is used to inhibit mosquito growth to prevent vector-borne diseases such as dengue, leishmaniosis, malaria and Japanese encephalitis and lindane is used to treat head lice in children [11].

OPPs are the class of agriculture pesticides most used worldwide. Besides their agricultural use, OPPs also have many domestic uses. Although OPPs are less persistent, most of them can cause harmful effects on humans [12]. Some OPPs, such as dimethoate, malathion and chlorpyrifos, have already been banned from commercialization in Europe, under Regulation EC No.1107/2009 [13].

BFRs are chemicals used to reduce the flammability of a variety of products, including electronics, vehicles, plastics and textiles. This group of chemicals consists of polybrominated diphenyl ethers (PBDEs) that comprise 209 congeners, polybrominated biphenyls (PBBs), tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCDD). Certain BFRs also have legislation that restricts or bans their use. Directive 2003/11/EC amends Directive 76/769/EEC and prohibits the sale of PentaBDE and OctaBDE (two commercial mixtures of PBDEs) in concentrations higher than 0.1% by mass [14]. Since 2006, all new electrical and electronic equipment should not contain PBBs and PBDEs [15,16]. In 2008, DecaBDE was also banned [17].

PCBs are a group of chlorinated hydrocarbons that include between one and ten chlorine atoms attached to a biphenyl moiety [18,19] and have 209 possible congeners. Since 1929, these chemicals have been widely used in various industrial materials such as hydraulic fluids, insulating oil in condensers and transformers and paints. Additionally, some paint manufacturing processes can produce PCB congeners as by-products [19,20]. Europe prohibited the manufacturing of PCBs in the late 1970s [19,21], the Stockholm Convention classified PCBs as persistent organic pollutants in 2001 [22] and the International Agency for the Research on Cancer (IARC) classified PCBs in Group I (carcinogenic to humans) [23].

SMs are chemicals used as fragrance additives in diverse household and personal care products, including perfumes, shampoos, lotions, deodorants, soaps and detergents. SMs can be divided into four major groups: nitro, polycyclic, macrocyclic and alicyclic musks. Nitro musks have been banned in many countries and replaced by polycyclic musks [24]. The EU forbids the use of the musks ambrette, moskene and tibetene in cosmetic products. The EU also limits the musks xylene and ketone in cosmetic products to less than 0.003–1% and 0.042–1.4%, respectively [25].

The major exposure routes for these chemicals are diet, occupational exposure, inhalation or absorption through the skin. These pollutants are linked to adverse health effects, including cancers, neurological, respiratory, immunological and reproductive disorders, thyroid dysfunction and diabetes [12,20,26–29].

These chemicals can be detected in shrimp and seawater samples all over the world [30]. Table S1 (Supplementary Material) presents some examples of contaminant levels found in Europe. The purpose of this study was to measure the presence of contaminants in two shrimp species, *Palaemon serratus* and *Palaemon varians*, and in the water from their habitat on the northwest (NW) Portuguese coast, contributing to the quality of the aquatic environment and to ensure food safety.

2. Results

2.1. Validation of the Analytical Methodology

The optimized procedure presented an adequate separation and quantification of the compounds with retention times in the range of 16.41–25.19 min (OCPs), 10.15–20.21 min (OPPs), 19.42–71.65 min (BFRs and PCBs) and 14.83–26.35 min (SMs). For all the compounds, the intraday and interday precisions were below 20%, and the results are summarized in Table S2. Intraday and interday precisions were 4.0–13.9% and 2.9–11.0% for OCPs, respectively, 6.7–9.9% and 5.4–15.8% for OPPs, respectively, 7.4–12.5% and 0.5–10.8% for BFRs, respectively, 3.3–7.7% and 1.0–4.9% for PCBs, respectively, and 7.4–13.5% and 4.1–13.2% for SMs, respectively. The results of the expanded combined uncertainty (Ur, tot) were 6.6–15.3% for OCPs, 11.3–38.0% for OPPs, 1.6–24.2% for BFRs, 5.2–15.3 for PCBs and 7.4–26.5% for SMs (Table S2). The values are in accordance with the EU guidance requirement of <50% [31].

2.1.1. Water Sampled in the Shrimps' Habitat

The optimized procedure was adequate for multiresidue analysis of various pollutants with a valuable separation and quantification of the compounds. The linearity range, coefficient of determination (R^2), method detection (MDL) and method quantification (MQL) limits, matrix effect (ME) and average recoveries for each group (OCPs, BFRs, PCBs, OPPs and SMs) for seawater samples are presented in Table S3. Good linearity was achieved for all the curves for the entire range of concentrations with R^2 higher than 0.99, except for BFR, TBECH (Table S3). The average recoveries presented in Table S3 ranged from 60–88% for OCPs with the exception of HCB (46%), 70–83% for OPPs, 86–93% for BFRs, 62–95% for PCBs and 65–77% for SMs. MDLs and MQLs were 0.006–0.013 ng/g wet weight (ww) and 0.020–0.044 ng/g ww for OCPs, 0.007–0.013 ng/g ww and 0.023–0.044 ng/g ww for OPPs, 0.004–0.031 ng/g ww and 0.013–0.104 ng/g ww for BFRs, 0.002–0.013 ng/g ww and 0.008–0.044 ng/g ww for PCBs and 0.009–0.013 ng/g ww and 0.029–0.045 ng/g ww for SMs, respectively. ME was calculated as described in Section 3.4, and values ranged between 16% and −109% (OCPs), 0% and 36% (OPPs), −9% and −70% (BFRs), −15% and −83% (PCBs) and 90% and 93% (SMs), as described in Table S3. For all the pollutants analyzed, 55.1% of the compounds suffered ion suppression, 18.4% ion enhancement, 24.5% were in the range of −20–20% and one presented a value of 0%.

2.1.2. Shrimp Samples

The chromatographic method was optimized for the edible tissues of shrimp samples. Three different QuEChERS methods were tested for OCPs, and the best QuEChERS was the AOAC (Table S4). Applying the selected QuEChERS (AOAC), different quantities of graphitized carbon black were tested in the clean-up for OCPs, the best results were obtained with 2 mg (Table S4). The linearity range, R^2, MDL, MQL, ME and average recoveries for each group of pollutants (OCPs, BFRs, PCBs, OPPs and SMs) are presented in Table S5. Good linearity was achieved in all analyte curves for the entire range of concentrations used, since R^2 values were higher than 0.99. The average recoveries ranged between 56–96% for OCPs, 60–91% for OPPs, 65–136% for BFRs, 73–88% for PCBs and 74–94% for SMs (Table S5). MDLs and MQLs were 0.95–2.89 ng/g ww and 3.16–9.63 ng/g ww for OCPs, 4.23–6.39 ng/g ww and 14.10–21.31 ng/g ww for OPPs, 1.08–3.70 ng/g ww and 3.59–12.33 ng/g ww for BFRs, 1.80–3.08 ng/g ww and 5.99–10.27 ng/g ww for PCBs and 1.33–3.51 ng/g ww and 4.42–11.71 ng/g ww for SMs, respectively. The complexity of food matrices with a large number of compounds can be problematic, interfering with the analytical signal causing ME. ME values were between −9% and −212% for OCPs, 1% and 29% for OPPs, 3% and −174% for BFRs, −16% and −107% for PCBs and 38% and 61% for SMs (Table S5). For all the compounds analyzed, 49.0% of the analytes suffered ion suppression, 21.6% ion enhancement and 29.4% were in the range of −20–20%, not considered the ME [32,33].

2.2. Pollutants

2.2.1. Pollutants in Water Sampled in the Shrimps' Habitat

Quantification of pollutants in seawater can be challenging because the levels are potentially low in this environmental matrix. The concentrations of the analyzed pollutants found in the different locations are reported in Table 1. All the results are presented as μg/L of water sampled in the shrimps' habitat. The OCPs found in seawater samples were α-HCH, lindane, δ-HCH, aldrin, dieldrin, DDE, DDD and DDT. However, only DDD and lindane were found above MDL. A DDD concentration of 0.012 μg/L was determined in the sample from aquaculture, in spring of 2018. Lindane was found in autumn samples from Ria de Aveiro 2017 (0.014 μg/L), Figueira da Foz 2017 (0.014 μg/L), Aveiro 2018 (0.021 μg/L) and Sado 2017 (0.023 μg/L). A previous study from 2002 also found the presence of OCPs in the Atlantic Ocean, in the Portugal zone, at 68 pg/L for α-HCH and 112 pg/L for lindane [34]. Chlorpyrifos was the only OPP found in the seawater samples analyzed, although below the MDL. A study from Zhong et al., in the North Sea reported concentrations between 27 and 86 pg/L for chlorpyrifos [35]. Regarding BFRs, BDE 28 and BTBPE were found, but only BTBPE was found above the MDL. A BTBPE concentration of 0.013 and 0.015 μg/L was found in spring samples from Ria de Aveiro 2018 and from aquaculture 2019, respectively. BFRs were detected previously in the Atlantic Ocean [36] (Table S1). BTBPE was detected previously in Korea Bays at a maximum of 0.021 ng/L [37]. PCB 28 was the only PCB found in seawater samples but was below the MDL. This is in accordance with another study in the Atlantic Ocean that found PCB 28 and other PCBs [38]. Concerning SMs, HHCB, AHTN and ketone were found in samples, although all below the MDL. A study that attempted to determine the presence of SMs in the Atlantic Ocean around Portugal did not detect any of the SMs analyzed, including HHCB, AHTN and ketone [39]. On the other hand, these three SMs were found in aquaculture sites from the Atlantic Ocean and Mediterranean Sea, with values between <MDL and 98.90 ng/L [40].

2.2.2. Pollutants in Shrimp Samples

Concentrations for the analyzed pollutants found in two shrimp species in different locations are summarized in Table 2. All the results are presented as ng/g ww. Despite the fact that several OCPs were detected in water, DDD was the only OCP found in shrimp samples. A concentration of 6.09 ng/g ww of DDD was found in a sample from Vila do Conde, autumn 2017. Persistent chemicals have been reported previously in shrimp samples from Europe, and a study from Belgium reported values of not detected (ND) to 0.81 ng/g ww for HCB, DDE and HCHs [41]. Another study from Belgium–Netherlands presented values of ND to 1.03 ng/g ww for DDE, DDD and DDT [42]. A report from Italy described values of ND to 1.02 ng/g ww for DDE, DDD, DDT and HCB [43]. OPPS and BFRs were not detected in any sample analyzed. Although BFRs have been described previously [44], as reported in Table S1, for OPPs, no report from Europe on the concentration in shrimps was found (using Web of Science database). Regarding PCBs, PCB 153 and PCB 180 were detected, but below the MDL, compared to a study from the Netherlands that reported a value of 0.117 ng/g ww for the sum of PCB 28, 52, 101, 108, 138, 153 and 180 [45]. Concerning SMs, HHCB, AHTN and ketone were detected. HHCB was detected in all the samples analyzed at concentrations ranging between 3.16 ng/g ww and 7.55 ng/g ww in spring from Aveiro 2019 and Ria de Aveiro 2018, respectively. AHTN was found in three samples from spring above the MDL, 2.64 ng/g ww in Matosinhos (shrimps with eggs) 2019 and, in Ria de Aveiro and Sado from 2018, the same value was obtained, 2.97 ng/g ww. The presence of eggs or shell does not significantly affect the quantity of pollutants. Ketone was observed to be present in half of the samples, in concentrations between 2.15 ng/g ww in Ria de Aveiro and 11.06 ng/g ww in Sado from spring 2018. The concentrations found in other studies are in the range of the values reported in this work. In shrimp samples from Asia, values for HHCB and AHTN ranged from 1.5–5.3 ng/g ww [46] and a further study, analyzing shrimp samples from local supermarkets in Spain, found values for HHCB and AHTN between 2.9 and 3.8 ng/g ww [47]. The sum and average of the SMs were

calculated considered MDL/$\sqrt{2}$ for concentrations below the MDL [48]. Comparing the average concentration values of spring and autumn for the three detected SMs, higher values were observed in spring. The average concentration value for HHCB was 4.89 ng/g ww in spring and 4.15 ng/g ww in autumn, and ketone presented values of 5.40 ng/g ww for spring and 4.55 ng/g ww for autumn. AHTN only presented values above the MDL in samples from spring, with an average of 2.86 ng/g ww. Evaluating the difference between the values for spring and autumn for the three SMs, we found that HHCB was significantly higher in spring samples ($p = 0.027$). Regarding the two shrimp species, the average for *P. serratus* was 4.64, 1.86 and 4.41 ng/g ww and for *P. varians* it was 4.40, 1.83 and 6.33 ng/g ww for HHCB, AHTN and ketone, respectively. Aquaculture shrimps have slightly lower values of HHCB and partially higher values of AHTN and ketone.

2.3. Bioaccumulation

The bioaccumulation degree can be reported as the bioaccumulation factor (BAF). For the calculation of the BAF, only the values detected for SMs were used, because these were found simultaneously in water and shrimp samples between 2017 and 2019. All SMs detected, HHCB, AHTN and ketone in seawater samples were below the MDL, whereas in shrimp samples HHCB and ketone were all above the MDL and three samples also presented AHTN above the MDL. For the pollutants below the MDL, the MDL was used to calculate the BAF [49]. The BAF values calculated are presented in Table 3. We observed bioaccumulation of all SMs detected in the biota samples. Regarding the average values for the three SMs detected, the BAF values are ranked as follows: ketone > HHCB > AHTN, ranging between 183.2 L/kg ww for *P. serratus* and 940.7 L/kg ww for *P. varians*. The BAF values regarding SMs in fish samples reported previously showed a wide range, from 93.3 L/kg ww to 6,456.5 L/kg ww, suggesting species-specific accumulation patterns [50,51].

2.4. Maximum Admissible Concentration

Pollutants such as OCPs, OPPs, PCBs and BFRs are already banned or have been restricted (with maximum levels legislated) [2,52–54]. The concentrations detected in this study for these pollutants were almost all < MDL, with the exception of lindane, DDD and BTBPE in seawater samples and DDD in shrimp samples. We can see that OCPs such as DDD and lindane, already banned for several years, can still be detected in the samples analyzed with values > MDL. BTBPE, detected in seawater samples, is an emerging BFR and, although it has been investigated, it is not yet legislated. The limited experimental data collected for BTBPE identified that this compound can accumulate in the body over time, due to the BAFs reported. These findings were included in a scientific opinion released by the European Food Safety Authority (EFSA) [55]. Regarding SMs, HHCB, ANTH and ketone were detected in shrimp samples > MDL. Although some legislation has been established, concerning the maximum concentration allowed of SMs in cosmetics [25], no regulation has been established for environmental or biota samples. The results of this study reported concentrations of SMs in shrimps and with BAF values higher than 100. These findings validate that specific legislation for SMs should be enforced for foodstuffs, such as fishery products.

Table 1. Levels for the pollutants found in water sampled in the shrimps' habitat (μg/L) from NW Portuguese coast; ND: not detected.

	Concentrations μg/L														
	OCPs								OPPs	BFRs		PCBs	SMs		
Location and Date of Sampling	**α-HCH**	**Lindane**	**δ-HCH**	**Aldrin**	**DDE**	**Dieldrin**	**DDD**	**DDT**	**Chlorpyrifos**	**BDE 28**	**BTBPE**	**PCB 28**	**HHCB**	**AHTN**	**Ketone**
Vila do Conde Autumn 2017	ND	ND	ND	<MDL	ND	ND	ND	ND	<MDL	ND	ND	ND	<MDL	<MDL	<MDL
Vila do Conde Spring 2018	<MDL	<MDL	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL	<MDL
Vila do Conde Autumn 2018	<MDL	<MDL	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Vila do Conde Spring 2019	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	ND
Matosinhos Autumn 2017	ND	ND	ND	ND	ND	ND	<MDL	<MDL	ND	ND	ND	ND	<MDL	<MDL	<MDL
Matosinhos Spring 2018	ND	< MDL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Matosinhos Autumn 2018	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Matosinhos Spring 2019	ND	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Aveiro Spring 2018	ND	<MDL	ND	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Aveiro Autumn 2018	ND	0.021	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Aveiro Spring 2019	ND	<MDL	<MDL	ND	ND	<MDL	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Ria de Aveiro Autumn 2017	ND	0.014	ND	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Ria de Aveiro Spring 2018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.013	ND	<MDL	<MDL	<MDL
Figueira da Foz Autumn 2017	ND	0.014	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Figueira da Foz Spring 2018	ND	ND	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Figueira da Foz Autumn 2018	ND	<MDL	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Figueira da Foz Spring 2019	ND	ND	ND	<MDL	<MDL	ND	ND	ND	ND	<MDL	ND	ND	<MDL	<MDL	<MDL
Aquaculture Autumn 2017	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	ND	ND	ND	<MDL	<MDL	ND
Aquaculture Spring 2018	ND	ND	<MDL	ND	ND	ND	0.012	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Aquaculture Autumn 2018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	ND	<MDL
Aquaculture Spring 2019	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	0.015	ND	<MDL	<MDL	<MDL

Table 1. *Cont.*

	Concentrations µg/L														
	OCPs								OPPs	BFRs		PCBs	SMs		
Location and Date of Sampling	**α-HCH**	**Lindane**	**δ-HCH**	**Aldrin**	**DDE**	**Dieldrin**	**DDD**	**DDT**	**Chlorpyrifos**	**BDE 28**	**BTBPE**	**PCB 28**	**HHCB**	**AHTN**	**Ketone**
Sado Autumn 2017	<MDL	0.023	<MDL	<MDL	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Sado Spring 2018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Sado Autumn 2018	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL
Sado Spring 2019	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	<MDL	<MDL	<MDL

Table 2. Levels for the pollutants found in shrimp samples (ng/g ww) from Portugal.

	Concentrations ng/g ww					
	OCPs	PCBs		SMs		
Location and Date of Sampling	**DDD**	**PCB 153**	**PCB 180**	**HHCB**	**AHTN**	**Ketone**
Vila do conde Autumn 2017	6.09	ND	ND	3.34	ND	3.59
Vila do Conde Spring 2018	ND	ND	ND	4.76	ND	ND
Vila do Conde Spring 2018_E	ND	ND	<MDL	5.26	ND	5.38
Vila do Conde Autumn 2018	ND	ND	ND	4.17	ND	ND
Vila do Conde Spring 2019	ND	ND	ND	4.34	<MDL	ND
Vila do Conde Spring 2019_E	ND	ND	ND	3.95	ND	ND
Matosinhos Autumn 2017	ND	<MDL	ND	3.49	<MDL	ND
Matosinhos Spring 2018_E	ND	ND	<MDL	4.95	ND	6.71
Matosinhos Autumn 2018	ND	ND	ND	5.78	ND	ND
Matosinhos Spring 2019	ND	ND	ND	5.92	<MDL	ND
Matosinhos Spring 2019_E	ND	ND	ND	4.30	2.64	ND
Aveiro Spring 2018	ND	ND	<MDL	5.38	<MDL	5.49
Aveiro Autumn 2018	ND	ND	ND	3.64	ND	ND
Aveiro Spring 2019	ND	ND	ND	3.16	<MDL	4.62
Ria de Aveiro Atumn 2017	ND	ND	ND	4.74	ND	ND
Ria de Aveiro Spring 2018	ND	ND	ND	7.55	2.97	2.15
Figueira da Foz Autumn 2017	ND	ND	ND	3.76	<MDL	4.52
Figueira da Foz Spring 2018	ND	ND	ND	4.75	ND	ND
Figueira da Foz Autum 2018	ND	ND	ND	5.30	ND	3.30
Figueira da Foz Spring 2019	ND	ND	ND	4.18	ND	3.96
Aquaculture Autumn 2017	ND	ND	ND	4.02	ND	7.41
Aquaculture Spring 2018	ND	ND	ND	6.02	ND	4.26
Aquaculture Autumn 2018	ND	ND	ND	4.46	<MDL	3.96
Aquaculture Spring 2019_S	ND	ND	ND	3.79	<MDL	4.95
Sado Autumn 2017	ND	ND	ND	3.17	<MDL	ND
Sado Spring 2018	ND	ND	ND	6.34	2.97	11.06
Sado Autumn 2018_S	ND	ND	<MDL	3.85	ND	ND
Sado Spring 2019_S	ND	<MDL	<MDL	3.58	<MDL	ND

ND: not detected; E: eggs; S: shell.

Table 3. Bioaccumulation factors (BAFs) for the SMs found in shrimps (L/kg ww).

Location and Date of Sampling	HHCB	AHTN	Ketone
Vila do conde Autumn 2017	249.4		305.2
Vila do Conde Spring 2018	355.7		
Vila do Conde Autumn 2018	312.0		
Vila do Conde Spring 2019	324.1	254.2	
Matosinhos Autumn 2017	260.9	254.2	
Matosinhos Spring 2018	369.9		570.6
Matosinhos Autumn 2018	432.4		
Matosinhos Spring 2019	442.8	254.2	
Aveiro Spring 2018	402.3	254.2	467.3
Aveiro Autumn 2018	272.5		
Aveiro Spring 2019	236.0	254.2	392.6
Ria de Aveiro Autumn 2017	354.6		
Ria de Aveiro Spring 2018	564.5	346.3	183.2
Figueira da Foz Autumn 2017	281.4	254.2	384.7
Figueira da Foz Spring 2018	354.9		
Figueira da Foz Autumn 2018	396.4		280.3
Figueira da Foz Spring 2019	312.7		336.7

Table 3. *Cont.*

Location and Date of Sampling	HHCB	AHTN	Ketone
Aquaculture Autumn 2017	300.6		
Aquaculture Spring 2018	450.3		362.1
Aquaculture Autumn 2018	333.7		336.4
Aquaculture Spring 2019	283.4	254.2	421.3
Sado Autumn 2017	237.1	254.2	
Sado Spring 2018	474.1	345.6	940.7
Sado Autumn 2018	288.0		
Sado Spring 2019	267.7	254.2	

2.5. Risk Assessment

The risk of exposure was evaluated for the detected SMs (HHCB, AHTN and ketone), the group of contaminants present with higher frequency in the analyzed samples. Data on safe SM consumption levels and their health risks for human population are very limited. However, to these SMs are attributed values for no observed adverse effect level (NOAEL) [56–58], as presented in Table 4. Based on this, the tolerable daily intake (TDI) was calculated. The values for maximum shrimp daily consumption per individual (28.82 g/day) and mean adult body weight in the Portuguese population (71.68 kg) were described in a previous work reporting data on the Portuguese population [59]. Only values of SM concentrations above the MDL were used to calculate the dietary exposure. The results described in Table 4 present values of exposure (median, 25th and 75th percentile) all below the TDI calculated, so it is unlikely that a potential health risk is present based on this risk assessment. These results are in accordance with previous works reporting risk assessment for SMs in seafood [46,47].

Table 4. NOAEL values and the estimated exposure to detected SMs from shrimp consumption among the Portuguese population.

	NOAEL	TDI Calculated	Median Exposure	P25 Exposure	P75 Exposure
SMs	**mg/kg bw/day**	**µg/kg bw/day**	**µg/kg bw/day**	**µg/kg bw/day**	**µg/kg bw/day**
HHCB	50	500	1.74	1.52	2.13
AHTN	5	50	1.19	1.06	1.20
Ketone	2.5	25	1.84	1.55	2.33

3. Materials and Methods

In this study, fourteen OCPs, six OPPs, twelve BFRs, thirteen PCBs and six SMs were included, in a total of 51 endocrine disruptors. Most of these compounds have high Log Kow which increases the potential to bioconcentrate in living organisms.

3.1. Sample Collection

P. serratus and *P. varians* shrimp species were collected in Portugal. *P. serratus* was collected along the NW Portuguese coast (namely in Vila do Conde, Matosinhos, Aveiro, Ria de Aveiro and Figueira da Foz) by local fishermen and *P. varians* (wild and aquaculture origin) was collected in the Sado estuary. *P. serratus*'s size ranged from 4–9 cm and the size of *P. varians* ranged from 2–4 cm. A total of 1 kg of shrimps was sampled in each location. The sampling was performed in autumn and spring between 2017 and 2019 in all the locations, according to the period allowed for shrimp capture by Portuguese law [60]. The edible portion of the shrimp was separated from the shell, except in some samples of *P. varians* in which separation was not possible, due to the small size of the shrimp; shrimps with eggs were stored separately. Twenty-eight samples were storage at −20 °C until analysis.

Surface water was collected in the same locations of shrimp samples and during the same period. A total of twenty-five samples were gathered: 4 in Vila do Conde, 4 in Matosinhos, 3 in Aveiro, 2 in Ria de Aveiro, 4 in Figueira da Foz and 8 in the Sado estuary (4 for aquaculture and 4 for wild shrimp). Samples were collected in clean glass bottles, previously rinsed with ultra-pure water and stored at −20 °C until extraction.

3.2. Reagents, Solvents and Materials

The detailed description of the reagents, solvents and materials used for the extraction and analysis of the studied contaminants is given in Table S6. For the contaminants analyzed by GC (Table S7), standard stock solutions of individual and mixed compounds were prepared in n-hexane with different concentrations and stored in amber colored vials at 4 °C prior to utilization.

3.3. Extraction Procedure

3.3.1. Water Sampled in the Shrimps' Habitat

Prior to extraction, the samples were filtered with ashless filter paper and the sample pH was adjusted to 2.5 using HCl 1 M. Internal standard (IS) was added to volumetric flasks (final concentrations of 100 µg/L for 4.4′-dichlorobenzophenone and 50 µg/L for 5′-fluoro-2,3′,4,4′,5-pentabromodiphenyl ether, TPP and AHTN-d3). The solid phase extraction (SPE) method was used for the extraction procedure. Strata C18-E cartridges (500 mg, 3 mL), in a vacuum system manifold (Chromabond, Düren, Germany), were previously cleaned with 5 mL of ethyl acetate:dichloromethane (1:1), and conditioned with 5 mL of methanol and 5 mL of ultrapure water. Subsequently, seawater samples were percolated through the cartridge. Then, the cartridges were rinsed with 10 mL of ultrapure water and dried under vacuum for 15 min. The elution was performed using 8 mL of ethyl acetate and 8 mL of dichloromethane. The cartridges were dried under vacuum. Anhydrous sodium sulfate (5 g) was added to the eluted samples (to remove all the existent water). In the end, 30 µL of *D*-(+)-gluconic acid δ-lactone at 5 mg/mL in acetonitrile (ACN)/ultrapure water (9:1) were added as an analyte protector. The extract was evaporated under a stream of nitrogen and the residues were reconstituted with 500 µL of *n*-hexane and injected into the chromatograph. OCPs, BFRs and PCBs were analyzed using gas chromatography with an electron capture detector (GC-ECD), OPPs by gas chromatography with a flame photometric detector (GC-FPD) and SMs using gas chromatography coupled with a mass spectrometric detector (GC-MS). Positive results were confirmed by GC-MS/MS. Details of the chromatography analysis are presented in the Supplementary Material (Section S1), GC-MS and MS/MS conditions for SMs are described in Table S8 [61,62] and GC-MS/MS conditions for the other compounds in Table S9 [63–65].

3.3.2. Shrimp

Optimization of the extraction procedure was performed with OCPs, by testing different QuEChERS (Original, AOAC and EN) and clean-up compositions (150 mg anhydrous magnesium sulfate, 50 mg PSA and 50 mg C18 with 2 mg graphitized carbon black or 5 mg graphitized carbon black) (Table S4). The optimized method was then tested and applied to all analyzed contaminants. Briefly, shrimp edible portions (shrimp muscle) were mechanically homogenized with a kitchen blender (Braun, Frankfurt, Germany), 5 g were weighed and the IS solution was added to a final concentration of 100 µg/L for 4.4′-dichlorobenzophenone and 50 µg/L for 5′-fluoro-2,3′,4,4′,5-pentabromodiphenyl ether, TPP and AHTN-d3. Next, 8 mL of ACN were added to the shrimp, followed by vortex homogenization for 3 min. QuEChERS AOAC was added and the mixture was again vortexed for 3 min and then centrifuged at 4000 rpm for 5 min. Afterwards, 1 mL of supernatant was transferred to a 2 mL tube containing the clean-up sorbents (150 mg anhydrous magnesium sulfate, 50 mg PSA, 50 mg C18 and 2 mg graphitized carbon black). The tubes were vortexed for 2 min followed by centrifugation at 4000 rpm for 5 min. An aliquot of the supernatant 300 µL (for OCP and OPPs) or 600 µL (for BFRs, PCBs and SMs)

was transferred to a vial and concentrated just to dryness using a gentle stream of nitrogen. The sample residue was reconstituted in 150 µL of n-hexane and injected into the GC for analysis. GC analysis of shrimp samples was conducted as described for seawater samples.

3.4. Method Validation

Repeatability was presented as intraday precision and intermediate as interday precision. Calibration curves were obtained for all contaminants analyzed in this study in shrimp or seawater fortified after extraction with standard solutions (matrix-matched calibration). In seawater samples, recoveries were calculated using spiked samples at three levels in triplicate, 0.06, 0.09 and 0.13 µg/L for all the compounds analyzed. The recoveries for shrimp samples were calculated using spiked samples at three levels, 16, 24 and 32 ng/g *w*/*w* for OCPs, 16, 28 and 40 ng/g *w*/*w* for OPPs and 8, 14 and 20 ng/g *w*/*w* for BFRs, PCBs and SMs, with each level in triplicate. The ME was calculated, as previously described [32], using standard solutions prepared in n-hexane compared to spiked blank samples. A positive result corresponds to an enhancement of the analytical response, whereas a negative value corresponds to a suppression effect. Values within the 20% signal variation are not considered as a ME [32,33]. The uncertainty was determined following the "top-down" approach, using validation data and the uncertainty of the purity of analytical standards. The coverage factor k of 2 and confidence level of 95% were applied for all the analytes [32].

3.5. Bioaccumulation Factor

The BAF can be measured as the ratio between concentration in aquatic species (shrimp µg/kg ww) and the concentration in ambient water (µg/L) [49]. The units of the BAF are mostly presented as L/kg [66]. A BAF greater than 1 indicates a potential of the organism to accumulate the pollutants, and values above 100 are considered significant [67].

3.6. Risk Assessment

A risk assessment was performed for a group of contaminants present in a higher frequency in the analyzed samples. The NOAEL [56–58] values were used to calculate a TDI by applying an uncertainty factor of 100 to account for the human variability and species differences [46]. The dietary exposure (µg/kg bw/day) of SMs was calculated according to the Equation (1) described previously [47].

$$Ei = \frac{Ci}{B} \times x \quad (1)$$

where *Ei* is the dietary exposure to the SM for individual (*i*) (µg/kg bw/day), *Ci* is the maximum shrimp daily consumption by individual (*i*) (g/day), *B* is the mean body weight of the adult Portuguese population (kg) and *x* is the concentration of SM in shrimp samples (µg/kg).

3.7. Statistical Analysis

The statistical analysis was performed using GraphPad Prism 6.01 software (La Jolla, CA, USA). Differences were considered significant when $p < 0.05$. Statistical significance of the difference between groups (autumn and spring) was evaluated by Student's *t*-test and a Wilcoxon test.

4. Conclusions

This study reports, for the first time, the analysis of trace organic pollutants in shrimp species collected from the NW Portuguese coast. Lindane, DDD and BTBPE were detected above the MDL in seawater samples. In shrimp samples, DDD, HHCB, AHTN and ketone were found above the MDL. The average concentrations of SMs in shrimp samples were higher in spring than in autumn. In addition, aquaculture shrimps had lower HHCB values and higher AHTN and ketone values than wild shrimp. Bioaccumulation of HHCB, AHTN

and ketone was confirmed using BAF calculation. The estimated exposure levels of SMs in shrimp samples were all below the estimated TDI, although these results need to be interpreted with caution due to the uncertainties of the limited data available. Although some of these pollutants have been banned for several years, they can still be detected in the environment today. Our results highlight the importance of continuous monitoring of pollutants in aquatic environments and marine organisms. For emerging pollutants like SMs, it is essential to establish specific legislation for aquatic environments and fishery products, such as shrimp, which is highly valuable in the human diet.

Supplementary Materials: The following are available online. Section S1: Gas chromatography Analysis description; Table S1: Concentration levels of the contaminants found in shrimp (ng/g ww) and seawater (µg/L) samples in previous studies in Europe; Table S2: Summary of uncertainties, intraday and interday precision obtained for OCPs, OPPs, BFRs, PCBs and SMs analytes; Table S3: Validation parameters using water sampled in the shrimp's habitat matrix matched calibration for the selected OCPs, OPPs, BFRs, PCBs and SMs; Table S4: QuEChERS and clean-up optimization; Table S5: Validation parameters using shrimp matrix matched calibration for the selected OCPs, OPPs, BFRs, PCBs and SMs; Table S6: Reagents, solvents, and materials used in the extraction of the samples and in the analysis of the studied contaminants; Table S7: Physic-chemical characteristics, molecular mass, and the supplier company of the studied contaminants; Table S8: GC-MS and MS/MS conditions for SMs analysis; Table S9: GC-MS/MS conditions for OCPs, OPPs, BFRs, PCBs analysis.

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Supplementary material

Validation and evaluation of selected organic pollutants in shrimp and seawater samples from the NW Portuguese coast

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Section 1

Gas chromatography Analysis description

Gas Chromatography – Electron Capture Detector

OCPs, BFRs and PCBs analysis were carried out with a Shimadzu GC-2010 (Kyoto, Japan) with an ECD detector, equipped with capillary Zebron-XLB column of 30 m (0.25 mm i.d., 0.25 µm film thickness, Phenomenex, California, USA). The carrying gas, helium, from Linde Sogás (purity ≥99.999%), flowed at 1.66 mL/min with a linear velocity of 36 cm/s. Whereas nitrogen (Linde Sogás, purity ≥99.999%) was employed as makeup gas at a flow rate of 30 mL/min. Injection was carried out in splitless mode, with an injector temperature of 250 °C and detector temperature was set at 300 ºC. The system was operated by GC-Solutions Shimadzu software. For OCPs determination, the column temperature programmed was the follow: start at 40 ºC (1 min hold), followed by increases of 20 ºC/min to 120 ºC (1 min hold), 10 ºC/min to 150 ºC (1 min hold), 10 ºC/min to 180 ºC (1 min hold), 20 ºC/min to 200 ºC (1 min hold) and 10 ºC/min to 290 ºC (2 min hold). For BFRs and PCBs analysis, the temperature program start at 40 ºC (1 min hold) followed by increases of 15 ºC/min to 120 ºC (1min hold), 10 ºC/min to 150 ºC (1 min hold), 10 ºC/min to 180 ºC (1 min hold), 20 ºC/min to 200 ºC (1 min hold), 2 ºC/min to 210 ºC (1 min hold), 2 ºC/min to 220 (2 min hold), 2 ºC/min to 260 ºC (2 min hold), 0.5 ºC/min to 270 ºC (2 min hold), 2ºC/min to 280 ºC.

Gas Chromatography - Flame Photometric Detector

OPPs analysis was carried out with a Shimadzu GC-2010 (Kyoto, Japan) with an FPD detector with phosphorus filter equipped with a TRB-5 column of 25 m (0.25 mm i.d., 0.18 µm film thickness, Teknokroma, Barcelona, Spain). The carrying gas, helium, from Linde Sogás (purity ≥99.999%), flowed at 0.89 mL/min with a linear velocity of 25.4 cm/s. The injector temperature was 250 °C and operated in splitless mode and the detector was maintained at 290 ºC. The system was operated by GC Solution Shimadzu software. The GC oven temperature was programmed for an initial temperature of 100 ºC (1 min hold), increasing at 20 ºC/min to 150 ºC (1 min hold), 2 ºC/min to 180 ºC (2 min hold) and finally at 20 ºC/min to 270 ºC (3 min hold).

