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## Cyclic imines identification and confirmation in European commercial shellfish and seaweed samples

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# SEAFOOD SAFETY

## NEW FINDINGS & INNOVATION CHALLENGES

Stakeholder Event & Open Science Meeting on Key Seafood Safety Developments by **ECsafeSEAFOOD**

ABSTRACTS BOOK

# BRUSSELS

25-26 January 2017

Royal Flemish Academy of Science and  
the Arts (KVAB) **BRUSSELS, BELGIUM**

Consumer needs and concerns Marine toxins in seafood and the environment  
Toxicity and modelling of seafood contaminants Evaluation of seafood  
monitoring data Rapid detection tools for environmental contaminants  
The future of seafood safety Communication outreach and education

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## Oral Presentations

### Safe seafood the consumers can trust: protection in the new Era

#### OP.01. Occurrence of flame retardants in European seafood and consumer risk assessment

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Flame retardants (FRs) are applied to materials (e.g. plastics, furniture or electronic devices) to increase their fire resistance. Polybromodiphenyl ethers (PBDEs) are the most used FRs. Since PBDEs are not bonded into plastics but blended, they can leach out and are found in the environment.

PBDEs are persistent and toxic, bioaccumulate and suffer long-range transport. They are considered persistent organic pollutants and their production has been banned in Europe and North America. Hence new brominated FRs are used as substitutes, as well as chlorinated FRs—dechloranes.

As part of the ECsafeSEAFOOD project, occurrence of FRs in European seafood and the risk for the consumers were assessed. More than 10 edible species (e.g. mackerel, mussel, salmon and tuna) were collected from more than 10 points in Europe including the Mediterranean Sea, the North Sea and the Atlantic Ocean.

The study monitored 8 PBDEs; HBCD; the emerging DBDPE, HBB and PBEB, and *syn*- and *anti*-Dechlorane Plus and Decs 602, 603 and 604. Lyophilized sample was spiked with internal standards, extracted by PLE, cleaned and purified by an acidic attack and SPE. Extracts were analysed by GC-MS/MS (recoveries 51-88 %, LODs 0.01-3.2 ng/g lipid weight (lw), LOQs 0.03-10.8 ng/g lw). HBCD was analysed by LC-MS/MS (recoveries 79-119?%, LODs 0.1-1.0 ng/g lw, LOQs 0.2-3.3 ng/g lw).

A risk assessment was carried out with the average daily intake calculated for several European countries according to the data collected about their typical diets. Both raw and cooked samples were analysed to assess the effect of cooking on the concentration of FRs.

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## **OP.02. Pharmaceuticals and endocrine disruptors in raw and cooked seafood from European markets: human health risk assessment**

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Seafood is consumed all over the world and in some countries it is the prime source of high-quality protein. Due to antropogenic activities, pharmaceuticals (PhACs) and endocrine disrupting compounds (EDCs) are present in aquatic environments. These compounds can be bioaccumulated in seafood and eventually found on markets. Monitoring the presence and levels of these contaminants in seafood is important, especially for those compounds for which no maximum residue limits in food stuff been established yet. Seafood may be an important route of human exposure to PhACs and EDCs, therefore, their potential risk to human health needs to be assessed. Besides, it is relevant to study the effect of cooking on contaminant levels since this process may increase the concentrations.

A European sampling survey of seafood was carried out within the ECsafeSEAFOOD project. Relevant commercial species were collected in different countries and, based on previous results, a list of priority PhACs and EDCs were analysed in raw and cooked samples.

In the majority of the samples PhACs were nor detected or below quantification limits, discarding a potential human risk through seafood ingestion. Regarding EDCs, methylparaben, triclosan, and bisphenol A were frequently detected with concentrations ranging from 0.28 to 183.8 ng/g dry weight. In addition, an increase in these contaminant levels was observed after cooking. A human health risk assessment was performed based on the occurrence of these contaminants, combined with seafood consumption data obtained from a pan-European consumer survey. Consumers from Spain had the highest EDCs intake from seafood consumption though the assessed intake was still below the tolerable weekly intake, and therefore no health concern from seafood consumption alone is foreseen. Other dietary and non-dietary sources of exposure may increase the exposure to these EDCs, but it is unlikely that this would imply a potential health risk to European consumers

**OP.03. Analysis and risk characterization of arsenic species in food supplements based on algae and marine animals.**

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60 different food supplements (FS) based on algae and marine animals (fish, molluscs and krill) were collected on the Belgian market and analysed for total arsenic (Astot), inorganic arsenic (Asi), and the organic arsenic species AB (arsenobetaine), DMA (dimethyl arsinat and MA (monomethyl arsonate). An exposure and risk assessment for the Belgian population was performed afterwards, based on the maximum recommended doses. Total As in the samples was analysed by ICP-MS (inductively coupled plasma - mass spectrometry). As speciation was conducted by HPLC-ICP-MS (high performance liquid chromatography). Astot concentrations were in the order algae > krill oils > mollusc extract > fish oil. The As species concentrations strongly differed among the samples. Remarkable were the relatively low species concentrations in the supplements based on marine animals. It can be assumed that arsenolipids, which were not determined in the present study, dominate in these samples which often consist of an oil fraction. Exposure to As was calculated for each FS by multiplying the concentration of these compounds with the maximal recommended dose. Risks related to the intake of arsenic species in the FS was evaluated by comparing the calculated exposure to the reference values suggested by ATSDR (2007), JECFA (2011) and EFSA (2009). If the reference value was a MRL-value, the conclusion 'concern' was given if the calculated exposure was higher than the MRL value. If the reference value was a BMDL-value, then a 'Margin-of-exposure' (MOE = BMDL/exposure) was calculated. The conclusion 'concern' was given when the MOE < 100. Regarding MA and DMA no (sub)chronic risk was present, and no risk for acute toxicity of Asi was detected. The conclusion 'concern' for chronic toxicity, was given in 19/60 samples.



#### **OP.04. MERLIN-Expo - new advanced tool for higher tier exposure assessment: Human exposure to seafood contaminated by dioxins and PCBs in Venice Lagoon**

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MERLIN-Expo is a new tool for integrated exposure assessment recently developed under "4FUN" FP7 project. MERLIN-Expo incorporates into an easy-to-use tool several advanced models simulating the fate of organic and inorganic contaminants in the environment and in human body. MERLIN-Expo software enables to apply uncertainty and sensitivity analysis, dynamic deterministic and probabilistic simulations in order to address different exposure and chemical fate problems.

The development and operational fusion of the advanced exposure assessment methodologies envisaged in MERLIN-Expo will have a significant impact in the long term on chemical safety management policies. More than 30 agencies in Europe are involved in chemicals exposure and risk evaluation. The main purpose of the present document is to introduce MERLIN-Expo, by showing a case study application, and to highlight its potential for being effectively integrated within the group of tools available to assess chemicals exposure for EU policy.

As case study, the application of MERLIN-Expo to assess long term ecological and human exposure to PCBs and PCDDs in Venice lagoon (Italy) will be presented. Literature data describing pollution historical trends in the lagoon were used as time-dependent model inputs. Three aquatic food models (Phytoplankton, Aquatic Invertebrate, Fish models) were implemented in MERLIN-Expo and coupled to a PBPK model to simulate chemical concentrations in humans after contaminated seafood dietary exposure. Modelling results were tested against available monitoring data to assess the tool reliability and applicability to real complex scenarios. Full chain exposure assessment was then complemented by uncertainty and sensitivity analysis.

## Marine toxins in seafood and the environment: developments in detection and prediction

### OP.05. Risks to shellfish food safety from Tetrodotoxins in the UK

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Tetrodotoxin (TTX) is the causative agent responsible for pufferfish poisoning, found mostly in tropical regions of the world in the organs of fish from the Tetradonotidae family. Clinical effects are similar to those of Paralytic Shellfish Poisoning, with symptoms including nausea, diarrhoea and vomiting, cardia arrhythmias and at higher levels respiratory failure, leading to death. TTXs have also been reported in a range of gastropods, and in two cases of bivalves from New Zealand and Japan. One human intoxication was recorded in 2007 in Europe following consumption of a contaminated sea snail. Until recently there has been no evidence for the accumulation of TTXs in bivalve molluscs within European waters, with the threat from these toxins deemed negligible. In 2013, however, we reported the first findings of Tetrodotoxins in bivalve molluscs anywhere in Europe, with evidence for TTX in mussels and oysters from the South of England. These appeared to be associated with the presence of *Vibrio* spp., in turn linked in the literature to direct production of TTX. Here we present the results from this initial study, together with follow up investigations to assess the prevalence of TTXs in bivalve molluscs from around the inshore waters of the UK. Shellfish samples were assessed between 2013 and 2016 inclusive. Hydrophilic interaction liquid chromatography with tandem mass spectrometry was used for detection of TTX and six related TTX congeners. The method was applied also to the detection of TTXs in vibrio cultures, confirming the apparent production of toxins by bacteria cultured from UK shellfish. Results from the subsequent screening study confirmed the continued presence of TTX in bivalves showing localised concentrations of the toxins during the warmer months in geographically specific areas of the country. The risks from these findings will be discussed.

**OP.06. Developments in the detection of known and unknown toxins in shellfish: tetrodotoxin as an example**

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Recent findings of the emerging toxin tetrodotoxin (TTX) in shellfish produced in The Netherlands led to public health concerns. In order to protect consumers a precautionary principle was applied and harvesting areas are closed when TTX is above the level of 20 µg/kg in shellfish. In the last decades within Europe several analytical chemical alternatives for the widely applied mouse bioassay (MBA) are implemented. Current trends involve UHPLC-MS/MS or targeted screening with LC-hrMS for (un)regulated toxins, such as TTX. Despite the chemical alternatives, alternatives based on the effect of a toxin are needed as these techniques are possible to detect unknown toxins or new risks. Animal-free in vitro cell based effect assays offer the same opportunity as the MBA, i.e. to detect unknown toxins and new risks. A neuroblastoma cell assay was optimized at our laboratory for the detection of various toxins in seafood samples. This easy, relatively cheap in vitro cell assay is used as a first screening to differentiate between blank and suspect samples. Only suspect samples are further investigated with analytical chemical tools. For the regulated toxins such targeted LC-MS/MS methods have been established, and can be applied in monitoring programs (e.g. if countries miss laboratories with cell culture facilities). However, the combination of effect screening with LC-MS/MS confirmation is very powerful. If unexplained results are obtained, i.e. suspect samples in the cell assay that cannot be confirmed by LC-MS/MS, a second stage of cell based assays may be applied. If suspect samples show a response in these assays, and thus suggest presence of an unknown, analytical tools (LC-hrMS) are used for identification. Results indicate that the complete proposed workflow, effect assays combined with analytical tools, will be able to bypass the MBA, the example of TTX will be used to show the applicability of the approach.

## **OP.07. High-Resolution Orbitrap Mass Spectrometry for the Identification of the Emerging Threats to Seafood Consumers in the Mediterranean Area**

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Research on marine biotoxins has shown a continuously changing toxin profile in the Mediterranean area with most of the toxin classes so far known being present in seafood collected along the Italian coastline. Starting from the late '80s with the first finding of okadaic acid in Adriatic mussels, a number of other classes of marine toxins have been found, including yessotoxins, pectenotoxins, saxitoxins, domoic acid, spirolides, palytoxins, and azaspiracids.

Liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS) presents great potential for the identification of all the above mentioned toxins as well as for the structural characterization of the unknowns, due to its high detection sensitivity and selectivity, stable mass accuracy, and large dynamic range. Some of the LC-HRMS methods and HRMS<sup>n</sup> based strategies used for gaining structural insights on the toxins of highest concern in the Mediterranean area are presented. A special focus is given to palytoxins, emerging toxins which are not regulated yet although there is evidence that they can accumulate in seafood thus entering the human food chain. A procedure including a one-step extraction, solid phase extraction (SPE) clean-up and LC-HRMS detection of individual palytoxins in mussels has been developed. The instrumental method allows an efficient chromatographic separation of individual compounds, including structural isomers, with good sensitivity, reproducibility and linearity in the range 14-1000 ng/mL in matrix. The whole procedure (sample preparation and LC-HRMS analysis) proved able to detect palytoxins in both spiked and natural mussel samples at levels as low as 70 ug/kg in crude mussel extracts and 15 ug/kg after SPE clean-up, well below the maximum level of 30 ug/kg suggested by EFSA. Although a full validation of the method is currently prevented by the unavailability of palytoxin(s) certified standards and reference material, this study constitutes the first unavoidable step in such a direction.



#### **OP.08. Cyclic imines identification and confirmation in European commercial shellfish and seaweed samples**

Maria Rambla-Alegre(1);Christopher O. Miles(2);Pablo de la Iglesia(1);Margarita Fernandez-Tejedor(3);Vera Barbosa(4);Alice Tediosi(5);Eneko Madorran(6);Tanja Calis(7);Michiel Kotterman(8);Kit Granby(9);Freek Van den Heuvel(10);Jorge Diogène(1)

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Cyclic imines (CIs) constitute a quite recently discovered group of marine biotoxins that act on neural receptors and that bioaccumulate in edible tissues of filter-feeding organisms. Cyclic imines have not been linked yet to human poisoning and are not regulated in Europe, although the European Food Safety Authority (EFSA) requires more data to perform conclusive risk assessment for consumers. CIs are grouped together due to the imino group functioning as their common pharmacore, responsible for acute neurotoxicity in mice.

Several commercial shellfish (96 samples) from eight different countries (Italy, Portugal, Slovenia, Spain, Ireland, Norway, Netherlands and Denmark) and seaweed samples of *Laminaria digitata* (oarweed) and *Saccharina latissima* (sugar kelp) from Norway were analysed for CIs. All samples were collected in the framework of the ECsafeSEAFOOD project. CIs concentrations in all the samples were analysed on a LC-3200QTRAP and LC-HRMS QExactive mass spectrometer.

In shellfish, two CIs, pinnatoxin G (PnTX-G) and 13-desmethylspirolide C (SPX-1) were found at low concentrations (0.1 to 12 µg/kg PnTX-G and 26 to 66 µg/kg SPX-1) and GYM-A was not detected in commercial samples (raw and processed samples). In summary, SPX-1 (n: 47) and PnTX-G (n: 96) were detected in 9.4% and 4.2% of samples, respectively, at concentrations higher than the LOQ, and in 7.3% and 30% of the samples at concentrations lower than the LOQ (25µg/kg for SPX-1 and 3µg/kg for PnTX-G), respectively.

The presence of pinnatoxin-G was unambiguously identified in the sugar kelp *Saccharina latissima* from Norway by LC-MS/MS. Further analysis of *S. latissima* and *L. digitata* were carried out and showed the presence either pinnatoxin G and/or some spirolides using LC-high resolution MS/MS, although their ratios varied markedly from sample to sample.

The evaluation of CIs in the environment has to pursue in order to assess properly their relevance and potential risks.

## Toxicity and modelling: tools and limitations

### **OP.09. Toxicity of emerging chemical contaminants evaluated in vivo with classic and alternative approaches using the zebrafish animal model**

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An accurate evaluation of the toxic effects of emerging chemical contaminants both at human and environmental level is the first step to achieve their effective regulation. Such evaluation should be performed in a realistic way, taking into account that contaminants are naturally present as mixtures.

In this work we employed the zebrafish animal model as a versatile tool relevant for both human and environmental toxicology. We tested the adverse effects of a mixture of PFCs in a combination naturally found in the aquatic environment. We performed this evaluation in zebrafish embryos using an assay considered alternative to the use of laboratory animals. The results obtained indicated that the mixture is more toxic than the individual compounds and proved that the assay is extremely valuable for evaluating the toxic effects of such contaminants in realistic conditions.

We then tested the toxic effects of microplastic as well as the potential of microplastic to affect the toxicity of a mixture of sorbed persistent organic pollutants and metals, including PFCs. To this end we used adult zebrafish and evaluated the dietary effect at organ level. Microplastic did not show toxic effect itself; however it increased greatly the adverse effect of the mixture of pollutants. Toxic effects were specifically evident in the liver.

The results obtained stress the importance of evaluating the toxicity of mixtures of chemicals in realistic condition to obtain reliable results useful for regulatory purposes; they also confirm that alternative tests complemented with assays with laboratory animals can provide a good input for risk characterization.

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## OP.10. Bioaccessibility of contaminants of emerging concern in raw and cooked commercial seafood species: insights for food safety risk assessment

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The health benefits of a diet based on seafood have been recognised due to the high levels of polyunsaturated n-3 fatty acids, essential elements and vitamins. Nevertheless, the accumulation of environmental contaminants of emerging concern (CEC) by seafood can be a concern for human health. These contaminants are strong candidates for future regulation, and risk-benefit assessment is essential to properly assess food safety issues of these CEC. The effect of the digestion on the availability of CEC for absorption by the intestinal epithelium (bioaccessibility) is essential in risk-benefit analysis, but the information is still scarce.