Gas Chromatography - Tandem Mass Spectrometry

SMs analysis were performed using a Trace GC Ultra gas chromatograph Polaris Q coupled with ion trap mass spectrometer (Thermo Fisher Scientific, Massachusetts, USA) operated in the electron impact ionization mode (70eV), equipped with Zebron ZB-5MSi column 30 m (0.25 mm i.d., 0.25 µm film thickness, Phenomenex,

California, USA). Operated by Xcalibur 1.3 software. Quantification was performed in selected ion monitoring (SIM) mode. The samples were injected through a splitless mode with helium from (Linde Sógas, purity ≥99.999%) as carrier gas at a constant flow rate of 1.3 mL/min. The injector was maintained at 240ºC. The GC oven temperature was programmed from an initial temperature of 80ºC, increasing at 10ºC/min to 160 ºC (10 min hold) and finally at 3 ºC/min to 200 ºC (1 min hold).

Table S1 - Concentration levels of the contaminants found in shrimp (ng/g ww) and seawater (µg/L) samples in previous studies in Europe.

			Σ concentrations (number of contaminants detected)					
	Location	**Date**	**OCPs**	**OPPs**	**BFRs**	**PCBs**	**SMs**	**Ref**
Shrimp	Scheldt Estuary (Belgian)	2001	ND - 2.61 (5)					[41]
	Tyrrhenian Sea in the Gulf of Naples	2003 - 2004	ND - 1.43 (4)					[43]
	Suppliers from Netherlands	2007 - 2008				0.117 (7)		[45]
	Scheldt estuary (Netherlands–Belgium)	2010	ND - 1.37 (3)		0.48 (3)			[42]
	Scheldt Estuary (Netherlands)	2001			35.05 – 47.49 (17)			[44]
	Establishments in Tarragona, Spain						11.5 (2)	[47]
	Atlantic Ocean, Portuguese Coast	2017 - 2018	ND - 6.09 (1)	ND	ND	ND	3.49 - 12.67 (3)	This study
	Atlantic Ocean, Aquaculture Portugal	2017 - 2018	ND	ND	ND	ND	3.17 - 20.37 (3)	
Seawater	Atlantic Ocean, Portugal	1997	0.000180 (2)					[34]
	Atlantic Ocean	2005				0.0000037 - 0.000220 (8)		[38]
	Atlantic Ocean	2009			ND - 0.0000072 (10)			[36]
	North Sea, Atlantic Ocean	2010	0.000065 (5)	0.000061 (1)				[35]
	Atlantic Ocean and Mediterranean Sea	2016					ND - 0.306 (4)	[40]
	Atlantic Ocean, Portugal	2017					ND (5)	[39]
	Atlantic Ocean, Portuguese Coast	2017 - 2018	ND - 0.021 (1)	ND	ND - 0.013 (1)	ND	ND	This study
	Atlantic Ocean, Aquaculture Portugal	2017 - 2018	ND - 0.023 (2)	ND	ND - 0.015 (1)	ND	ND	

Table S2 - Summary of uncertainties, intraday and interday precision obtained for OCPs, OPPs, BFRs, PCBs and SMs analytes.

Analyte	ucm	ur,repro (%)	Br (%)	ur,cm (%)	ur,ref (%)	u r,tot (%)	Ur,tot (%)	Intraday (%)	Interday (%)
α-HCH	0.7	3.4	-5.26	1.4	0.3	6.4	12.9	11.4	4.0
HCB	0.6	3.0	-0.66	1.2	0.3	3.3	6.6	9.7	2.9
β-HCH	1.0	3.8	3.07	2.0	0.3	5.3	10.5	4.9	10.1
Lindane	1.0	4.2	-1.56	2.1	0.3	4.9	9.9	13.9	7.6
δ-HCH	0.5	1.8	-3.65	1.0	0.3	4.2	8.3	12.7	11.0
Aldrin	0.6	3.1	1.07	1.3	0.3	3.5	7.0	5.3	2.9
α-endosulfan	1.4	6.1	-0.83	2.7	0.3	6.7	13.4	4.0	6.0
DDE	0.7	3.2	-0.06	1.4	0.3	3.5	7.0	8.7	4.4
Dieldrin	0.8	3.3	1.14	1.5	0.3	3.8	7.7	6.7	4.6
Endrin	0.8	3.0	1.59	1.5	0.3	3.7	7.4	4.0	6.6
DDT	1.3	6.1	-3.69	2.6	0.3	7.6	15.3	5.4	11.3
DDD	1.1	4.2	-0.68	2.1	0.3	4.8	9.6	4.2	5.2
β-endosulfan	1.4	5.7	0.24	2.9	0.3	6.4	12.8	6.7	6.0
Methoxychlor	1.0	4.8	-2.78	2.1	0.3	5.9	11.8	4.9	7.4
Dimethoate	1.6	7.6	-5.01	3.2	0.3	9.7	19.4	9.9	8.7
Chlorpyrifos-methyl	1.3	6.0	-0.93	2.7	0.3	6.7	13.3	7.4	7.9
Parathion-methyl	0.9	4.2	-3.21	1.8	0.3	5.6	11.3	9.4	5.4
Malathion	2.0	9.3	-1.77	4.1	0.3	10.3	20.6	8.2	9.5
Chlorpyrifos	1.9	8.3	-0.04	3.7	0.3	9.1	18.2	6.7	8.1
Chlorfenvinphos	3.5	16.5	-6.37	6.9	0.3	19.0	38.0	8.1	15.8
TBECH	1.3	5.3	5.05	2.5	0.3	7.7	15.5	7.4	6.6
BDE 28	1.6	7.2	9.22	3.2	0.3	12.1	24.2	10.6	5.9
PBT	0.5	2.5	2.85	1.1	0.3	4.0	7.9	12.4	2.2
PBEB	0.9	4.0	3.17	1.7	0.3	5.4	10.8	11.4	3.4
BDE 47	0.7	2.9	4.66	1.3	0.3	5.6	11.3	9.9	2.7
BDE 100	0.7	3.1	2.83	1.3	0.3	4.4	8.9	10.4	2.6
BDE 99	0.1	0.6	0.38	0.3	0.3	0.8	1.6	8.0	0.5
TBB	0.7	3.1	3.37	1.3	0.3	4.8	9.6	8.5	2.6
BDE 153	0.7	3.2	-1.73	1.3	0.3	3.9	7.8	12.3	2.8
BDE 154	1.2	5.7	-2.37	2.5	0.3	6.6	13.2	12.5	5.8
BDE 183	1.4	5.7	-3.29	2.8	0.3	7.2	14.3	11.3	6.8
BTBTE	1.0	4.5	-0.08	2.0	0.3	5.0	10.0	10.7	10.8
PCB 28	0.4	1.6	4.97	0.7	0.3	5.3	10.5	3.8	2.4
PCB 52	0.5	2.4	4.84	1.0	0.3	5.5	11.0	4.6	1.9
PCB 101	0.9	3.7	6.40	1.8	0.3	7.6	15.3	7.7	4.9
PCB 77	0.6	2.6	5.56	1.1	0.3	6.2	12.5	5.3	2.1
PCB 118	0.8	3.5	-0.73	1.6	0.3	3.9	7.8	3.3	3.4
PCB 114	0.8	3.5	5.71	1.5	0.3	6.8	13.7	6.1	3.0
PCB 153	0.4	1.8	5.96	0.8	0.3	6.3	12.6	7.0	1.5

PCB 138	0.6	2.7	6.25	1.2	0.3	6.9	13.8	3.9	2.1
PCB 126	0.4	1.7	3.71	0.8	0.3	4.2	8.4	7.2	1.6
PCB 156	0.3	1.6	1.86	0.7	0.3	2.6	5.2	6.6	1.4
PCB 157	0.6	2.7	3.29	1.1	0.3	4.4	8.8	4.8	2.1
PCB 180	0.2	1.2	2.92	0.5	0.3	3.2	6.4	6.2	1.0
PCB 169	0.6	2.5	2.79	1.2	0.3	4.0	7.9	5.0	2.3
celestolide	1.3	6.6	-0.99	2.7	0.3	7.2	14.4	11.3	8.3
ambrette	0.8	3.4	-7.12	1.6	0.3	8.1	16.1	10.8	13.0
HHCB	1.7	7.5	-0.73	3.3	0.3	8.2	16.4	11.5	13.2
xylene	1.3	6.7	-11.11	2.7	0.3	13.2	26.5	11.6	7.3
AHTN	0.6	2.4	2.58	1.1	0.3	3.7	7.4	13.5	5.0
ketone	0.6	3.1	-6.78	1.3	0.3	7.6	15.1	7.4	4.1

*u*cm- standard uncertainty of mean measured; *u*r,repro – within-lab reproducibility; *B*r - relative bias; *u*r,cm – uncertainty of systematic error; *u*r,ref – uncertainty of purity of analytical standard; *u*r,tot - relative combined measurement uncertainty; *U*r,tot - expanded combined measurement uncertainty.

Table S3 - Validation parameters using water sampled in the shrimp's habitat matrix matched calibration for the selected OCPs, OPPs, BFRs, PCBs and SMs.

		Calibration range (µg/L)	Regression equation (µg/L)	Linearity ($R^{2)}$	MDL (µg/L)	MQL (µg/L)	ME	Recoveries
OCPs	α-HCH	0.01 - 0.13	y= 132893779x + 1021457	0.997	0.008	0.026	-88%	60%
	HCB	0.01 - 0.13	y= 49374711x + 796376	0.993	0.013	0.044	-102%	46%
	β-HCH	0.01 - 0.13	y= 12377955x + 189596	0.994	0.011	0.036	-76%	87%
	Lindane	0.01 - 0.13	y= 113396890x + 1965367	0.995	0.009	0.031	-63%	72%
	δ-HCH	0.01 - 0.13	y= 104216354x + 1075640	0.995	0.010	0.033	-109%	84%
	Aldrin	0.01 - 0.13	y= 65240137x + 301274	0.998	0.006	0.021	-74%	75%
	α-endosulfan	0.01 - 0.13	y= 47758982x + 284167	0.993	0.011	0.037	-52%	81%
	DDE	0.01 - 0.13	y= 39964569x + 19073440	0.991	0.013	0.042	-24%	80%
	Dieldrin	0.01 - 0.13	y= 60109710x + 401794	0.994	0.011	0.036	-32%	79%
	Endrin	0.01 - 0.13	y= 42515476x + 196073	0.992	0.012	0.040	16%	81%
	β-endosulfan	0.01 - 0.13	y= 94365717x + 776534	0.998	0.006	0.020	18%	85%
	DDD	0.01 - 0.13	y= 25634192x + 60787	0.993	0.011	0.037	-83%	82%
	DDT	0.01 - 0.13	y= 166281349x + 1600524	0.996	0.009	0.030	50%	84%
	Methoxychlor	0.01 - 0.13	y= 76145243x + 766864	0.992	0.012	0.040	86%	88%
OPPs	Dimethoate	0.01 - 0.13	y= 38512480x + 40798	0.996	0.010	0.033	36%	73%
	Chlorpyrifos-methyl	0.01 - 0.13	y= 38352460x + 141861	0.991	0.013	0.044	8%	70%
	Parathion-methyl	0.01 - 0.13	y= 37254725x + 117188	0.991	0.013	0.044	33%	77%
	Malathion	0.01 - 0.13	y=38867381x + 166301	0.997	0.009	0.030	12%	81%
	Chlorpyrifos	0.01 - 0.13	y= 35730243x + 112607	0.998	0.007	0.023	0%	76%

	Chlorfenvinphos	0.01 - 0.13	y= 20001864x + 26362	0.998	0.007	0.023	17%	83%
BFRs	TBECH	0.01 - 0.13	y = 14104536x + 909102	0.952	0.031	0.104	-9%	93%
	BDE 28	0.01 - 0.10	y= 81490036x + 858650	0.995	0.011	0.036	-20%	86%
	PBT	0.01 - 0.13	y= 64432907x + 343442	0.997	0.007	0.023	-10%	88%
	PBEB	0.01 - 0.13	y= 87862395x + 809774	0.995	0.011	0.036	-35%	87%
	BDE 47	0.01 - 0.13	y= 97516663x + 1702848	0.993	0.013	0.043	-70%	93%
	BDE 100	0.01 - 0.10	y= 103919127x + 376275	0.997	0.007	0.024	-58%	89%
	BDE 99	0.01 - 0.10	y= 98103175x + 466692	0.998	0.006	0.019	-36%	91%
	TBB	0.01 - 0.10	y= 70242770x + 1151459	0.998	0.007	0.022	-37%	91%
	BDE 153	0.01 - 0.10	y= 104926351x + 388905	0.997	0.007	0.022	-45%	90%
	BDE 154	0.01 - 0.10	y= 45652538x + 604600	0.993	0.010	0.034	-36%	86%
	BDE 183	0.01 - 0.10	y= 92242191x + 539132	0.999	0.004	0.013	-22%	88%
	BTBPE	0.01 - 0.13	y= 64760812x + 605681	0.992	0.012	0.042	25%	87%
PCBs	PCB 28	0.01 - 0.13	y= 102933267x + 1325126	0.998	0.007	0.023	-15%	62%
	PCB 52	0.01 - 0.13	y= 63106100x + 971630	0.992	0.012	0.040	-18%	76%
	PCB 101	0.01 - 0.13	y= 97975532x + 3013827	0.994	0.010	0.035	-20%	84%
	PCB 77	0.01 - 0.13	y= 101036917x + 754781	0.991	0.013	0.044	-41%	82%
	PCB 118	0.01 - 0.13	y= 126880256x + 1312831	0.996	0.009	0.029	-20%	82%
	PCB 114	0.01 - 0.13	y= 332139764x + 5533491	0.995	0.009	0.031	-32%	86%
	PCB 153	0.01 - 0.13	y= 136028816x + 1810527	0.995	0.010	0.033	-28%	86%
	PCB 138	0.01 - 0.10	y= 109538813x + 871840	0.999	0.002	0.008	-83%	81%
	PCB 126	0.01 - 0.13	y= 127729214x + 4143415	0.996	0.008	0.028	-45%	85%
	PCB 156	0.01 - 0.13	y= 261852734x + 3309590	0.998	0.006	0.021	-49%	87%
	PCB 157	0.01 - 0.13	y=228228539x + 2963534	0.992	0.012	0.041	-74%	87%
	PCB 180	0.01 - 0.13	y= 213945518x + 2986104	0.997	0.007	0.023	-56%	87%
	PCB 169	0.01 - 0.13	y= 150438200x + 2032588	0.992	0.013	0.042	-47%	95%
SMs	HHCB	0.01 - 0.13	y= 3018360x + 19270	0.994	0.013	0.045	93%	72%
	xylene	0.01 - 0.13	y= 638375x - 3424	0.992	0.013	0.042	90%	65%
	AHTN	0.01 - 0.10	y= 2589662x - 623	0.996	0.009	0.029	90%	68%
	Ketone	0.01 - 0.13	y= 14411585x - 2912	0.993	0.012	0.039	91%	77%

Table S4 - QuEChERS and clean-up optimization.

	Recoveries (50 µg/L)				
	QuEChERS			Clean-up graphitized carbon	
OCPs	Original	AOAC	EN	2 mg	5 mg
α-HCH	105%	**102%**	96%	**50%**	47%
HCB	84%	**85%**	75%	**37%**	28%
β-HCH	83%	**77%**	75%	**62%**	60%
Lindane	98%	**94%**	107%	**81%**	72%
δ-HCH	132%	**104%**	144%	**91%**	73%

Aldrin	89%	**85%**	79%	**58%**	54%
α-endosulfan	80%	**77%**	76%	**68%**	66%
DDE	96%	**89%**	83%	**68%**	66%
Dieldrin	102%	**97%**	93%	**70%**	70%
Endrin	129%	**127%**	122%	**70%**	68%
β-endosulfan	121%	**113%**	121%	**68%**	64%
DDD	111%	**105%**	107%	**74%**	75%
DDT	91%	**92%**	91%	**71%**	73%
Methoxychlor	142%	**131%**	131%	**75%**	69%

Table S5 - Validation parameters using shrimp matrix matched calibration for the selected OCPs, OPPs, BFRs, PCBs and SMs.

		Calibration range (ng/g ww)	Regression equation (ng/g ww)	Linearity ($R^{2)}$	MDL (ng/g ww)	MQL (ng/g ww)	ME	Recoveries
OCPs	α-HCH	2.67 - 26.67	y= 26293x - 35928	0.997	1.52	5.07	-19%	96%
	HCB	2.67 - 26.67	y= 149710x + 57140	0.999	0.95	3.16	-100%	71%
	β-HCH	2.67 - 26.67	y= 7882x + 251167	0.997	1.54	5.15	-9%	83%
	Lindane	2.67 - 26.67	y= 23984x -22164	0.999	0.96	3.19	39%	91%
	δ-HCH	2.67 - 26.67	y= 18146x - 30396	0.996	1.91	6.35	-174%	56%
	Aldrin	2.67 - 26.67	y= 224426x + 108435	0.998	1.27	4.24	-135%	72%
	α-endosulfan	2.67 - 26.67	y= 59890x - 60884	0.997	1.56	5.21	47%	74%
	DDE	2.67 - 26.67	y= 50029x - 62724	0.997	1.54	5.13	57%	84%
	Dieldrin	2.67 - 26.67	y= 187368x - 80901	0.998	1.19	3.97	-212%	80%
	Endrin	2.67 - 26.67	y= 140881x - 64321	0.998	1.14	3.78	-95%	76%
	β-endosulfan	2.67 - 26.67	y= 44034x - 49111	0.998	1.39	4.63	11%	79%
	DDD	2.67 - 26.67	y= 38451x - 42109	0.998	1.43	4.77	35%	88%
	DDT	2.67 - 26.67	y= 56480x + 11441	0.995	2.03	6.78	14%	81%
	Methoxychlor	2.67 - 26.67	y= 16121x - 18222	0.991	2.89	9.63	-15%	83%
OPPs	Dimethoate	8.00 - 80.00	y= 23236x + 17097	0.995	6.39	21.31	29%	91%
	Chlorpyrifos-methyl	8.00 - 80.00	y= 20376x + 1149	0.998	4.23	14.10	6%	86%
	Parathion-methyl	8.00 - 80.00	y= 20453x - 10154	0.997	4.55	15.17	17%	60%
	Malathion	8.00 - 80.00	y= 19019x - 5561	0.997	4.51	15.02	7%	74%
	Chlorpyrifos	8.00 - 80.00	y= 15881x - 93	0.997	4.86	16.21	1%	86%
	Chlorfenvinphos	8.00 - 64.00	y= 7963x - 6454	0.994	5.79	19.31	12%	89%
BFRs	TBECH	4.00 - 40.00	y= 43101x + 140736	0.996	2.77	9.24	8%	91%
	BDE 28	4.00 - 40.00	y= 127797x + 316589	0.993	3.70	12.33	-29%	85%
	PBT	4.00 - 40.00	y= 94172x + 436079	0.996	2.84	9.46	-15%	65%
	PBEB	4.00 - 40.00	y= 108866x + 275105	0.997	2.41	8.04	-38%	66%
	BDE 47	4.00 - 40.00	y= 69674x + 34024	0.993	3.58	11.93	-174%	136%
	BDE 100	4.00 - 40.00	y= 81935x + 134040	0.998	1.87	6.22	-99%	87%

	BDE 99	4.00 - 40.00	y= 89632x + 522952	0.997	2.20	7.34	-72%	89%
	TBB	4.00 - 40.00	y= 49674x + 391723	0.998	1.89	6.32	-58%	86%
	BDE 153	4.00 - 40.00	y= 76402x + 151733	0.999	1.08	3.59	-92%	79%
	BDE 154	4.00 - 40.00	y= 84795x + 147501	0.998	2.03	6.76	-82%	80%
	BDE 183	4.00 - 40.00	y=72951x + 135649	0.999	1.28	4.27	-71%	76%
	BTBPE	4.00 - 40.00	y=51196x + 219525	0.998	1.91	6.38	3%	72%
PCBs	PCB 28	4.00 - 40.00	y= 104162x + 256379	0.996	2.62	8.75	-16%	80%
	PCB 52	4.00 - 40.00	y= 55416x + 227287	0.996	2.72	9.08	-26%	88%
	PCB 101	4.00 - 40.00	y= 80515x + 238679	0.997	2.53	8.44	-20%	82%
	PCB 77	4.00 - 40.00	y= 57279x + 233121	0.995	3.08	10.27	-34%	76%
	PCB 118	4.00 - 40.00	y= 70935x + 176582	0.997	2.60	8.65	-29%	76%
	PCB 114	4.00 - 40.00	y= 124222x + 350178	0.996	2.63	8.77	-29%	77%
	PCB 153	4.00 - 40.00	y= 87313x + 189294	0.997	2.31	7.71	-55%	82%
	PCB 138	4.00 - 40.00	y= 70795x + 208002	0.997	2.46	8.22	-93%	80%
	PCB 126	4.00 - 40.00	y= 70839x + 267188	0.997	2.52	8.40	-47%	73%
	PCB 156	4.00 - 40.00	y= 104655x + 176793	0.998	1.80	5.99	-86%	82%
	PCB 157	4.00 - 40.00	y= 81029x + 109421	0.998	1.88	6.27	-107%	84%
	PCB 180	4.00 - 40.00	y= 80140x + 271232	0.997	2.21	7.36	-87%	85%
	PCB 169	4.00 - 40.00	y= 50547x + 305253	0.997	2.44	8.14	-90%	82%
SMs	Celestolide	4.00 - 40.00	y= 579x - 817	0.999	1.48	4.94	38%	77%
	Ambrette	4.00 - 40.00	y= 154x + 355	0.995	3.36	11.21	55%	74%
	HHCB	4.00 - 40.00	y= 537x - 621	0.996	3.16	10.53	56%	79%
	MX	4.00 - 40.00	y= 129x + 666	0.994	3.41	11.38	58%	85%
	AHTN	4.00 - 40.00	y=522x + 651	0.998	2.18	7.28	45%	82%
	MK	4.00 - 40.00	y= 309x + 127	0.999	1.33	4.42	61%	94%

Table S6 - Reagents, solvents, and materials used in the extraction of the samples and in the analysis of the studied contaminants.

Reagents, Solvents and materials		**Supplier company**	**City**	**Country**
Acetonitrile	chromatographic grade	Merck	Darmstadt	Germany
Methanol	chromatographic grade	Scharlau	Barcelona	Spain
Ethyl acetate	Purity ≥ 99.8%	Merck	Darmstadt	Germany
Dichloromethane	chromatographic grade	Honeywell	Indiana	USA
n-Hexane	chromatographic grade	Merck	Darmstadt	Germany
D-(+)-Gluconic acid δ-lactone	Assay ≥ 99.0%	Sigma Aldrich	Darmstadt	Germany
QuEChERS extraction kit AOAC	6 g magnesium sulfate and 1.5 g sodium acetate	Agilent tecnologies	California	USA
QuEChERS extraction kit EN	1 g sodium chloride, 4 g magnesium sulfate, 1g sodium citrate and 0.5 g sodium hydrogencitrate sesquihydrate	Agilent tecnologies	California	USA
QuEChERS extraction kit original	1 g sodium chloride, 4 g magnesium	Agilent tecnologies	California	USA

Sodium sulfate anhydrous		Merk	Darmstadt	Germany
Graphitized carbon black		Agilent tecnologies	California	USA
Dispersive solid phase AOAC clean up	150 mg anhydrous magnesium sulphate, 50 mg PSA and 50 mg C18	Agilent tecnologies	California	USA
Hydrochloric acid (HCl)	37%	Carlo Erba	Rodano	Italy
Deionized water	Resistivity of 15.0 MΩ.cm	Milipore	Molsheim	France
	Produced in a Elix apparatus			
Ultrapure water	Resistivity of 18.2 MΩ.cm	Millipore	Molsheim	France
	Produced using a Simplicity 185 system			
Strata C18-E (500 mg, 3 mL)		Phenomenex	California	USA

Table S7 - Physico-chemical characteristics, molecular mass, and the supplier company of the studied contaminants.

	Contaminants		Log Kow	CAS	Molecular weight (g/mol)	Supplier company
OCPs	α- hexachlorocyclohexane	α-HCH	3.80 [a]	319-84-6	290.8	Sigma Aldrich (Darmstadt, Germany)
	β- hexachlorocyclohexane	β-HCH	3.78 [a]	118-74-1	284.8	
	δ- hexachlorocyclohexane	δ-HCH	4.14 [a]	319-85-7	290.8	
	lindane		3.72 [a]	58-89-9	290.8	
	hexachlorobenzene	HCB	5.73 [b]	319-86-8	290.8	
	1,1,1 trichloro-2,2- bis-(p-chlorophenyl) ethane)	*o,p'*-DDT	6.91 [b]	309-00-2	364.9	
	2,2-bis(p-chlorophenyl)-1,1-dichloroethylene	*p,p'*-DDE	6.51 [b]	959-98-8	406.9	
	dichlodiphenyldichloro-ethane	*p,p'*-DDD	6.02 [b]	72-55-9	318.0	
	aldrin		6.50 [b]	60-57-1	380.9	
	dieldrin		5.40 [b]	72-20-8	380.9	
	endrin		5.20 [b]	33213-65-9	406.9	
	α- endosulfan		3.83 [b]	72-54-8	320.0	
	β- endosulfan		3.62 [b]	50-29-3	354.5	
	methoxychlor		5.08 [b]	72-43-5	345.7	
OPPs	dimethoate		0.78 [b]	60-51-5	229.3	Sigma Aldrich (Darmstadt, Germany)
	chlorpyrifos-methyl		4.31 [b]	5598-13-0	322.5	
	parathion-methyl		2.86 [b]	298-00-0	263.2	
	malathion		2.36 [b]	121-75-5	330.4	
	chlorpyrifos		4.96 [b]	2921-88-2	350.6	
	chlorfenvinphos		3.81 [b]	470-90-6	359.6	
BFRs	2,4,4′-tribromodiphenyl ether	BDE 28	5.94 [c]	41318-75-6	406.89	Isostandards Material, S.L. (Madrid, Spain)
	2,2′,4,4′- tetrabromodiphenyl ether	BDE 47	6.81 [c]	41318-75-6	405.8	
	2,2′,4,4′,5-pentabromodiphenyl ether	BDE 99	7.32 [c]	87-83-2	485.6	
	2,2′,4,4′,6-pentabromodiphenyl ether	BDE 100	7.24 [c]	85-22-3	499.6	
	2,2′,4,4′,5,5′-hexabromodiphenyl ether	BDE 153	7.90 [c]	59080-40-9	627.6	

	2,2′,4,4′,5,6′-hexabromodiphenyl ether	BDE 154	7.82 [e]	189084-64-8	563.6	
	2,2′,3,4,4′,5′,6-heptabromodiphenyl ether	BDE 183	8.27 [e]	32534-81-9	563.6	
	1,2-dibromo-4-(1,2-dibromoethyl)-cyclohexane	TBECH	4.82 [d]	3322-93-8	427.80	
	pentabromotoluene	PBT	6.25 [d]	68631-49-2	643.5	
	pentabromoethylbenzene	PBEB	6.76 [d]	207122-15-4	643.5	
	2-ethylhexyl 2,3,4,5-tetrabromobenzoate	TBB	7.73 [d]	207122-16-5	721.4	
	1,2-bis(2,4,6-tribromophenoxy) ethane	BTBPE	8.31 [d]	37853-59-1	687.6	
PCBs	2,4,4′-Trichlorobiphenyl	PCB 28	5.67 [e]	7012-37-5	257.5	Techno Spec S.L. (Barcelona, Spain)
	2,2′,5,5′-Tetrachlorobiphenyl	PCB 52	5.84 [e]	35693-99-3	292.0	
	2,2′,4,5,5′-Pentachlorobiphenyl	PCB101	6.38 [e]	37680-73-2	326.4	
	3,3′,4,4′-Tetrachlorobiphenyl	PCB 77	6.36 [e]	32598-13-3	292.0	
	2,3′,4,4′,5′-Pentachlorobiphenyl	PCB 118	6.74 [e]	31508-00-6	326.4	
	2,3,4,5,4'-Pentachlorobiphenyl	PCB 114	6.65 [e]	74472-37-0	326.4	
	2,2′,4,4′,5,5′-Hexachlorobiphenyl	PCB 153	6.92 [e]	35065-27-1	360.9	
	2,2′,3,4,4′,5′-Hexachlorobiphenyl	PCB 138	6.83 [e]	35065-28-2	360.9	
	3,3',4,4',5'-Pentachlorobiphenyl	PCB 126	6.89 [e]	57465-28-8	326.4	
	2,3,3′,4,4′,5-Hexachlorobiphenyl	PCB 156	7.18 [e]	38380-08-4	360.9	
	2,3,3',4,4',5'-Hexachlorobiphenyl	PCB 157	7.18 [e]	69782-90-7	360.9	
	2,2′,3,4,4′,5,5′-Heptachlorobiphenyl	PCB 180	7.36 [e]	35065-29-3	395.3	
	3,4,5,3',4',5'-Hexachlorobiphenyl	PCB 169	7.42 [e]	32774-16-6	360.9	
SMs	galaxolide	HHCB	5.90 [b]	13171-00-1	244	LGC Standards (Middlesex, UK)
	tonalide	AHTN	5.70 [b]	83-66-9	268	
	ketone		4.30 [b]	12222-05-5	258	
	xylene		3.12 [b]	81-15-2	297	
	celestolide		5.70 [b]	21145-77-7	258	
	ambrette		4.00 [b]	81-14-1	294	
IS	4.4'- dichlorobenzophenone			90-98-2	251.1	Sigma Aldrich (Darmstadt, Germany)
	triphenylphosphate			115-86-6	326.3	
	5′- fluoro-2,3′,4,4′,5-pentabromodiphenyl ether			446254-80-4	564.7	Isostandards Material, S.L. (Madrid, Spain)
	AHTNd3			1396967-82-0	261	LGC Standards (Middlesex, UK)

[a] National Toxicology Program, 2011. Hexachlorocyclohexane (technical grade), and other hexachlorocyclohexane isomers. Rep. Carcinog. 12, 256–258

[b] Hazardous Substances Data Bank (HSDB)

[c] Braekevelt, E. et al., 2003. Direct measurement of octanol-water partition coefficients of some environmentally relevant brominated diphenyl ether congeners. Chemosphere. 51, 563-567

[d] Dong, L. et al., 2021. New understanding of novel brominated flame retardants (NBFRs): Neuro(endocrine) toxicity. Ecotoxicology and Environmental Safety. 208, 111570

[e] IARC, 2016. Polychlorinated biphenyls. IARC monographs on the evaluation of carcinogenic risks to humans. 107, 9-500.

Table S8 - GC-MS and MS/MS conditions for SMs analysis.

SMs	CAS	Molecular weight (g/mol)	RT (min)	Quantifier ion (m/z)*	Qualifier ions (m/Z)*	MRM transitions (m/z)*
Celestolide	13171-00-1	244	14.83	229	173, 244	229 → 173
Ambrette	83-66-9	268	20.09	253	91, 223	253 → 91
HHCB	12222-05-5	258	20.74	243	213, 258	243 → 213
Xylene	81-15-2	297	21.29	282	265, 297	282 → 265
AHTN	21145-77-7	258	21.34	243	187, 258	243 → 187
Ketone	81-14-1	294	26.35	279	191, 294	279 → 191
AHTN-d3 (IS)	1396967-82-0	261	21.23	246	160, 190	n.a

n.a. - not applicable; * Dong et al., 2014 and Kuklenyik et al., 2007.

Table S9 - GC-MS/MS conditions for OCPs, OPPs, BFRs, PCBs analysis.

		EDC or FPD	MS/MS
	Compounds	RT (min)	MRM transitions (m/z)*
OCPs	α-HCH	16.41	183 → 179
	HCB	16.67	284 → 214
	β-HCH	17.18	183 → 179
	Lindane	17.34	183 → 179
	δ-HCH	17.99	183 → 179
	Aldrin	20.12	263 → 191
	α-endosulfan	21.90	195 → 170
	DDE	22.35	318 → 281
	Dieldrin	22.49	243 → 211
	Endrin	22.98	245 → 173
	β-endosulfan	23.17	195 → 170
	DDD	23.27	235 → 165
	DDT	23.36	235 → 165
	Methoxychlor	25.19	227 → 197
OPPs	Dimethoate	10.15	125 → 62
	Chlorpyrifos-methyl	13.98	286 → 241
	Parathion-methyl	14.12	263 → 246
	Malathion	16.76	173 → 99
	Chlorpyrifos	17.13	314 → 258
	Chlorfenvinphos	20.21	267 → 159
BFRs	TBECH	22.07	267 → 79
	BDE 28	28.11	408 → 248
	PBT	28.43	486 → 326
	PBEB	29.92	500 → 406
	BDE 47	36.72	486 → 326
	BDE 100	43.76	564 → 404
	BDE 99	45.80	564 → 404

	TBB	46.35	421 → 393
	BDE 153	51.59	644 → 484
	BDE 154	54.85	644 → 484
	BDE 183	67.00	720 → 562
	BTBPE	71.65	359 → 278
PCBs	PCB 28	19.42	256 → 186
	PCB 52	20.64	292 → 255
	PCB 101	24.52	326 → 256
	PCB 77	26.52	292 → 222
	PCB 118	28.03	326 → 256
	PCB 114	28.70	326 → 256
	PCB 153	29.45	360 → 290
	PCB 138	31.40	360 → 290
	PCB 126	32.07	326 → 256
	PCB 156	35.24	360 → 290
	PCB 157	35.67	360 → 290
	PCB 180	36.56	394 → 324
	PCB 169	38.18	360 → 290

* Fernandes et al., 2011; Sapozhnikova, 2018 and Sapozhnikova and Lehotay, 2013.

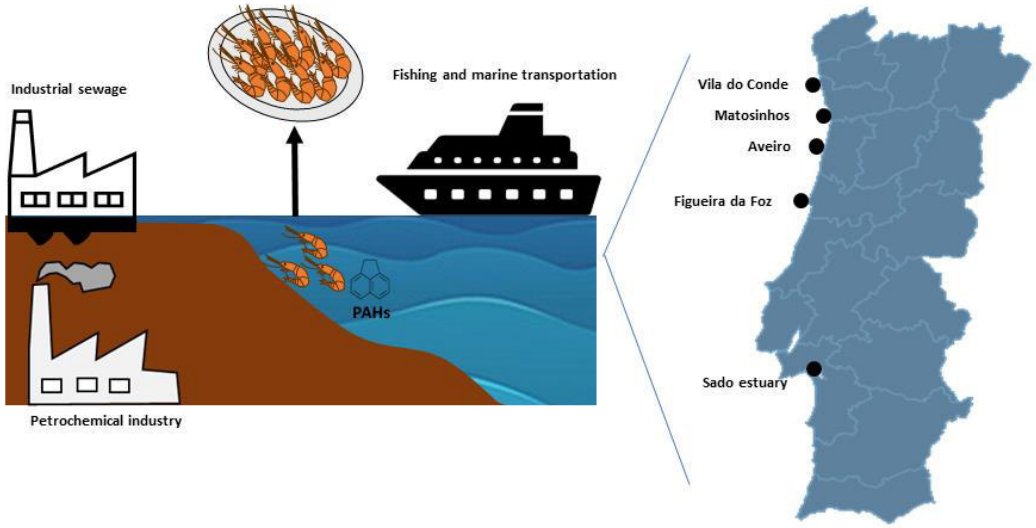
2.2. Seasonal and spatial comparison of polycyclic aromatic hydrocarbons among decapod shrimp from coastal Portugal

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Seasonal and spatial comparison of polycyclic aromatic hydrocarbons among decapod shrimp from coastal Portugal

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Statement of contribution

The contribution of the candidate, Maria Luz Maia, in this work includes the accomplishment of the sample preparation, data evaluation, statistical analysis and written of the original draft.