In this context, a standardized *in vitro* digestion method was used to assess the bioaccessibility of CEC in raw and cooked seafood, including seaweeds, bivalves, crustaceans and fish. The CEC and others contaminants addressed in this work include toxic elements (e.g. MeHg), perfluorinated compounds (PFCs; e.g. PFOS and PFOA), brominated flame retardants (BFRs; e.g. BDE47, BDE100,  $\alpha$ -HBCD), pharmaceuticals and personal care products (PPCPs; e.g. venlafaxine, methylparaben and octocrylene) and marine biotoxins (e.g. okadaic acid, azaspiracids and tetrodotoxin).

CEC bioaccessibility varied according to compound, species and cooking procedure. For example, MeHg revealed low bioaccessibility in all species (1 - 60 %), and steaming decreased MeHg bioaccessibility. Low bioaccessibility was also observed for BDE47 and BDE100 (< 45 %), while PFCs and PPCPs revealed higher bioaccessibility percentages (between 71 and 95 %). A decrease was observed in PBDEs and venlafaxine bioaccessibility after steaming. Okadaic acid and azaspiracids bioaccessibility varied between 55 and 80 %, but decreased after steaming, and low tetrodotoxin (15 %) bioaccessibility was observed in pufferfish. These data are essential for accurate risk assessment of CEC in seafood that will enable drawing up maximum permissible concentrations for CEC at the European level

## OP.11. Bioavailability of emerging contaminants in seafood

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Current legislation on maximum permissible levels of contaminants in seafood considers concentrations found in raw products only. However, the majority of seafood are further processed before consumption, that may result in an increase or decrease of contaminant levels. Furthermore, upon human digestion, substances can precipitate or be transformed chemically, affecting the amount of contaminant potentially accessible for absorption in the intestine (bioaccessibility). Finally, specific receptors and transport mechanisms across intestinal epithelial cells can further affect the final bioavailable amount of contaminants that enters systemic circulation.

In the present work we evaluated the bioavailability of toxic metals (iAs, MeHg), biotoxins (tetrodotoxin, azaspiracid-1), PFCs, musks and UV filters for their transport across human normal epithelial cells H4. For this purpose, H4 cells were grown on microporous membranes until reaching proper transepithelial resistance. Bioaccessible fractions of digested seafood (lean fish, fatty fish and molluscs), spiked with contaminants, were applied to the apical (= lumenal) side of cells, and the transport of the contaminants across epithelia to the basolateral (= towards the body) was evaluated upon incubation.

Contaminants had varying rates of cross-cell transport. Interestingly, transport also depended on the digested food matrix, as well as on the concentration of spiked contaminants, raising a question about proper experimental setup to measure the transport. Nonetheless, information on bioavailability and contaminant-food matrix interactions are important variables that affect exposure of consumers to seafood contaminants and should be included in risk assessment.

### Acknowledgments

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**OP.12. The influence of microplastic inclusion in feed on carryover of environmental pollutants from feed to seabass and salmon**

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Marine litter and microplastic particles cause a man-made pollution of the oceans. Microplastic particles occur in large quantities throughout the seawater column where it is being ingested by fish, when confused with prey items or when the fish drink the water. Environmental pollutants will sorb to the microplastic particles according to their physical chemical properties, with apolar compounds having the highest affinity to the predominant type of microplastic found in the oceans, i.e. low density polyethylene (LD-PE). The aims of the present controlled trials were 1) to study whether the presence of micro-plastic associated with feed may alter the bioaccessibility of emerging seafood contaminants and 2) to determine toxicokinetic parameters i.e. the carryover from feed to fish fillets, important to assess the safety of aquaculture products. The transfer of contaminants were measured and the toxicokinetics modelled from feeding trials where microplastic particles (inclusion 2% of 125-200 µm LD-PE) were added either with contaminants sorbed to microplastic or as clean particle inclusion into contaminated feed, and compared to a contaminated diet without particles. The study included Atlantic salmon (*Salmo salar*), that is one of the main farmed seafood products consumed in Europe and with production in Northern Europe, while European seabass (*Dicentrarchus labrax*) is produced in Southern Europe, where it also is a highly consumed seafood product. The contaminants studied include the brominated flame retardants HBCD, PBDEs; PCBs and methyl mercury. The inclusion of 2 % microplastic particles in feed did influence the toxicokinetics of some contaminants, however in nature, where chemical transfer from microplastic ingestion by fish is overwhelmed by that from natural feed and water, no significant changes are expected.

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## Rapid detection tools for seafood safety

### **OP.13. Electrochemical immunosensors for the detection of marine toxins: tetrodotoxins in puffer fish and azaspiracids in mussels**

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Marine toxins present in the environment are relevant for food safety issues. Researchers are currently putting special emphasis on the development of biosensors for their detection. Compared to conventional techniques, biosensors can provide advantages in terms of sensitivity, specificity, rapidity, design versatility, simplicity, portability, cost-effectiveness and multiplexed configurations.

Tetrodotoxins are potent neurotoxins present in puffer fish but have been recently reported in shellfish. Azaspiracids, are lipophilic toxins that may be present in shellfish. In the frame of the ECsafeSEAFOOD project, immunosensors for the detection of tetrodotoxins and azaspiracids have been developed. On the one hand, the biosensor for tetrodotoxins is based on the immobilization of the antigen on dithiols self-assembled on gold electrodes, followed by a competitive indirect assay. On the other hand, the biosensor for the detection of azaspiracids is based on the immobilisation of the antibody on magnetic microparticles, followed by a competitive direct assay. The use of self-assembled monolayers for antigen immobilisation or protein G-coated magnetic beads as antibody immobilisation supports provides orientations appropriate for the subsequent antigen-antibody affinity interaction, and reduces or avoids non-specific binding and matrix effects. In both approaches, chronoamperometric measurements on 8-electrode arrays have been performed. The analysis of naturally-contaminated puffer fish tissues and mussel extracts, and the good agreement found when compared with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have demonstrated the applicability of the developed immunosensors.

These results pave the way towards the implementation of compact, reliable, inexpensive and automated electrochemical biosensors for high throughput sample analysis in food safety monitoring programs.

#### **OP.14. New recognition elements for the selective detection of environmental contaminants in seafood**

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When making a biosensor, a critical issue is to develop an appropriate sensing layer where the target analyte recognition occurs. In this work 3 different biomaterials (nanobodies, aptamers and molecular imprinted polymers) have been used for sensing environmental contaminants.

1. Nanobodies are single domain antibodies obtained from phage display libraries, which are comprised exclusively of the variable domain of the heavy chain (VHH). A highly selective nanobody for TBBPA detection has been implemented in a Surface Plasmon Resonance (SPR) system. The reported assay, showed an IC<sub>50</sub> for TBBPA of 0.40 ng/mL and negligible cross reactivity (<0.1%) with other tested analogues.

2. Molecular imprinted polymers (MIPs) are generated by co-polymerization of a functional monomer with a cross-linking monomer in the presence of a template molecule. In this work perfluorinated compounds have been used as templates for the preparation of microspheres with affinity towards PFOS and PFOA. After their characterization one MIP was selected due to its high number of active sites and the imprinted effect achieved. In this sense this MIP showed a high selectivity for PFOA detection.

3. Aptamers are small oligonucleotide acid molecules with strong affinity and specificity towards various molecular targets, based on their unique three-dimensional structure. The use of an aptamer for the development of an SPR method for arsenic detection in seafood is reported. A biofunctionalized layer using p-arsanilic acid was created on a gold chip and the different parameters of the method have been optimized. The method showed a limit of detection below 10 ng/mL in water samples.

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### **OP.15. A novel nanoarray for enhancing seafood security**

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The state-of-the-art in natural marine biotoxin analysis in seafood is quite diverse with progress in moving away from the antiquated mouse bioassays. Future bioanalytical methods suitable for the industry require cost-effective approaches to compete with multi-analyte laboratory based analytical approaches such as mass spectrometry. Advancements are required whereby biosensors should detect diverse groups of contaminants as a single test. The difficulties of this approach with nanoarray platforms arise with regulatory limits and assay design. For marine and cyanobacterial toxins antibody based novel sensor methods offer model solutions. For natural toxins the design and application of broad specificity antibodies on multiplex and nanoarray platforms using a single combinational sample preparation offers this opportunity. Broad specificity antibodies to the marine and freshwater toxin targets have been produced and characterised. A planar waveguide immunoassay platform for the multiplex detection of these toxins has been developed. Toxin-protein conjugates were spotted onto sensor slides and molecular interactions between antibodies and conjugates were measured using secondary antibodies labelled with a fluorescent dye. The assays were optimised with regards sensitivity. The speed of the assay was optimised from 45 min, by studying the reaction kinetics, until a fully completed test could be performed within 15 minutes. The sensitivity (IC<sub>50</sub>) for each toxin group has been illustrated as 0.06, 0.42, 1.86, 1.40 and 0.19 ng/mL for saxitoxin, okadaic acid, domoic acid, microcystins and cylindrospermopsin in water samples. To date the assay also demonstrates high suitability for toxin detection in seafood samples in ten minutes.



**OP.16. Real time monitoring of sea contaminants by an autonomous lab-on-a-chip biosensor: The SEA-on-a-CHIP project.**

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Chemical contamination of estuarine and coastal areas is a highly complex issue with negative implications for the environment and human health (through the food chain) and related coastal industries such as fisheries. Early warning systems that can provide extreme sensitivity with exquisite selectivity are required.

SEA-on-a-CHIP aims to develop a miniaturized, autonomous, remote and flexible immunosensor platform based on a fully integrated array of micro/nano-electrodes and a microfluidic system in a lab-on-a-chip configuration combined with electrochemical detection for real time analysis of marine waters in multi-stressor conditions.

The platform will be able to perform the measure of eight representative compounds simultaneously, including contaminants affecting aquaculture production and also those produced by this industry, but it is easy adaptable to other target compounds or other situations required by early warning systems for coastal waters contamination analysis. The system will be built in order to work with one-month autonomy and measuring in real time at least twice per day. The platform will enable an early detection of contamination in aquaculture exploitations and coastal areas, in support of sea industry, environmental and human health protection, as well as the implementation of the Marine Strategy Framework Directive (MSFD).

This presentation will introduce the SEA-on-a-CHIP project and will provide a brief summary of achievements after three years of project, as the performance of the SEA-on-a-CHIP second prototype.

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## Identification and characterization of seafood contaminants

### OP.17. Identification of an optimized protocol for extraction and characterization of microplastics in seafood products

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Pollution by microplastics is a major concern nowadays. Indeed, these plastics particles (<5 mm) are found worldwide in oceans. Ingestion by marine species have been broadly described. However, microplastics can also represent an emerging risk for human consumers when eating seafood products that potentially contain these contaminants. To date, a few studies investigated the levels of contamination collected *in situ*. The most important step for microplastics identification is to use solvent(s) or chemical(s) allowing reliable tissue digestion without degrading plastics polymers. Indeed, no standardized protocol is defined despite the need of international bodies such as OSPAR or EFSA. Moreover, international recommendations, such as the one of OSPAR, use protocol with nitric acid which have been shown to degrade polyamide. The purpose of this work was to test six existing protocols on fifteen plastics polymers and their impact on plastic integrity using weighting, microscopic visualization, pyrolysis-GC/MS and Raman spectroscopy. Moreover, digestion efficiencies were assessed on different seafood: mussels, crabs and fish using microscopic observation and determination of the removal of organic matter. A single protocol had no incidence on plastics polymers and led to good digestion efficiencies on all three seafood products. The protocol using KOH 10% with incubation at 60°C for 24h appeared to be the best compromise for extraction and identification of microplastics in seafood products. The selected protocol is now applied in a study to determine the prevalence of microplastics in the major seafood products consumed in France.

**OP.18. Prevalence, abundance and species diversity of anisakid nematode larvae in Atlantic cod are correlated with geographic area and fish size**

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Atlantic cod (*Gadus morhua*) is among the commercially most important fish species on the world market. It is marketed fresh, frozen or processed. Infection of the edible parts (fillets and liver) with ascaridoid nematodes has long been known.

In the present study, 755 cods were sampled in the Barents-, Baltic- or North Sea between 2012 and 2014. The presence of ascaridoid was investigated by applying the UV-press method or the artificial digestion method, on viscera and fillets.

The following species were identified from a subsample of nematodes from cod caught in the Baltic- and North Sea: *Anisakis simplex* s.s., *A. pegreffii*, *Pseudoterranova decipiens*, *P. krabbei*, *Contracaecum osculatum* and *Hysterothylacium aduncum*. The cod sampled in this study presented a diversity of anisakid nematodes with a majority of fish displaying a mixed infection.

Infection parameters varied greatly among the sampling regions. For example, between all sampling areas, the prevalence varied for whole fish between 22 and 100 % and for fillets between 15 and 90 %. Intensity varied greatly between sampling areas, as well. Moreover, maximum intensity of worms in the fillets was not always reflecting highest prevalence. However, intensity and prevalence correlated significantly and positively with fish length and there was some evidence of seasonal infection patterns. Whereas 46 % of cod had muscle infection, the majority had parasites mainly in the ventral part of the fillet. This observation is of importance for the processing of the fish. Indeed, the trimming of the ventral part of the cod fillet would allow the almost total elimination of anisakids. The present study demonstrated the variable presence of anisakid parasites in the different parts of cod, including the fillets. However, the trimming of the fillets could greatly reduce the risk associated with these parasites.

### **OP.19. Effect of cooking on levels of contaminants of emerging concern in commercial seafood**

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Seafood is a major route for human exposure to environmental contaminants of emerging concern, such as brominated flame retardants (BFRs), perfluorinated compounds (PFCs), toxic element species (inorganic arsenic (iAs) and organic mercury (MeHg)), polycyclic aromatic hydrocarbons (PAHs) and microplastics. However, there is still a lack of monitoring programmes targeting the assessment of these contaminants in seafood, as well as deeper knowledge on the effect of culinary processing on these contaminants levels in seafood. The present study aims to evaluate the effect of culinary processing on several contaminants of emerging concern in seafood consumed in Europe. A minimum of 25 specimens from fish, crustaceans and cephalopods, and 50 bivalve specimens were prepared using common household steaming practices. In most cases an increase in contaminant levels was found after cooking. The contaminant levels of toxic metals, mainly iAs and MeH, increased in most seafood species (83%); whereas the highest increase of contaminant levels were observed within BFRs, specifically PBEB and  $\sum$ MeO-PBDEs. These results clearly indicate that culinary processing may affect the levels of contaminants, with more than 100% increase in cooked samples of sole, mackerel, seabream, plaice, octopus, hake, tuna, mussels, brown crab, salmon and cod. The increase in contaminant levels after cooking still represents no potential health risk for consumers. Nonetheless, the effect of culinary processing should be integrated in monitoring programmes leading to food risk assessment, in order to avoid over/underestimation of risks for consumer health and to enhance their confidence in seafood consumption.

**Key words:** seafood, priority contaminants, steaming

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## Emerging approaches for future seafood safety

### OP.20. Can seafood safety be compromised in the ocean of tomorrow?

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Anthropogenic activities have contributed to two environmental challenges: remarkable chemical contamination and dramatic climate change. Both stressors strongly affect marine ecosystems and are expected to worsen in the future, threatening marine species' welfare and survival, and ultimately consumers' health. Marine organisms inhabiting polluted areas are surrounded by a diversity of pollutants from different chemical groups (e.g. flame retardants, toxic elements, perfluorinated compounds). Some of these contaminants have been recently defined as substances of emerging concern since they currently lack regulation and their toxicological effects to both seafood species and humans still require further understanding. In this way, assessing possible implications of climate change is even more imperative. Hence, this work aims to assess the effects of climate change (warming and acidification) on the bioaccumulation and elimination of mixtures of emerging contaminants (dechloranes 602, 603 and 604, PFOS, PFOA, TBBPA and iAs), using commercially important bivalve species (*Mytilus galloprovincialis* and *Ruditapes philippinarum*) as biological models. Overall, PFOS revealed the highest bioconcentration factor, followed by TBBPA > PFOA > iAs. Despite variations associated to the behaviour and specific chemical properties of each compound, data showed that the bioaccumulation and elimination mechanisms of emerging contaminants are remarkably affected by both temperature and acidification. For instance, increased temperatures promoted higher bioconcentration of TBBPA, but also lower bioconcentration of PFOA and PFOS. On the other hand, acidification promoted the bioconcentration of PFOS, but impaired the bioconcentration of PFOA and iAs. Furthermore, when both climate stressors were combined, the bioconcentration of TBBPA and PFOA further increased, but further decreased for iAs. Finally, data also allowed concluding that 20 days of depuration is not enough for a complete elimination of most contaminants. This study evidenced the need to integrate climate change effects in future seafood risk assessment and to develop mitigation strategies, thus ensuring consumers safety.