Seasonal and spatial comparison of polycyclic aromatic hydrocarbons among decapod shrimp from coastal Portugal

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous global pollutants that are lipophilic, bioaccumulative and can be both endocrine disruptors and carcinogens. In this study, we measured 18 PAHs among decapod shrimp samples (*Palaemon* spp.) collected during the spring and autumn from multiple locations along the Portuguese coastal, including local markets and aquaculture farms. ΣPAH concentrations in samples ranged from 3.14 – 320.48 ng/g (ww) with the highest value in this range detected in a sample provided from an aquaculture facility. Fifteen of the 18 scanned PAHs were present in shrimp samples. However, detectable concentrations (0.07 ng/g ww) of the carcinogenic benzo-a-pyrene occurred for a single sample only and approached method detection limits. No significant differences in shrimp ΣPAH concentrations were evident between the spring and autumn seasons. Target hazard quotient estimation suggested low probability of adverse health effects to consumers through consumption of shrimp from the study collection locations.

Keywords

Polycyclic aromatic hydrocarbons; Aquatic environment; Coast pollution; *Palaemon serratus*; *Palaemon varians*; Atlantic Ocean.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a class of persistent organic pollutants generated from production and use of petroleum-derived substances and during the incomplete combustion of organic materials, such as, oil, gas, coal, wood, tobacco or garbage (ATSDR 1995; Bolden et al. 2017). These chemicals with two or more fused benzene rings typically occur naturally and usually not as single compounds, but as complex mixtures (Zhang et al. 2015). PAHs are being released into the environment and currently more than 100 PAHs are known (Xia et al. 2012), with 16 of these being classified as priority pollutants by the Environmental Protection Agency (EPA) (USEPA 2005). Due to the characteristics of these chemicals, including persistence, long-range transport, and hydrophobicity, they are extensively distributed in air, sediments, water, and biota (Zhang et al. 2015; Sun et al. 2016). PAHs can enter in the aquatic environment via several pathways including petroleum contamination by spills, surface runoff, and industrial sewage discharge (Zhang et al. 2015; Sun et al. 2016). These chemicals accumulate in the environment and can bioaccumulate in biota, such as marine organisms, some consumed, as seafood. Consequently, diets become a meaningful path of human contamination by PAHs to add to occupational exposure of the population, namely smokers. Therefore, PAHs in the last years have been under evaluation by the Scientific Committee on Food (SCF) in the International Program on Chemical Safety (IPCS), and by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) (Scientific Committee on Food 2002; IPCS 2009). Concerning human exposure, PAHs have been described to be carcinogenic, mutagenic, and teratogenic (IARC 2002, 2010). These compounds were also reported to have endocrine disruptor capacity (WHO 2013) and long term exposure can lead to proliferation of mutated cells resulting in cancer growth (Das et al. 2017). Furthermore, PAHs are related to reproductive disorders and adverse neurobehavioral effects (Schroeder 2011; Bolden et al. 2017). Thus, it is important to quantify the magnitude and composition of PAH profiles in the environment and in popular foods that represent a potential source of human exposure. Legislation of PAHs only comprises the maximum acceptable levels for muscle meat of smoked fishery products of 2.0 ng/g for benzo-a-pyrene and 12.0 ng/g for the sum of benzo-a-pyrene, benz-a-anthracene, benzo-b-fluoranthene and chrysene (Commission Directive (EU) 2015). Several studies have been performed to explore PAHs contamination in marine organisms. Shrimp, a shellfish considered a delicacy to the human population, have been extensively studied all over the world (Maia et al. 2020).

Shrimps are the most consumed seafood in the world, highly consumed by Portuguese population and can be a healthy complement to our diet, nevertheless it can also be a source of environmental contaminants due to the pollution of several coastal areas. Hence the assessment of contaminants in shrimp is crucial to secure that seafood such as shrimp consumed by Portuguese population are safe. This study aims to investigate the occurrence of PAHs in Portuguese shrimp samples. For that purpose, the concentrations of 18 PAHs (16 US EPA priority pollutants (USEPA 2005), benzo-j-fluoranthene recommended by European Directive, 2004/107/EC (European Union 2004) and dibenzo-a,l-pyrene, considered to be extremely toxic and 100-200 times more tumorigenic than benzo-a-pyrene (Cavalieri et al. 1994) were investigated, from wild shrimp and from aquaculture operations along the Portuguese coast. Different species of shrimp were collected in several locations, covering different years and seasons. The variation of concentrations between seasons, sampling locations and species were evaluated. The risk assessment, via shrimp consumption for the adult Portuguese population, was estimated. To our knowledge, this is the first study at least in the last two decades that analyze PAHs as an environment pollutant in the edible inner portion of the shrimp species in a wide geographical and temporal space in Portuguese coast.

Material and methods

The PAHs extraction method was previously described elsewhere (Ramalhosa et al. 2009). Briefly, 5 g of shrimp were mechanically homogenized and 10 mL of acetonitrile were added, followed by 3 min of vortex. QuEChERS original was added, and the mixture was again vortex for 3 min followed by centrifugation at 3500 rpm during 5 min. Then, 5 mL of extract was evaporated with gentle stream of nitrogen, resuspended with 333 µL of acetonitrile, obtaining a concentration factor of 15 times. The extracts were filtrated through a 0.22 µm PTFE syringe filter and analyzed using high performance liquid chromatography (HPLC) with photodiode array (PDA) and fluorescence (FLD) detectors in series.

The detailed description of the sample's characterization (Table SM1) and collection, reagents and materials used for the extraction and analysis of the studied PAHs [naphthalene (Naph), acenaphthylene (Acy), acenaphthene (Ace), fluorene (Flu), phenanthrene (Phe), anthracene (Ant), fluoranthene (Fln), pyrene (Pyr), benz-a-anthracene (BaA), chrysene (Chry), benzo-b-fluoranthene (BbFt), benzo-k-fluoranthene (BkFt), benzo-a-pyrene (BaP), dibenz-a,h-anthracene (DBa,hA), benzo-g,h,i-perylene (Bg,h,iP), indeno-

1,2,3-cd-pyrene (InP), benzo-j-fluoranthene (BjFt) and dibenzo-a,l-pyrene (DBa,lP)], liquid chromatography analysis and risk assessment through Target Hazard Quotient (THQ) calculation are given in supplementary material.

Statistical analysis: All the results are presented as arithmetic means obtained from at least duplicates. The D'Agostino-Pearson Omnibus Normality Test was applied to verify whether the distribution of the variables was normal. Statistical significance of the difference among the groups was assessed by one-way analysis of variance (ANOVA test). To test the statistical differences between seasons, locations of sampling, species and if the samples were from aquaculture or wild, the samples with eggs or shell were excluded. The differences among means were considered statistically significant when $p < 0.05$ and a full summary of statistical results are provided in Table SM5.

Results and discussion

Validation of the methodology

The optimized procedure presented an efficient separation and quantification of the compounds with retention times in the range of 11.52 to 31.78 min, as shown in Fig. SM1. The retention times, linearity range, coefficient of determination (R2), matrix effect (ME), recoveries and method detection (MDL) and method quantification (MQL) limits for each of the 18 studied PAHs are presented in Table SM3. Calibration curves were obtained using at least six mixed matrix matched standards containing all the PAHs and all the curves showed good linearity over the entire range concentrations with coefficient of determination (R2) higher than 0.99 with exception of Acy due to the lower sensibility of this compound. The recoveries were calculated using shrimp samples spiked at three levels in triplicate (Table SM2).

The average of the recoveries ranged between 51% and 84% for Acy and Phe, respectively (Table SM3). Food matrices are complex with large number of compounds that can interfere in the analytical signal, providing ME (Kwon et al. 2012). The ME can decrease detection response or increased analytical signal, being problematic in the analysis of contaminants such as PAHs. To evaluate the ME in shrimp samples PAHs standard solutions (prepared in acetonitrile) were compared to spiked blank samples, as described previously (Nagyová and Tölgyessy 2019). The ME values obtained for the 18 PAHs are presented in Table SM3. Some PAHs were more affected by ME namely Naph, Acy, and Ace. Acy and Ace are the compounds that presented lower recoveries too, these could be related to the fact that Naph,

Ace, and Acy are the most volatile PAHs (Adami et al. 2000). MDLs and MQLs were calculated by considering the slope of the matrix match calibration curve and the residual standard deviation of the regression line (EMA 1995).

The MDLs and MQLs obtained ranged between 0.01 to 2.63 and 0.05 to 8.76 ng/g ww for Chry and Ace respectively, with exception of Acy that presented higher values. Regarding BaP the values of MDL and MQL were, respectively, 0.03 and 0.11 ng/g ww, so this method is adequate for shrimp analysis since the maximum level allowed is 2 ng/g (Commission Directive (EU) 2015), and the method has sufficiently low limits giving it the ability to be used in monitoring purposes.

PAHs in shrimp samples

PAH concentrations were measured in muscle tissues of shrimp captured from the Portuguese coast (Table SM4). The results for PAH levels were corrected using the average recovery determined for each PAH and the sum of PAHs were calculated considered method detection limit (MDL)/√2 for concentrations below MDL (Hornung and Reed 1990). Fifteen of the eigtheen study PAHs (Naph, Ace, Flu, Phe, Ant, Fln, Pyr, BaA, Chry, BbFt + BjFt, BkFt, BaP, DBa,LP, and Bg,h,iP) were detected in at least one sample, while Phe (Group 3- Unclassifiable as carcinogenic in humans (IARC 2010)) was detected in all samples. Compounds like Acy, Ace, BaA, DBa,hA, and InP were not detected in any shrimp samples.

Among the PAHs analyzed in this study the one that presented higher values was Naph (which is classified by IARC as possible carcinogenic to humans – Group 2B (IARC 2010)) with exception for the sample of Matosinhos spring of 2018 with shrimp eggs (MSp18-e). These results are in agreement with other studies that also analyzed PAHs in shrimp samples (Kao et al. 2014; Wilson et al. 2014; Dosunmu et al. 2016; Sun et al. 2016; Fogaça et al. 2018; Wickliffe et al. 2018; Olayinka et al. 2019). In most of the analyzed samples, Naph, Phe, Fln, and Pyr were detected. Naph and Phe are low molecular weight PAHs (LMW-PAHs) and Fln and Pyr are high molecular weight PAH (HMW-PAHs). LMW-PAHs are described as natural occurring PAHs (petrogenic origin), while HMW-PAHs are produced during combustion processes (pyrolytic origin) (Adami et al. 2000; Adeleye et al. 2016). LMW-PAHs concentrations, ranged between 2.77 ng/g ww in Matosinhos sample (MSp18-e) and 320.03 ng/g ww in Sado estuary sample (SSp19a-s). LMW-PAHs were predominant in all investigated sampling sites (Vila do Conde, Matosinhos, Aveiro, Figueira da Foz, Sado estuary and also in Bangladesh and India), due to Naph presenting higher

concentrations, which are considerably higher than the other PAHs detected in this study (Fig. 1).

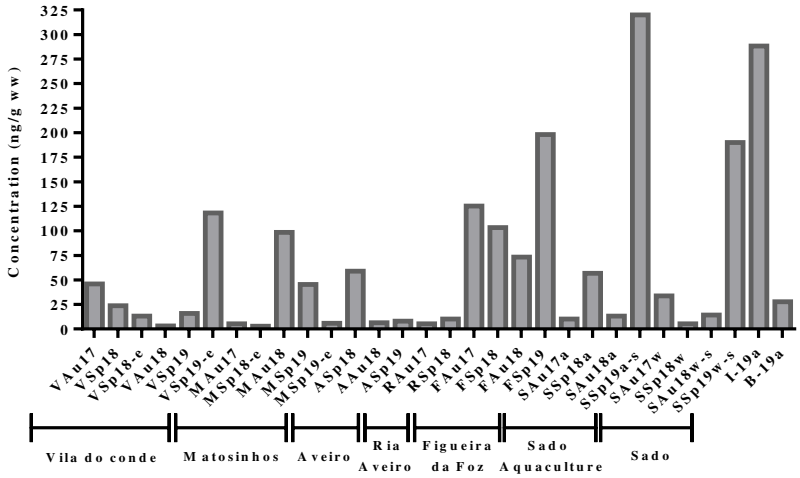


Fig. 1 Composition pattern of LMW-PAHs in shrimp samples

The values found for HMW-PAHs ranged between 0.11 ng/g ww for Ria de Aveiro sample (RSp18) and 0.89 ng/g ww for Bangladesh sample (B-19a), as presented in Fig. 2.

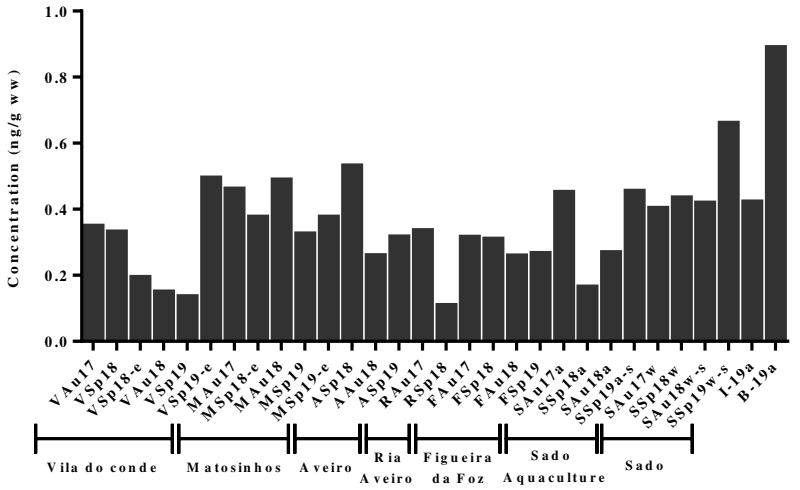


Fig. 2 Composition pattern of HMW-PAHs in shrimp samples

The known carcinogen BaP (Group 2A (IARC 2010)) was detected in only one sample (Asp18) and at a concentration (0.07 ng/g) below the maximum guidance level of 2 ng/g. Regarding the sum of BaA, BaP, Chry and BbFt+BjFt the values ranged between 0.021 and

1.77 ng/g ww, all below the maximum legislated of 12 ng/g (Commission Directive (EU) 2015), although the sum values may be overestimated due to the co-elution of BbFt and BjFt, the values are far below the maximum level.

The ΣPAHs ranged between 3.14 ng/g ww from Matosinhos spring of 2018 for shrimp with eggs (MSp18-e) and 320.48 ng/g ww in sample from Sado aquaculture from spring of 2019 with shell (SSp19a-s). Higher values of ΣPAHs were found in shrimps collected in spring of 2019, 320.48 ng/g ww (Sado aquaculture with shell), 198.47 ng/g ww (Figueira da Foz), 190.68 ng/g ww (Sado with shell) and in shrimp sample of India 288.74 ng/g ww. In the last years, some studies reported the presence of PAHs in shrimps over the world with the analysis of 16 EPA PAHs (Fogaça et al. 2018; Shilla and Routh 2018; Fernando et al. 2019; Soltani et al. 2019). Only one of these studies were from a European country, more specific Portugal (Fogaça et al. 2018), however samples were purchased at the local market, so shrimp origin was Equator and Vietnam and not Portuguese coast. The present study detected more PAHs comparing with the study of Fogaça et al. that only detected Flu among their samples.

The higher number of PAHs was detected in the sample collected from Sado in Spring of 2019 analyzed with shell (SSp19w-s) and in the foreign sample from Bangladesh (B-19a), both with thirteen PAHs, seven above MDL. Regarding aquaculture the highest number of detected PAHs was found in a sample from Sado in Autumn of 2017 (Sau17a) with eight PAHs detected. Bangladesh shrimps presented a ΣPAHs of 25.90 ng/g ww and India 285.98 ng/g ww. A previous study in India reported a value of 4.14 ng/g ww for the sum of six PAHs (Ant, Fln, Pyr, Chry, BaP and Bg,h,iP) (Amaraneni 2006). These two samples (India and Bangladesh) were the only ones, amongst the samples analyzed, in which Chry was detected, this PAH are classified as Group 3 - Unclassifiable as carcinogenic in humans (IARC 2010).

Sampling seasons

Comparing seasons of sampling, Spring and Autumn (Fig. 3), can be observed that the higher values were found in Spring (320.48 ng/g ww in aquaculture from Sado (SSp19a-s), 198.47 ng/g ww in Figueira da Foz (FSp19), 190.68 ng/g ww in Sado (SSp19w-s), 118.66 ng/g ww in Vila do Conde (VSp19-e), 59.55 ng/g ww in Aveiro (Asp18) and 10.32 ng/g ww in Ria de Aveiro (RSp18) with exception of samples from Matosinhos. It can be noticed that in Spring of 2019 the samples have higher values of ΣPAHs, reaching the maximum level in

the aquaculture sample from Sado estuary. The higher values obtained in shrimps from Sado estuary in 2019 Spring, could be explained by a transitory contamination probably from chimney washing of ships anchored in the Sado or from the rainwater system of the factories that are next to the river reported by the media (JN 2019). Although these differences in PAH concentrations were present, they were not statistically significant ($p = 0.122$).

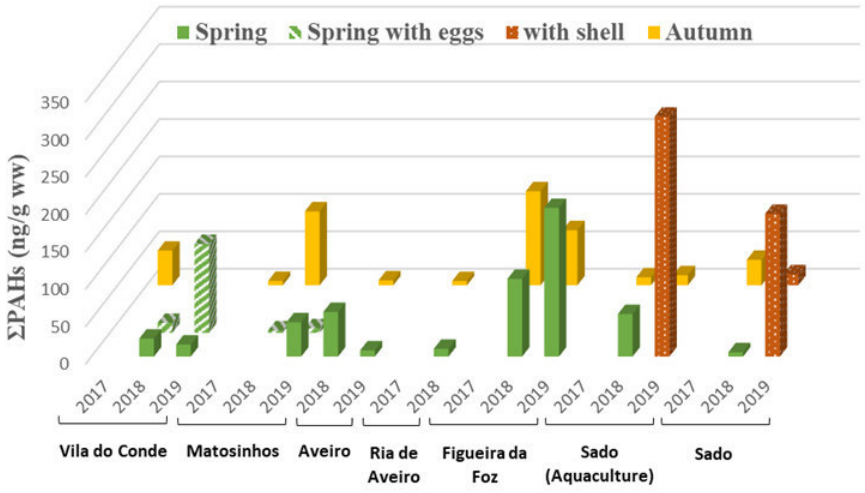


Fig. 3 Concentration of ΣPAHs in shrimp samples from different sites collected in distinct seasons

Location of sampling

Regarding the location of the sampling, statistically significantly differences were observed between the different locations (Table SM5). The average values of ΣPAHs in Figueira da Foz were high comparing with the other sampling locations, considering the data analyzed 73.61 ng/g ww in Autumn of 2018 to 198.47 ng/g ww in Spring of 2019, this could be explained by the intensive shipping and fishing in the area. Even though the sampling site of Matosinhos is in the proximity to a petrochemical refinery established in the area the values ranged from 3.14 ng/g ww in Spring of 2018 in a sample with eggs to 98.82 ng/g ww in Autumn of 2018. These values are lower than the values reported in previous studies in locations near to a petrochemical industry (Dosunmu et al. 2016; Sun et al. 2016). A study performed in shrimp samples from Daya Bay, China, shown values for ΣPAHs ranged from 110 ng/g ww to 300 ng/g ww (Sun et al. 2016). In shrimps from Imo River, Nigeria the values ranged between 180.81 ng/g ww and 940.73 ng/g ww (Dosunmu et al. 2016). Vila do Conde sampling site presented values between 3.39 ng/g ww in 2018 (Vau18) and 118.66 ng/g ww in 2019 (VSp19-e), these values may be related to highly industrialized area along Ave River

that flows to Atlantic Ocean in Vila do Conde. The values found for the ΣPAHs were very similar between Aveiro and Ria de Aveiro and ranged between 5.69 ng/g ww in 2017 (RAu17) and 10.32 ng/g ww in 2018 (RSp18). The exception was the sample from Aveiro in Spring of 2018 where was detected a higher value of Naph comparing with the other samples of this location achieving a ΣPAHs of 59.55 ng/g ww.

Species

Two shrimp species were analyzed, *P. serratus* and *P. varians*, the mean values found for ΣPAHs, were 48.67 ng/g ww for *P. serratus* and 80.82 ng/g ww for *P. varians*. *P. varians* specie was only present in Sado estuary, so the location of the sampling in addition to the differences between species can explain the variations between these two groups. As presented in Table SM5 the difference between species were significantly (p = 0.028). In the case of *P. varians* aquaculture and wild shrimps were sampled, when comparing these two groups not significantly differences were observed (p = 0.441).

Shrimp with eggs

Samples of shrimps with eggs were collected from Vila do Conde and Matosinhos during Spring of 2018 and 2019. Considering the hydrophobic characteristic of PAHs, it can be hypothesized that in samples with eggs (high content of lipids) a higher value of PAHs can be obtained. A previous work analyzed muscle shrimp and shrimp eggs separately, the lipid content found was 1.5% and 7.3 – 9.6% respectively and total PAH ranged between 0.6 – 10.9 ng/g dry weight to 12.1 – 89.7 dry weight in muscle and eggs, respectively (Carls et al. 2016). Despite these findings, the differences between the samples with and without eggs were not significant (p = 0.286), only one sample with eggs from Vila do Conde (VSp19-e) presented a higher value comparing with the sample without the eggs, the content of PAHs found was 7 times higher.

Shrimp with shell

Some samples of shrimp were analyzed with shell that is rich in chitin a precursor of chitosan. Several publications reported the chitin and chitosan as possible low cost biosorbents to adsorptive removal of PAHs (Crisafully et al. 2008; Tran et al. 2015). Regarding the samples of *P. varians* from Sado estuary analyzed with and without shell, the results show a significant difference between these groups (p = 1.64 x 10^{-5}). The samples with shell presented the higher values 190.68 ng/g ww for Sado in Spring 2019 (SSp19w-s)

and 320.48 ng/g ww for Sado aquaculture in Spring 2019 (SSp19a-s), with exception of sample from Sado in Autumn 2018 (Sau18w-s) that presented a lower value of 14.72 ng/g ww. However, with these data, it is not possible to confirm the accumulation of PAHs by sorption in shrimp shell *in vivo* and further studies are needed.

Human exposure and risk assessment

Public health levels of concern (LOC) for individual PAHs established by the Food and Drug Agency and the National Oceanic and Atmospheric Administration organizations (FDA; NOAA 2010) were used to compare to the maximum concentration levels found in this study (Table SM6). The individual levels of PAHs measured in this study were far below the LOC reported and maximum concentrations detected in shrimp samples were often at least 100 times lower than the respective LOC value (Table SM6).

Regarding human exposure concerns, the risk assessment was performed, as described previously (Conte et al. 2016; Zhu et al. 2016). The health risk from shrimp consumption by Portuguese population were evaluated based on the THQ. This quotient indicates the ratio between exposure and oral reference doses from USEPA (USEPA 2021). Hazard Index (HI) was determined as the sum of the THQ calculated for the individual PAHs. If THQ or HI values were less than 1, it is unlikely to have health risk of exposure from (individual or combined, respectively). Conversely if the value is above 1 a potential risk of adverse health effects from the exposure may occur, increasing the probability as the THQ or HI value increases (USEPA 2000). THQ calculated using equation S1 (supplementary material), for Naph, Flu, Ant, Fln, Pyr and BaP were all below 1 and HI calculated from the sum of these PAHs were also all below 1 for the different species analyzed (Table SM7), suggest that there is minimal risk of chronic health effects for humans as associated with the PAH contamination of shrimp collected from various locations along the Portuguese coast.

Conclusion

PAHs have a harmful impact on human health due to their potential cytotoxicity, mutagenicity and carcinogenicity. Thus, determining their concentrations in common and popular food sources such as shrimp is important for protecting human health. This study reports, for the first time, the PAHs levels of contamination on different shrimp species collected in Portugal. Fourteen from the eighteen PAHs were present in Portuguese shrimp samples, including 12 of the 16 EPA priority compounds. The same shrimp species from different sampling locations and dates show differing magnitudes and patterns of PAH

contamination. This finding could indicate that the surrounding environment seems to be more important to PAHs accumulation than the specific species of shrimp (Maia et al. 2020), so possibly shrimps can be indicators of variations in environmental pollution. The THQ and HI values calculated for Naph, Flu, Ant, Fln, Pyr and BaP via shrimp consumption suggests a low probability of developing adverse health effects. The results of this research highlight the importance of monitoring PAHs in aquatic organisms valuable in human diets ensuring the safety of consumed seafood.

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Declarations

Conflicts of interest/Competing interests – The authors have no conflict of interests to declare

Author contributions

Maria Luz Maia: Investigation, Formal analysis, Visualization and Writing - original draft; Paula Paíga: Investigation and Formal analysis; Maria João Ramalhosa: Methodology; Supervision; Validation and Writing - review & editing; Cristina Delerue-Matos: Resources and Writing - review & editing; Valentina Fernandes Domingues: Supervision; Validation and Writing - review & editing; Conceição Calhau: Validation and Writing - review & editing.

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Supplementary material

Seasonal and spatial comparison of polycyclic aromatic hydrocarbons among decapod shrimp from coastal Portugal

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Material and methods

Sample collection: Two shrimp species were collected to perform this study, *Palaemon serratus* and *Palaemon varians*. The specie *P. serratus* was collected by a local fisherman in four sampling sites along the Northwest Portuguese coast at two miles from the coast (Vila do Conde, Matosinhos, Aveiro and Figueira da Foz) and the specie *P. varians* was collected in Sado estuary (wild and aquaculture samples). *P. serratus's* size ranged from 4–9 cm and the size of *P. varians* ranged from 2–4 cm. A total of 1 kg of shrimps was sampled in each location. The sampling in all the locations were performed twice (autumn and spring) for two years, according to the period allowed for shrimp capture in Portuguese law (DRE 2000). In order to compare the results obtained from shrimp collected in Portuguese coast with some shrimp species sold in Portugal, two samples of shrimp from other countries were purchased in a local supermarket, *Penaeus vannamei* (India aquaculture) and *Penaeus monodon* (Bangladesh aquaculture). In some samples the edible portion of the shrimp were separated from the shell, in other samples it was not possible to separate the shell due to the small size of the shrimp; shrimps with eggs were stored separately. Thirty samples were collected and frozen at -20 ºC until analysis and their characteristics are described in Table SM1.

Reagents and materials: Certified EPA 610 mixture standards containing 16 polycyclic aromatic hydrocarbons, naphthalene (Naph) 1000 µg/mL, acenaphthylene (Acy) 2000 µg/mL, acenaphthene (Ace) 1000 µg/mL, fluorene (Flu) 200 µg/mL, phenanthrene (Phe) 100 µg/mL, anthracene (Ant) 100 µg/mL, fluoranthene (Fln) 200 µg/mL, pyrene (Pyr) 100 µg/mL, benz-a-anthracene (BaA) 100 µg/mL, chrysene (Chry) 100 µg/ mL, benzo-b-fluoranthene (BbFt) 200 µg/mL, benzo-k-fluoranthene (BkFt) 100 µg/mL, benzo-a-pyrene (BaP) 100 µg/mL, dibenz-a,h-anthracene (DBa,hA) 200 µg/mL, benzo-g,h,i-perylene (Bg,h,iP) 200 µg/mL, and indeno-1,2,3-cd-pyrene (InP) 100 µg/mL were purchased from Supelco (Bellefonte, PA, USA) and the individual standards of benzo-j-fluoranthene (BjFt) and dibenzo-a,l-pyrene (DBa,lP) both with 2000 µg/mL were also obtained from Supelco (Bellefonte, PA, USA). The purities of all PAHs were between 97.9% and 99.9%. Working mixed standard solutions containing all the PAHs were prepared by dilution of the stock solutions with acetonitrile and stored at -20 ºC. Acetonitrile (assay 99.99%) was purchased from VWR Chemicals (Fontenay-sous-Bois, France). QuEChERS extraction kit original method, containing 4 g magnesium sulfate, 1 g sodium chloride with 50 mL tubes was obtained from Agilent Technologies (California). Deionized water (resistivity of 15.0 MΩ.cm)

was produced using a Elix apparatus (Millipore, Molsheim, France) and purified (resistivity of 18.2 MΩ.cm) using a Simplicity 185 system (Millipore). The eluents used in the chromatographic analysis were filtered through a 0.22 µm nylon membrane filter 47 mm (Fioroni Filters, Ingré, France) using a Dinko D-95 vacuum pump (Barcelona, Spain) and degassed for 15 min in an ultrasonic bath (Selecta P, Barcelona, Spain). The standards and the sample extracts were filtered through a 0.22 µm PTFE syringe filter, 13 mm (Specanalitica, Carcavelos, Portugal).

Liquid chromatography analysis: Chromatographic separation was performed using a Shimadzu LC system (Shimadzu Corporation, Kyoto, Japan) equipped with a degasser (DGU-20A5R), a pump (LC 20AD), an autosampler (SIL 20AHT), a PDA (SPD-M20A) and a FLD (RF-10AXL) detectors, a system controller (CBM-20A), and a LabSolution software version 5.82 (Shimadzu, Kyoto, Japan) for control and data processing.

Separation of the analyzed PAHs was performed in a C18 column (CC 150/4 Nucleosil 100-5 C18 PAH; 5 µm particle size and with 150 x 4.0 mm (length x internal diameter)) from Macherey–Nagel (Duren, Germany). The oven temperature was set at 30 ºC and an injection volume of 20 µL was used. Ultra-pure water (A) and acetonitrile (B) were the eluents used. Chromatographic program started with 50% of eluent A and B maintained during 5 min. Then, a linear increase to 100% of eluent B was made in 15 min and remain during 16 min. After, 2 min was set to the mobile phase return to the initial composition. The total run time was of 45 min with a flow rate of 0.8 mL/min.

Detection of the studied PAHs was performed using PDA and FLD detectors connected in series. Only for Acy the analytical signal was registered in the PDA detector because this PAH has limited fluorescence. For the remaining 17 PAHs the analytical signal was registered in FLD detector. In the later detector, to achieve better sensitivity, each PAH was detected at its optimum excitation/emission wavelength pair. Therefore, 260/315 nm was used for the detection of Naph, Ace, and Flu; 260/366 nm was used for the detection of Phe; 260/430 nm was used for the detection of Ant, Fln, Pyr, BaA, Chry, BbFt, BjFt, BkFt, BaP, DBa,hA, Bg,h,iP, and DBa,lP; and 290/505 nm was used for the detection of InP. Acyanalysis was performed at 229 nm in PDA detector.

Risk assessment: The values of Target Hazard Quotient (THQ) were calculated using the equation 1, described previously (Conte et al. 2016; Zhu et al. 2016),

$$\mathrm{THQ} = \frac{C \times (Ef \times Ed \times F)}{W \times T \times RfD} \times 10^{-3} \qquad \text{(S1)}$$

where, C is the concentration of PAH in shrimp samples (µg/g); Ef is the exposure frequency (estimated consumption of one time per week, 52 days/year); Ed is the exposure duration (80 years, average lifetime expectancy Portugal); F is the amount of shrimp ingested (28.8 g/person/day, maximum value reported, (Jacobs et al. 2017)); W is the mean body weight of Portuguese adult population (71.68 kg; (Jacobs et al. 2017)); T is the average exposure time (365 days/year x number of exposure years, in this study 80 years); RfD is the Reference Dose for Oral Exposure (mg/kg/day); 10^{-3} is the unit conversion factor (Conte et al. 2016; Zhu et al. 2016).

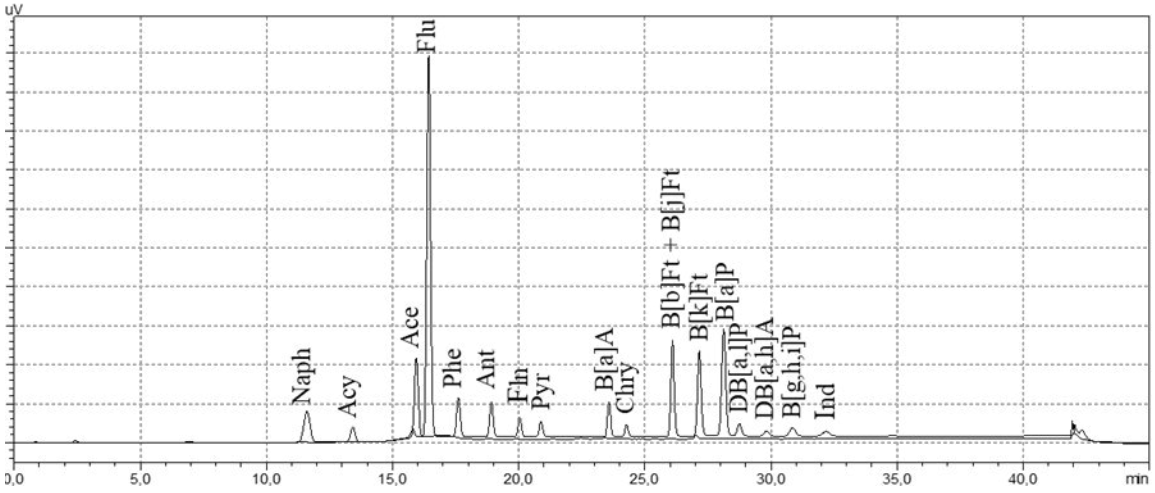


Fig. SM1 LC overlay chromatograms of a standard solution of PAHs mixture containing 18 PAHs (Naph-207.79 µg/L, Acy-706.58 µg/L, Ace-207.79 µg/L, Flu-41.00 µg/L, Phe-20.74 µg/L, Ant-20.76 µg/L, Fln-40.87 µg/L, Pyr-20.07 µg/L, BaA-20.38 µg/L, Chry-20.38 µg/L, BbFt + BjFt-81.60 µg/L, BkFt-20.68 µg/L , BaP-20.78 µg/L, DBa,lP-40.00 µg/L, DBa,hA-41.43 µg/L, Bg,h,iP-41.43 µg/L, Ind-20.80 µg/L) obtained in the FLD and in the PDA detectors

Table SM1 Characterization of shrimp samples

Sampling location	Date of sampling	Specie	Aquiculture/ wild	Shell	Eggs	Code
Vila do conde	Autumn 2017	*Palaemon serratus*	wild			VAu17
	Spring 2018		wild			VSp18
	Spring 2018		wild		x	VSp18-e
	Autumn 2018		wild			VAu18
	Spring 2019		wild			VSp19
	Spring 2019		wild		x	VSp19-e
Matosinhos	Autumn 2017	*Palaemon serratus*	wild			MAu17
	Spring 2018		wild		x	MSp18-e
	Autumn 2018		wild			MAu18
	Spring 2019		wild			MSp19
	Spring 2019		wild		x	MSp19-e
Aveiro	Spring 2018	*Palaemon serratus*	wild			ASp18
	Autumn 2018		wild			AAu18
	Spring 2019		wild			ASp19
Ria de Aveiro	Autumn 2017	*Palaemon serratus*	wild			RAu17
	Spring 2018		wild			RSp18
Figueira da Foz	Autumn 2017	*Palaemon serratus*	wild			FAu17
	Spring 2018		wild			FSp18
	Autumn 2018		wild			FAu18
	Spring 2019		wild			FSp19
Sado estuary	Autumn 2017	*Palaemon varians*	aquaculture			SAu17a
	Autumn 2017		wild			SAu17w
	Spring 2018		aquaculture			SSp18a
	Spring 2018		wild			SSp18w
	Autumn 2018		aquaculture			SAu18a
	Autumn 2018		wild	x		SAu18w-s
	Spring 2019		aquaculture	x		SSp19a-s
	Spring 2019		wild	x		SSp19w-s
India	purchased 2019	*Penaeus monodon*	aquaculture			I-19a
Bangladesh	purchased 2019		aquaculture			B-19a

V-Vila do Conde; M-Matosinhos; A-Aveiro; R-Ria Aveiro; F-Figueira da Foz; S-Sado estuary; Au-Autumn; Sp-Spring; 17- 2017; 18-2018; 19-2019; e-eggs; a-aquaculture; w-wild; s-shell; I-India; B-Bangladesh.