## **OP.21. Climate change as potential driver of emerging risks for seafood safety**

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(1) EFSA

According to the General Food Law, the European Food Safety Authority (EFSA) is required to identify emerging risks in the fields within its mission. EFSA has developed a methodological framework for identification of emerging risk, starting from a preliminary identification of priority emerging issues identified through knowledge networking activities. The long term anticipation of emerging risks includes the identification of drivers. Drivers are the underlying natural or human-induced factors that directly or indirectly cause emerging risks. Climate change is one of the identified drivers for several reasons, among which the impact on the occurrence and toxicity of toxin producing phytoplankton, bacteria and pathogenic viruses.

Starting from 2010, EFSA has assessed several signals of potential emerging issues for seafood safety, mainly related to biotoxins and microbiological hazards. Collection and generation of new data, risk characterization, review and analysis of occurrence, exposure and toxicity have followed up in relation to the dinoflagellate *Gambierdiscus* (at the origin of ciguatera food poisoning), cyanobacteria, norovirus and *vibrio paraheamolyticus*. Preliminary data show that environmental conditions associated to climate changes favour the occurrence of cyanobacteria but the effect on their toxicity is still not clear.

The ongoing data collection and generation will be useful for the future development, calibration and validation of models predicting the occurrence and toxicity of these organisms, considering realistic climate change scenarios. It is also necessary, in concert with EU Member States bodies and International organisations, to monitor the present distribution of toxigenic/pathogenic organisms. Risk assessment methods may need to be reviewed in order to consider the possible impact of climate changes.

## **OP.22. Phycoremediation of diflubenzuron, lindane, copper and cadmium potential by *Laminaria digitata***

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Pesticide residues and heavy metals represent a serious environmental concern due to their persistency and potential multi-organ toxicity in both human and wildlife. Therefore, there is a need to implement sustainable remediation methods with reasonable costs to effectively remove these contaminants. The use of macroalgae to remove, degrade or render harmful contaminants in aquatic systems may provide a cost-efficient solution.

The aim of this study was to examine the possibilities for utilization of the brown seaweed *Laminaria digitata* to remove pesticides (diflubenzuron and lindane) and toxic elements (cadmium and copper) in an integrated recirculation aquaculture system. The impact of the process was evaluated by assessing contaminant levels in macroalgae, mussel edible tissue and seawater during 120 h exposure.

*Laminaria digitata* was effective to reduce some organic pollutants (pesticides) and toxic elements in mussels (*Mytilus galloprovincialis*) and seawater when simulating highly contaminated environments. Indeed, in the presence of this algae, levels of diflubenzuron decreased by 70% in mussels after 120 h of exposure. However, this algae was not efficient to reduce lindane, Cu and Cd levels in mussels. In contrast, the levels of the three contaminants were reduced in seawater, remaining only 4, 33 and 42 % of initial levels after 4, 48 and 120 h, respectively, in the presence of the algae and mussels.

Overall, *Laminaria digitata* may allow us to develop “green” methodologies for bioremediation of organic pollutants in an integrated recirculation aquaculture system.

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### OP.23. Effects of depuration on metal levels of bivalve species

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Bivalves are sedentary and filter-feeding organisms that can concentrate pathogenic microorganisms and chemical contaminants from their environment, where toxic elements are the main concern due to their harmful effects. Depuration is only currently mandatory in the EU to diminish pathogenic microorganisms' levels in bivalves harvested for human consumption in polluted waters in order to ensure healthy and safe products for commercialization. However, the efficacy of depuration to eliminate chemical contaminants is still poorly understood, despite few studies reported lower levels of some toxic metals in bivalves after depuration. In this context, the main objective of this research was to evaluate the effectiveness of depuration on reduction of the levels of toxic metals (Hg, Cd, Pb and As) of bivalve species (*Ruditapes philippinarum*, *Mytilus galloprovincialis* and *Scrobicularia plana*) from contaminated estuarine waters. Results evidenced that depuration was effective in reduction of levels of toxic elements (mainly Pb) in the three species, but particularly in *S. plana* after 2 and 8 days (39 and 60%, respectively). This species is currently declared unfit for human consumption due to the high levels of Pb, often found above the Maximum Permissible Limits (MPLs; 1.5 mg/kg). The levels of other toxic elements were always well below the MPLs (0.5 and 1.0 mg/kg for Hg and Cd, respectively) and the maximum allowable levels for total As (86 mg/kg) in all bivalve species, despite the depuration reduced Hg (32%; after 6 days), Cd (38%; after 8 days) and As (19%; after 4 days) levels in *R. philippinarum* as well as 10% of As (after 4 days) in *S. plana*. In conclusion, depuration may be employed as an excellent mitigation strategy to reduce toxic elements levels (e.g. lead) in contaminated bivalves to acceptable values for human consumption.

#### **OP.24. Effects of industrial processing on regulated and emerging contaminant levels in seafood**

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(1) Technical University of Denmark (DTU), National Food Institute (2) DanSalmon (3) DTU, National Food Institute

Mitigation of contaminants in industrial processing was studied for prawns (cooked and peeled), halibut (cold smoked) and salmon (cold smoked and trimmed). Raw prawns had significantly higher cadmium, chromium, iron, selenium and zinc content in autumn than in spring, while summer levels typically were intermediate. Peeling raw prawns increased mercury concentration but reduced the concentration of all other elements including inorganic arsenic, total arsenic, chromium, zinc, selenium but especially cadmium, copper and iron ( $p < 0.05$ ), however interaction between seasons and processing was observed.

Non-toxic organic arsenic in raw halibut and salmon did not transform to carcinogenic inorganic arsenic during industrial smoking. Hence inorganic arsenic was low ( $< 3$  ng/g wet weight) in both raw and smoked salmon (farmed, Norway,  $N=4$  pairs) and halibut (wild caught, Greenland,  $N=10$  pairs) fillets rich in organic arsenic (up to 9041 ng/g salmon and 744 ng/g halibut, wet weight). Processing salmon did not significantly change any levels (calculated both per wet weight, dry weight or lipid content). Smoking decreased total arsenic (17%) and increased PCB congeners in halibut (wet weight). However PFOS, PCB and PBDE congeners were not different in processed halibut when corrected for water loss or lipid content.

#### **Acknowledgments**

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## Posters

### Safe seafood the consumers can trust: protection in the new Era

#### P.01. Seaweeds as animal feed: A risk assessment of arsenic exposure to humans

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It is a well-known phenomena that seaweeds, particularly the Phaeophyceae, are capable of accumulating toxic elements to levels which may prove hazardous to public health when consumed directly. A number of papers have been published in the recent past highlighting the potential for cytotoxic cancer causing potential of *Hizikia fusiformis*– due to its natureally high retention of inorganic arsenic. However, little is known about the potential for human toxicity as a result of consuming livestock and their products which are fed diets containing appreciable amounts of seaweed meal.

The introduction of seaweed into livestock diets has been shown to increase antioxidant activity in animals while also providing valuable vitamins and minerals, with the use of seaweed meal in livestock diets a common occurrence. However, seaweeds used in animal feed are known to contain high levels (~40 mg/kg) of arsenic which may have the potential to carry-over into animal muscle and produce, and therefore pose a threat to human health.

In this study, we have looked at a 6-year data set of monthly arsenic levels taken from seaweed meal sold as animal feed. A risk assessment tool was used to estimate the potential for arsenic carry-over in animal tissue and produce, and the potential for human exposure as a result of consuming livestock products. It has also been determined that different fraction sizes of seaweed meal have an effect on arsenic levels.

This study aims to determine the potential risk to humans consuming animal products which have the potential to be contaminated by arsenic, as a result of the introduction of seaweed animal feed naturally containing high levels of arsenic into the livestock diet.



## **P.02.Tackling frauds: strategies to improve seafood safety and quality**

Serena Meistro(1);Marzia Pezzolato (1);Valentina Audino (1);Katia Varello (1);Elisa Baioni (1);Valentina Cambiotti (1);Pierluigi Acutis (1);Elena Maria Bozzetta(1)

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Seafood fraud is a serious global problem. Selling fish products as fresh when they have actually been frozen/thawed is a common fraudulent practice. Moreover, fish intended for raw consumption must be previously frozen according to Regulations EC 853/2004 and EU 1276/2011, in order to protect consumers. Thus, besides being a commercial fraud, non compliance to this rule also represents a sanitary fraud. Since 2008 we have been evaluating the most performing techniques in distinguishing fresh from frozen fish in order to make available reliable tools both for official control plans and for self-control activities of suppliers. A histological method, with performances assessed on 35 fish species (sensitivity 90.70% - C.I. 82.49-95.9%, specificity 92.59% - C.I. 75.71-99.09%) and showing the best predictive power when compared to spectroscopic techniques, was set up and is now applied in monitoring programmes, performed by regulatory authorities and food business operators (FBO). For marinated fish we could assess the same method reliability; for smoked products, preliminary data are encouraging. A specific monitoring plan, implemented in North-Western Italy in order to estimate how widespread the fraud phenomenon was among restaurants and retailers, showed that the proportion of storage mislabelling was up to 27% in 2014 and up to 12% in 2015. Only a tight collaboration with FBO and control authorities will allow to correctly consider all the processing conditions that have to be distinguished from true frauds. However some major challenges have to be considered: in particular, cephalopods and crustaceans cannot be correctly classified as fresh or frozen/thawed adopting nor the histological approach neither other methods with the expected validity up to date. Thus we recently applied a FFFS (front-face fluorescence spectroscopy) technique and a proteomic approach to cephalopods and a NIRS technique (near infrared spectroscopy) also to crustaceans: our promising results obtained will be further investigated.

### **P.03. UV-filters and musk fragrances in European seafood: Occurrence and risk assessment**

Sara Cunha(1);Laura Trabalón(2);Silke Jacobs(3);Vera Liane Barbosa(4);Margarita Fernandez-Tejedor(5);Kit Granby(6);Wim Verbeke(7);Christiaan Kwadijk(8);Federico Ferrari(9);Johan Robbens(10);Isabelle Sioen(11);António Marques(12);Martí Nadal(13)

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In the last few years, the utilization of personal care products (PCPs) such as musk fragrances and UV-filters has rapidly increased, leading to a rise of potential deleterious effects in marine biota. Even at low doses, PCPs may cause synergic toxicity effects and cumulative stress in exposed organisms.

This study, conducted in the framework of the ECsafeSEAFOOD FP7 project is focused on the monitoring of UV-filters and musk fragrances in seafood at European scale. Additionally, the potential risks for human health associated with the exposure to these contaminants in seafood were assessed for consumers from different European countries. Fifty five samples of seafood consumed in Europe were collected, covering different habitats, extra-EU origin or EU production, and wild or farmed organisms, and were analyzed by gas-chromatography tandem mass spectrometry.

Nine of the eleven UV-filter compounds analyzed, namely 2-ethylhexyl salicylate, homosalate, 3-4-ethylbenzylidenecamphor, octocrylene, 2-ethylhexyl 4-methoxycinnamate, benzophenone 3, benzophenone 1, 2,2'-dihydroxy-4,4'-dimethoxybenzophenone and isoamyl-4-methoxycinnamate were detected in a wide range of samples. Apparently, no correlation was found between the content of UV-filters and location.

The content of 10 different musks was also determined in the 55 seafood samples. Galaxolide (HHCB) and HHCB-Lactone, a transformation product of HHCB, were the most abundant compounds in most of the samples. Musk concentrations ranged between 1 and 100 µg/L, being lower than those found in literature.

The human exposure to musks and UV-filters, estimated from the concentration values in seafood and the daily consumption of each seafood species, were far below toxicological reference values. Although the sampling was representative of the European seafood consumption, it must be highlighted that these results must be considered as a “first screening”. Future research should ensure larger numbers of analyzed samples and integrate the effect of household culinary preparation.

## Marine toxins in seafood and the environment: developments in detection and prediction

### P.04. Development of New Biosensors to detect Ciguatoxins

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Ciguatoxins are lipid-soluble polyether compounds produced by dinoflagellates from the genus *Gambierdiscus spp.*. Ciguatoxins are mostly found in tropical and subtropical zones; however, within the last decade, they have been identified in fishes caught in European waters, notably in Madeira<sup>1</sup> and Canary Islands<sup>2</sup>, while *Gambierdiscus spp.* have also been found both in the NE Atlantic Ocean<sup>3</sup> and in the Mediterranean Sea<sup>4</sup>. These toxins bind to Voltage Gated Sodium Channels at the surface of human sensory neurons where they remain, causing Ciguatera Fish Poisoning with a variety of gastrointestinal, cardiovascular and neurological symptoms (paresthesia, ataxia, cold allodynia), including persistent neurological effects. Ciguatera is the major cause of food poisonings by seafood worldwide, with an estimated 50 000 to 500 000 victims per year. However, there is so far no simple and quick way of detecting these toxins in contaminated samples. Currently, only heavy and expensive laboratory methods are available to detect them: LC-MS/MS, receptor-binding assays by competition with radiolabeled compounds, and neuroblastoma cell-based assays performed on mammalian neurons<sup>5</sup>. We have started to engineer biosensors based on the detection of a transcriptional signal in the yeast model *Saccharomyces cerevisiae*. This unicellular eukaryotic model is well-known and easy to genetically modify, grows fast and presents a very good conservation of signaling pathways with higher eukaryotes. We present a series of genetically modified yeast strains which allow us to follow the activation of specific signaling pathways responding linearly to ciguatoxin exposure. This pre-exploratory project received a seed-funding by CNRS (PEPs project, Océasafe).

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**P.05. “EUROCIGUA, Risk Characterization of Ciguatera Food Poisoning in Europe”  
GP/EFSA/AFSCO/2015/03**

Ana canals(1)

(1) AECOSAN

Since 2008, autochthonous ciguatera food poisoning (CFP) outbreaks have been reported in Spain (Canary Islands) and in Portugal (Madeira). In the Canary Islands the fish genus *Seriola* was involved in many of the outbreaks. *Gambierdiscus* spp., microalgae responsible for CFP, was also detected in the waters of the Madeira and Canary Islands. In order to characterize the risk of CFP in the European Union, the European Food Safety Authority (EFSA) and fourteen European organizations have signed a Framework Partnership Agreement (FPA) that is co-funded by EFSA aimed to characterize the risk of Ciguatera Food Poisoning in Europe (EuroCigua). The main goal of Specific Agreement (SA) no.1 is the management and the scientific coordination of the project. It is coordinated by the Spanish Agency for Consumer Affairs Food Safety and Nutrition (AECOSAN). The SA no.2 aims to determine the incidence of ciguatera in Europe and the epidemiological characteristics of cases, and it is coordinated by the Institute of Health Carlos III (ISCIII). The assessment of the presence of ciguatoxin in food and the environment is the main objective of SA no.3, coordinated by the Institute for Research and Technology in Food and Agriculture (IRTA). Finally, the SA no.4 is coordinated by the University of Vigo (UVigo), whose main target is to develop and validate methods for the detection, quantification and confirmation of the presence of ciguatoxin contaminated specimens. This innovative project joint efforts of research organizations, epidemiology institutions and competent authorities on food safety from several European Member States. The project has strong links to European institutions and international research institutions across the world in order to use this experience for the characterization of this intoxication in Europe as well as its impact in public health. The project started in June 2016 and will run for 4 years.

**P.06. Dinoflagellates of the genus *Ostreopsis* in the Southern Catalan coast (Mediterranean Sea) and Reunion Island (Indian Ocean)**

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(1) IRTA (2) Università degli Studi di Napoli Federico II (3) Hydro Réunion (4) Marine Monitoring, Institute of Agriculture and Food Research & Technology (IRTA), Sant Carles de la Ràpita

Blooms of *Ostreopsis* cf. *ovata* have been reported in the Mediterranean Sea. Outbreaks of this benthic dinoflagellate may be a risk to human health through direct contact and also related to the presence of palytoxins in shellfish. The routine monitoring programs of toxic phytoplankton in shellfish growing areas are usually designed to detect toxin-producing plankton species but not benthic microalgae. A survey was conducted during 3 years along the southern Catalan coast to characterize the population trends and evaluate toxin production in wild populations. Results confirmed the presence of toxin producing *Ostreopsis* cf. *ovata* which were phylogenetically grouped within the Atlantic/Mediterranean clade. Toxin production was in the range of <1- 25 pg palytoxin-like compounds eq/cell. Toxin content was negatively correlated with cell abundance. The strains isolated during the survey produced OVTX-a, OVTX-b, OVTX-b, OVTX-c, isomers OVTX-d and -e, and isoPLTX, the different growth conditions tested did not have effect on the toxin profile. Sea urchins obtained from this area did not contain palytoxin like compounds. During the survey conducted in Reunion Island *Ostreopsis* cf. *ovata* was also detected, these strains grouped within the Indo-Pacific clade and did not produce palytoxin like compounds.