Table SM2 Spiking levels for all PAHs analyzed (ng/g ww)

PAHs	Spiking 1	Spiking 2	Spiking 3
Naph	5	10	21
Acy	18	35	71
Ace	5	10	21
Flu	1	2	4
Phe	0.5	1	2
Ant	0.5	1	2
Fln	1	2	4
Pyr	0.5	1	2
BaA	0.5	1	2
Chry	0.5	1	2
BbFt+BjFt	2	4	8
BkFt	0.5	1	2
BaP	0.5	1	2
DBa,lP	1	2	4
DBa,hA	1	2	4
Bg,h,iP	1	2	4
InP	0.5	1	2

Table SM3 Validation parameters using matrix matched standards for the selected PAHs

PAHs	Retention time	Calibration range (ng/g ww)	Regression equation (ng/g ww)	Linearity (R^2)	Recoveries (%)	ME (%)	MDL (ng/g ww)	MQL (ng/g ww)
Naph	11.52	0.35 - 27.71	y= 9591x + 21098	0.9998	76	-88.36	0.56	1.86
Acy	13.28	23.48 - 94.21	y= 2732x - 45461	0.9800	51	-60.60	15.98	53.28
Ace	15.86	0.35 - 27.71	y= 59862x - 2810	0.9958	52	-27.33	2.63	8.76
Flu	16.34	0.07 - 5.47	y= 1682234x - 87573	0.9988	70	-10.85	0.28	0.94
Phe	17.51	0.03 - 2.77	y= 360924x - 14936	0.9991	84	-3.26	0.11	0.36
Ant	18.81	0.03 - 1.39	y= 304735x - 5172	0.9989	77	-5.06	0.06	0.22
Fln	19.92	0.07 - 5.45	y= 89579x - 3582	0.9998	76	0.38	0.09	0.29
Pyr	20.75	0.03 - 2.68	y= 140974x - 303	0.9999	76	0.46	0.04	0.12
BaA	23.44	0.03 - 2.72	y= 297729x - 1066	0.9999	71	2.49	0.03	0.09
Chry	24.11	0.03 - 2.04	y= 98707x + 216	1.0000	72	-6.80	0.01	0.05
BbFt+BjFt	25.93	0.14 - 10.88	y= 211120x - 7733	0.9999	69	0.78	0.12	0.39
BkFt	26.96	0.03 - 2.76	y= 792116x - 5160	0.9999	69	-1.14	0.03	0.11
BaP	27.90	0.03 - 2.77	y= 1152739x - 9892	0.9999	66	1.83	0.03	0.11
DBa,lP	28.54	0.07 - 5.33	y= 73356x - 254	0.9999	65	1.15	0.05	0.16
DBa,hA	29.54	0.07 - 5.52	y= 29222x - 163	0.9998	71	-8.32	0.08	0.28
Bg,h,iP	30.53	0.07 - 5.50	y= 54435x - 2209	0.9999	56	-16.43	0.07	0.25
InP	31.78	0.03 - 2.77	y=79794x + 1831	0.9994	65	5.64	0.08	0.26

ME-matrix Effect; MDL-method detection limit; MQL-method quantification limit.

Table SM4 Levels of PAHs achieved in shrimp samples (ng/g ww) by LC

	Concentrations PAHs (ng/g ww)																
Sample	**Naph**	**Ace**	**Flu**	**Phe**	**Ant**	**Fln**	**Pyr**	**BaA**	**Chry**	**BbFt + BjFt**	**BkFt**	**BaP**	**DBa,lP**	**Bg,h,iP**	**ΣPAHs**	**ΣBaA; BaP; Chry;BbFt+BjFt**	**Number of PAHs >MDL**
VAu17	43.60	< MDL	< MDL	0.25	< MDL	0.14	< MDL			0.14	< MDL	< MDL			46.29	0.16	5
VSp18	21.30	< MDL	< MDL	0.26		0.15	0.07			< MDL	< MDL				23.95	0.08	4
VSp18-e	10.79	< MDL	< MDL	0.25	< MDL	< MDL				< MDL	< MDL	< MDL			13.33	0.11	2
VAu18	0.95	< MDL	< MDL	0.24		0.13					< MDL				3.39		3
VSp19	13.62	< MDL	< MDL	0.20	< MDL	0.11					< MDL				16.05		3
VSp19-e	115.83	< MDL	< MDL	0.29		0.21	0.12			< MDL	< MDL	< MDL	< MDL		118.66	0.11	4
MAu17	3.09	< MDL	< MDL	0.22	< MDL	0.14	< MDL			0.16	< MDL	< MDL	0.09		5.87	0.18	6
MSp18-e	< MDL	< MDL	< MDL	0.27	< MDL	0.14	0.10			< MDL	< MDL	< MDL			3.14	0.11	3
MAu18	95.97	< MDL	< MDL	0.27	< MDL	0.17	0.07			0.14	< MDL		0.08		98.82	0.14	7
MSp19	43.03	< MDL	< MDL	0.30	< MDL	0.13	0.09			< MDL	< MDL				45.75	0.08	4
MSp19-e	3.20	< MDL	< MDL	0.41	0.15	0.13	0.11			< MDL	< MDL	< MDL			6.20	0.11	5
ASp18	56.65	< MDL	< MDL	0.28	< MDL	0.11	0.13			< MDL		0.07	0.12		59.55	0.16	6
AAu18	3.77	< MDL	0.31	0.32	0.11	0.14	0.07				< MDL	< MDL			6.62	0.02	6
ASp19	5.59	< MDL	< MDL	0.26	< MDL	0.14	0.05			< MDL	< MDL	< MDL			8.26	0.11	4
RAu17	2.96	< MDL	< MDL	0.30	< MDL	0.13	0.10			< MDL	< MDL				5.69	0.08	4
RSp18	9.72		< MDL	0.25	< MDL					< MDL	< MDL				10.32	0.08	2
FAu17	123.04	< MDL	< MDL	0.25		0.13				0.16	< MDL				125.66	0.16	5
FSp18	101.04	< MDL	< MDL	0.25	< MDL	0.13	0.07			< MDL		< MDL			103.70	0.11	4
FAu18	71.00	< MDL	< MDL	0.25	< MDL	< MDL	0.07			< MDL	< MDL	< MDL			73.61	0.11	3
FSp19	195.91	< MDL	< MDL	0.24		0.15	0.09				< MDL				198.47		4
SAu17a	7.84	< MDL	< MDL	0.30	0.07	0.09	0.11			0.15	< MDL		0.08		10.71	0.15	8
SSp18a	53.82	< MDL	0.30	0.49	0.17					0.14		< MDL			56.81	0.16	6
SAu18a	10.79	< MDL	< MDL	0.24	< MDL	0.16				< MDL	< MDL				13.40	0.08	3
SSp19a-s	317.73	< MDL	< MDL	0.20	< MDL		0.05	< MDL		< MDL	< MDL			0.27	320.48	0.11	4
SAu17w	31.33	< MDL	< MDL	0.23	< MDL	< MDL	0.08			0.15	< MDL		0.08		34.06	0.15	6
SSp18w	2.88	< MDL	< MDL	0.29	< MDL	0.20	0.11	< MDL		< MDL	< MDL				5.70	0.11	4
SAu18w-s	11.91	< MDL	< MDL	0.34		0.15	0.13			< MDL	< MDL	< MDL			14.72	0.11	4
SSp19w-s	187.67	< MDL	< MDL	0.19	0.10	0.09	0.08	< MDL		< MDL	0.03	< MDL		0.33	190.68	0.13	7
I-19a	285.98	< MDL	< MDL	0.28		0.18	0.10		0.02	< MDL	< MDL	< MDL			288.74	0.12	5
B-19a	25.90		0.46	1.34	< MDL	0.24	0.16	< MDL	0.04	< MDL	< MDL	< MDL		0.31	28.63	0.16	7

MDL– method detection limit; BbFt and BjFt were co-eluted together.

Table SM5 Statistical analysis data

One Way ANOVA	*F*	*P value*	*F critic*
Seasons (Autumn/Spring)*	2.43	1.22×10^{-1}	3.95
Sampling locations*	**32.40**	**4.35×10^{-18}**	**2.33**
Species (*P. serratus*/*P. varians*)*	**4.98**	**2.84×10^{-2}**	**3.96**
Aquaculture/wild*	0.62	4.41×10^{-1}	4.41
With eggs/without eggs	1.20	2.86×10^{-1}	4.30
With shell/without shell	**26.27**	**1.64×10^{-5}**	**4.17**

* Excluing data with eggs and shell

Table SM6 Level of concern (LOC) for PAHs and their maximum concentration (Cmax) detected in the shrimp samples

PAHs	Cmax (ng/g)	LOC (ng/g)
Naph	317.73	123,000
Flu	0.46	246,000
Phe	1.34	1,846,000
Ant	0.17	1,846,000
Fln	0.24	246,000
Pyr	0.16	185,000
Chry	0.04	132,000
BbFt + BjFt	0.16	1320
BkFt	0.03	13,200
BaP	0.07	132

Table SM7 THQ and HI calculated for PAHs through the consumption of different shrimp species

	THQ						HI
Shrimp species	**Naph**	**Flu**	**Ant**	**Fln**	**Pyr**	**B(a)P**	
Palaemon serratus	**1×10^{-4}**	**9×10^{-7}**	**4×10^{-7}**	**4×10^{-7}**	**3×10^{-7}**	**2×10^{-7}**	**1×10^{-4}**
Palaemon varians	**2×10^{-4}**	**9×10^{-7}**	**3×10^{-7}**	**4×10^{-7}**	**3×10^{-7}**	-	**2×10^{-4}**
Penaeus vannamei	**8×10^{-4}**	-	-	**5×10^{-7}**	**3×10^{-7}**	-	**8×10^{-4}**
Penaeus monodon	**7×10^{-5}**	**1×10^{-6}**	-	**7×10^{-7}**	**5×10^{-7}**	-	**8×10^{-5}**

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Chapter 3

Effects of Brominated Flame Retardants on lipid metabolism

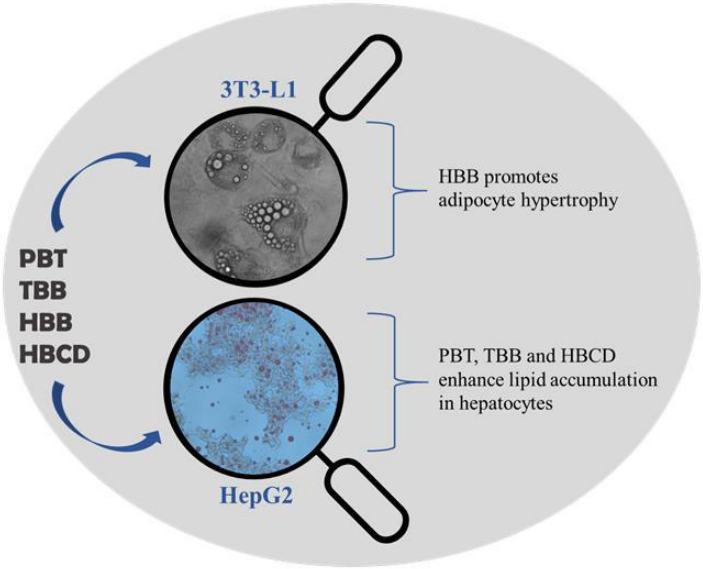
Impact of Brominated Flame Retardants on lipid metabolism: an in vitro approach

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Statement of contribution

The contribution of the candidate, Maria Luz Maia, in this work includes the accomplishment of cell culture, *in vitro* assays, statistical analysis and written of the original draft.

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Impact of brominated flame retardants on lipid metabolism: An *in vitro* approach☆

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ABSTRACT

Brominated flame retardants (BFRs) are chemicals employed to lower the flammability of several objects. These endocrine disruptor chemicals are lipophilic and persistent in the environment. Due to these characteristics some have been restricted or banned by the European Union, and replaced by several new chemicals, the novel BFRs (NBFRs). BFRs are widely detected in human samples, such as adipose tissue and some were linked with altered thyroid hormone levels, liver toxicity, diabetes and metabolic syndrome in humans. However, the disturbance in lipid metabolism caused by BFRs with emphases to NBFRs remains poorly understood. In this study, we used a pre-adipocyte (3T3-L1) cell line and a hepatocyte (HepG2) cell line to investigate the possible lipid metabolism disruption caused by four BFRs: hexabromobenzene (HBB), pentabromotoluene (PBT), 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) and hexabromocyclododecane (HBCD). For that purpose, proliferation and Oil Red *O* assays, as well as, medium fatty acids profile evaluation using Gas chromatography and RNA extraction for quantitative RT-PCR assays were performed. We detected a significant reduction in the proliferation of pre-adipocytes and an increased lipid accumulation during differentiation caused by HBB. This BFR also lead to a significant increased expression of IL-1β and decreased expression of PGC-1α and adiponectin. Nevertheless, PBT, TBB and HBCD show to increase lipid accumulation in hepatocytes. PBT also display a significant increase of PPARγ gene expression. Lipid accumulation in the cells can occur by diverse mechanisms depending on the BFR. These results highlight the importance of endocrine disruptor compounds in obesity etiopathogeny.

1. Introduction

Brominated flame retardants (BFRs) are used in high quantities in electronics, vehicles, clothes, toys and plastics to reduce flammability (Kim et al., 2014). BFRs are a vast and complex chemical family that can be divided primarily in three groups, polybrominated biphenyls and diphenyl ethers (PBBs and PBDEs), tetrabromobisphenol A (TBBP-A) and hexabromocyclododecane (HBCD). Been considered persistent in the environment, the use of some of them have been banned or restricted by the European Union (Covaci et al., 2011; EFSA, 2021). Consequently, this led to the appearance and usage in several products of novel BFRs (NBFRs), as an alternative to the previously banned ones. Representatives of this group are hexabromobenzene (HBB), pentabromotoluene (PBT) and 2-ethylhexyl-2,3,4,5-tetrabromobenzoate (TBB) (Covaci et al., 2011). In general, BFRs have the ability to accumulate in animal adipose tissue with potentially biomagnification through the food chain (Kim et al., 2014; Taheran et al., 2017) and are capable of interfering with normal hormonal signaling, acting as endocrine disruptors (EDs) (Hoppe and Carey, 2007). BFRs have been extensively identified in environmental and human samples, for instance blood, breast milk and adipose tissue (Hoppe and Carey, 2007; Taheran et al., 2017). The major exposure routes to these chemicals are dietary sources, occupational

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exposure, indoor dust ingestion and inhalation of indoor air (Covaci et al., 2011; Kim et al., 2014). Several animal experiments have reported associations among the exposure to BFRs and adverse health effects. Alterations in thyroid hormone homeostasis, reproductive health effects, neurobehavioral changes and developmental neurotoxicity were associated to BFRs mixtures exposure in rats (Kim et al., 2014). A study from Hoppe et al. demonstrates that the daily exposure of rats to penta-PBDE alters insulin metabolism of isolated adipocytes, increases lipolysis and decreases glucose oxidation. Which are features associated with metabolic obesity, insulin resistance and type 2 diabetes (Hoppe and Carey, 2007). Other findings showed that PBBs and PBDEs reduced thyroid plasma T4 hormone, enhanced hepatic phospholipids, induced hepatocytic swelling, liver neoplastic nodules, necrosis, and carcinomas (Annamalai and Namasivayam, 2015; ATSDR, 2004). A more recent study demonstrated that PBDE-209 can disrupt liver tissue structure, impairing lipid metabolism and leading to an increase of the liver tissue weight, body fat and body weight (Alimu et al., 2021). In human studies, levels found in human serum from PBDE-153 and PBB-153 exposure were linked with diabetes and metabolic syndrome with different relationship dose-response (Lim et al., 2008). Furthermore, dietary exposure to HBCD and PBDEs were found to be associated with the risk of diabetes type 2 (Ongono et al., 2019). PBDEs exposure in humans were also linked to alterations on hormone levels of thyroid, reproductive toxicity, liver toxicity and developmental neurotoxicity (Annamalai and Namasivayam, 2015).

As mentioned, the exposure to BFRs have been associated to the increased risk of metabolic syndrome, obesity or diabetes development. Hence, it is of utmost importance to study the disturbance in lipid metabolism caused by BFRs, with emphases to NBFRs that remains poorly understood.

Our goal was to evaluate the modulation of lipid accumulation after exposure to BFRs in adipocytes and in other metabolic organ, the liver. The fatty acids analysis allow to explore the crosstalk between adipocyte and hepatocytes, since their release are a key in these relation with possible impact in metabolic dysfunction (Azzu et al., 2020; Jung and Choi, 2014).

With that purpose pre-adipocyte and hepatocyte cell lines widely used to study adipocyte/hepatocyte function and lipid metabolism were tested using *in vitro* assays suitable for the compounds screening. These studies were performed to explain the lipid metabolism disruption mechanisms of four BFRs: PBT, HBB, TBB and HBCD.

2. Materials and methods

2.1. Cells, chemicals and reagents

The murine 3T3–L1 cell line and the human HepG2 cell line were acquired from the American Type Culture Collection through LGC Promochem (Barcelona, Spain). Antibiotic–antimycotic solution, Dulbecco's modified Eagle's medium (DMEM), Minimum essential medium eagle (MEME), Krebs–Ringer Hepes, Bicarbonate buffer, Tris–HCl, trypsin-EDTA, oil red *O*, sulforhodamine B (SRB), Oleic acid and primers for RT-PCR analysis were acquired from Sigma-Aldrich Chemicals (St Louis, MO). Fetal bovine serum (FBS) was acquired to Gibco (Paisley, UK). Dimethyl sulfoxide (DMSO), gelatin, trichloroacetic acid (TCA) and Triton X-100 were acquired from Merck (Darmstadt, Germany). Anhydrous sodium sulfate and sodium hydroxide were acquired from Pronalab (Lisbon, Portugal); boron trifluoride-methanol (BF_3) 14% in methanol and butylated hydroxytoluene (BHT) (≥99%) were acquired from Sigma-Aldrich (St. Louis, USA); methanol was from VWR Chemicals Prolabo (Fontenay-sous-Bois, France); n-hexane (99%) was acquired from Merck (Darmstadt, Germany); and sodium chloride (99.5%) was purchased from Panreac (Barcelona, Spain). NZyol was obtained from NZYTech, Lisbon, Portugal.

2.2. Cell culture conditions

The murine cell line 3T3-L1 was cultured in a humidified atmosphere (37 °C, 5% CO_2), using DMEM, supplemented with 1.5 g/L $NaHCO_3$, 1% (v/v) antibiotic–antimycotic and 10% (v/v) FBS at pH 7.2. A differentiation protocol was applied to 3T3-L1 preadipocytes to obtain adipocytes. Briefly, two days after achieving full confluence (day 0 of differentiation), 3T3-L1 preadipocytes were exposed to a differentiation cocktail [(DEX (0.25 μM), IBMX (0.5 mM) and insulin (10 μg/mL)] in DMEM. This cocktail was removed after an additional 2 days, and cells were thereafter maintained in DMEM with insulin (10 μg/mL). The medium was renewed each 2–3 days. Adipocytes were used for the experiments when around 80% of control cells were differentiated (12 days after differentiation induction).

The human cell line HepG2 was cultured in a humidified atmosphere (37 °C in 5% CO_2). Cells were grown in MEME, supplemented with 2.2 g/L $NaHCO_3$, 5.96 g/L Hepes, 1% (v/v) antibiotic–antimycotic and 10% (v/v) FBS at pH 7.4. Every 2–3 days the culture medium was replaced and every 7 days the cell culture was split (when 90–95% of confluence as reached). The subculture was performed enzymatically with 0.25% trypsin-EDTA for 1 min at 37 °C, split 1:4 to in plastic culture dishes (21 cm^2; 60 mm; Orange Scientific, Belgium).

For cell proliferation experiments, cell cultures were seeded on 96-well plates (0.32 cm^2; Orange Scientific) in 200 μL culture medium. For Oil Red *O* assays, cells were seeded on 24-well plates (1.65 cm^2; Orange Scientific) in a volume of 0.5 mL culture medium including different concentrations of the tested BFRs, solved in culture medium (control cells growth in the presence of DMSO in culture media). DMSO was used as the compound's vehicle.

The chosen concentrations of BFRs were based on previous cell-line studies and on the levels detected in the human population for endocrine disruptors (Norberto et al., 2017; Pestana et al., 2014).

2.3. Cell treatments with BFRs

Cells were incubated 24 h with BFRs for short-term exposure experiments, specifically with HBCD, PBT, TBB and HBB in culture medium. For long-term exposure (chronic) cells were treated for at least 4 weeks with 250 nM of each BFR. For the experiment control, cells grown in medium with 0.1% of DMSO, instead of BFRs.

2.4. Cell viability

3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay was previously described (Norberto et al., 2017) and performed to test cell viability. Cells were exposed to BFRs in a range of 10–500 nM for 24 h, after which were incubated with 50 μL of MTT solution [(5 mg/mL of phosphate buffered saline (PBS)] for 2 h. Next the cells were dissolved in 300 μL of DMSO after removing the medium. Absorbance was read at 540 nm and corrected with 620 nm. The results for cell viability were presented as a percentage of the control.

2.5. Proliferation assay

SRB assay was assessed as described (Faria et al., 2010; Marques et al., 2013). After 24 h of treatment with 50–250 nM concentrations of BFRs was added 25 μL of ice-cold 50% (w/v) TCA to the preadipocytes incubation medium (control was medium with 0.1% of DMSO). Cells were fixed in the dark for 1 h at 4 °C. To remove TCA, the plates were washed with tap water, then were air-dried and stained using 0.4% (w/v) SRB dissolved in 1% acetic acid for 15 min. Following incubation, to eliminate residual dye, the plates were rinsed four times with 1% acetic acid, air-dried and then, using 200 μL of 10 mM Tris base solution (pH 10.5), the bound dye was solubilized. The absorbance was determined in a microplate reader at 490 nm (Thermo Electron Corporation, Multiskan Ascent; Waltham, MA, USA). The results for proliferation

assays were presented as a percentage of the control.

2.6. Oil red O assays

Oil Red *O* assay was performed according to (Marques et al., 2013). On the final day of differentiation protocol (day 12) for 3T3-L1 cell line and after 4 weeks of treatment (chronic with or without the presence of oleic acid) for HepG2 in the presence of the tested compounds, the culture medium was removed. PBS were used to wash the cells before the fixation with 50% (w/v) TCA for 1 h, then were washed again with PBS and distilled water. The cells were then stained using 0.3% oil red *O* solution [in isopropanol/distilled water (3:2)] for 15 min, then were washed 3 times using distilled water and were air-dried. The Oil red *O* in the cells was recovered in 300 μL of DMSO, the absorbance was measured on a microplate reader at 490 nm. Results were normalized using trypan blue method to count the number of cells.

2.7. Medium fatty acids profile determination by gas chromatography – flame ionization detector (GC-FID)

For this assay, a previous described method was performed with few modifications (Sousa et al., 2018). Sodium methoxide (NaOMe) is applied as a mechanism to form fatty acid methyl esters (FAME). A known standard mixture (Sigma 47,885-U Supelco 37 Component FAME Mix, USA) was used to identify the FAME by comparison. One mL of the medium was used for this assay and 20 μL (100 μg/L) of internal standard (C13:0) was added, then 5 mL of NaOMe (0.5 M) was also added and all were vortexed. The samples were warmed (10 min at 100 °C) and cooled for 5 min. After, 5 mL of BF_3 was added and the samples were warmed this time for 30 min at 100 °C and cooled again for 5 min. Next, 1 mL of BHT in n-hexane (0.02%) and 2 mL of sodium chloride (58 g/L) were added. Samples were vortexed and centrifuged for 5 min at 3000 rpm. The top layer was recovered and dried using anhydrous sodium sulfate. Then, it was removed 400 μL which were evaporated to dryness using nitrogen flow. Finally, the samples were rediluted in 100 μL of n-hexane for Gas Chromatography (GC) analyses that were executed using a Shimadzu GC-2010 equipped with a FID and a Shimadzu AOC-20i autoinjector. An Agilent J&W Cp-Sil 88 capillary column (60 m × 0.25 mm ID, 0.20 μm) from Santa Clara, USA were used for FAME separation. Regarding the operating conditions: the split–splitless injector in split mode (split ratio of 1:50) and the injection volume of 1.5 μL were used. The injector and detector temperatures were maintained at 250 °C and 270 °C, respectively. The temperature program: 100 °C for 1 min, which was enhanced at 3 °C/min to 170 °C, hold for 2 min, then increased 0.5 °C/min to 180 °C, hold for 1 min, than 0.5 °C/min to 185 °C, hold for 3 min, than 0.5 °C/min to 200 °C, hold for 1 min; carrier gas: He, 30 mL/min; detector gas flows: H2, 40 mL/min; air, 400 mL/min. Data acquisition and analysis were achieved using a Shimadzu software for GC systems. The results are presented in μg/L and variation between fatty acid profile in medium after cell treatment with BFRs and after treatment with control. Concentration for each fatty acid was obtained by the ratio fatty acid/C13:0 signal for each peak and the known C13:0 concentration.

2.8. RNA extraction and quantitative RT-PCR assays

Total RNA was extracted from cells (3T3-L1 and HepG2) as previously described (Norberto et al., 2017) using NZyol isolation reagent, according to manufacturer's instructions (NZY Tech1, Lisbon, Portugal). RNA extracted from cells were dissolved in RNase-free water and stored at −20 °C prior to RT-PCR. A NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE) was used to assess spectrophotometrically the total RNA concentration, then samples were treated with DNaseI (Promega RQ1 RNase-free DNase) to prevent contamination with genomic DNA. For cDNA synthesis, the manufacturer's instructions of NZY First-strand cDNA synthesis kit (NZY Tech1, Lisbon, Portugal) were followed and the reverse transcription was done with 1 μg of total RNA. After the resulting cDNA was treated with NZYRnase H (NZY Tech1, Lisbon, Portugal). A LightCycler 96 system (Roche, Portugal) was used to perform the quantitative RT-PCR. The cDNA amplification was performed using 1 μL of cDNA 25 ng/μL, 9 μL of PCR mix containing primers (0.2 μM final concentration) and 5 μL of SYBR Green mix (FastStart Essential DNA Green master mix, Roche). The primer sets used for 3T3-L1 and HepG2 and Cycling conditions are described in Table 1. Data was analyzed with LightCycler 96 software. The gene expression was determined using $2^{-\Delta Ct}$ method as described (Schmittgen and Livak, 2008).

2.9. Statistical analysis

All results are presented as arithmetic means and standard error of mean (SEM) and were obtained from at least duplicates. The D'Agostino-Pearson Omnibus Normality Test was applied to verify whether the distribution of the variables was normal. Statistical significance of the difference among treatments and control was assessed by Student's t-test. Statistical significance of the difference among various groups was assessed by one-way analysis of variance (ANOVA test) using the Tukey test. The statistical analysis was achieved using Graph Pad Prism 6.01 software (La Jolla, CA, USA). The differences among means were considered significant when $p < 0.05$.

3. Results

3.1. BFRs impact in a pre-adipocyte cell line (3T3-L1)

3.1.1. Proliferation analysis

Cells were exposed to each studied BFR in concentration range from 50 nM to 250 nM. Cell proliferation data is shown in Fig. 1. PBT, TBB and HBCD do not significantly affect preadipocytes proliferation, measured by protein quantification. However, HBB diminished ($p <$ 0.0001 for 100 nM and 250 nM) cell proliferation, the result was more pronounced for the highest concentration (Fig. 1d).

3.1.2. Lipid accumulation analysis by oil red O

Cells were exposed to each studied BFR in concentration range from 50 nM to 250 nM during differentiation. Adipocyte's differentiation measured by lipid accumulation is shown in Fig. 2. Lipid accumulation was not affected by TBB (Fig. 2b). In the case of PBT, higher lipid accumulation occurs when cells were treated with the lower tested concentration (50 nM), with a significative result ($p = 0.0002$) (Fig. 2a). For HBCD and HBB there is a U shape dose-response profile. Regarding HBCD, the intermediate concentration (100 nM) decreased lipid accumulation, $p = 0.02$ (Fig. 2c). Despite the generalized increased of lipid accumulation by HBB, the lower and higher concentrations present the higher effect, p values of $p = 0.0003$ and $p < 0.0001$, respectively (Fig. 2d).

3.1.3. Fatty acids analysis

Cells were exposed to each studied BFR in a concentration of 250 nM during differentiation. The medium was collected at day 3 and 11 of the differentiation procedure. The fatty acids composition of the 3T3-L1 medium itself is shown in Table S1 and the absolute concentration values of fatty acids found for the different treatments are presented in Table S2. The variation (Δ) between the fatty acids profile in the medium of cells treated with BFRs and cells treated with control is represented in Fig. 3. 3T3-L1 medium does not contain short-chain fatty acids (SCFA), so their presence in the cell medium can result from adipocytes lipolysis. Instead, some polyunsaturated fatty acids (PUFA) such as C20:5 n3 and C22:6 n3 were no longer detected in medium after treatments (Tables S1 and S2).

Comparing to cells treated with control, PBT and TBB treatments decreased SCFA concentration in the medium at day 11, whereas with

Table 1
Primer sequences and cycling conditions.

Cell line	Primer	Sequence (5' - 3')	Denaturation	Annealing	Elongation	cycles
3T3-L1	mHPRT1	GCCCCAAAATGGTTAAGGTT CAAGGGCATATCCAACAACA	95 °C; 10s	58 °C; 10s	72 °C; 10s	45
	mPPAR-α	CCCTGAACATCGAGTGTCGAA TTCGCCGAAAGAAGCCCTTA		62 °C; 10s		
	mPPAR-γ	GGTGTGATCTTAACTGCCGGA GCCCAAACCTGATGGCATTG		62 °C; 10s		
	mCIDEA	GGCCGTGTTAAGGAATCTGC GTATGTGCCCGCATAGACCA		60 °C; 10s		
	mPGC-1α	TGGATGAAGACGGATTGCCC AGAGCTTCTTAAGTAGAGACGGC		60 °C; 10s		
	mIL-1β	GTGCTGTCGGACCCATATGA AGGCCACAGGTATTTTGTCGT		60 °C; 10s		
	MCP-1	GCCCCACTCACCTGCTGCTACT CCTGCTGCTGGTGATCCTCTTGT		65 °C; 10s		
	Adiponectin	GTTGCAAGCTCTCCTGTTCC TCTCCAGGAGTGCCATCTCT		59 °C; 10s		
HepG2	hHPRT1	TGCTGACCTGCTGGATTACA TTTATGTCCCCTGTTGACTGG		59 °C; 10s		
	hPPAR-α	GCCTAAGGAAACCGTTCTGTG AGGACGATCTCCACAGCAAAT		60 °C; 10s		
	hPPAR-γ	ACAGATGTGATCTTAACTGTCGGA CCCAAACCTGATGGCATTATGAG		61 °C; 10s		
	hC/EBPα	TATAGGCTGGGCTTCCCCTT AGCTTTCTGGTGTGACTCGG		60 °C; 10s		
	hPGC-1α	ACCACAAACGATGACCCTCC TGGTTTGGCTTGTAAGTGTTGTG		61 °C; 10s		

Hypoxanthine-guanine phosphoribosyltransferase 1 (HPRT1), used as housekeeping gene.

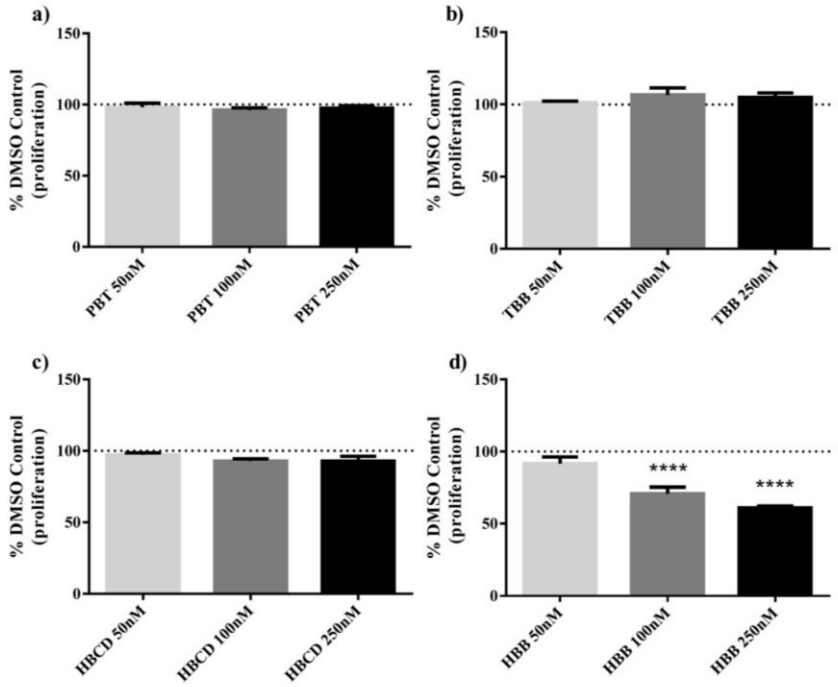


Fig. 1. Effect of a) PBT, b) TBB, c) HBCD and d) HBB on preadipocytes proliferation. Cells were treated with the BFRs for 24 h on the culture medium. Cell proliferation was determined after staining cellular protein with sulforhodamine B. Columns correspond to mean of the percentage of control ± SEM. Significantly different from control (DMSO), ****$p < 0.0001$.

TBB treatment SCFA concentration has increased at day 3. For HBB and HBCD treatments an increase in monounsaturated fatty acids (MUFA) concentration at day 11 was observed, furthermore treatment with HBB has increased long chain fatty acids (LCFA) concentration.

Regarding the differences within each cell treatment between day 3 and day 11 (Fig. 3), it was observed that PBT treatment led to a decrease

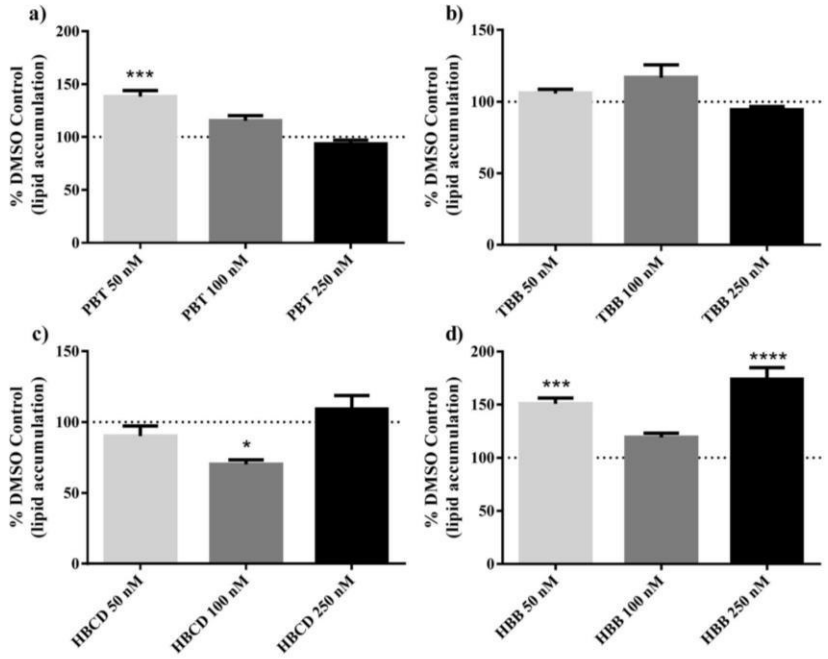


Fig. 2. Effects of a) PBT, b) TBB, c) HBCD and d) HBB on adipocytes differentiation measured by Oil Red *O*. Cells were treated with the BFRs on the culture medium for 12 days during differentiation. Lipid accumulation was determined after staining cellular lipids with Oil Red *O*. Columns correspond to mean of the percentage of control ± SEM. Significantly different from control (DMSO), $^{*}p < 0.05$, $^{***}p < 0.001$, $^{****}p < 0.0001$.