**P.07. In vitro bioaccessibility of the hydrophilic marine biotoxins domoic acid and saxitoxins in raw and steamed shellfish**

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Domoic acid (DA) and Saxitoxins (STX) are potent marine neurotoxins responsible for the human amnesic shellfish poisoning (ASP) and paralytic shellfish poisoning (PSP), respectively. These toxins are hydrophilic compounds that may be easily released from seafood matrices during digestion. However, to date bioaccessibility studies have not been carried and the amount of toxins ingested in food has been considered equal to the amount of toxins available for uptake by the human body. In this study, DA and STX fractions released from the food matrix into the digestive fluids (bioaccessibility) were assessed using a static *in vitro* digestion model. Contaminated mussels, cockles and clams, collected from the Portuguese coast were used to assess bioaccessibility in raw and steamed shellfish. In addition to assessing bioaccessibility of the toxins, conversion of PSP toxins during the digestion process were examined. This study provides relevant new data that can improve and lead to more accurate food safety risk assessment studies concerning these toxins.



## **P.08. Harmful Algal Blooms, Climate Change and Human Health**

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Harmful Algal Bloom (HAB) species produce potent natural toxins which are detrimental to both animal and human health. People are exposed to the toxins through consuming contaminated seafood (particularly shellfish) as well as contact with contaminated water and aerosols. Impacts from these biotoxins include gastrointestinal, respiratory and possibly neurodegenerative diseases (both acute and chronic), and in severe cases, death. HABs are increasing in aquatic ecosystems worldwide which may be due to natural and anthropogenic climate change, and changing non-climatic environmental factors (e.g. nutrient loading). These changes could affect future algal bloom frequency, composition and spatio-temporal distribution and, in the UK, may lead to the permanent establishment of historically uncommon HAB species. This could pose an increasing threat to human health from multiple routes of exposure, including the consumption of biotoxin-contaminated seafood. It is therefore important to understand the relationship between HABs, climate and other environmental changes, and human health to be able to project future changes in HAB events to prevent and mitigate health and socio-economic impacts.

A scoping review of the topic has been undertaken to map the existing literature and identify knowledge and research gaps. In addition, we will use outputs from a hydrodynamic, biogeochemical coupled model for the northwest Atlantic shelf (NWS AMM7), observational *in situ* and satellite oceanographic data, and UK HAB species monitoring datasets to investigate the links between observed and modelled environmental variability and specific HAB-species occurrences. In the future, UK hospital episode statistics linked with the HAB monitoring data will be used to explore the potential associations between HABs and human health impacts. Finally, climate projections for the UK (UKCP09 based on medium emissions scenarios) will be used to identify projected changes to key environmental variables directly linked to algal bloom development and thus to HABs and human health risks.

**P.09. Cell based assay and LC-MS/MS for the identification and quantification of ciguatoxins in fish. Application to the risk characterization of ciguatera**

Jorge Diogène(1); Lucia Soliño(1); Jean Turquet(2); Saha Widgy(3); Anthony Pautonnier(3); Christopher Loeffler(4); Harold Flores Quintana(4); Virginie Hossen(5); Patricia Leroy(6); Eric David(7); Pierre Velge(5); Sylviane Dragacci(6); Sophie Krys(6); Laia Reverté(1); Maria Rambla(1)

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Ciguatera fish poisoning (CFP) is the most common food-borne illness related to the consumption of reef fish in tropical and subtropical areas. The causative agents are toxins belonging to the ciguatoxins (CTXs) group. The neuroblastoma-2a (N2a) cell assay and liquid chromatography-tandem mass spectrometry (LC-MS/MS) were used to assess composite cytotoxicity of samples of fish involved in ciguatera, a strategy that allows to screen fish at risk and identify and quantify the presence of CTXs.

This approach was used to study 120 lionfish samples collected from the surrounding waters of Guadeloupe (n = 60), Saint Barthélemy Islands (n = 55) and Saint Martin (n = 5). Twenty-seven of these samples exhibited CTX-like activity by the N2a assay. Ciguatoxin (CTX) was confirmed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in multiple samples that presented highest composite toxicity levels by N2a. Those fish found to contain CTXs were all from Saint Barthélemy.

A second example was the application of this strategy to establish the lowest observable adverse effects level (LOAEL) obtained from 8 ciguatera events involving 21 individuals in Guadeloupe (French West Indies). For 12 of these events, the presence of CTXs was indicated in meal remnants and in uncooked fish by the mouse bioassay (MBA). Caribbean ciguatoxins (C-CTXs) were confirmed by LC-MS/MS analysis. Based on toxin intakes, the LOAEL was estimated at 4.2 ng P-CTX-1 eq./individual corresponding to 48.4 µg P-CTX-1 eq.kg<sup>-1</sup> body weight (bw). Although based on limited data, these results are consistent with the conclusions of the European Food Safety Authority (EFSA) opinion which indicates that a level of 0.01 µg P-CTX-1 eq.kg<sup>-1</sup> fish, regardless of source, should not exert effects in sensitive individuals when consuming a single meal.

This strategy is presently being used routinely for the identification of CTXs in numerous samples.

**P.10. In vitro investigation on the human metabolism of the lipophilic phycotoxin SPX-1 using liquid chromatography hyphenated with high resolution Orbitrap mass spectrometry**

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13-desmethyl spirolide C (SPX-1) is a marine lipophilic biotoxin produced by dinoflagellates which can contaminate shellfish. Although effects on human health have not been described, data on the behavior of this toxin after human ingestion, including its metabolism, are scarce. Therefore, the aim of this work was to investigate the metabolic fate of SPX-1 using external metabolic activation systems. First, using rodent and human liver S9 fractions, phase I reactions were screened. The combination of phase II conjugation reactions with phase I were also screened with rodent S9. The investigation was conducted in two steps: first the decrease of the parent compound was measured via a LC/HRMS quantitative method and then the formation of metabolites was studied via the metabolite searching software MetWorks®. Results showed that almost all SPX-1 disappeared after only phase I reactions and that at least one hydroxylated metabolite could be detected for both rat and human S9. When phase I and II reactions were performed concomitantly, almost all SPX-1 also disappeared but four metabolites, one being undescribed, could be detected. These results need to be confirmed by i) increasing the selectivity with phase I enzymes using human liver microsomes ii) screening the metabolites formed using a competent hepatic cell line. The identification of the metabolic pathways involved is a key issue for phycotoxin kinetics and toxicity evaluation.

#### **P.24. Azaspiracids and other “Emerging” Algal Toxins in Crabs at Frøya, Norway**

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Brown crabs (*Cancer Pagurus*) were held in tanks and dosed with toxin-free blue mussel homogenate spiked with either pure azaspiracid-1 (AZA1) in 2013 or the cyclic imine pinnatoxin G (PnTxG) in 2014. The crabs were humanely sacrificed periodically up to two weeks after dosing, dissected, and analysed for toxins and metabolites by LC-HRMS. A selection of the crabs were also analysed by ELISA for total AZAs and for the cyanobacterial microcystin group of toxins.

The hepatopancreas (“brown meat”) of all crabs in both studies were heavily contaminated with AZAs, but no significant amounts of AZAs were detected in the white meat. The AZA metabolite profiles resembled those normally found in blue mussels, although several new metabolites were detected and tentatively identified. Analysis of the naturally-contaminated crabs from 2014 indicated a half-life for AZA1 of ca 1 month. The average concentration of AZA1 in the crabs’ hepatopancreata was 55 µg/kg, but levels varied widely (4–147 µg/kg, SD = 31 µg/kg), and cooking modified the AZA toxin-profile, making assessment of consumers’ potential exposure to AZAs via crabs challenging.

No pinnatoxins or metabolites were detected in crabs dosed with PnTxG, indicating either low uptake or rapid excretion/metabolism. These results are in accord with earlier studies on the uptake by crabs of cyclic imines of the spirolides class,<sup>1</sup> and suggest that cyclic imines do not accumulate in crabs.

Microcystins were detected at low levels (average 30 µg/kg, range 7–46 µg/kg, SD = 12 µg/kg) in all 12 hepatopancreas samples tested. These toxins are usually associated with freshwater cyanobacteria, and this finding is not yet confirmed and the origin of the contamination is being investigated. However, microcystins have previously been detected in marine crabs in western Canada.<sup>2</sup>

## Toxicity and modelling: tools and limitations

### **P.11. Toxicity of selected emerging seafood contaminants measured by in vitro cell culture models**

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Seafood products may contain emerging contaminants of natural and anthropogenic origin. Many of these substances are not regulated and maximum permissible amounts have not been set yet for various reasons. Contaminants may be present in low quantities with limited detections methods available and there may be a lack of knowledge about potential toxicity.