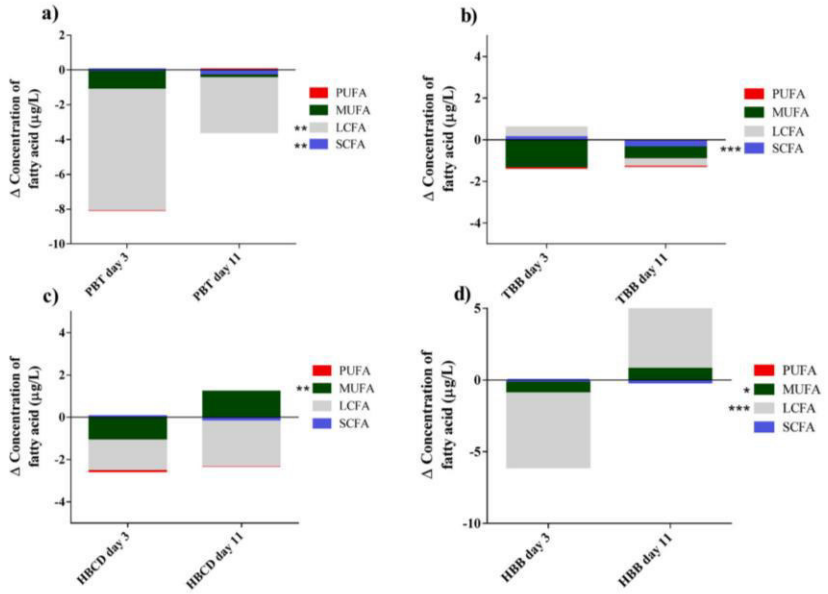


Fig. 3. Effects of BFRs in fatty acid composition of 3T3-L1 medium. Variation (between DMSO control and BFRs) of the concentration of SCFA, LCFA, MUFA and PUFA for a) PBT, b) TBB, c) HBCD, and d) HBB. Columns correspond to variation of the concentration of fatty acids. Significantly differences, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

of SCFA ($p = 0.008$) and an increase of LCFA ($p = 0.002$) concentrations in the medium (Fig. 3a), TBB also led to a decrease of SCFA ($p = 0.0004$) concentration (Fig. 3b). In cells treated with HBCD, there were an increase in MUFA ($p = 0.009$) concentration (Fig. 3c) in medium and HBB increased LCFA ($p = 0.0004$) and MUFA ($p = 0.02$) concentration (Fig. 3d) from day 3 to day 11.

3.1.4. Gene expression analysis

Cells were exposed to each studied BFR in a concentration of 250 nM during differentiation. At the end of the differentiation procedure, the cells were collected for RNA extraction. The effect of BFR compounds tested on gene expression is described in Fig. 4. The BFRs tested increased PPARα expression, except for HBCD (Fig. 4a), with a significant result for PBT ($p = 0.04$). On the other hand, a decreased PPARγ expression was observed, except for PBT (Fig. 4b). All compounds, except for HBCD, decreased CIDEA expression, with a significant result ($p = 0.02$) for TBB (Fig. 4 c). All BFRs tested increased IL-1β gene expression, with significative differences for PBT ($p = 0.005$), TBB ($p = 0.03$) and HBB ($p = 0.01$) present in Fig. 4e. PBT ($p = 0.01$) and HBB ($p = 0.02$) significantly decreased PGC-1α (Fig. 4d) expression and additionally HBB ($p = 0.03$) significantly decreased adiponectin gene expression (Fig. 4g).

3.1.5. BFRs impact in a hepatocyte cell line (HepG2)

Cells were exposed to each BFR studied in concentrations that ranged from 10 nM to 500 nM in order to study cell viability. The results showed that cell viability was not altered by any BFR (data not shown).

3.1.6. Lipid accumulation analysis by oil red O

Cells were exposed to each studied BFR in a concentration of 250 nM during at least 4 weeks (chronic exposure), results are presented in Fig. 5. BFRs led to an increase of lipid accumulation in HepG2, except for HBB. Oleic acid was used as positive control, to increase lipid accumulation in the cells. The results presented for PBT, TBB and HBCD showed a significantly increase in lipid accumulation between control and the treatments ($p = 0.002$, $p < 0.0001$ and $p = 0.0008$, respectively). In the case of PBT, when cells were co-treated with PBT and oleic acid (rich lipid environment), a synergistic effect on lipid accumulation was observed, increasing the lipid accumulation on cells (data not shown).

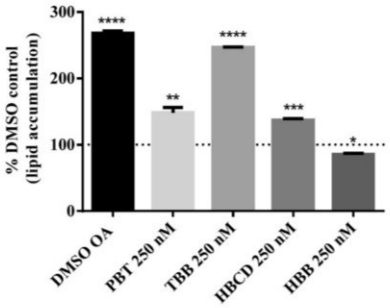


Fig. 5. Effects of PBT, TBB, HBCD and HBB on HepG2 lipid accumulation measured by Oil Red *O*. Cells were treated with the BFRs on the culture medium for more than 4 weeks, oleic acid (OA) was used as positive control. Lipid accumulation was determined after staining cellular lipids with Oil Red *O*. Columns correspond to mean of the percentage of control ± SEM. Significantly differences, $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

3.1.7. Fatty acids analysis

Cells were exposed to each studied BFR in a concentration of 250 nM during at least four weeks. Then the medium was collected at day 2 and 7 for one week. Tables S3 and S4 presented the fatty acids composition of the HepG2 medium itself and absolute concentration values of fatty acids found for the different treatments, respectively. Concerning differences between treatments and control, it was observed that HBB treatment led to a decrease in concentration of MUFA at day 2 and PUFA at day 7. Comparing the same treatment between day 2 and 7 (Fig. 6), the results show that, PBT treatment led to a decrease of LCFA ($p = 0.01$) concentration (Fig. 6a), while TBB led to an increase of LCFA ($p = 0.01$) concentration in the medium between day 2 and day 7 (Fig. 6b). Whereas HBB treatment led to an increase of MUFA ($p = 0.04$) and a

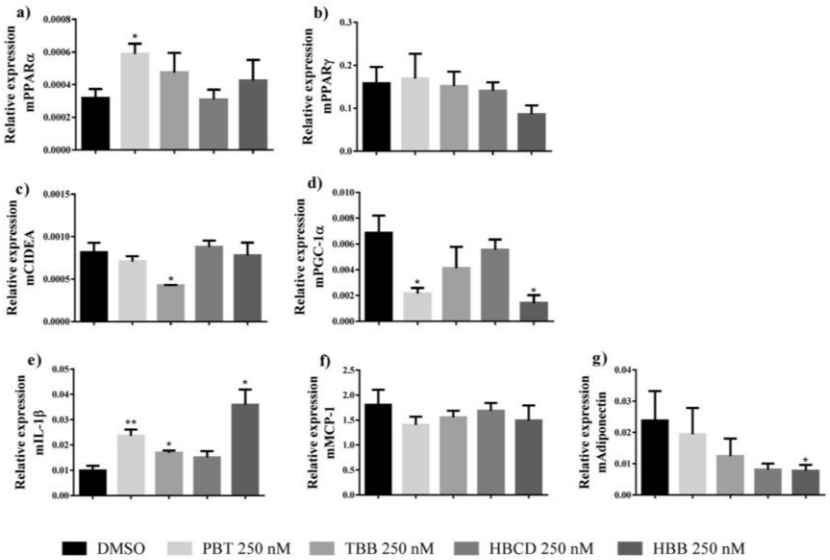


Fig. 4. Effects of BFR compounds exposure during adipocyte differentiation procedure on gene expression, a) PPARα, b) PPARγ, c) CIDEA, d) PGC-1α, e) IL-1β, f) MCP-1, g) Adiponectin. Cells were treated with 250 nM of each BFR and collected at day 12, after differentiation. Gene expression is represented as total expression normalized with HPRT ± SEM. Significantly different from control (DMSO), $*p < 0.05$, $**p < 0.01$.

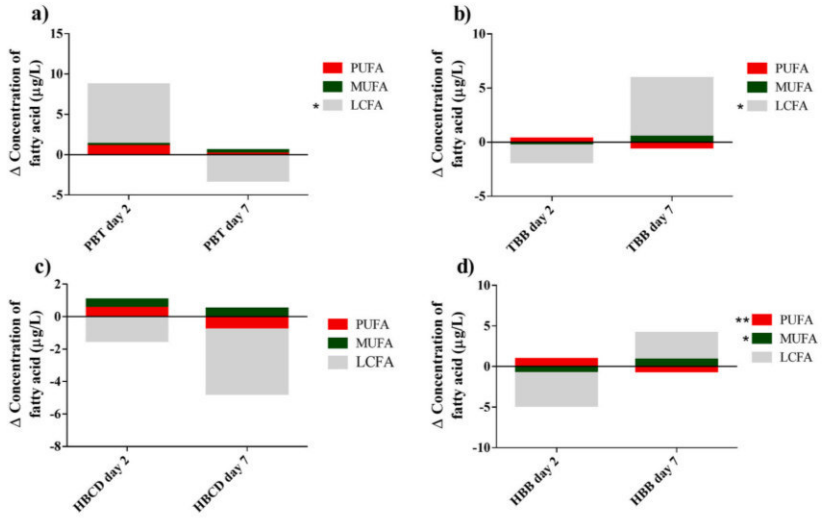


Fig. 6. Effects of BFRs in fatty acid composition of HepG2 medium. Variation (between DMSO control and BFRs) of the concentration of LCFA, MUFA and PUFA for a) PBT, b) TBB, c) HBCD and d) HBB. Columns correspond to variation of the concentration of fatty acids. Significantly differences, $*p < 0.05$, $**p < 0.01$.

decrease of PUFA ($p = 0.008$) concentrations in the medium between day 2 and day 7 (Fig. 6d).

3.1.8. Gene expression analysis

Cells were chronically exposed to each studied BFR in a concentration of 250 nM. The effects of BFR compounds tested on gene expression are described in Fig. 7. PBT, TBB and HBB showed a slight tendency to decrease PPARα expression (Fig. 7a). PBT and HBCD appear to increase PPARγ gene expression, with a significant difference for PBT ($p = 0.01$), while TBB ($p = 0.02$) and HBB decrease PPARγ gene expression Fig. 7b. HBCD demonstrated a tendency to decrease C/EBPα gene expression (Fig. 7c). Regarding the expression of PGC-1α, TBB seems to decrease and HBCD appears to slightly increase gene expression (Fig. 7d).

4. Discussion

BFRs implication in lipid metabolism and their ability to cause metabolic dysfunction were investigated in this study. For that purpose, two cell lines where used: a pre-adipocyte cell line to explore the possible lipid accumulation in adipose tissue and a hepatocyte cell line to investigate the effects of this excessive lipid accumulation in other tissues, in this case the liver. HBB exposure resulted in a significantly

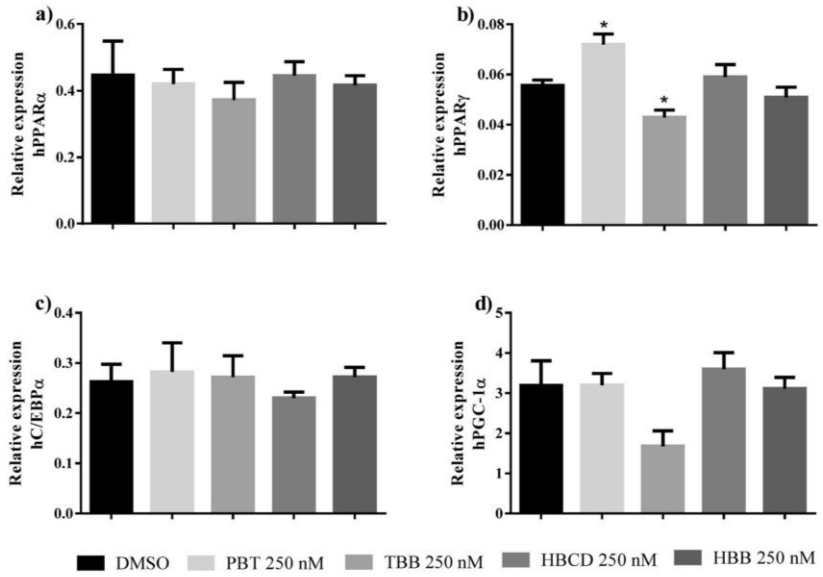


Fig. 7. Effects of chronic exposure of hepatocytes to BFR compounds on gene expression, a) PPARα, b) PPARγ, c) c/EBPα, d) PGC-1α. Cells were treated with 250 nM of each BFR. Gene expression is represented as total expression normalized with HPRT ± SEM. Significantly different from control (DMSO), $*p < 0.05$.

decrease of preadipocytes proliferation and increase in lipid accumulation (adipocytes differentiation). The capacity to promote adipocyte differentiation was described previously by other authors using PBDE 47 (Yang et al., 2018) and PBDE 99 (Armstrong et al., 2020). These findings may reflect a hypertrophy of the adipocytes (less adipocytes with more lipid accumulation). Moreover, adipocyte hypertrophy has been described as one of the main features of obesity and an early hypertrophy of these cells can be related to the impairment of mitochondrial function and increased oxidative stress (Baldini et al., 2021; Yang et al., 2018). At day 3 of adipocyte differentiation, all the BFR-treated cells had fewer fatty acids in the medium than control cells, suggesting that the cells were either internalizing more or releasing less. At the end of the differentiation (day 11), probably because of the excessive accumulation of fatty acids in the early stage of the differentiation or the use of medium glucose instead of fatty acids, HBCD and HBB-treated cells medium had more MUFA and LCFA compared to control cells, respectively. Next, we evaluate the modulative effect of the exposure to BFRs on adipocyte function key genes and confirm the outcome (lipid accumulation) performed using oil red *O* method. In accordance, gene expression at day 11 corroborated the adipocyte hypertrophy phenotype of HBB-treated cells, since a significant increased expression of IL-1β and decreased expression of PGC-1α and adiponectin were observed. IL-1β is expressed in response to stress and can indicate cell dysfunction, which can affect other organs as an inflammatory response. Hence, the increased production of adipokine IL-1β can be associated with development of insulin resistance and metabolic syndrome (Wood et al., 2009). Additionally, decreased adiponectin expression could indicate alterations in the endocrine function of the cell and their deficiency were described to be related to the predisposition to insulin resistance and diabetes (Kadowaki et al., 2006). Furthermore, this decrease of adiponectin expression could lead to a reduced activation of PPARs (α and β) (Kallwitz et al., 2008) and compromise the insulin signaling, contributing to insulin resistance. Indeed, TBB, HBCD and HBB-treated cells had a decrease in PPARγ gene expression, although not statistically significant. PPARγ is described to promote lipogenic pathways by stimulating fatty acid uptake (Baldini et al., 2021), justifying the alteration in lipid profile in the medium. PGC-1α expression is implicated in metabolic processes that maintain lipid balance and its decreased expression is linked to the decline of fatty acids transport and lipid metabolizing enzymes (Supruniuk et al., 2017). Its downregulation could impair lipids transport and lipids oxidation in the mitochondria, leading to lipid accumulation, stress and metabolic dysfunction. Similar to HBB-treated cells, cells treated with PBT showed an increase in IL-1β and a decrease in PGC-1α gene expression, alongside with a tendency to a decrease in adiponectin gene expression. In addition to these common pathways for disfunction, PBT also induced a significant increase in PPAR-α expression, responsible for the regulation of several critical genes for lipid and lipoprotein metabolism (Yoon, 2009).

When adipose tissue loses its ability to accommodate the excess of energy, by excessive lipid accumulation or other disruptive stimulus (e.g. endocrine disruptors), it becomes dysfunctional leading to ectopic accumulation (lipotoxicity) (Guilherme et al., 2008; Jorge-Galarza et al., 2016). This accumulation in other metabolic organs, as the liver, compromises the metabolic hemostasis contributing to obesity-related comorbidities (Wada et al., 2007).

Lipid accumulation on hepatocytes using HepG2 cell line after prolonged treatment with BFRs were analyzed. Apart from HBB, all other studied BFRs were able to induce lipid accumulation in hepatocytes. Interestingly, HBB that previously induced pronounced effects in lipid accumulation in adipocytes, showed no effect in hepatocytes. When functioning correctly, hepatocytes can metabolize lipids in the cell and/or include them in lipoproteins to be transported to other tissues. On the other hand, their dysfunction could lead to hepatic lipid accumulation with an increased toxicity to the cell (Geng et al., 2021). Some chemical pollutants considered EDs, such as BFRs, were previously reported to increase lipid accumulation (Hao et al., 2019; Huc et al., 2012; Sun et al., 2021; Xiang et al., 2018). Regarding hepatocytes findings of fatty acid medium composition, at day 7 HBB and HBCD decreased PUFA concentration comparing to the control and PBT decreased LCFA concentration in the medium from day 2 to day 7 of the treatment. This could be indicative of more internalization or less release of lipids by the treated cells. Moreover, PPARγ gene expression was increased after PBT treatment along with an increase in lipid accumulation. PPARs are involved in the regulation of lipid metabolism since lipids are biological PPAR ligands. Specifically, PPARγ is involved in insulin signaling pathways, increasing insulin sensitivity, triglyceride storage regulation and in promoting lipogenic pathways by stimulating fatty acid uptake (Baldini et al., 2021). Which suggests that the lipid accumulation in PBT-treated hepatocytes is mediated by PPARγ. Accordingly, co-treatment with PBT and oleic acid (rich lipid environment) led to a higher increase of lipid accumulation, probably by an increase in fatty acid uptake.

5. Conclusion

In conclusion, a disturbance in lipid metabolism caused by BFRs exposure was observed. HBB seems to act in lipid accumulation through adipocyte hypertrophy, while PBT, TBB and HBCD appear to have a higher impact in hepatocyte lipid accumulation. The present study provides evidence that BFRs, and more specifically NBFRs, could increase the risk of metabolic dysfunction of adipose tissue with impact on the liver metabolism. Hence, emphasizing the importance to continue investigating the effects of BFR on lipid metabolism, particularly the less studied ones as the NBFRs, either individually or in mixtures.

Author statement

Maria Luz Maia: Investigation, Formal analysis, Visualization and Writing – original draft. Sara Sousa: Investigation and Formal analysis. Diogo Pestana: Conceptualization; Methodology; Supervision; Validation and Writing – review & editing. Ana Faria: Conceptualization; Methodology; Supervision; Validation and Writing – review & editing. Diana Teixeira: Validation and Writing – review & editing. Cristina Delerue-Matos: Resources and Writing – review & editing. Valentina Fernandes Domingues: Validation and Writing – review & editing. Conceição Calhau: Conceptualization, Validation, Supervision, Resources and Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118639.

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Supplementary data

Impact of Brominated Flame Retardants on lipid metabolism: an *in vitro* approach

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Table S1
Fatty acids composition (µg/L) of the 3T3-L1 medium (DMEM).

Fatty acids		µg/L
SCFA	C12:0	0.26
	C14:0	1.35
	C15:0	0.60
	C16:0	12.01
	C17:0	0.47
	C18:0	14.77
	C20:0	0.18
MUFA	C16:1 n-7	1.18
	C18:1 n-9 c	8.37
PUFA	C18:2 n-6 c	1.78
	C20:3 n-6	0.70
	C20:4 n-6	1.96
	C20:5 n-3	0.46
	C22:6 n-3	1.48

Table S2
Fatty acid composition (µg/L) of 3T3-L1 medium after each cell treatment (0.1% DMSO, or 250nM of each BFRs) during 3T3-L1 differentiation.

		Day 3					Day 11				
Fatty acids/Cell treatment		DMSO	PBT	TBB	HBCD	HBB	DMSO	PBT	TBB	HBCD	HBB
SCFA	C4:0	ND	0.10	0.17	0.10	ND	0.16	ND	ND	0.13	0.14
	C8:0	0.15	0.07	0.10	0.09	0.13	0.24	0.21	0.16	0.17	0.12
LCFA	C14:0	0.67	0.65	0.67	0.66	0.71	0.83	0.78	0.79	0.72	0.74
	C15:0	0.57	0.45	0.39	0.41	0.41	0.55	0.50	0.48	0.67	0.57
	C16:0	8.39	7.12	7.89	7.75	7.87	8.84	8.74	8.10	9.23	9.61
	C17:0	0.38	0.32	0.31	0.32	0.35	0.45	0.45	0.47	0.50	0.47
	C18:0	9.42	3.92	10.62	8.86	4.82	8.89	5.85	9.34	6.27	15.21
MUFA	C16:1 n-7	1.05	0.92	0.92	0.96	1.05	1.21	1.13	0.98	1.22	1.13
	C18:1 n-9c	7.73	6.85	6.61	6.83	6.98	7.45	7.37	7.11	8.64	8.30
PUFA	C18:2 n-6 c	1.60	1.55	1.53	1.49	1.59	1.59	1.60	1.53	1.57	1.67

*ND-not detected

Table S3
Fatty acids composition (µg/L) of the HepG2 medium (MEME).

Fatty acids		µg/L
LCFA	C14:0	1.34
	C15:0	0.59
	C16:0	11.11
	C17:0	0.53
	C18:0	15.90
MUFA	C16:1 n-7	1.29
	C18:1 n-9 c	8.48
PUFA	C18:2 n-6 c	1.81
	C20:3 n-6	0.87
	C20:4 n-6	2.21
	C20:5 n-3	0.52
	C22:6 n-3	1.63

Table S4
Fatty acid composition (µg/L) of HepG2 medium collected 2 and 7 days after cells were treated at least four weeks at 0.1% DMSO or 250nM of each BFRs.

		Day 2					Day 7				
Fatty acids/Cell treatment		DMSO	PBT	TBB	HBCD	HBB	DMSO	PBT	TBB	HBCD	HBB
LCFA	C14:0	1.04	1.04	1.00	0.97	1.17	1.00	1.10	0.97	0.87	1.04
	C15:0	ND	0.60	0.67	0.73	0.67	0.60	0.60	0.68	0.82	0.51
	C16:0	10.34	10.38	10.21	10.48	9.74	8.83	8.92	8.53	9.44	8.40
	C18:0	10.83	17.59	8.63	8.61	6.39	14.69	6.77	15.78	5.35	13.90
MUFA	C16:1 n-7	1.36	1.41	1.35	1.50	1.47	1.21	1.33	1.08	1.03	1.06
	C18:1 n-9 c	7.14	7.38	7.06	7.51	6.52	6.72	6.79	7.11	7.18	7.48
PUFA	C18:2 n-6 c	1.69	2.12	1.72	1.83	1.54	1.87	2.01	1.67	1.59	1.55
	C20:4 n-6	2.09	1.99	1.67	1.91	2.60	2.18	1.94	1.79	1.64	1.67
	C22:6 n-3	1.46	1.41	1.42	1.27	1.23	1.05	0.96	0.84	0.90	1.02

*ND-not detected

Chapter 4

Fatty acids and minerals composition of Portuguese coast shrimps

Minerals and fatty acids profile of Northwest Portuguese Coast Shrimps

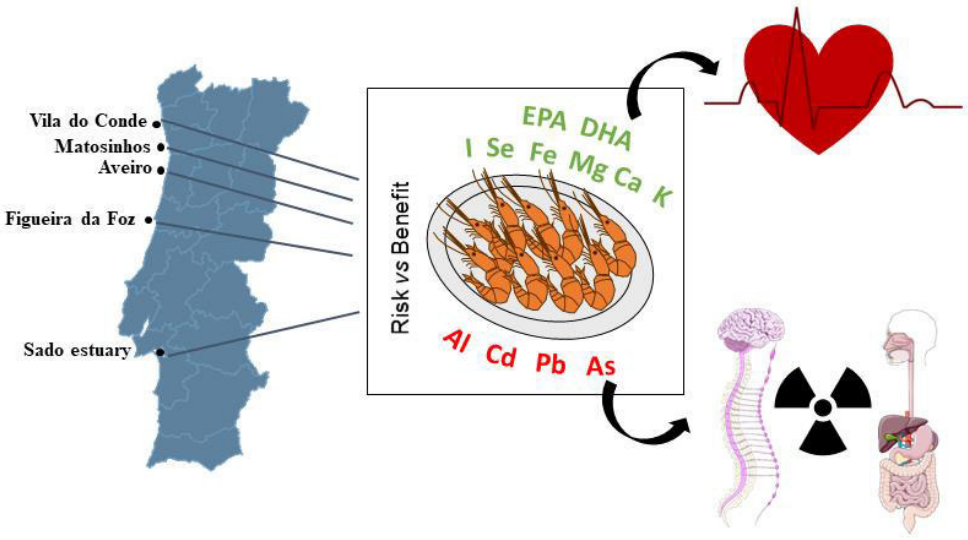
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Minerals and fatty acids profile of Northwest Portuguese Coast Shrimps

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In press



Statement of contribution

The contribution of the candidate, Maria Luz Maia, in this work includes the accomplishment of sample preparation, GC-FID, ICP-MS and FAAS determinations, data analysis and written of the original draft.

Minerals and fatty acids profile of Northwest Portuguese Coast Shrimps

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Highlights

Two shrimp species from the Northwest Portuguese coast were analyzed

Shrimp species analyzed presented low IA, IT, HH and ω-6/ω-3 ratios

Shrimp can be a good dietary source of EPA + DHA, Cu, Se, Fe, Mg, Na, K, Ca, I and Cr

Abstract

Two shrimp species were sampled in different seasons and locations on Portuguese coast. Their fatty acid content and 28 essential, probably essential and potentially toxic/non-essential elements were quantified. A 100 g serving of shrimp muscle from the Portuguese coast provides 78% of the recommended daily intake. Both species presented low ω-6/ω-3 ratio, 0.15 for *P. serratus* and 0.23 for *P. varians,* respectively. The quality indices presented also good values for shrimp samples, hypocholesterolemic/hypercholesterolemic ratio (HH) was 2.60 for *P. serratus* and 2.55 for *P.varians*. The index of thrombogenicity (IT) were 0.24 and 0.25 for *P. serratus* and *P. varians*, respectively and index of atherogenicity (IA) was 0.41 and 0.43 for *P. serratus* and *P. varians*, respectively. The Mg, Na, K, Ca, I, Cr, Cu and Se in shrimp samples analyzed can provide more than 5% of the Recommended Dietary Allowance (RDA)/ Adequate Intake (AI). The best nutritional Ca/Mg ratio was found in *P. serratus* (1.7). Portuguese shrimp has proven to be a good dietary source of EPA, DHA and some essential elements, also Target Hazard Quotient (THQ) and risk-benefit evaluation shown that there is no significant danger associated with the consumption of Portuguese shrimp.

Keywords

Shrimp; fatty acids; trace elements; macro elements; daily intake; risk assessment; risk-benefit ratio.

1. Introduction

Seafood is highly appreciated and indicated as part of a balanced human diet, being consumed all over the world and considered high quality food. The seafood market has grown over the years, making seafood an important source of nutrients for the human population. Shrimp is one of the most consumed crustaceans worldwide, these can be filter feeders and a food source for superior animals and can be a healthy complement to human diet (Aguzzi et al., 2005; Mercogliano et al., 2020).

According to available data from the Food and Agriculture Organization (FAO) database, a total of 10,624,124 tons of shrimp were consumed in 2018, compared to the 9,357,845 tons in 2014 (FAO, 2021). The amount consumed per capita in the world was 1.3 kg in 2014 and 1.4 kg in 2018. Data for Portugal show a greater growth in shrimp consumption between 2014 and 2018, specifically from 1.8 kg to 2.5 kg/per capita (FAO, 2021). With regard to the capture of shrimp species in Portugal, in 2019 it reached 106 tons. This amount provided revenues of 3,292 thousand euros (INE, 2020), highlighting the importance of shrimp in the global economy.

Shrimp is mainly composed of a large amount of water (approximately 75%) and protein (approximately 80% of the dry weight) (Dayal et al., 2013). The fat and calorie content are very low. The very low lipid content can be divided into phospholipids (65-70%), cholesterol (15-20%) and total acylglycerols (10-20%). Furthermore, 32% of total lipids are polyunsaturated fatty acids (PUFA), of which about 64% are ω-3 PUFA and 33% are ω-6 PUFA. These values usually indicate a high nutritional quality. The ω-3 PUFA component is mainly represented by eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (AlFaris et al., 2022; Dayal et al., 2013). The PUFA/SFA and ω-6/ω-3 ratios are the most used indicators to assess the nutritional value of foods (Chen and Liu, 2020). Shrimp is also a good source of essential nutrients, such as B-complex vitamins, vitamin A and E, astaxanthin, macrominerals (phosphorus and calcium), and trace elements (selenium, copper, zinc and iodine) (Dayal et al., 2013). According to the World Health Organization (WHO), trace elements can be classified into three groups (WHO, 1996): essential elements, probably essential elements and potentially toxic elements.

Although shrimp is a good source of nutrients, they can also accumulate potentially toxic trace elements from the surrounding environment. Shrimps can be found close to the seafloor of most coasts and estuaries. Aquatic ecosystems have been threatened over the years by the input of contaminants, which leads to a decrease in the quality of the aquatic

environment and the diversity of species, in particular of economically relevant species (Nascimento et al., 2016). Even though trace elements are naturally occurring hydrosphere constituents, human actions, over time, changed the geochemical cycle, which increases their environmental concern. Some trace elements are required in small amounts as components of enzymes vital for healthy biota development, but these can also be toxic in high concentrations (Silva et al., 2016).

Heavy metals are a group of trace pollutants that can accumulate in aquatic organisms, such as shrimp, and be bioconcentrated along the food chain, reaching humans through their diet and posing health risks (Araújo et al., 2016; Di Beneditto et al., 2022). Over time, heavy metals can accumulate in organs such as the kidney and liver. Lead and cadmium usually target the kidneys and nervous system. Consumption of foods with high levels of arsenic can lead to loss of brain function, paralytic syndrome, cardiovascular collapse and increased risk of some cancers (Gheysari and Rahimi, 2015). In addition, the International Agency for Research on Cancer (IARC) has classified cadmium and arsenic as Group 1 – carcinogenic to humans (IARC, 2012, 2011) and lead as Group 2A - probably carcinogenic to humans (IARC, 2006). In light of this, the European Union has set maximum levels of heavy metals in food. For crustaceans, the maximum level for cadmium and lead is 0.5 mg/kg wet weight (ww) (European Commission, 2001).

This study aimed to characterize the fatty acid profile and the levels of essential, probably essential, and potentially toxic/non-essential elements of two shrimp species, *Palaemon serratus* and *Palaemon varians*, from the NW Portuguese Coast. In addition, the contribution of shrimp consumption to the daily intake of several essential and probably essential elements was estimated and a risk assessment and risk-benefit assessment was carried out for the consumption of shrimp by the Portuguese adult population with regard to potentially toxic/non-essential elements. The monitoring of these parameters in terms of environment and human health is of particular importance and to our knowledge, this is the first study at least in the last decade that performed these analyses in the edible portion of *P. serratus* and *P. varians* species in a wide geographical and temporal space in Portuguese coast.

2. Material and Methods

2.1 Chemicals and solutions

Dichloromethane and n-hexane (99%) were purchased from Merck (Darmstadt, Germany) and methanol from VWR Chemicals Prolabo (Fontenay-sous-Bois, France). Sodium

hydroxide and anhydrous sodium sulfate were obtained from Pronalab (Lisbon, Portugal). Sodium chloride (99.5%) was acquired from Panreac (Barcelona, Spain). Butylated hydroxytoluene (BHT) (≥ 99%), boron trifluoride-methanol (BF_3) (14% in methanol), and 37 Component FAME (fatty acid methyl esters) Mix were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Internal standard, Tridecanoic acid (C13:0) was from Fluka (Buchs, Switzerland).

Ultrapure water resistivity of 18.2 MΩ cm, was from a Simplicity 185 water purification system (Millipore, Molsheim, France). Standard solutions of Na, K, Ca and Mg and lanthanum nitrate were from Panreac Química SA (Barcelona, Spain) and 65% HNO_3 was from Sigma-Aldrich (Steinheim, Germany). Ammonium hydroxide solution (ACS reagent, 28-30% NH_3 basis) was acquired from Fluka (Seelze, Germany). Tetramethylammonium hydroxide, TMAH 25% w/w was from Alfa Aesar (Karlsruhe, Germany). The multi-element standard solution used to prepare Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) calibration solutions was PlasmaCAL SCP-33-MS, from SCP Science (Baie-D'Urfé, Quebec, H9X 4B6, Canada), and the internal standards solution was ICP-MS-200.8-IS-1 solution (100 mg/L of ^{89}Y, ^{141}Pr, ^{159}Tb and ^{175}Lu), obtained from AccuStandard® (New Haven, Connecticut, USA). Tellurium (^{125}Te) isotope were from Sigma-Aldrich (St. Louis, Missouri, USA).

Dogfish liver (DOLT-4) and skimmed milk powder (ERM®-BD151) certified reference materials were obtained from the National Research Council Canada (Ottawa, Ontario, Canada) and Institute for Reference Materials and Measurements (Geel, Belgium), respectively.

2.2 Sampling

P. serratus samples were gathered along the NW Portuguese coast (namely in Figueira da Foz, Ria de Aveiro, Aveiro, Matosinhos and Vila do Conde) by local fishermen. *P. varians* samples (wild and of aquaculture origin) were gathered in the Sado estuary. At each location a total of 1 kg of shrimp was collected, the size ranged between 4 - 9 cm and 2 - 4 cm for *P. serratus* and *P. varians*, respectively. Sampling was carried out in autumn and spring between 2017 and 2019, according to the period allowed for the capture (DRE, 2000). The shell was separated from the edible portion of the shrimp, and shrimp with eggs were stored separately. Thirty-two samples were stored at -80 ºC until analysis.

2.3 Total lipid content and fatty acids composition

The total lipid content (TLC) of shrimp samples was extracted by the Folch procedure (Folch et al., 1957), using dichloromethane instead of chloroform as previously described (Ozório et al., 2012). For extraction, 1 g of each shrimp sample was used. The TLC evaluation was performed in duplicate.

The lipid residue obtained was then dissolved in 3 mL of dichloromethane, and the fatty acid profile of shrimp was determined according to base-catalyzed transesterification method (Bondia-Pons et al., 2007), and sodium methoxide (NaOMe) was used as a catalyst to form FAME (Sousa et al., 2018). C13:0 was used as an internal standard.

2.3.1 Gas Chromatography-Flame Ionization Detection (GC-FID)

Analyses were carried out using a GC-2010 gas chromatograph from Shimadzu (Kyoto, Japan) equipped with an FID, a Shimadzu AOC-20i autoinjector and An Agilent J&W CP-Sil 88 capillary column (100 m x 0.25 mm I.D., 0.20 µm film thickness). The supplementary material describes the operating conditions.

2.3.2 Nutritional Quality Indices

The nutritional quality of the fatty acids was evaluated taking into account the following indices: index of atherogenicity (IA), index of thrombogenicity (IT), and hypocholesterolemic/hypercholesterolemic ratio (HH), calculated according to the respective equations indicated below (1) – (3). In addition, Polyunsaturated/Saturated Fatty Acid (PUFA/SFA) and ω-6/ω-3 ratios were also calculated (Chen and Liu, 2020).

$$IA = \frac{C12{:}0 + 4 \times C14{:}0 + C16{:}0}{\Sigma MUFA + \Sigma\omega 3 + \Sigma\omega 6} \qquad (1)$$

$$IT = \frac{C14{:}0 + C16{:}0 + C18{:}0}{0.5 \times MUFA + 0.5 \times n6PUFA + 3 \times n3PUFA + n3/n6} \qquad (2)$$

$$HH = \frac{cis\ C18{:}1 + \Sigma PUFA}{C12{:}0 + C14{:}0 + C16{:}0} \qquad (3)$$

2.4 Macrominerals and trace element determination

2.4.1 Microwave digestion

Microwave-assisted wet digestion of shrimp samples was performed using a Microwave Accelerated Reaction System (MARS-X, 1500; from CEM, Matthews, NC, USA) equipped

with a 14-position carousel and pressure and temperature sensors. Approximately 0.5 g of each shrimp sample was weighed into a Teflon microwave vessel, and 10 mL of HNO3 was added. Then, the microwave-assisted digestion was run at 1200 W, with a ramp rate of 5 °C/min to 50 °C and a 10 min hold; then ramped at 10 ºC/min to 100 ºC with a 10 min hold; and finally ramped at 10 ºC/min to 150 ºC with a 15 min hold. During the digestion procedure, temperature and pressure were checked in a control vessel. After the microwave-assisted digestion and cooling, 2,5 mL of the sample solution was immediately added to 20 mL of ammonium hydroxide:water (1:1) mixture, for iodine analysis (Lawrence H. P. et al., 2013). All samples were analyzed in duplicate. All material used for ICP-MS analyses were soaked in 10% HNO3 for 24 h, washed with ultra-pure water and dried prior to use.

2.4.2 Sample solutions analysis

The macrominerals Na, Mg, K and Ca were determined by flame atomic absorption spectrometry (FAAS) using a AAnalyst™ 200 spectrometer (PerkinElmer, Waltham, MA, USA), as described by (Cabrita et al., 2016).

Trace-elements were determined by ICP-MS using an iCAP Q (Thermo Fisher Scientific, Waltham, MA, USA), using the operational parameters described by (Cabrita et al., 2016; Leite et al., 2017). ^{7}Li, ^{27}Al, ^{47}Ti, ^{51}V, ^{52}Cr, ^{55}Mn, ^{57}Fe, ^{59}Co, ^{60}Ni, ^{65}Cu, ^{66}Zn, ^{75}As, ^{82}Se, ^{85}Rb, ^{88}Sr, ^{107}Ag, ^{111}Cd, ^{118}Sn, ^{121}Sb, ^{133}Cs, ^{137}Ba, ^{208}Pb and ^{209}Bi isotopes were monitored for analytical determination, with ^{89}Y, ^{141}Pr, ^{159}Tb and ^{175}Lu isotopes as internal standards. For iodine determination, the ^{127}I isotope was monitored, with ^{125}Te as internal standard.

2.4.3 Method validation

The method detection limit (MDL) and method quantification limit (MQL) were estimated from 8 measurements of the digestion blanks.

Certified reference materials (DOLT-4 and ERM®-BD151) were used for method performance control. The certified materials (0.5 g) were digested and analyzed simultaneously with the shrimp samples and under the same conditions.

2.4.4 Contribution to recommended element intake

The percent daily value (%DV) of essential and probably essential elements resulting from shrimp consumption was determined based on the content of the element in the samples, a serving dose of 100 g of shrimp and the Recommended Dietary Allowance (RDA) or

Adequate Intake (AI) values (NIH, 2021) for adult men with 31-50 years old, weighing 70 kg. The RDA or AI are presented in Table 1.

Table 1. RDA and AI values for 11 essential and probably essential elements.

Element	Mg	Na	K	Ca	I	Fe	Cr	Cu	Mn	Se	Zn
RDA or AI (mg/day)	420	1500*	3400*	1000	0.15	8	0.035*	0.90	2.30*	0.055	11

*AI values

2.4.5 Risk assessment

The Target Hazard Quotient (THQ) values were calculated using the equation 4, described previously (Zhu et al., 2016).

$$\text{THQ} = \frac{C \times (Ef \times Ed \times F)}{W \times T \times RfD} \times 10^{-3} \qquad (4)$$

Describing the formula, C is the mean of potentially toxic element content in shrimp (mg/kg); Ef is the exposure frequency (52 days/year, estimated consumption once a week); Ed is the exposure duration (80 years, average lifetime expectancy Portugal); F is the amount of shrimp ingested (28.8 g/person/day, the maximum reported value (Jacobs et al., 2017); W is the mean body weight of Portuguese adults (71.68 kg, (Jacobs et al., 2017)); T is the mean exposure time for noncarcinogens (365 days/year x number of years of exposure, 80 years in this study); R*f*D is the reference dose for oral exposure (USEPA, 2021); and 10^{-3} is the unit conversion factor (Zhu et al., 2016).

2.4.6 Risk-benefit assessment

Hazard quotient for risk-benefit ratio was expressed as the dimensionless hazard quotient HQEFA. The values were calculated using the equation 5, described previously (Gladyshev et al., 2009; Kosker, 2020).

$$\text{HQEFA} = \frac{(Refa \times C)}{(Cf \times RfD \times W)} \times 10^{-3} \qquad (5)$$

Describing the formula, Refa is the recommended daily dose of essential fatty acids (EPA + DHA) (mg/day); C is the mean of potentially toxic element content in shrimp (mg/kg); Cf is the mean amount of essential fatty acids (EPA + DHA) found in shrimp (mg/100 g); R*f*D is the reference dose for oral exposure (USEPA, 2021); W is the mean body weight of

Portuguese adults (71.68 kg, (Jacobs et al., 2017)); and 10^{-3} is the unit conversion factor. The Refa value used was 250 mg per day (EFSA, 2012). For the calculations described in section 2.4.5 and 2.4.6, the values obtained for all the elements analyzed were used directly, with exception of As, for which a conversion factor was used. Particularly marine species are well-known to contain high concentrations of As, and shellfish is described to contain a maximum level of inorganic As of about 6% of the total As (EFSA, 2009). This factor is relevant since organic As is not as toxic as inorganic forms of As (Kosker, 2020). For As calculations, the inorganic As (the toxic form) was assumed to be 6% of the total concentration found (EFSA, 2009).

2.5 Statistical analysis

Results were obtained from at least duplicates. Data were expressed as mean ± standard deviation (SD). The statistical significance of the difference between two groups was assessed by nonparametric Mann-Whitney test. Kruskal–Wallis one-way analysis of variance was used to compare differences between more than two groups. To evaluate the correlation between the elements and fatty acids content, the Pearson correlation coefficient was used. Statistical analysis was performed using SPSS Inc., version 22.0 (SPSS Inc., Chicago, IL, USA). Differences between means were considered significant when $p < 0.05$. For statistical calculations, results below the MDL or MQL were assumed as MDL/√2 or MQL/√2, a commonly used procedure for imputing these results (Hornung and Reed, 1990).

3. Results and discussion

Data describing the detailed fatty acid profile (mg/100g) for the two shrimp species from different locations are reported in Tables S1 – S3. The fatty acid ratios and the lipid quality indices are presented in Table 2. The main fatty acids found in shrimp muscle tissue were palmitic (C16:0), stearic (C18:0), oleic (C18:1 n-9 c), EPA (C20:5 n-3) and DHA (C22:6 n-3) acids (Tables S1-S3), a prevalence similar to that was observed in a previous work (Pires et al., 2018).

Table 2. Fatty acids (mg/100g ± SD) and quality indices in *P. serratus* and *P. varians* samples; E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		ΣSFA	ΣMUFA	ΣPUFA	Σω3	Σω6	PUFA/SFA	ω6/ω3	IA	IT	HH
Palaemon serratus	Vila do Conde	Autumn 2017	158.3 ± 0.4	90.4 ± 0.3	204.2 ± 0.7	182.4 ± 0.6	21.8 ± 0.1	1.29	0.12	0.43	0.24	2.45
		Spring 2018	183 ± 16	114 ± 7	265 ± 17	225 ± 14	40 ± 3	1.45	0.18	0.38	0.21	2.83
		Spring 2018 E	166 ± 11	111 ± 10	228 ± 22	192 ± 18	36 ± 3	1.37	0.19	0.38	0.23	2.81
		Autumn 2018	186 ± 13	112 ± 9	223 ± 16	190 ± 13	33 ± 3	1.20	0.17	0.42	0.26	2.54
		Spring 2019	164 ± 6	93 ± 4	217 ± 17	189 ± 15	28 ± 2	1.32	0.15	0.43	0.24	2.51
		Spring 2019 E	158.0 ± 0.5	114 ± 6	222 ± 23	192 ± 21	30 ± 2	1.40	0.16	0.38	0.22	2.80
	Matosinhos	Autumn 2017	263 ± 61	157 ± 35	348 ± 54	317 ± 49	31 ± 6	1.32	0.10	0.42	0.23	2.56
		Spring 2018	177 ± 4	111 ± 4	264 ± 4	225 ± 3	39.2 ± 0.7	1.49	0.17	0.37	0.21	2.93
		Spring 2018 E	154 ± 39	109 ± 29	212 ± 56	176 ± 48	36 ± 8	1.38	0.20	0.38	0.23	2.86
		Autumn 2018	154 ± 2	72 ± 2	189 ± 12	167 ± 11	22 ± 1	1.23	0.13	0.44	0.25	2.48
		Spring 2019	184 ± 38	106 ± 24	254 ± 54	222 ± 47	32 ± 7	1.38	0.14	0.41	0.23	2.65
	Aveiro	Spring 2018	319 ± 30	195 ± 15	435 ± 38	367 ± 33	68 ± 5	1.36	0.18	0.40	0.24	2.64
		Autumn 2018	154 ± 14	66 ± 6	189 ± 20	159 ± 17	30 ± 3	1.22	0.19	0.43	0.25	2.46
		Spring 2019	130 ± 15	72 ± 10	167 ± 26	147 ± 23	20 ± 3	1.28	0.14	0.42	0.25	2.57
	Ria de Aveiro	Autumn 2017	140 ± 4	51 ± 1	189 ± 3	164 ± 3	24.91 ± 0.02	1.35	0.15	0.47	0.24	2.29
		Spring 2018	143 ± 42	62 ± 20	190 ± 57	164 ± 49	26 ± 7	1.33	0.16	0.44	0.24	2.40
		Spring 2018 E	308 ± 4	165 ± 3	365 ± 5	307 ± 5	58.2 ± 0.1	1.19	0.19	0.46	0.27	2.23
	Figueira da Foz	Autumn 2017	213 ± 34	104 ± 15	251 ± 42	225 ± 38	26 ± 4	1.18	0.12	0.46	0.26	2.28
		Spring 2018	141 ± 2	86 ± 17	191 ± 6	170 ± 5	21.6 ± 0.9	1.35	0.13	0.40	0.22	2.78
		Spring 2018 E	237 ± 40	149 ± 28	282 ± 48	243 ± 41	38 ± 7	1.19	0.16	0.41	0.25	2.59
		Autumn 2018	160 ± 8	97 ± 7	225 ± 18	197 ± 16	28 ± 2	1.40	0.14	0.39	0.22	2.78
		Spring 2019	155 ± 36	83 ± 21	192 ± 48	170 ± 42	22 ± 5	1.24	0.13	0.43	0.25	2.46
Palaemon varians	Sado	Autumn 2017 A	197 ± 15	71 ± 7	265 ± 17	223 ± 15	42 ± 2	1.35	0.19	0.41	0.21	2.73
		Spring 2018 A	183 ± 43	98 ± 23	247 ± 58	213 ± 50	34 ± 7	1.35	0.16	0.39	0.21	2.74
		Spring 2018 A E	237 ± 12	117 ± 7	262 ± 16	228 ± 14	34 ± 2	1.11	0.15	0.45	0.25	2.35
		Autumn 2018 A	176 ± 31	88 ± 19	233 ± 49	173 ± 37	61 ± 12	1.32	0.35	0.45	0.25	2.54
		Spring 2019 A	258 ± 12	12 ± 8	244 ± 18	195 ± 14	49 ± 4	0.94	0.25	0.49	0.32	2.11
		Autumn 2017 W	182 ± 30	92 ± 16	227 ± 45	179 ± 37	48 ± 8	1.25	0.27	0.39	0.24	2.85
		Spring 2018 W	158 ± 23	76 ± 13	198 ± 28	172 ± 24	26 ± 4	1.26	0.15	0.39	0.21	2.77
		Spring 2018 W E	262 ± 24	123 ± 12	298 ± 40	258 ± 35	40 ± 5	1.14	0.16	0.41	0.23	2.57
		Autumn 2018 W	216 ± 6	96.5 ± 0.8	308 ± 6	226 ± 5	82 ± 1	1.42	0.36	0.43	0.24	2.69
		Spring 2019 W	159 ± 20	72 ± 9	163 ± 12	130 ± 9	33 ± 3	1.02	0.25	0.49	0.31	2.19

The elements analyzed in the 32 shrimp samples were divided into three classes: essential elements (Ca, Na, K, Mg, Cr, Fe, Co, Cu, Zn, Se, I); probably essential elements (V, Mn, Ni); and potentially toxic/non-essential elements (Li, Al, Ti, As, Rb, Sr, Ag, Cd, Sn, Sb, Cs, Ba, Pb, Bi). The validation of the methodology is presented in Tables S4 and S5, results are presented in Table S6 (essential macrominerals), Table S7 (essential trace elements), Table S8 (probably essential trace elements) and Table S9 and S10 (potentially toxic/non-

essential trace elements) of the supplementary material. Results were expressed as mg/kg ww.

3.1 Sampling sites along the NW Portuguese coast

To compare the samples from different sampling sites Kruskal-Wallis one way test was performed and significant differences were observed for Cu, Fe, Zn, V, Mn, Co, I, Ca Li, Al, Ti, Sr, Ag, As, Pb, Sb, Cs, Ba and Bi ($p < 0.001$), for DHA, Cr, Ni, Na and Cd ($p < 0.01$) and for Σω-6, Rb and Sn ($p < 0.05$). For EPA (C20:5 n-3) DHA (C22:6 n-3), SFA, Σmonounsaturated fatty acids (MUFA), ΣPUFA and Σω-3 the highest values were found in samples from Aveiro, whereas the highest content of Σω-6 was found in the samples from Sado estuary (Table 2).

For the essential macrominerals (Table S6) it was observed that in shrimp samples the Mg content was higher in Figueira da Foz (334 mg/kg). The Na and Ca contents were higher in the Sado estuary samples (3350 and 2100 mg/kg, respectively), and the K content was higher in Figueira da Foz (5310 mg/kg). Regarding essential trace elements (Table S7), Cr was found above MQL only in samples from the Sado estuary (0.10 – 0.14 mg/kg). For Fe, Co, Cu, Zn and Se, the values were also higher in the Sado estuary (63 mg/kg, 0.035 mg/kg, 13 mg/kg, 11.9 mg/kg and 0.247 mg/kg, respectively), for I the higher value was found in Vila do Conde (0.48 mg/kg). Regarding probable essential elements (Table S8), for V, Mn and Ni, the highest values were found in the samples from the Sado estuary (0.21 mg/kg, 1.33 mg/kg and 0.19 mg/kg, respectively). Concerning potentially toxic/non-essential elements (Tables S9 – S10), higher contents of Li, Al, Ti, Sr and Cd were observed in the aquaculture samples from Sado estuary (0.14 mg/kg, 90 mg/kg, 10.7 mg/kg, 32 mg/kg and 0.006 mg/kg, respectively), whereas Ba and Pb contents were higher in wild samples from Sado estuary (0.3 mg/kg and 0.087 mg/kg, respectively); As, Ag and Cs contents were higher in samples from Figueira da Foz (11.2 mg/kg, 0.09 mg/kg and 0.018 mg/kg, respectively); Rb and Bi were higher in samples from Aveiro (1.362 mg/kg and 0.033 mg/kg, respectively); and Sn and Sb were higher in samples from Ria de Aveiro (0.021 mg/kg and 0.005 mg/kg, respectively).

3.2 Shrimp species comparison (*P. serratus* vs *P. varians*)

In order to compare the samples from the two shrimp species analyzed, nonparametric Mann-Whitney test was performed. The two shrimp species studied showed the same mean concentration of TLC of 1.3 g/100g (Table S11), a value within the previously reported range

for TLC in shrimp samples: 0.5 – 1.9 g/100g (Li et al., 2011). Comparing the mean value for DHA, this was significantly higher in *P. serratus*, while the mean value for Σω-6 was higher in *P. varians* ($p < 0.01$) presented in Figure S1. Regarding the essential trace elements, *P. varians* samples (Figure 1) presented significantly higher mean content of V, Cr, Mn, Fe, Co, Ni, Cu, Zn, I, Na and Ca.

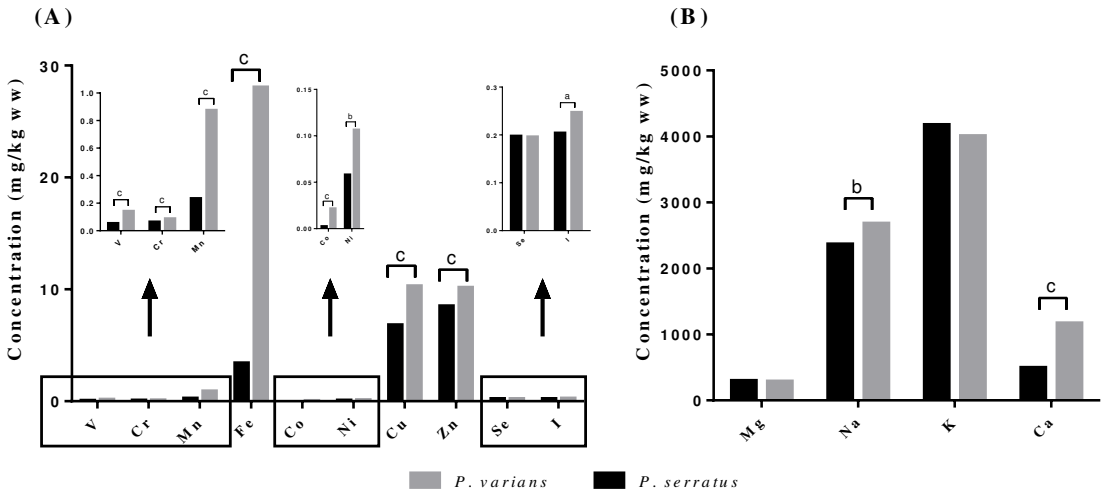


Figure 1. Concentration of essential elements in the two shrimp species *P. serratus* and *P. varians*. Essential trace elements (A), Macro-minerals (B). Different letters (a, b and c) in the bars of each element indicate significant differences between the two shrimp species at $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively.

P. varians is the shrimp species from the Sado estuary, the sampling site with higher values of most of these elements. Therefore, it is possible to assume that the differences in the element content may come from the habitat rather than the species itself. Potassium was the most abundant macro-mineral in the two shrimp species analyzed. The mean concentration of essential and probably essential elements (Figure 1) followed the order of K > Na > Ca > Mg > Zn > Cu > Fe > Mn > I > Se > Cr > Ni > V > Co for *P. serratus* and K > Na > Ca > Mg > Fe > Cu > Zn > Mn > I > Se > V > Ni > Cr > Co for *P. varians*. The difference in the order of the elements between the two species was the position of Zn, Fe, Cr and V. Regarding potentially toxic/non-essential elements, As, Cd, Ag, Sb, Cs and Bi were found in significantly higher levels in *P. serratus* samples, while *P. varians* showed significantly higher levels of Li, Ba, Al, Ti and Sr (Figure 2). The mean concentration of potentially toxic/non-essential elements in shrimp samples followed the order As > Sr > Ti > Al > Rb > Ag > Ba > Li > Pb > Bi > Cs > Sn > Sb > Cd for *P. serratus* samples, while for *P. varians* the order was Al > Sr > Ti > As > Rb > Ba > Pb > Ag > Li > Bi > Cs > Sn > Cd > Sb.

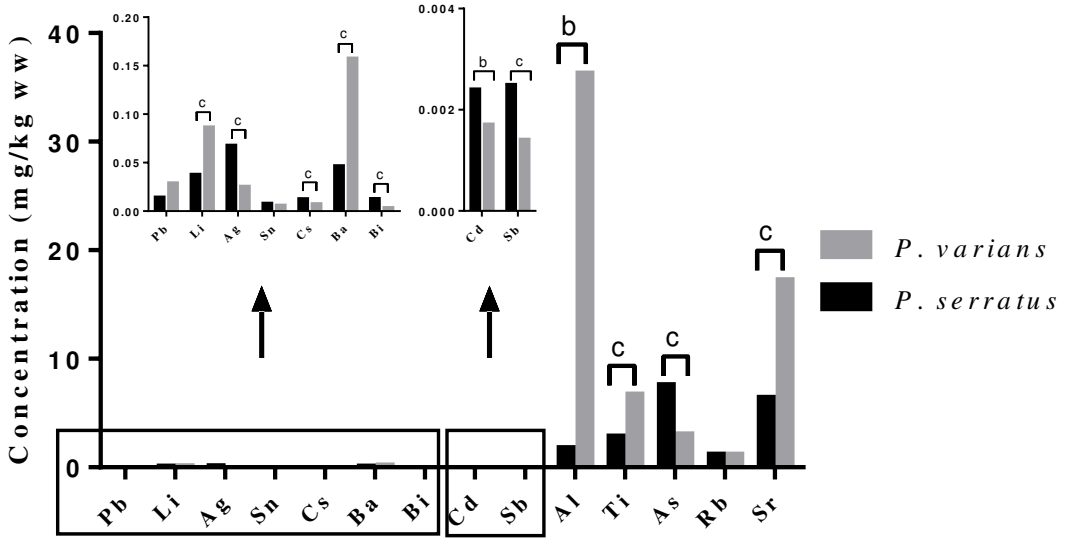


Figure 2. Levels of non-essential/toxic elements in the two shrimp species *P. serratus* and *P. varians*. Different letters (b and c) in the bars of each element indicate significant differences between the two shrimp species at $p < 0.01$ and $p < 0.001$, respectively.

3.3 Sampling seasons comparison (Spring vs Autumn)

Regarding the differences between spring and autumn, the statistical analysis was performed by nonparametric Mann-Whitney test, the ΣMUFA content (Table 2) was higher in spring samples ($p < 0.05$). Within species, for *P. varians* samples, the Σω-6 content was significantly higher in autumn ($p < 0.01$), while for *P. serratus* the Σω-6 content was significantly higher in spring ($p < 0.05$). When comparing the shrimp samples with and without eggs (found only in spring), it was found that ΣSFA, ΣMUFA ($p < 0.001$) and Σω-6 ($p < 0.05$) contents were higher in samples with eggs. As previously described, shrimp eggs are rich in C16:0, C18:0, C16:1 n-7, C18:1 n-9 c and C18:2 n-6 (Narciso and Morais, 2001). In our study, these fatty acids were significantly higher in the shrimp samples with eggs ($p < 0.05$, $p < 0.01$, $p < 0.01$, $p < 0.01$ and $p < 0.05$, respectively) contributing to the higher ΣSFA, ΣMUFA and Σω-6 values. Regarding essential and probably essential elements (Tables S6 – S8), significant differences were observed for Cu, Se and K. Cu and Se mean values were higher in spring samples, while K was higher in autumn samples ($p < 0.01$). Higher mean content was observed in spring for Zn ($p < 0.05$) and in autumn for V ($p < 0.01$). Regarding the presence of eggs in the shrimp, Cu was higher in shrimps with eggs, while K was higher in shrimps without eggs, in agreement with the results for Cu and K reported for the different seasons. Regarding potentially toxic/non-essential elements, *P. serratus* samples presented significant differences for As, Rb, Sn and Bi. As and Bi mean content

were higher in spring samples ($p < 0.001$ and $p < 0.05$, respectively), while Rb and Sn were higher in autumn ($p < 0.05$). Concerning *P. varians* samples, As, Cd and Rb presented significant differences between seasons. As and Rb mean content were higher in spring ($p < 0.001$), while Cd was higher in autumn ($p < 0.05$). When considering the presence of eggs in shrimp samples, no significant differences were observed.

3.4 Elements comparison with literature

The results obtained in this study for essential and probably essential elements were compared with values from a national food composition database (INSA, 2019) and some previous studies (Biswas et al., 2021; Bogard et al., 2015; El Gendy et al., 2015; Gokoglu et al., 2008; Liu et al., 2017; Rao, K. R. P.; Sreeramulu and K.; Sreedhar, 2016; Shi et al., 2015) presented in Table S12. The content of the elements available for comparison (Fe, Zn, Cu and Mn) obtained in this work for *P. serratus* were well within the values reported for the same species (Gokoglu et al., 2008). Comparing the different species, some differences in the elements content were observed. These differences can be influenced by the habitat (sampling location). In general, the content values obtained in this study were within or close to the range reported for Mg, Fe, Zn, Se, Cu, Mn, I and Cr (Bogard et al., 2015; El Gendy et al., 2015; Gokoglu et al., 2008; INSA, 2019; Rao, K. R. P.; Sreeramulu and K.; Sreedhar, 2016; Shi et al., 2015).

The intake of Mg and Ca have showed potential benefits to prevent metabolic syndrome dysfunctions when the Ca/Mg is close to 2 (Rosanoff, 2010). In our study, the mean Mg content was similar between the two shrimp species, although the mean Ca content was higher in *P. varians* compared to *P. serratus* (Table S12). This is reflected in the Ca/Mg ratio, which was significantly higher ($p < 0.001$) in *P. varians* (4.1) compared to *P. serratus* (1.7). Based on these results, the best nutritional ratio exists in *P. serratus* (1.7).

For Na and K, the values obtained in this work were above the previously reported ones presented in Table S12. The Na content was higher than that reported in the National food database. These results are not a good nutritional finding due to the importance of reducing salt consumption. The biological interactions of Na and K are interesting because they play an important role in the development of hypertension and cardiovascular disease (CVD). The Na/K ratio (more than individual Na or K intake) has been associated with an increased risk of CVD (Cook et al., 2010). According to the AI values recommended for Na and K, a healthy ratio most be ≤0.6 (Cook et al., 2010). Regarding our results, the mean values of

the Na/K ratio were slightly below the recommended value (0.57) for *P. serratus* and slightly above (0.67) for *P. varians*, ($p < 0.001$). For Co, Ni and V, the values determined in this work were below the ones reported previously presented in Table S12. Except for K and Mg, all the other essential and probably essential elements analyzed presented higher concentrations in *P. varians* (Figure 1 and Table S12). Comparing the species origin (wild or aquaculture), the results are very similar. Both samples (wild and aquaculture) were collected in the Sado estuary. As the sampling sites in the Sado estuary were close to each other, perhaps the elements added to the water for shrimp aquaculture could reach to wild ones, which may explain why this specie had a higher content of almost all the essential elements analyzed.

Table S13 provides a comparison between the results obtained for the most studied potentially toxic/non-essential elements in our study and in previous reports (Di Beneditto et al., 2022; El Gendy et al., 2015; Giri and Singh, 2015; Gokoglu et al., 2008; Kaymacı and Altun, 2016; Olgunoglu, 2015; Raissy, 2016). The mean for As and Al contents obtained in this study were higher than previously reported values (Di Beneditto et al., 2022; Giri and Singh, 2015; Olgunoglu, 2015; Raissy, 2016). The release of Al and As to the aquatic environment can occur both by natural and anthropogenic ways. Regarding the content of Sr, Cd and Sn, the mean values obtained in the present study were below the previously reported data (Di Beneditto et al., 2022; El Gendy et al., 2015; Giri and Singh, 2015; Gokoglu et al., 2008; Kaymacı and Altun, 2016; Raissy, 2016). The Pb content obtained was in the range of some reported values (Di Beneditto et al., 2022; Giri and Singh, 2015). The European Commission set a maximum limit of 0.5 mg/kg ww for Pb and Cd content levels in crustaceans (European Commission, 2001). As shown in Tables S9 and S10, all samples presented values below the legislated maximum.

3.5 Nutritional quality indices and ratios

The PUFA/SFA ratio is one of the most used to assess the nutritional value of foods. Values reported in the literature ranged between 0.5 - 1.6 for fish and between 0.2 - 2.1 for shellfish (Chen and Liu, 2020). Dayal et al. consider that a healthy diet must have at least a PUFA/SFA ratio of 0.54 or above (Dayal et al., 2013). In this study, as shown in Table 2, all wild samples analyzed showed a PUFA/SFA ratio above 1, which indicates a good nutritional value of *P. serratus* and *P. varians*, and these values are in the range of previously reported works (Chen and Liu, 2020). The only exception was the aquaculture sample collected in the spring of 2019 (0.94); however, the value is well above the recommended

value of 0.54. The mean value of the PUFA/SFA ratio for *P. serratus* was 1.31, while for *P. varians* it was 1.22 (Figure S1). Regarding the sampling locations, the highest value of 1.49 was found in Matosinhos.

ω-3 and ω-6 are important fatty acids in the diet, although Western diets are deficient in ω-3, which leads to increased ω-6/ω-3 ratio, which can have some adverse consequences, such as chronic diseases when the ratio is greater than 10 (Simopoulos, 2008). In the present study, all shrimp samples showed ω-6/ω-3 ratios below 1, with the mean value being 0.18 and the range 0.10 - 0.36. As the Σω-3 content in shrimp is very high, the consumption of Portuguese shrimp may help to reduce the ω-6/ω-3 ratio in the diet. In addition, *P. serratus* presented significantly lower values (mean 0.15) when compared to *P. varians* (mean 0.23), $p < 0.001$ (Figure S1). ω-3 fatty acids are essential nutrients, humans are unable to produce them, so they have to be provided by the diet (Simopoulos, 2008). Thus, it is important to consume foods high in EPA and DHA. The European Food Safety Authority (EFSA) has recommended a daily intake of 250 mg of EPA+DHA (EFSA, 2012). A 100 g serving of muscle of shrimp from the Portuguese coast provides 195 mg of EPA plus DHA (average) (78% of the recommended daily intake).

Quality index IA demonstrates the association between the main SFA and the main classes of unsaturated fatty acids (UFA). SFA are considered pro-atherogenic (promote lipid adhesion to blood vessels wall cells), and UFA are considered anti-atherogenic (inhibit platelet aggregation and decrease cholesterol and phospholipids levels, preventing the development of micro and macro coronary diseases) (Soares et al., 2021). Lower values of IA indicate better nutritional quality. The IA values calculated in this study (equation 1) indicate a healthier index compared to previously reported values for shrimp samples. The mean value determined in this study for *P. serratus* was 0.41 and for *P. varians* was 0.43 (Figure S1), whereas previously reported values ranged between 0.71 and 0.82 (Chen and Liu, 2020). The IT quality index indicates the thrombogenic potential (tendency to form clots in blood vessels), using the association between SFA, considered pro-thrombogenic (increases thrombus formation), and MUFA and PUFA, considered anti-thrombogenic (decreases thrombus formation) (Soares et al., 2021). Consequently, it is beneficial for cardiovascular health to consume foods with lower IT (Chen and Liu, 2020). The mean values for IT determined in this study (equation 2) were 0.24 and 0.25 for *P. serratus* and *P. varians*, respectively (Figure S1, which are in agreement with previously reported values (0.21 and 0.30) for shrimp samples (Chen and Liu, 2020). Lower IA and IT values in foods

are associated with better nutritional quality, and their consumption can help reduce the risk of coronary disease (Chen and Liu, 2020). The HH quality index is associated with CVD risk. This ratio indicates the relationship between hypo- and hypercholesterolic fatty acids, essential in lipoprotein metabolism, for the transport of plasma cholesterol (Paszczyk and Łuczyńska, 2020). In this study, the values for HH index were 2.60 and 2.55 for *P. serratus* and *P. varians*, respectively (Figure S1), and these are in the range of previously reported values for shellfish species (0.21 – 4.75) (Chen and Liu, 2020).

3.6 Daily value of elements

The RDA and AI (NIH, 2021) define quantitative recommendations of essential elements for the general population to remain healthy. These recommended values (RDA and AI) can be used to determine daily values, as described in section 2.4.4, and the results are described in Figure 3.

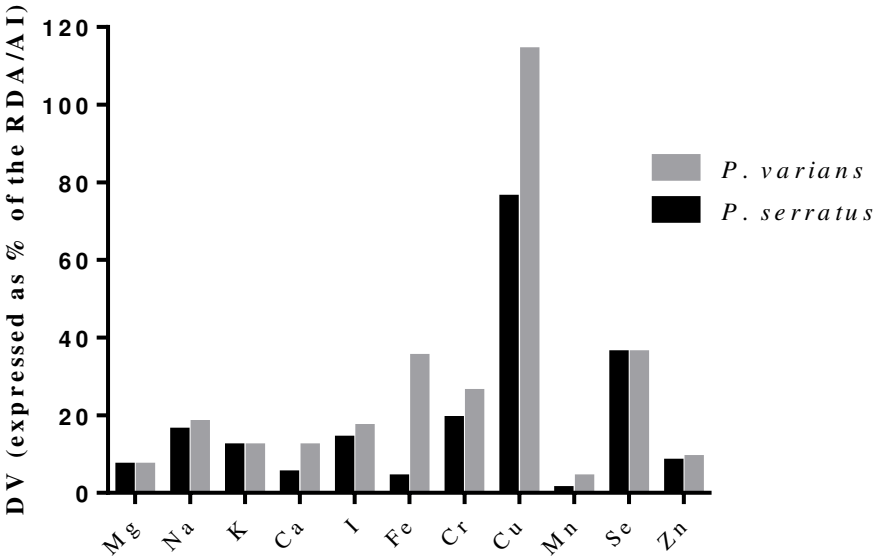


Figure 3. Daily value (DV%) of one serving dose (100 g) of shrimp for essential and probably essential elements.

Overall, shrimp can be a valuable food source of the elements evaluated (Figure 3), except for Mn (only between 1 – 4% of the RDA/AI) and Fe in *P. serratus* (which provides only 4% of the RDA/AI). For all other elements (Mg, Na, K, Ca, I, Cr, Cu and Se; and Fe in *P. varians*) shrimp can provide more than 5% of the RDA/AI. For some elements the contribution may be dependent on the shrimp species. For Cu, *P. varians* may represent 114% of the RDA (exceeding the recommended daily value), while *P. serratus* may represent 76% of the RDA.

Regarding Fe, *P. varians* accounts for 35% of the RDA, but *P. serratus* represents only 4% of the RDA.

3.7 Risk assessment

The elements of the group of potentially toxic/non-essential elements are one of the most important forms of marine pollution due to their accumulation and toxicity to aquatic organisms, such as shrimp (Rao, K. R. P.; Sreeramulu and K.; Sreedhar, 2016). The THQ for Cd, Pb, As, Al, Ag, Sr, V, Sb and Ba was calculated using the reported R*f*D for these elements (USEPA, 2021), equation 4.

Table 3. THQ and HI for potentially toxic/non-essential elements resulting from shrimp consumption.

		THQ						
		P. serratus					*P. varians*	
Element	R*f*D	Vila do Conde	Matosinhos	Aveiro	Ria de Aveiro	Figueira da Foz	Wild Sado	Aquaculture Sado
Cd	**0.001**	0.0001	0.0001	0.0001	0.0001	0.0002	0.00007	0.0001
Pb	**0.004**	0.0001	0.0005	0.00006	0.0003	0.00009	0.0005	0.0004
As	**0.0003**	0.1	0.09	0.08	0.05	0.09	0.04	0.03
Al	**0.0004**	0.2	0.3	0.03	0.2	0.4	5.4	5.0
Ag	**0.005**	0.0008	0.0008	0.0008	0.0006	0.0009	0.0003	0.0003
Sr	**0.6**	0.0005	0.0006	0.0005	0.0008	0.0006	0.002	0.002
V	**0.009**	0.0003	0.0004	0.0005	0.0004	0.0002	0.0009	0.001
Sb	**0.0004**	0.0003	0.0003	0.0003	0.0005	0.0004	0.0002	0.0002
Ba	**0.2**	0.00001	0.00001	0.000008	0.000008	0.00002	0.00005	0.00004
HI		0.3	0.4	0.1	0.3	0.5	5.4	5.0

* Arsenic calculation was made by assuming the inorganic As 6% of the total concentration.

The Hazard Index (HI) is calculated as the sum of THQ for all elements shown in Table 3. THQ represents the risk of adverse health effects of a particular element, while HI represents the potential risk of the exposure to the combined elements (Zhu et al., 2016). If the THQ and HI values are less than 1, it is assumed that there is no adverse health risk due to exposure to the elements (alone or in combination, respectively). If it is above 1, a potential risk may arise from the exposure (USEPA, 2000). The THQ and HI values determined for the two shrimp species are presented in Table 3 and were calculated using the average values for each sampling location. The THQ value for Al in *P. varians* were found to be above 1. Although, the value for Al was above 1, this element is practically not absorbed by the gastrointestinal tract, not posing a particular risk. Regarding HI, the values were above 1 also for *P. varians*, due to the high values of Al.

3.8 Risk-benefit assessment

The HQEFA for Cd, Pb, As, Al, Ag, Sr, V, Sb and Ba was calculated using the reported R*f*D for these elements (USEPA, 2021) and the recommended daily dose of EPA + DHA (EFSA, 2012), equation 5. For these results, if HQEFA < 1, means the consumption of shrimp species studied is beneficial to health, on the other hand if HQEFA > 1 suggests that shrimp consumption may pose a risk. The HQEFA values determined for the two shrimp species in the different sampling locations (average values) are presented in Table 4. The HQEFA value was found to be above 1 for Al in *P. varians*. However as described previously, this element may not pose a particular risk. Moreover, for all the other calculations the HQEFA was below 1.

Table 4. Risk-benefit ratio (HQEFA) for the two shrimp species analyzed.

		HQEFA						
		P. serratus					*P. varians*	
Element	**RfD**	**Vila do Conde**	**Matosinhos**	**Aveiro**	**Ria de Aveiro**	**Figueira da Foz**	**Wild Sado**	**Aquaculture Sado**
Cd	**0.001**	0.00004	0.00004	0.00004	0.00004	0.00006	0.00002	0.00004
Pb	**0.004**	0.00005	0.0001	0.00002	0.00009	0.00003	0.0001	0.0001
As	**0.0003**	0.03	0.03	0.03	0.02	0.03	0.01	0.01
Al	**0.0004**	0.05	0.1	0.009	0.08	0.1	1.7	1.6
Ag	**0.005**	0.0003	0.0003	0.0003	0.0002	0.0003	0.00009	0.0001
Sr	**0.6**	0.0002	0.0002	0.0002	0.0003	0.0002	0.0005	0.0006
V	**0.009**	0.0001	0.0001	0.0002	0.0001	0.00007	0.0003	0.0003
Sb	**0.0004**	0.0001	0.0001	0.0001	0.0002	0.0001	0.00007	0.00006
Ba	**0.2**	0.000004	0.000004	0.000002	0.000003	0.000007	0.00002	0.00001

* Arsenic calculation was made by assuming the inorganic As 6% of the total concentration.

3.9 Correlations between fatty acids and elements

The data described showed a strong Pearson correlation (0 .5 < | r | > 1) and $p < 0.05$ between the levels of fatty acids and elements (Table 5). The fatty acid profile of shrimp is essentially dependent on their food sources (Gatune et al., 2014). The levels of fatty acids with odd numbers of carbon atoms, C15:00 and C17:00, correlate with a high number of element concentrations, with positive and negative correlations. C15:0 and C17:0 fatty acids are considered phytoplankton biomarkers as they are unique to individual groups of algae (Taipale et al., 2013). In aquatic food webs, most fatty acids are synthesized by phytoplankton and bacteria before being transferred via herbivorous invertebrates to fish and crustaceous. A possible explanation for these positive correlations is that these elements may act as enzyme co-factors or play a role as positive modulators. Negative correlations can be explained by toxic elements such as As or a role as negative modulators.

Some potentially toxic/non-essential elements (Al, As, Li, Ti, Sr, Ag, and Pb) showed an unexpected positive correlation with the levels of some fatty acids. One possible explanation is that these elements inhibit downstream metabolism, increasing these fatty acid levels.

Table 5. Positive and negative strong correlations between fatty acids and elements.

Fatty acids	Elements
Positive correlation	
C14:0	Cu, Fe, Ni, Zn, Li, Ti, Mn, Co, Sr and Ca
C15:0	Cu, Fe, Ni, Zn, Li, Ti, Mn, Co, Sr, Ca, Cr, Al, V and Ba
C17:0	Cu, Fe, Ni, Zn, Li, Ti, Mn, Co, Sr, Ca, Cr, Al, V and Ba
C16:1 n-7	Fe, Cr, Li, Al, Mn, Co and I
C18:1 n-9 c	As
C18:1 n-9 t	Fe, Pb, Ni, Cr, Li, Al, Mn and Co
C18:2 n-6 c	Cu, Ni, Zn, Ti, Sr and Ca
C18:3 n-3	I
C20:1n-9	As and Ag
C22:0	Pb, Cr and Al
C22:6 n-3 (DHA)	Cs
Σω-6	Zn, Sr and Ca
Negative correlation	
C15:0	As and Ag
C17:0	As, Ag and Cs
C20:1 n-9	Mn, Co and Ba

4. Conclusion

In general, the results obtained in this study showed that shrimp from NW Portuguese coast have an average TLC of 1.3 g/100 g and that palmitic, stearic, oleic, EPA and DHA acids as the main fatty acids. Shrimp species differed significantly in terms of DHA and Σω-6 content, although both had low IA, IT, HH and ω-6/ω-3 ratio, which represents good nutritional quality. Regarding the elemental composition, the shrimp species showed significant differences for the content of Na, Ca, Cr, Fe, Co, Cu, Zn, I, V, Mn and Ni (essential and probably essential elements) and As, Cd, Ag, Sb, Cs, Bi, Li, Ba, Al, Ti and Sr content (potentially toxic/non-essential elements). Thus, the daily value for essential elements resulting from the consumption of 100g of Portuguese shrimp was estimated, and shrimp can be a good nutritional source of EPA + DHA, Cu, Se, Fe, Mg, Na, K, Ca, I and Cr. The THQ, HI and risk-benefit ratio values calculated shown that there is no significant threat associated with Portuguese shrimp consumption for adult population.

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CRediT authorship contribution statement

Maria Luz Maia: Conceptualization, Methodology, Validation, Investigation, Writing - original draft. **Agostinho Almeida**: Conceptualization, Validation, Supervision, Writing - review & editing. **Cristina Soares**: Methodology, Writing - review & editing. **Luís M. S. Silva**: Statistical analysis. **Cristina Delerue-Matos**: Resources, Writing - review & editing. **Conceição Calhau**: Writing - review & editing. **Valentina Fernandes Domingues**: Conceptualization, Validation, Supervision, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary material

Minerals and fatty acids profile of Northwest Portuguese Coast Shrimps

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Gas Chromatography-Flame Ionization Detection (GC-FID):

Operating conditions were as follows: split mode, with a split ratio of 1:10 and an injection volume of 1 µL. Injector and detector temperatures were maintained at 250 and 260 °C, respectively. A flow rate of 30 mL/min of helium as a carrier gas (Linde Sógas, purity ≥ 99.999%), 40 mL/min of hydrogen and 400 mL/min of air was used. The column thermal gradient was as follow: initial temperature of 80 °C, which was increased at 4 °C/min to 220°C; hold for 5 min, then increase at 2 ºC/min to 228 ºC; hold for 0.5 min, then increase at 2ºC/min to 230 ºC; hold for 0.5 min, then increase at 4ºC/min to 240 ºC; hold for 12 min. FAME were identified by comparison with a known standard FAME Mix and quantified using the software GC solution for GC systems (Shimadzu). The quantification of FAME in mg/100g of shrimp was obtained using the known concentration and area of the used internal standard (C13:0).

Table S1. Saturated fatty acid profile of the two shrimp species analyzed (mg/100g ± SD); E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year	SFA										
		C10:0	C12:0	C14:0	C15:0	C16:0	C17:0	C18:0	C20:0	C21:0	C22:0	C24:0
Palaemon serratus	**Vila do Conde** Autumn 2017	0.091±0.006	0.337±0.005	6.07±0.07	2.56 ± 0.01	102.9±0.35	5.081±0.005	37.2±0.2	2.090±0.009		1.205±0.006	0.78 ± 0.01
	Spring 2018	1.2 ± 0.08	0.61 ± 0.01	6.9 ± 0.3	3.7 ± 0.4	115 ± 10	5.6 ± 0.6	42 ± 3	3.0 ± 0.5	0.7 ± 0.1	2.7 ± 0.6	2.6 ± 0.1
	Spring 2018 E	3.4 ± 0.2	0.49 ± 0.02	6.6 ± 0.7	3.4 ± 0.4	102 ± 6	5.1 ± 0.2	40 ± 3	1.553±0.005	0.306 ± 0.002	1.6 ± 0.2	1.0 ± 0.1
	Autumn 2018	1.7 ± 0.2	0.54 ± 0.03	6.2 ± 0.5	2.9 ± 0.3	114 ± 9	4.9 ± 0.5	51 ± 3	1.88 ± 0.06	0.32 ± 0.03	1.2 ± 0.1	1.48 ± 0.07
	Spring 2019	1.1 ± 0.1	0.318±0.006	6.7 ± 0.8	2.807±0.008	107 ± 3	5.1 ± 0.2	38 ± 2	1.33 ± 0.06	0.3065±0.0006	1.1 ± 0.1	1.1 ± 0.2
	Spring 2019 E	0.9 ± 0.6	0.40 ± 0.08	7 ± 1	2.7 ± 0.2	101 ± 3	4.6 ± 0.1	38 ± 4	1.6 ± 0.1			1.3 ± 0.1
	Matosinhos Autumn 2017	5 ± 2	0.77 ± 0.09	10.4± 0.9	3.3 ± 0.6	168 ± 38	8 ± 2	61 ± 16	4 ± 1		1.9 ± 0.3	1.2 ± 0.5
	Spring 2018	2.0 ± 0.9	0.48 ± 0.04	7.3 ± 0.1	3.8 ± 0.3	110 ± 3	6.0 ± 0.2	42.3±0.2	1.90 ± 0.02	0.26 ± 0.08	1.97 ± 0.07	1.09 ± 0.02
	Spring 2018 E	1.2 ± 0.7	0.5 ± 0.1	6 ± 2	2.8 ± 0.8	95 ±25	5 ± 1	38 ± 8	1.6 ± 0.6		1.6 ± 0.4	1.3 ± 0.6
	Autumn 2018	0.5 ± 0.1	0.49 ± 0.05	6 ± 1	2.6 ± 0.2	90 ± 2	4.3 ± 0.2	41 ± 6	1.366±0.007	6 ± 4	1.2 ± 0.2	0.8 ± 0.2
	Spring 2019		0.5 ± 0.1	7 ± 1	3.2 ± 0.6	119 ±26	6 ± 1	42 ± 8	2.0 ± 0.4	0.56 ± 0.04	1.8 ± 0.6	2.3 ± 0.3
	Aveiro Spring 2018	0.22 ± 0.06	0.74 ± 0.04	10.3± 0.8	5.5 ± 0.6	211 ± 25	10 ± 1	72 ± 2	3.67 ± 0.05		3.5 ± 0.2	1.83 ± 0.05
	Autumn 2018	2.2 ± 0.2	0.34 ± 0.04	5.2 ± 0.4	3.6 ± 0.3	89 ± 7	5.4 ± 0.3	37 ± 5	1.2 ± 0.1	0.200 ± 0.007	0.66 ± 0.04	0.8 ± 0.1
	Spring 2019	0.20 ± 0.03	0.30 ± 0.02	4.2 ± 0.6	2.0 ± 0.3	83 ± 12	3.7 ± 0.7	34 ± 1	1.3 ± 0.3	0.323 ± 0.04	1.2 ± 0.2	0.6 ± 0.1
	Ria de Aveiro Autumn 2017	1.4 ± 0.3	0.29 ± 0.01	5.5 ± 0.3	3.58 ± 0.01	90 ± 5	4.7 ± 0.1	30.3±0.2	1.47 ± 0.04		1.1 ± 0.1	1.09 ± 0.07
	Spring 2018	1.049±0.007	0.30 ± 0.05	5 ± 1	2.9 ± 0.7	89 ±26	4 ± 1	35 ±11	1.7 ± 0.5		1.7 ± 0.6	1.6 ± 0.6
	Spring 2018 E	9.1 ± 0.9	0.94 ± 0.03	12.0± 0.5	6.78 ± 0.04	194 ± 2	8.14 ± 0.03	71 ± 2	2.32 ± 0.02		1.76 ± 0.06	2.1 ± 0.1
	Figueira da Foz Autumn 2017	2.1 ± 0.1	0.33 ± 0.02	8 ± 1	4.7 ± 0.6	133 ± 21	7.1 ± 0.9	54 ± 10	1.8 ± 0.3	0.281 ± 0.001	1.1 ± 0.2	1.0 ± 0.3
	Spring 2018	0.8 ± 0.1	0.39 ± 0.01	5.48±0.03	3.08 ± 0.09	88 ± 4	4.4 ± 0.1	35 ± 1	1.425±0.006	0.33 ± 0.07	1.1 ± 0.3	1.6 ± 0.1
	Spring 2018 E	11.1 ± 0.1	0.8 ± 0.1	8 ± 1	4.6 ± 0.9	142 ± 24	7 ± 1	59 ±11	1.9 ± 0.4		1.89 ± 0.4	1.1 ± 0.3
	Autumn 2018	0.3 ± 0.2	0.51 ± 0.02	6.6 ± 0.7	3.5 ± 0.3	99 ± 8	5.7 ± 0.6	40 ± 2	1.5 ± 0.1	0.26 ± 0.01	1.5 ± 0.2	1.54 ± 0.05
	Spring 2019	1.1 ± 0.4	0.241±0.003	5.2 ± 0.9	3.0 ± 0.7	99 ±25	5 ± 1	37 ± 7	1.6 ± 0.3	0.40 ± 0.09	1.4 ± 0.5	1.17 ± 0.02
Palaemon varians	**Sado** Autumn 2017 A	0.15 ± 0.04	0.21 ± 0.05	7.7 ± 0.5	19 ± 2	106 ± 8	18 ± 2	41 ± 3	1.79 ± 0.06	0.36 ± 0.03	1.2 ± 0.1	1.67 ± 0.1
	Spring 2018 A	0.8 ± 0.1	0.34 ± 0.04	7 ± 2	14 ± 4	13 ±25	13 ± 3	40 ± 9	1.5 ± 0.3	0.5 ± 0.1	0.9 ± 0.2	1.621±0.005
	Spring 2018 A E	8.8 ± 0.9	0.58 ± 0.03	10.7± 0.5	18.2 ± 0.2	127 ± 7	16.1 ± 0.2	51 ± 4	1.6 ± 0.2		1.7 ± 0.2	1.5 ± 0.1
	Autumn 2018 A		0.40 ± 0.03	11 ± 2	10 ± 2	102 ±19	10 ± 2	38 ± 6	1.6 ± 0.3	0.30 ± 0.05	1.5 ± 0.4	0.8 ± 0.1
	Spring 2019 A	2.3 ± 0.1	0.47 ± 0.08	8.6 ± 0.4	14 ± 1	143 ± 9	16 ± 1	67.1±0.3	2.8 ± 0.1		2.5 ± 0.2	1.6 ± 0.1
	Autumn 2017 W		0.206±0.003	7 ± 1	12 ± 3	95 ±18	14 ± 3	42 ± 5	1.8 ± 0.4		0.9 ± 0.2	1.0 ± 0.1
	Spring 2018 W		0.23 ± 0.02	6.0 ± 0.8	11 ± 1	81 ±14	11 ± 2	36 ± 4	1.35 ± 0.07		0.96 ± 0.03	1.5 ± 0.4
	Spring 2018 W E	7.8 ± 0.3	0.35 ± 0.04	9 ± 1	21 ± 1	135 ±13	18 ± 1	56 ± 6	2.0 ± 0.3	0.53 ± 0.01	11 ± 1	1.4 ± 0.3
	Autumn 2018 W	0.28 ± 0.02	0.68 ± 0.03	12.6± 0.9	11.7 ± 0.1	123 ± 3	12.7 ± 0.2	52 ± 3	1.74 ± 0.06		0.991±0.005	0.99 ± 0.02
	Spring 2019 W	0.200±0.005	0.183± 0.05	6.0 ± 0.3	7.4 ± 0.9	90 ± 9	9 ± 1	43 ± 8	1.6 ± 0.2		0.98 ± 0.07	0.9 ± 0.1

Table S2. Monounsaturated fatty acids profile of the two shrimp species analyzed (mg/100g ± SD); E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		MUFA						
			C14:1 n-5	C16:1 n-7	C18:1 n-9 t	C18:1 n-9 c	C20:1n-9	C22:1 n-9	C24:1 n-9
Palaemon serratus	Vila do Conde	Autumn 2017	2.7 ± 0.2	19.567±0.004	0.93 ± 0.03	63.3 ± 0.3	3.1 ± 0.2	0.3102±0.0009	0.4617±0.0008
		Spring 2018	4.3 ± 0.4	20 ± 1	3 ± 1	79 ± 5	3.9 ± 0.5	0.7 ± 0.1	3 ± 1
		Spring 2018 E	3.4 ± 0.7	24 ± 3	1.59 ± 0.02	78 ± 6	3.1 ± 0.3	0.3928±0.0005	0.586 ± 0.003
		Autumn 2018	0.8 ± 0.1	24 ± 2	1.4 ± 0.1	83 ± 6	2.5 ± 0.4	0.23 ± 0.02	0.57 ± 0.02
		Spring 2019	4.1 ± 0.4	15.5 ± 0.9	1.02 ± 0.04	69 ± 2	3.1 ± 0.3	0.23 ± 0.02	0.55 ± 0.04
		Spring 2019 E	3.7 ± 0.4	22 ± 2	1.25 ± 0.05	83 ± 3	3.5 ± 0.7	0.39 ± 0.05	0.566 ± 0.007
	Matosinhos	Autumn 2017	7 ± 2	33 ± 6	1.2 ± 0.3	110 ± 26	4.0 ± 0.9	0.47 ± 0.03	1.05 ± 0.01
		Spring 2018	3.6 ± 0.4	20.1 ± 0.8	1.29 ± 0.04	80 ± 4	4.4 ± 0.7	0.66 ± 0.02	1.4 ± 0.1
		Spring 2018 E	3.9 ± 0.2	20 ± 6	0.959±0.003	80 ± 22	2.8 ± 0.8	0.34 ± 0.06	0.8 ± 0.2
		Autumn 2018	3.8 ± 0.1	14.3 ± 0.6	0.7 ± 0.1	50.4 ± 0.7	2.5 ± 0.3	0.219 ± 0.002	0.42 ± 0.05
		Spring 2019	0.710 ± 0.009	19 ± 4	1.1 ± 0.3	82 ± 19	2.6 ± 0.7	0.25 ± 0.02	0.79 ± 0.08
	Aveiro	Spring 2018	8.9 ± 0.5	27 ± 2	1.6 ± 0.1	151 ± 11	5.0 ± 0.2	0.500 ± 0.007	1.14 ± 0.04
		Autumn 2018	2.4 ± 0.3	15 ± 1	0.94 ± 0.07	45 ± 4	1.9 ± 0.1	0.112 ± 0.007	0.233 ± 0.006
		Spring 2019	0.4383±0.0006	11 ± 2	0.6 ± 0.1	57 ± 8	1.7 ± 0.2	0.19 ± 0.02	0.49 ± 0.06
	Ria de Aveiro	Autumn 2017	2.03 ± 0.08	15.4 ± 0.2	0.463±0.001	30.9 ± 0.9	1.3 ± 0.2	0.17 ± 0.02	0.37 ± 0.03
		Spring 2018	1.4 ± 0.4	19 ± 6	0.7 ± 0.2	38 ± 13	2.0 ± 0.6	0.3 ± 0.1	0.8 ± 0.2
		Spring 2018 E	6.77 ± 0.09	57.0 ± 0.6	2.21 ± 0.09	95 ± 2	3.5 ± 0.2	0.35 ± 0.03	0.486 ± 0.008
	Figueira da Foz	Autumn 2017	3.6 ± 0.4	26 ± 4	0.90 ± 0.02	70 ± 11	2.7 ± 0.3	0.171 ± 0.004	0.63 ± 0.02
		Spring 2018	0.7 ± 0.1	13.4 ± 0.2	0.55 ± 0.01	69 ± 17	1.6 ± 0.2	0.28 ± 0.02	0.6 ± 0.1
		Spring 2018 E	6 ± 1	27 ± 5	1.3 ± 0.2	110 ± 21	3.5 ± 0.7	0.46 ± 0.01	0.8 ± 0.1
		Autumn 2018	3.65 ± 0.06	20 ± 2	0.8 ± 0.1	69 ± 5	2.96 ± 0.07	0.258 ± 0.008	0.61 ± 0.03
		Spring 2019	3.0 ± 0.2	12 ± 3	0.7 ± 0.2	63 ± 18	2.6 ± 0.6	0.15 ± 0.06	0.7 ± 0.2
Palaemon varians	Sado	Autumn 2017 A	2.9 ± 0.4	21 ± 2	0.7 ± 0.1	44 ± 4	1.37 ± 0.08	0.244 ± 0.002	0.32 ± 0.02
		Spring 2018 A	5 ± 1	31 ± 7	2.7 ± 0.6	56 ± 13	1.5 ± 0.2	0.27 ± 0.06	0.53 ± 0.02
		Spring 2018 A E	6 ± 1	41 ± 2	3.37 ± 0.07	64 ± 4	1.64 ± 0.08	0.67 ± 0.02	0.45 ± 0.02
		Autumn 2018 A	2.0 ± 0.6	26 ± 5	1.5 ± 0.2	55 ± 12	2.1 ± 0.5	0.3 ± 0.1	0.324 ± 0.004
		Spring 2019 A	4.7 ± 0.4	36 ± 3	1.506±0.005	78 ± 5	1.73 ± 0.01	0.39 ± 0.02	0.33 ± 0.07
		Autumn 2017 W	2.34 ± 0.5	22 ± 5	0.9 ± 0.2	65 ± 11	1.8 ± 0.5	0.19 ± 0.05	0.225 ± 0.001
		Spring 2018 W	2.8 ± 0.2	24 ± 4	2.1 ± 0.5	44 ± 9	1.9 ± 0.2	0.30 ± 0.04	0.36 ± 0.03
		Spring 2018 W E	4.8 ± 0.5	41 ± 4	3.0 ± 0.3	72 ± 7	0.77 ± 0.05	0.53 ± 0.02	0.8 ± 0.2
		Autumn 2018 W	4.3 ± 0.1	29.4 ± 0.6	1.40 ± 0.06	58.3 ± 0.2	2.1113±0.0003	0.42 ± 0.05	0.485 ± 0.002
		Spring 2019 W	1.67 ± 0.3	21 ± 3	0.72 ± 0.05	47 ± 6	1.57 ± 0.09	0.178 ± 0.003	0.17 ± 0.05

Table S3. Polyunsaturated fatty acids profile of the two shrimp species analyzed (mg/100g ± SD); E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		PUFA							
			C18:2 n-6 c	C18:3 n-3	C20:2 n-6	C20:3 n-3	C20:3 n-6	C20:4 n-6	C20:5 n-3	C22:6 n-3
Palaemon serratus	Vila do Conde	Autumn 2017	7.22 ± 0.02	4.88 ± 0.04	2.21 ± 0.03	1.41 ± 0.01	0.294 ± 0.005	12.05 ± 0.05	99.6 ± 0.4	76.5 ± 0.1
		Spring 2018	17 ± 1	5.7 ± 0.2	3.7 ± 0.4	2.3 ± 0.3	0.56 ± 0.04	18.6 ± 0.9	128 ± 8	89 ± 6
		Spring 2018 E	15 ± 1	5.2 ± 0.8	2.7 ± 0.4	1.6 ± 0.3	0.46 ± 0.05	18 ± 2	111 ± 11	74 ± 6
		Autumn 2018	11.2 ± 0.9	6.34 ± 0.04	3.0 ± 0.2	1.7 ± 0.1	0.55 ± 0.01	18 ± 2	107 ± 7	75 ± 6
		Spring 2019	12.00 ± 0.85	4.3 ± 0.4	3.0 ± 0.2	1.3 ± 0.2	0.37 ± 0.03	13 ± 1	103 ± 8	81 ± 6
		Spring 2019 E	12.0 ± 0.6	4.3 ± 0.6	2.770 ± 0.008	1.42 ± 0.05	0.7 ± 0.4	15 ± 1	109 ± 13	77 ± 7
	Matosinhos	Autumn 2017	10 ± 2	5.3 ± 0.6	3.3 ± 0.7	2.4 ± 0.6	0.55 ± 0.08	16 ± 3	173 ± 24	136 ± 24
		Spring 2018	19.5 ± 0.4	5.9 ± 0.5	3.56 ± 0.06	1.7 ± 0.1	0.34 ± 0.01	15.8 ± 0.3	128 ± 2	89 ± 1
		Spring 2018 E	20 ± 4	5 ± 1	2.9 ± 0.6	1.4 ± 0.4	0.5 ± 0.1	13 ± 3	99 ± 27	72 ± 19
		Autumn 2018	9.3 ± 0.6	4.3 ± 0.3	2.00 ± 0.06	1.16 ± 0.04	0.32 ± 0.04	10.4 ± 0.5	91 ± 6	71 ± 4
		Spring 2019	15 ± 3	5 ± 1	3.5 ± 0.6	1.5 ± 0.2	0.45 ± 0.03	13 ± 3	119 ± 26	97 ± 20
	Aveiro	Spring 2018	37 ± 3	8.9 ± 0.3	5.7 ± 0.6	2.3 ± 0.1	0.62 ± 0.04	24 ± 2	194 ± 16	162 ± 17
		Autumn 2018	12 ± 1	9 ± 1	2.0 ± 0.3	1.3 ± 0.1	0.54 ± 0.03	15 ± 2	85 ± 9	64 ± 6
		Spring 2019	10 ± 2	2.7 ± 0.6	2.2 ± 0.3	0.9 ± 0.1	0.175 ± 0.001	8 ± 1	76 ± 12	67 ± 10
	Ria de Aveiro	Autumn 2017	12.3 ± 0.5	12.7 ± 0.1	1.618 ± 0.004	1.5 ± 0.1	0.479 ± 0.005	10.5 ± 0.6	84 ± 3	66.2 ± 0.3
		Spring 2018	12 ± 3	16 ± 5	2.1 ± 0.6	4 ± 1	0.7 ± 0.2	11 ± 3	91 ± 27	53 ± 16
		Spring 2018 E	29.93 ± 0.07	42 ± 1	4.2 ± 0.1	7.9 ± 0.3	2.01 ± 0.08	22.06 ± 0.03	173 ± 3	83.4 ± 0.6
	Figueira da Foz	Autumn 2017	11 ± 1	6 ± 1	3.0 ± 0.3	1.5 ± 0.3	0.32 ± 0.02	12 ± 2	115 ± 19	102 ± 17
		Spring 2018	9.1 ± 0.4	3.8 ± 0.2	2.36 ± 0.06	1.0 ± 0.1	0.30 ± 0.02	9.9 ± 0.3	88 ± 2	77 ± 3
		Spring 2018 E	16 ± 3	6 ± 1	3.6 ± 0.7	1.7 ± 0.3	0.6 ± 0.1	18 ± 3	132 ± 20	104 ± 19
		Autumn 2018	11 ± 1	5.4 ± 0.6	3.04 ± 0.07	1.3 ± 0.1	0.37 ± 0.04	13.4 ± 0.9	103 ± 9	87 ± 7
		Spring 2019	10 ± 2	4 ± 1	2.5 ± 0.6	1.0 ± 0.3	0.28 ± 0.01	9 ± 2	85 ± 21	81 ± 20
Palaemon varians	Sado	Autumn 2017 A	18 ± 2	8.9 ± 0.6	2.1 ± 0.2	1.1 ± 0.1	0.62 ± 0.02	21.2 ± 0.5	141 ± 9	73 ± 5
		Spring 2018 A	14 ± 3	11 ± 3	2.9 ± 0.5	1.23 ± 0.09	0.9 ± 0.2	15 ± 3	135 ± 32	66 ± 16
		Spring 2018 A E	15 ± 1	12.2 ± 0.3	3.19 ± 0.07	1.6 ± 0.2	1.019 ± 0.002	15 ± 1	137 ± 8	77 ± 5
		Autumn 2018 A	45 ± 8	7 ± 2	5 ± 1	1.3 ± 0.5	0.4 ± 0.1	11 ± 3	122 ± 25	42 ± 9
		Spring 2019 A	27 ± 2	12.1 ± 0.8	2.7 ± 0.2	2.3 ± 0.3	0.66 ± 0.05	18 ± 1	99 ± 7	82 ± 6
		Autumn 2017 W	29 ± 5	7 ± 2	2.0 ± 0.2	0.9 ± 0.1	0.43 ± 0.06	16 ± 3	123 ± 25	48 ± 10
		Spring 2018 W	11 ± 2	9 ± 1	1.6 ± 0.3	1.1 ± 0.2	0.5 ± 0.1	13 ± 1	107 ± 15	55 ± 8
		Spring 2018 W E	18 ± 2	13 ± 2	3.2 ± 0.2	1.7 ± 0.3	0.93 ± 0.08	17 ± 2	153 ± 21	90 ± 12
		Autumn 2018 W	62 ± 1	9.5 ± 0.2	5.18 ± 0.07	1.37 ± 0.01	0.489 ± 0.005	14.2 ± 0.1	160 ± 4	55.4 ± 0.8
		Spring 2019 W	18 ± 2	7.7 ± 0.4	1.7 ± 0.2	1.41 ± 0.01	0.44 ± 0.02	12.3 ± 0.9	76 ± 4	45 ± 5

Table S4. Recoveries of the certified reference materials DOLT-4 and ERM®-BD151 (mean ± SD).

	Certified value (mg/kg)	Determined value (mg/kg)	Recoveries (%)
DOLT-4			
As	9.66 ± 0.62	8.80 ± 0.29	91 ± 3
Cd	24.3 ± 0.8	25.1 ± 0.2	103.1 ± 0.8
Cu	31.2 ± 1.1	33.4 ± 0.4	107 ± 1
Fe	1833 ± 75	2024 ± 6	110.4 ± 0.3
Pb	0.16 ± 0.04	0.15 ± 0.01	97 ± 9
Ni	0.97 ± 0.11	0.94547 ± 0.00006	97.471 ± 0.006
Se	8.3 ± 1.3	7.3 ± 0.3	88 ± 4
Zn	116 ± 6	113 ± 1	97.8 ± 0.6
ERM-BD151			
I	1.78 ± 0.17	1.55 ± 0.04	87 ± 2

Table S5. MDL and MQL for macrominerals, essential, probably essential and non-essential/toxic trace elements.

Elements	MDL (mg/kg)	MQL (mg/kg)
As	0.006	0.06
Cd	0.0004	0.001
Cu	0.01	0.04
Fe	0.4	1
Pb	0.006	0.02
Ni	0.02	0.07
Se	0.03	0.08
Zn	0.1	0.3
Cr	0.03	0.1
Li	0.002	0.007
Al	0.3	0.8
Ti	0.03	0.09
V	0.003	0.008
Mn	0.007	0.02
Co	0.001	0.003
Rb	0.003	0.01
Sr	0.005	0.02
Ag	0.004	0.01
Sn	0.001	0.004
Sb	0.0002	0.0005
Cs	0.002	0.005
Ba	0.009	0.03
Bi	0.001	0.004
I	0.002	0.02
Ca	0.0001	0.0003
Na	0.0001	0.0002
Mg	0.00001	0.00002
K	0.0001	0.0004

Table S6. Content (mean ± SD, mg/kg ww) of four essential macrominerals in the analyzed shrimp samples and Na/Ka and Ca/Mg ratios; E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		Macro-elements (mg/kg)				Na/K	Ca/Mg
			Mg	Na	K	Ca		
Palaemon serratus	Vila do Conde	Autumn 2017	330 ± 30	2200 ± 200	4600 ± 300	430 ± 20	0.47	1.33
		Spring 2018	270 ± 10	1990 ± 30	3900 ± 100	400 ± 10	0.51	1.47
		Spring 2018 E	280 ± 4	2008 ± 4	4020 ± 10	380 ± 30	0.50	1.36
		Autumn 2018	290 ± 10	2480 ± 40	4200 ± 80	540 ± 80	0.59	1.84
		Spring 2019	302 ± 3	2470 ± 60	4200 ± 200	470 ± 20	0.58	1.57
		Spring 2019 E	280 ± 9	2300 ± 100	3900 ± 100	350 ± 20	0.58	1.23
	Matosinhos	Autumn 2017	311 ± 9	1820 ± 30	4570 ± 1	400 ± 100	0.40	1.18
		Spring 2018	280 ± 10	2100 ± 100	3700 ± 200	560 ± 10	0.57	1.97
		Spring 2018 E	287 ± 1	2180 ± 10	3600 ± 100	470 ± 20	0.60	1.62
		Autumn 2018	308 ± 9	2880 ± 80	4100 ± 200	580 ± 10	0.70	1.88
		Spring 2019	300 ± 10	2640 ± 80	3800 ± 100	560 ± 20	0.69	1.87
	Aveiro	Spring 2018	291 ± 4	2320 ± 20	4440 ± 80	490 ± 90	0.52	1.68
		Autumn 2018	272 ± 3	2162 ± 8	4080 ± 20	517 ± 2	0.53	1.90
		Spring 2019	290 ± 20	2500 ± 100	4340 ± 300	360 ± 30	0.57	1.26
	Ria de Aveiro	Autumn 2017	300 ± 30	2300 ± 100	4500 ± 300	430 ± 60	0.51	1.45
		Spring 2018	320 ± 20	3000 ± 200	4500 ± 400	730 ± 80	0.66	2.26
		Spring 2018 E	310 ± 20	2500 ± 200	3900 ± 300	800 ± 100	0.65	2.46
	Figueira da Foz	Autumn 2017	334 ± 1	1834 ± 9	5310 ± 20	270 ± 10	0.35	0.81
		Spring 2018	294 ± 5	2360 ± 40	4000 ± 90	370 ± 10	0.59	1.25
		Spring 2018 E	290 ± 10	2640 ± 50	4000 ± 100	420 ± 30	0.65	1.43
		Autumn 2018	297 ± 2	2610 ± 20	3920 ± 80	650 ± 30	0.67	2.17
		Spring 2019	330 ± 10	2860 ± 70	4100 ± 100	830 ± 50	0.69	2.49
Palaemon varians	Sado	Autumn 2017 A	280 ± 10	2690 ± 90	4300 ± 300	900 ± 100	0.62	3.13
		Spring 2018 A	262.2 ± 0.2	2370 ± 10	4060 ± 70	1000 ± 100	0.58	3.93
		Spring 2018 A E	270 ± 20	2520 ± 50	3790 ± 20	940 ± 40	0.66	3.51
		Autumn 2018 A	320 ± 10	2830 ± 7	3640 ± 30	2100 ± 100	0.78	6.44
		Spring 2019 A	330 ± 20	3350 ± 50	4120 ± 40	1200 ± 100	0.81	3.66
		Autumn 2017 W	250 ± 10	2600 ± 200	3800 ± 200	990 ± 70	0.68	3.93
		Spring 2018 W	260 ± 3	2400 ± 80	4120 ± 90	1050 ± 30	0.58	4.05
		Spring 2018 W E	280 ± 10	2500 ± 100	3800 ± 100	1200 ± 200	0.667	4.264
		Autumn 2018 W	324 ± 3	2890 ± 90	3900 ± 100	1608 ± 7	0.74	4.97
		Spring 2019 W	290 ± 10	2640 ± 40	4500 ± 200	700 ± 100	0.59	2.59

Table S7. Content (mean ± SD, mg/kg ww) of seven essential trace elements in the analyzed shrimp samples; E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		Essential elements (mg/kg)						
			Cr	Fe	Co	Cu	Zn	Se	I
Palaemon serratus	Vila do Conde	Autumn 2017	< MQL	3.3 ± 0.7		6.7 ± 0.6	8.9 ± 0.9	0.13 ± 0.04	0.48 ± 0.03
		Spring 2018	< MQL	1.58 ± 0.02		6.2 ± 0.1	7.4 ± 0.1	0.183 ± 0.001	0.18 ± 0.08
		Spring 2018 E	< MQL	2.0 ± 0.1		7.1 ± 0.7	8.2 ± 0.7	0.19 ± 0.01	0.186 ± 0.002
		Autumn 2018	< MQL	2.0 ± 0.7		7.1 ± 0.2	9.32 ± 0.01	0.18 ± 0.03	0.122 ± 0.005
		Spring 2019	< MQL	2.6 ± 0.3		7.5 ± 0.2	8.86 ± 0.07	0.246 ± 0.045	0.18 ± 0.03
		Spring 2019 E	< MQL	2.5 ± 0.3	0.005 ± 0.003	7.8 ± 0.5	7.9 ± 0.2	0.17 ± 0.03	0.16 ± 0.02
	Matosinhos	Autumn 2017	< MQL	9 ± 3		6.13 ± 0.01	8.1 ± 0.3	0.173 ± 0.005	0.22 ± 0.04
		Spring 2018	< MQL	2.69 ± 0.06		6.1 ± 0.7	9.37 ± 0.07	0.21 ± 0.04	0.096 ± 0.008
		Spring 2018 E	< MQL	4 ± 1	0.007 ± 0.003	7.3 ± 0.3	8.4 ± 0.3	0.201 ± 0.009	0.19 ± 0.03
		Autumn 2018	< MQL	2.2 ± 0.2		7.3 ± 0.3	8.4 ± 0.4	0.19 ± 0.02	0.0791 ± 0.0007
		Spring 2019	< MQL	4.7 ± 0.7		6.9 ± 0.9	7.8 ± 0.3	0.21 ± 0.01	0.17 ± 0.06
	Aveiro	Spring 2018	< MQL	< MQL		6.79 ± 0.34	8.60 ± 0.05	0.245 ± 0.002	0.11 ± 0.05
		Autumn 2018	< MQL	2 ± 1		6.3 ± 0.3	8.58 ± 0.03	0.176 ± 0.003	0.06 ± 0.02
		Spring 2019	< MQL	< MQL		6.1 ± 0.4	7.6 ± 0.4	0.18 ± 0.04	0.06 ± 0.01
	Ria de Aveiro	Autumn 2017	< MQL	2.25 ± 0.03		5.1 ± 0.4	7.8 ± 0.8	0.19 ± 0.02	0.16 ± 0.02
		Spring 2018	< MQL	4.4 ± 0.8	0.009 ± 0.002	7 ± 1	10 ± 1	0.199 ± 0.008	0.38 ± 0.07
		Spring 2018 E	< MQL	4.70 ± 0.08	0.011 ± 0.002	9 ± 1	10.4 ± 0.7	0.2281 ± 0.0034	0.46 ± 0.02
	Figueira da Foz	Autumn 2017	< MQL	5.7 ± 0.6		5.4 ± 0.7	9 ± 1	0.17 ± 0.06	0.33 ± 0.03
		Spring 2018	<MDL	2.53 ± 0.02		6.62 ± 0.08	7.567 ± 0.007	0.21 ± 0.02	0.37 ± 0.09
		Spring 2018 E	< MQL	4.3 ± 0.8		7.3 ± 0.7	8.1 ± 0.2	0.27 ± 0.03	0.22 ± 0.02
		Autumn 2018	< MQL	6 ± 3		6.48 ± 0.06	7.9 ± 0.3	0.206 ± 0.008	0.12 ± 0.03
		Spring 2019	< MQL	3.7 ± 0.2		7.3 ± 0.6	7.9 ± 0.7	0.23 ± 0.01	0.19 ± 0.02
Palaemon varians	Sado	Autumn 2017 A	< MQL	2.6 ± 0.4	0.0089 ± 0.0001	9.4 ± 0.3	10.4 ± 0.5	0.19 ± 0.02	0.17 ± 0.03
		Spring 2018 A	0.108 ± 0.004	35 ± 2	0.02433 ± 0.00005	8.193 ± 0.003	9.1 ± 0.2	0.188 ± 0.003	0.21 ± 0.01
		Spring 2018 A E	0.14 ± 0.02	63 ± 14	0.035 ± 0.005	9. ± 0.2	9.2 ± 0.6	0.19 ± 0.02	0.35 ± 0.05
		Autumn 2018 A	< MQL	35 ± 6	0.0299 ± 0.0009	11.3 ± 0.3	10.4 ± 0.4	0.247 ± 0.007	0.21 ± 0.02
		Spring 2019 A	< MQL	5 ± 2	0.0142 ± 0.0006	13 ± 1	10.6 ± 0.3	0.171 ± 0.009	0.18 ± 0.04
		Autumn 2017 W	< MQL	2.55 ± 0.08	0.0058 ± 0.0004	8.805 ± 0.005	10.0 ± 0.3	0.175 ± 0.002	0.31 ± 0.05
		Spring 2018 W	< MQL	36 ± 5	0.026 ± 0.002	9.1 ± 0.4	9.7 ± 0.2	0.217 ± 0.004	0.24 ± 0.02
		Spring 2018 W E	0.14 ± 0.06	57 ± 5	0.032 ± 0.001	9.1 ± 0.4	9.3 ± 0.3	0.179 ± 0.005	0.42 ± 0.06
		Autumn 2018 W	0.10 ± 0.02	41 ± 15	0.030 ± 0.005	12 ± 2	11.9 ± 0.2	0.21 ± 0.01	0.204 ± 0.009
		Spring 2019 W	< MQL	3.6 ± 0.2	0.015 ± 0.002	12.1 ± 0.1	10.7 ± 0.3	0.204 ± 0.006	0.198 ± 0.007

Table S8. Content (mean ± SD, mg/kg ww) of three probably essential trace elements in the analyzed shrimp samples; E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		Probable essential elements (mg/kg)		
			V	Mn	Ni
Palaemon serratus	Vila do Conde	Autumn 2017	<MDL	0.28 ± 0.01	0.09 ± 0.02
		Spring 2018	0.03 ± 0.01	0.20 ± 0.02	< MQL
		Spring 2018 E	0.089 ± 0.004	0.24 ± 0.05	< MQL
		Autumn 2018	0.04 ± 0.03	0.22 ± 0.02	< MQL
		Spring 2019	0.14 ± 0.02	0.221 ± 0.002	< MQL
		Spring 2019 E	< MQL	0.17 ± 0.01	0.069 ± 0.008
	Matosinhos	Autumn 2017	0.096 ± 0.008	0.29 ± 0.02	0.071 ± 0.02
		Spring 2018	0.05 ± 0.02	0.20 ± 0.01	0.13 ± 0.03
		Spring 2018 E	0.014 ± 0.008	0.22 ± 0.01	0.0936 ± 0.0004
		Autumn 2018	0.10 ± 0.05	0.197 ± 0.007	< MQL
		Spring 2019	0.09 ± 0.02	0.24 ± 0.03	< MQL
	Aveiro	Spring 2018	0.10 ± 0.06	0.19 ± 0.01	< MQL
		Autumn 2018	< MQL	0.209 ± 0.004	0.07 ± 0.02
		Spring 2019	0.12 ± 0.02	0.16 ± 0.01	< MQL
	Ria de Aveiro	Autumn 2017	0.05 ± 0.03	0.22 ± 0.03	< MQL
		Spring 2018	0.05 ± 0.04	0.32 ± 0.03	< MQL
		Spring 2018 E	0.07 ± 0.01	0.39 ± 0.03	< MQL
	Figueira da Foz	Autumn 2017	0.04 ± 0.03	0.25 ± 0.02	< MQL
		Spring 2018	<MDL	0.24 ± 0.02	< MQL
		Spring 2018 E	0.094 ± 0.008	0.253 ± 0.006	< MQL
		Autumn 2018	0.04 ± 0.02	0.25 ± 0.01	< MQL
		Spring 2019	< MQL	0.25 ± 0.02	< MQL
Palaemon varians	Sado	Autumn 2017 A	0.08 ± 0.01	0.36 ± 0.02	< MQL
		Spring 2018 A	0.16 ± 0.01	0.95 ± 0.06	0.11 ± 0.03
		Spring 2018 A E	0.21 ± 0.01	1.23 ± 0.08	0.18 ± 0.02
		Autumn 2018 A	0.122 ± 0.003	1.205 ± 0.009	0.15 ± 0.08
		Spring 2019 A	0.21 ± 0.03	0.6 ± 0.1	< MQL
		Autumn 2017 W	0.04 ± 0.02	0.41 ± 0.07	< MQL
		Spring 2018 W	0.106 ± 0.003	1.0 ± 0.1	0.09 ± 0.02
		Spring 2018 W E	0.17 ± 0.03	1.33 ± 0.08	0.16 ± 0.02
		Autumn 2018 W	0.16 ± 0.07	1.2 ± 0.2	0.19 ± 0.06
		Spring 2019 W	0.19 ± 0.06	0.48 ± 0.03	< MQL

Table S9. Content (mean ± SD, mg/kg ww) of seven potentially toxic/non-essential elements in the analyzed shrimp samples; E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		Potentially toxic/non-essential elements (mg/kg)						
			Li	Al	Ti	As	Rb	Sr	Ag
Palaemon serratus	Vila do Conde	Autumn 2017	0.038 ± 0.002	3 ± 1	2.53 ± 0.08	7.0 ± 0.8	1.2 ± 0.1	5.0 ± 0.5	0.073 ± 0.005
		Spring 2018	0.0284 ± 0.0005	< MQL	2.15 ± 0.09	9.3 ± 0.5	1.08 ± 0.02	4.7 ± 0.3	0.071 ± 0.009
		Spring 2018 E	0.0297 ± 0.0008	0.9 ± 0.6	2.1 ± 0.2	9.9 ± 0.4	1.12 ± 0.03	4.6 ± 0.9	0.070 ± 0.009
		Autumn 2018	0.0389 ± 0.0007	< MQL	3.12 ± 0.5	6.3 ± 0.3	1.163 ± 0.006	8 ± 2	0.059 ± 0.001
		Spring 2019	0.040 ± 0.002	0.9 ± 0.2	2.53 ± 0.06	8.5 ± 0.1	1.19 ± 0.08	6.3 ± 0.5	0.07 ± 0.01
		Spring 2019 E	0.037 ± 0.001	1.2 ± 0.2	2.0 ± 0.1	10.2 ± 0.4	1.08 ± 0.06	4.1 ± 0.5	0.069 ± 0.006
	Matosinhos	Autumn 2017	0.05 ± 0.01	4.1 ± 0.7	2.4 ± 0.6	8.2 ± 0.1	1.20 ± 0.05	5 ± 2	0.055 ± 0.001
		Spring 2018	0.033 ± 0.001	1.4 ± 0.5	3.0 ± 0.1	8.7 ± 0.4	1.06 ± 0.05	7.1 ± 0.6	0.07 ± 0.01
		Spring 2018 E	0.036 ± 0.002	2.5 ± 0.7	2.705 ± 0.007	9.2 ± 0.1	1.03 ± 0.01	5.3 ± 0.2	0.076 ± 0.008
		Autumn 2018	0.0436 ± 0.0009	< MQL	3.21 ± 0.07	4.7 ± 0.2	1.10 ± 0.07	7.8 ± 0.7	0.071 ± 0.005
		Spring 2019	0.050 ± 0.006	3.3 ± 0.7	3.1 ± 0.1	8.514 ± 0.005	0.98 ± 0.04	7.7 ± 0.2	0.071 ± 0.009
	Aveiro	Spring 2018	0.031 ± 0.002	<MDL	2.5 ± 0.2	8.2 ± 0.4	1.18 ± 0.05	5.1 ± 0.2	0.075 ± 0.005
		Autumn 2018	0.0221 ± 0.0009	<MDL	2.98 ± 0.06	4.21 ± 0.02	1.362 ± 0.003	7.08 ± 0.05	0.07 ± 0.02
		Spring 2019	0.027 ± 0.001	<MDL	2.0 ± 0.1	9.3 ± 0.5	1.29 ± 0.08	4.3 ± 0.3	0.064 ± 0.004
	Ria de Aveiro	Autumn 2017	0.035 ± 0.002	< MQL	2.5 ± 0.4	4.7 ± 0.5	1.2 ± 0.1	6 ± 1	0.0401 ± 0.0008
		Spring 2018	0.036 ± 0.002	1.9 ± 0.2	4.0 ± 0.3	4.7 ± 0.4	1.3 ± 0.1	9 ± 1	0.051 ± 0.006
		Spring 2018 E	0.0295 ± 0.0006	2.4 ± 0.5	4.2 ± 0.4	4.1 ± 0.3	1.16 ± 0.05	11 ± 1	0.055 ± 0.005
	Figueira da Foz	Autumn 2017	0.0474 ± 0.0006	4.2 ± 0.7	2.0 ± 0.1	6.4 ± 0.3	1.4 ± 0.1	2.8 ± 0.5	0.058 ± 0.002
		Spring 2018	0.0366 ± 0.0003	1.6 ± 0.8	2.1 ± 0.1	9.2 ± 0.1	1.085 ± 0.007	4.4 ± 0.4	0.09 ± 0.03
		Spring 2018 E	0.050 ± 0.005	3.4 ± 0.8	2.522 ± 0.008	11.2 ± 0.4	1.02 ± 0.02	6.0 ± 0.7	0.069 ± 0.003
		Autumn 2018	0.049 ± 0.005	3 ± 1	3.58 ± 0.07	5.08 ± 0.05	1.033 ± 0.008	9.1 ± 0.3	0.08 ± 0.01
		Spring 2019	0.053 ± 0.003	2 ± 1	4.5 ± 0.1	8.3 ± 0.3	1.10 ± 0.04	11 ± 2	0.087 ± 0.008
Palaemon varians	Sado	Autumn 2017 A	0.0490 ± 0.0003	< MQL	4.6 ± 0.5	1.96 ± 0.05	1.16 ± 0.02	12 ± 2	0.023 ± 0.002
		Spring 2018 A	0.086 ± 0.004	46 ± 3	6.00 ± 0.05	2.62 ± 0.07	1.22 ± 0.06	15 ± 1	0.020 ± 0.001
		Spring 2018 A E	0.14 ± 0.03	90 ± 22	6.28 ± 0.06	2.26 ± 0.02	1.1855 ± 0.0006	13 ± 1	0.025 ± 0.002
		Autumn 2018 A	0.090 ± 0.007	38 ± 10	10.7 ± 0.4	2.07 ± 0.01	0.96 ± 0.01	32 ± 2	0.0371 ± 0.0001
		Spring 2019 A	0.069 ± 0.002	< MQL	6.9 ± 0.8	6.05 ± 0.04	1.26 ± 0.04	20 ± 3	0.029 ± 0.001
		Autumn 2017 W	0.0435 ± 0.0004	<MDL	5.2 ± 0.5	1.8 ± 0.1	1.01 ± 0.04	14 ± 1	0.0211 ± 0.0002
		Spring 2018 W	0.09 ± 0.01	52 ± 7	6.2 ± 0.6	2.98 ± 0.02	1.27 ± 0.03	15 ± 1	0.021 ± 0.002
		Spring 2018 W E	0.13 ± 0.01	84 ± 13	7.7 ± 0.6	2.24 ± 0.03	1.17 ± 0.04	18 ± 4	0.022 ± 0.002
		Autumn 2018 W	0.12 ± 0.03	51 ± 24	9 ± 2	2.02 ± 0.06	1.0 ± 0.1	24 ± 4	0.035 ± 0.004
		Spring 2019 W	0.050 ± 0.001	1.6 ± 0.1	4.2 ± 0.3	6.3 ± 0.3	1.35 ± 0.06	10 ± 1	0.025 ± 0.002

Table S10. Content (mean ± SD, mg/kg ww) of seven potentially toxic/non-essential elements in the analyzed shrimp samples; E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		Potentially toxic/non-essential elements (mg/kg)						
			Cd	Sn	Sb	Cs	Ba	Pb	Bi
Palaemon serratus	Vila do Conde	Autumn 2017	0.0021 ± 0.0008	0.016 ± 0.004	0.0027 ± 0.0002	0.0121 ± 0.0006	< MQL	< MQL	0.0047 ± 0.0007
		Spring 2018	< MQL	< MQL	0.0024 ± 0.0003	0.0129 ± 0.0006	0.07 ± 0.02	<MDL	0.0147 ± 0.0003
		Spring 2018 E	0.00271 ± 0.00005	< MQL	0.0022 ± 0.0002	0.0130 ± 0.0004	0.07 ± 0.02	< MQL	0.0080 ± 0.0004
		Autumn 2018	0.0022 ± 0.0006	0.00592 ± 0.00007	0.0013 ± 0.0002	0.013 ± 0.002	0.09 ± 0.04	<MDL	0.0055 ± 0.0004
		Spring 2019	0.0019 ± 0.0004	0.02 ± 0.02	0.0030 ± 0.0009	0.012 ± 0.002	< MQL	< MQL	0.008 ± 0.001
		Spring 2019 E	0.003 ± 0.001	< MQL	0.0027 ± 0.0008	0.012 ± 0.002	< MQL	< MQL	0.0126 ± 0.0007
	Matosinhos	Autumn 2017	0.00153 ± 0.00003	0.0120 ± 0.0007	0.003 ± 0.001	0.015 ± 0.002	0.05 ± 0.01	0.032 ± 0.006	0.0085 ± 0.0007
		Spring 2018	0.0027 ± 0.0003	< MQL	0.0019 ± 0.0002	0.0124 ± 0.0002	< MQL	< MQL	0.01410 ± 0.00003
		Spring 2018 E	0.0025 ± 0.0009	0.004 ± 0.004	0.0024 ± 0.0007	0.014 ± 0.001	< MQL	0.0375 ± 0.0008	0.0180 ± 0.0004
		Autumn 2018	0.0016 ± 0.0002	0.00130 ± 0.00003	0.0014 ± 0.0002	0.0104 ± 0.0001	0.09 ± 0.02	< MQL	0.0101 ± 0.0004
		Spring 2019	0.0022369 ± 0.0000007	0.0042 ± 0.0008	0.0019 ± 0.0005	0.011 ± 0.002	< MQL	0.06 ± 0.01	0.013 ± 0.003
	Aveiro	Spring 2018	0.003 ± 0.001	< MQL	0.0025 ± 0.0005	0.0139 ± 0.0002	0.04 ± 0.01	<MDL	0.033 ± 0.003
		Autumn 2018	0.002 ± 0.001	0.008 ± 0.008	0.0021 ± 0.0006	0.01148 ± 0.00007	< MQL	<MDL	< MQL
		Spring 2019	0.002 ± 0.001	<MDL	0.0019 ± 0.0003	0.012 ± 0.002	< MQL	<MDL	0.028 ± 0.002
	Ria de Aveiro	Autumn 2017	0.0030 ± 0.0007	0.007 ± 0.002	0.0026 ± 0.0004	0.012 ± 0.003	< MQL	< MQL	<MDL
		Spring 2018	0.002 ± 0.002	0.021 ± 0.007	0.005 ± 0.002	0.013 ± 0.002	< MQL	0.03 ± 0.03	< MQL
		Spring 2018 E	< MQL	0.005 ± 0.001	0.0034 ± 0.0008	0.0126 ± 0.0003	0.05 ± 0.04	< MQL	< MQL
	Figueira da Foz	Autumn 2017	0.0027 ± 0.0009	0.0141 ± 0.0008	0.003 ± 0.001	0.018 ± 0.002	< MQL	<MDL	0.01202 ± 0.00009
		Spring 2018	0.0039 ± 0.0002	0.02 ± 0.02	0.0024 ± 0.0002	0.0137 ± 0.0005	0.2 ± 0.1	<MDL	0.0232 ± 0.0002
		Spring 2018 E	0.0025 ± 0.0004	0.010 ± 0.003	0.00220 ± 0.00006	0.013 ± 0.001	< MQL	0.014 ± 0.003	0.03 ± 0.02
		Autumn 2018	0.0055 ± 0.0002	0.019 ± 0.005	0.0027 ± 0.0009	0.014 ± 0.001	< MQL	<MDL	0.015 ± 0.001
		Spring 2019	0.0024 ± 0.0005	< MQL	0.0022 ± 0.0003	0.013 ± 0.001	0.084 ± 0.003	<MDL	0.0183 ± 0.0006
Palaemon varians	Sado	Autumn 2017 A	0.00140 ± 0.00004	0.004 ± 0.001	0.0010 ± 0.0003	< MQL	0.07 ± 0.02	<MDL	< MQL
		Spring 2018 A	0.0013 ± 0.0008	0.0065 ± 0.0009	0.0014 ± 0.0007	0.009 ± 0.001	0.14 ± 0.01	0.040 ± 0.003	< MQL
		Spring 2018 A E	0.0021 ± 0.0001	0.013 ± 0.003	0.0020 ± 0.0003	0.015 ± 0.003	0.20 ± 0.03	0.071 ± 0.003	< MQL
		Autumn 2018 A	0.006 ± 0.003	0.005 ± 0.001	0.0010 ± 0.0002	0.006805 ± 0.000009	0.162 ± 0.008	< MQL	< MQL
		Spring 2019 A	< MQL	0.004 ± 0.003	0.0008 ± 0.0001	< MQL	0.07 ± 0.03	<MDL	0.0072 ± 0.0001
		Autumn 2017 W	0.0026 ± 0.0003	< MQL	0.00072 ± 0.00009	< MQL	0.2 ± 0.1	<MDL	<MDL
		Spring 2018 W	< MQL	0.006 ± 0.003	0.002 ± 0.001	0.010 ± 0.001	0.16 ± 0.06	0.044 ± 0.003	< MQL
		Spring 2018 W E	< MQL	0.011 ± 0.006	0.0028 ± 0.0008	0.014 ± 0.001	0.3 ± 0.1	0.087 ± 0.003	< MQL
		Autumn 2018 W	< MQL	0.008 ± 0.002	0.0017 ± 0.0001	0.009 ± 0.002	0.21 ± 0.01	0.020 ± 0.007	< MQL
		Spring 2019 W	< MQL	< MQL	0.00085 ± 0.00002	< MQL	0.09 ± 0.05	<MDL	0.00878 ± 0.00004

Table S11. Total lipid content (TLC) of the two shrimp species analyzed (g/100g ± SD); E-eggs; A-aquaculture; W-wild.

Specie	Location of the sampling, season and year		TLC
Palaemon serratus	Vila do Conde	Autumn 2017	1.25 ± 0.07
		Spring 2018	1.55 ± 0.07
		Spring 2018 E	2 ± 0
		Autumn 2018	1 ± 0
		Spring 2019	1.25 ± 0.07
		Spring 2019 E	1.25 ± 0.07
	Matosinhos	Autumn 2017	1.7 ± 0.2
		Spring 2018	1.7 ± 0.8
		Spring 2018 E	0.9 ± 0.3
		Autumn 2018	1 ± 1
		Spring 2019	1.25 ± 0.07
	Aveiro	Spring 2018	1.9 ± 0.1
		Autumn 2018	1.1 ± 0.1
		Spring 2019	1.2 ± 0.2
	Ria de Aveiro	Autumn 2017	1.4 ± 0.2
		Spring 2018	1.2 ± 0.3
		Spring 2018 E	1.0 ± 0.1
	Figueira da Foz	Autumn 2017	1.5 ± 0.1
		Spring 2018	1.35 ± 0.07
		Spring 2018 E	1.1 ± 0.2
		Autumn 2018	0.65 ± 0.07
		Spring 2019	1.2 ± 0.4
Palaemon varians	Sado	Autumn 2017 A	1 ± 0
		Spring 2018 A	1 ± 0
		Spring 2018 A E	1.2 ± 0.3
		Autumn 2018 A	1 ± 0
		Spring 2019 A	2 ± 0
		Autumn 2017 W	1 ± 0
		Spring 2018 W	1.05 ± 0.07
		Spring 2018 W E	1.3 ± 0.3
		Autumn 2018 W	1.6 ± 0.8
		Spring 2019 W	1.15 ± 0.07
			1.30 ± 0.01

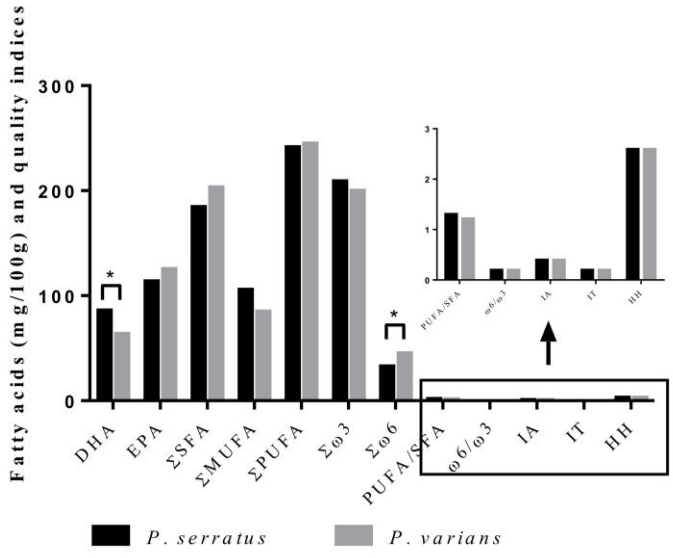


Figure S1. Fatty acids and quality indices in *P. serratus* and *P. varians* samples, * $p < 0.05$.

Table S12. Concentration of 14 essential and probably essential elements (mg/kg ww): comparison with a national database and previously reported data.

	Our study		(INSA, 2019)	(Gokoglu et al., 2008)	(Rao, K. R. P.; Sreeramulu and K.; Sreedhar, 2016)	(Bogard et al., 2015)	(El Gendy et al., 2015)	(Shi et al., 2015)	(Liu et al., 2017)	(Biswas et al., 2021)
Elements	***P. serratus***	***P. varians***	**Specie not identified**	***P. serratus***	***Plesionika spinipes***	***Harina chingri***	***Penaeus semisulcatus***	***Argis lar***	***Penneropenaeus chinensis***	***Penaeus monodon***
Na	2367	2678	1900	-	-	850	-	-	-	-
Ca	495	1170	870	-	-	5500	-	-	-	-
K	4176	4008	1800	-	-	2100	-	-	-	-
Mg	298	286	300	-	-	450	-	-	-	-
Fe	4	28	18	2	28	27	-	-	-	220
Zn	8	10	3	6	36	13	17	11	-	81
Se	0.2	0.2	-	-	-	0.4	-	-	-	-
Cu	7	10	-	6	4	5	5	2	-	-
Co	0.008	0.02	-	-	0.3	-	0.5	-	-	-
Mn	0.2	0.9	-	0.3	-	6	-	-	-	35
I	0.21	0.25	-	-	-	0.26	-	-	-	-
Ni	0.06	0.1	-	-	-	-	0.8	-	-	0.08
V	0.06	0.1	-	-	-	-	-	-	1.6	-
Cr	0.07	0.09	-	-	-	-	-	0.1	-	0.24

Table S13. Mean concentrations of 6 potentially toxic/non-essential elements (mg/kg ww): comparison with previously reported data.

	This study		(Gokoglu et al., 2008)	(El Gendy et al., 2015)	(Raissy, 2016)	(Kaymacı and Altun, 2016)	(Giri and Singh, 2015)	(Olgunoglu, 2015)	(Di Beneditto et al., 2022)
Elements	***P. serratus***	***P. varians***	***P. serratus***	***Penaeus semisulcatus***	***Penaeus merguiensis***	***Red Shrimp***	***Penaeus indicus***	***Aristeus antennatus***	***Penaeus brasiliensis***
Al	2	36	-	-	-	-	-	0.58	-
As	8	3	-	-	0.18	-	0.37	-	2.94
Sr	6	17	-	-	-	-	56	-	-
Cd	0.002	0.002	0.88	1.57	0.19	0.22	0.07	-	0.009
Sn	0.008	0.006	-	-	-	7.65	-	-	-
Pb	0.01	0.03	-	2.33	0.33	0.95	0.04	-	0.02

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Chapter 5

Conclusions and future perspectives

Conclusions

In the last decades, environmental pollution has been recognized as a major concern, particularly in coastal areas due to the rapid development of these regions. Oceans are known to be important in the transport and fate of environmental pollutants, such as OCPs, OPPs, BFRs, PCBs, PAHs, SMs and heavy metals, being a reservoir for many of them. Despite several of these pollutants are nowadays prohibited or restricted, these can still be detected in the environment, due to lipophilic and persistent properties.

In the present work, analytical methodologies were implemented for the determination of several environmental pollutants in shrimp samples and water from their habitat, as well as the evaluation of nutritional properties by the analysis of minerals and fatty acids. The spatial and seasonal occurrence of environmental pollutants and alterations in nutritional composition were investigated. Additionally, the possible effects of environmental pollutants, specifically BFRs, in lipid metabolism were explored.

The extraction of all environmental pollutants evaluated in Portuguese shrimps with exception of heavy metals was achieved by applying QuEChERS methods. The analysis was performed using GC with electron capture detector (ECD) for OCPs, PCBs and BFRs, GC with tandem mass spectrometry (MS/MS) for SMs, GC with flame photometric detector (FPD) for OPPs. All positive results were confirmed by GC-MS/MS. HPLC with photodiode array (PDA) and fluorescence (FLD) detectors in series was used for PAHs monitoring. In the case of heavy metals, microwave-assisted wet digestion was applied and analyzed using ICP with mass spectrometry (MS). The determination of OCPs, OPPs, PCBs, BFRs and SMs in water from the shrimp's habitat was performed by applying solid phase extraction (SPE) method with C18-E cartridges in a vacuum system manifold and analyzed by GC as described for shrimp samples.

The results from this work showed the presence of OCPs (Lindane and DDD) and BFRs (BTBPE) in water from shrimps' habitats.

Concerning shrimp samples, OCPs (DDD), SMs (HHCB, AHTN and ketone), PAHs (Naph, Ace, Flu, Phe, Ant, Fln, Pyr, BaA, BbFt, BkFt, BaP, Bg,h,iP, BjFt and Da,lP) and heavy metals (such as arsenic, cadmium, lead and aluminum) were found. PAHs and heavy metals were not evaluated in water samples. For the environmental pollutants with legislated maximum values, the concentrations found in this study were below the legislated values. Nevertheless, some of the environmental pollutants found have no legislation regarding

maximum allowable values, emphasizing the need of more monitoring studies to reinforce existing legislation.

Considering seasonal variations of the environmental pollutants found in shrimp samples, the average concentrations of the SMs and PAHs and the concentration of arsenic detected were observed to be higher in spring than in autumn. These results may be explained by the increase in physiological activity and growth rate of shrimps during spring resulting in higher environmental pollutants accumulation. Regarding the location of the shrimp sampling, differences were observed for PAHs and some heavy metals concentrations. PAHs and arsenic were higher in Figueira da Foz, this could be justified by the intensive shipping and fishing in the area, lead and aluminum were higher in Sado estuary, more stationary waters. The values of SMs exposure estimated were below the tolerable daily intake (TDI), so it is improbable that a potential health risk occurs from SMs ingestion through shrimp consumption. For PAHs and heavy metals, the target hazard quotient (THQ) was calculated, only aluminum presented values above 1, which may represent the potential risk of adverse health effects through shrimp consumption. However, as aluminum is poorly absorbed by the gastrointestinal tract, it does not present a particular risk.

The presence of these pollutants in food, such as shrimps emphasizes the need to examine the possible outcomes of the exposure to them. For that, a pre-adipocyte and hepatocyte cell lines were used to examine adipocyte/hepatocyte function and lipid metabolism by applying *in vitro* assays suitable for BFRs screening. BFRs exposure caused a disturbance in lipid metabolism and different effects were observed between the BFRs investigated. HBB seems to lead to adipocyte hypertrophy, whereas PBT, TBB and HBCD appear to cause lipid accumulation in hepatocytes. These findings suggest that BFRs could increase the risk of metabolic dysfunction of adipose tissue with a possible impact on liver metabolism.

The beneficial aspect of shrimp consumption was also evaluated through the characterization of the fatty acid profile and the levels of essential and probably essential minerals. Minerals were extracted and analyzed as described for heavy metals. Regarding the determination of fatty acids, the extraction was performed by the Folch procedure followed by base-catalyzed derivatization and analyzed using GC with flame ionization detection (FID). Portuguese shrimps analyzed presented good nutritional quality represented by the low values in the nutritional indices and ratios, index of atherogenicity (IA), index of thrombogenicity (IT), hypocholesterolemic/hypercholesterolemic ratio (HH) and ω-6/ω-3 ratio. The content of sodium, calcium, chromium, iron, cobalt, copper, zinc,

iodine, vanadium, manganese, and nickel presented significant differences between the two shrimp species analyzed, being the highest values present in *P. varians*, the shrimp specie from Sado estuary. The sampling sites in the Sado estuary were close to each other, so its possibly that some elements added to the water for shrimp aquaculture could reach to wild ones, explaining why this specie had a higher content of almost all the essential elements analyzed. The consumption of Portuguese shrimp can be a good nutritional source of EPA + DHA, copper, selenium, iron, manganese, sodium, potassium, calcium, iodine. The assessment of risk-benefit ratio for the two shrimp species analyzed, concerning heavy metals (risk) and EPA + DHA (benefit) confirms the good quality of Portuguese shrimps.

Overall, the present study confirmed the presence of environmental pollutants in Portuguese shrimps (representing a risk of their consumption) and the existence of good amounts of essential minerals and fatty acids (representing the benefit of their consumption). Although pollutants levels found were generally quite low, the accumulation and the synergy of different pollutants could pose risks. So, the results emphasize the importance of constant monitoring of pollutants in marine environments and organisms, ensuring the safety of consumed Portuguese shrimps.

Future perspectives

Even though the findings of this work, some banned pollutants are being replaced for several new alternatives with unknown impacts on the environment, therefore further studies and the continuous monitorization of Portuguese coast are essential. In addition, the large consumption of shrimps generates considerable amounts of shellfish waste, which although biodegradable should be studied as a raw material for new products promoting a circular economy.

Lastly, to improve the knowledge regarding the condition of Portuguese coast and the consequent risk-benefit to human Portuguese population, further studies need to be performed.

Therefore, some proposals for future work are:

-The analysis of other contaminants, such as phthalates, antibiotics and microplastics in shrimp samples from Portuguese coast;

-Besides the parent compounds, metabolites and transformation products should be also included in monitoring studies. In this way, a more embracing knowledge of environmental

contamination could be acquired, contributing to future improvements in the legislation (environmental control and food safety);

-Determine the antioxidant and antimicrobial capacity of shrimp shell waste (shrimp waste valorization) to develop new valorized by-products;

-Extend the analysis of contaminants to different species of different trophic levels and highly consumed by the Portuguese population, such as, Atlantic chub mackerel (*Scomber colias*). Being a secondary consumer in the trophic chain, this fish feeds on, for example, zooplankton, cephalopods and crustaceans) and can be found in the Atlantic Ocean, including the Portuguese west coast;

-Extend the *in vitro* studies to other environmental pollutants, such as synthetic musks and mixtures of contaminants;

-Develop a risk assessment based on more data of species and contaminants;

-Develop a more extensive risk-benefit assessment.