In the present work we have analyzed several seafood contaminants with limited information on toxicity, i.e. toxic metals (iAsIII, iAsV, MeHg), perfluorinated compounds (PFCs), brominated compounds (TBBPA) and musks (HHCB, HHCB lactone). Human cell lines in vitro were used for the evaluation of their cytotoxicity, induction of apoptosis, ROS production and genotoxicity (H2AX phosphorylation). In addition, some typical mixtures of contaminants found in seafood have been tested for their combined effects. A special attention was put on stabilization of hydrophobic compounds in water based cell culture media.

Results showed that in the set tested, metals are the most toxic, followed by PFCs, while TBBPA and musks have a limited toxicity. Heavy metals showed high toxicity at concentrations as low as 1 mg/l. The lowest concentration at which toxic effects of PFCs were observed was 15 mg/l. According to our results further studies of these contaminants are needed to avoid potential food safety issues in the future.

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## P.12. Carryover of methylmercury from feed to seabass and salmon

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Contamination of food generally has a negative impact on the quality and may imply a risk to human health. Mercury (Hg) is one of the most hazardous compounds in our environment and is released from the earth's crust by both natural and anthropogenic processes. The mercury species 'methylmercury' is highly toxic, because affects the function of enzymes, easily crosses the blood-brain and the placenta barriers and is toxic to the nervous system (especially the developing brain). It bioaccumulates and biomagnifies through the aquatic food chain. Methylmercury is the most common mercury species in fish and humans are also mainly exposed to methylmercury from consumption of fish and other seafood.

The aims of the present controlled fish feeding trials were to study the carryover from feed to fish fillets (at low spike levels (1x background level of methylmercury) and to determine toxicokinetic parameters.

The study included Atlantic salmon (*Salmo salar*), which is one of the main farmed seafood product consumed in Europe and with production in Northern Europe as well as European seabass (*Dicentrarchus labrax*) produced in Southern Europe, where it is a highly consumed seafood product.

The weight gain of the fish, their feed intake, feed and fish fillet contaminant level were determined to model the uptake and elimination of methylmercury. The toxicokinetics for feed with low levels of methylmercury (41-75 ng/g) showed high assimilation and low elimination.

### Acknowledgments

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## Rapid detection tools for seafood safety

### **P.13. Quantification of viable but non culturable cells of *Listeria monocytogenes* using real-time PCR, on seafood processing environment samples.**

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Listeriosis is caused by the food-borne pathogen *Listeria monocytogenes*, which can be found in seafood and processing plants. Furthermore, it can form biofilms with died, viable and viable but non culturable cells (VBNC), which make it resistant against cleaning and disinfection operations. The microbiological techniques detected only viable cells and classical real-time PCR technique (RT-PCR) detected total bacterial population (died, viable and VBNC). The VBNC state was an important sanitary problem because the bacteria were potentially pathogen in favorite conditions. The aim of this study was the optimization of a RT-PCR method using an intercalating agent of DNA, propidium monoazide (PMA) to quantify viable and VBNC of *L. monocytogenes* in seafood environment samples. *NovF / NovR* primer pairs targeting the *hlyA* virulence gene, was selected from a panel of 14 primer pairs. The method specificity to detect *L. monocytogenes* demonstrated among 16 *L. monocytogenes* strains and 59 strains of other bacteria genus or other *Listeria* species. An optimal PMA concentration has been identified as 50  $\mu$ M. Once this method validated, it has been used to detect/quantify *L. monocytogenes* in seafood environment samples before and after cleaning and disinfectant operations. In parallel, the samples were analyzed by microbiological methods to quantify/detect viable cells. The results showed that after cleaning and disinfectant operations, VBNC *L. monocytogenes* cells can persist in seafood and processing plants.

#### **P.14. New monitoring tool for aquaculture management and seafood safety**

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Seafood safety is strongly dependent on the quality of seawater. The vast majority of farming sites are located in coastal areas that can be affected by several types of pollutants released to the environment by anthropogenic or natural sources and by biotoxins from harmful algal blooms. A lot of contaminants, wide spread in the environment, are taken up by aquatic organisms, entering into the trophic chain and ultimately affecting consumers' health.

In this context, real-time measurements, automatic detection and monitoring are fundamental tools to ensure the compliance with new marine legislation, protection of consumers, as well as the marine environment preservation.

The SEA-on-a-CHIP device is an autonomous, miniaturized on site analytical system for real-time monitoring of harmful contaminants in seawater, developed to be used as an early warning system in aquaculture facilities.

The device is being developed to simultaneously analyse eight representative contaminants of emerging concern, presenting accumulation and toxicity problems, among which: endocrine disruptors, persistent organic pollutants, marine biotoxins, antibiotics and pesticide.

The system provides an extremely sensitive and selective detection of the target compounds through an indirect competitive immunoassay combined with electrochemical detection.

Following the prototype development during the project, the biosensor system has been tested through comparison with conventional analytical chemistry, in order to validate its performance for in situ environmental and aquaculture related applications. Several trials and case studies have been carried out in laboratory, controlled conditions and real environment, for all the selected target compounds.

Along the project and particularly during the last year, achievements and results has been disseminated involving end users and stakeholders in public meetings, publishing supporting audio video material, developing an exploitation plan and a proof of concept in terms of industrial manufacturability and commercialization of the device.

This work is supported by the FP7 SEA-on-a-CHIP project No. 614168.

**P.15. Molecular tools for the detection of larval zoonotic parasites of the genera *Anisakis* and *Pseudoterranova* (Nematoda: Anisakidae) and their allergens, in marine fish of commercial value**

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Larval stages of parasitic nematodes of the genera *Anisakis* and *Pseudoterranova* have been reported as responsible of human infections (anisakidosis) caused by the consumption of raw, undercooked marine fish, harbouring zoonotic anisakid larvae. Species-specific primers/probes was developed based on the mtDNA *cox2* gene, to be used in RT-PCR, for the DNA detection of the most frequent species of the genera *Anisakis* and *Pseudoterranova* occurring in European fish. Specific primers/probes were designed, so far, for five anisakid species of the genera *Anisakis* (i.e. *A. pegreffii*, *A. simplex* (s. s.), and *Pseudoterranova* (i.e. *P. decipiens* (s. s.), *P. bulbosa* and *P. krabbei*). They were labeled with different fluorescent colors, able to detect and identify those anisakid species. The RT-PCR primers/probes, to be used also in multiplex assays, exhibited a high level of specificity, sensitivity and repeatability. It represents a rapid and inexpensive method to identify anisakids in fish fillets.

In addition, a qRT-PCR assay was developed to examine the expression levels of mRNA of some genes coding for antigenic/allergenic proteins of the zoonotic species *Anisakis pegreffii*, under different temperature conditions and time intervals. A differential production of those antigens/allergens released by larvae - *in vitro* cultured - was observed at different intervals of temperatures and time. Possible "biomarkers" of sensitivity to high temperatures represented by some allergens with a major role in human IgE-immune response, have been observed.

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#### **P.16. Application of smart TTI labels for seafood safety monitoring in the cold chain**

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Time Temperature Integrators (TTI) are simple, inexpensive devices that indicate an easily measurable, time-temperature change, directly dependent on the temperature history of the food product they are attached to. The objective of the study was to develop enzymatic TTIs tailored to monitor specific safety requirements of seafood.

The colour change of the enzymatic TTI (M-Check Point®, VITSAB, Malmo, Sweden) is the result of a controlled enzymatic hydrolysis by a microbial lipase (*Rhizopusoryzae* lipase) of a lipid substrate (methylmyristate for M-type and a mixture of trilaurin and tripalmitin for LP-type TTI). Mathematical models which describe the effect of enzyme concentration and storage temperature on the response of the enzymatic TTI in the range 0-30°C were developed. Fish of the *Scombridae* family were inoculated with *Morganella morganii*. Selected TTIs were attached on fish packages and stored at controlled isothermal (0-20°C) and variable temperature conditions. Histamine concentration at predetermined times was estimated based on the response of the TTI and was compared to actual measured histamine formation. Oysters (*Grassostrea gigas*) were inoculated with *Vibrio parahaemolyticus*, placed in plastic trays with attached TTIs and stored at controlled isothermal conditions (15-30°C) and at variable conditions. Microbial load at predetermined times was estimated based on the response of the TTI and was compared to actual measured enumeration. The comparisons between actual and predicted by the TTI indices were based on accuracy and bias factors.

The results of the study indicate that the selected TTIs can be a powerful and a cost effective tool in validating improved handling and storage of seafood products. A TTI based system can be used for a realistic control of the chill chain and efficient management of shelf-life, quality changes, and risk in seafood products.

## Identification and characterization of seafood contaminants

### P.17. Quality of Pointhead flounder and Japanese sandfish

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Pointhead flounder and Japanese sandfish are primarily consumed as salted dry fish. As the final quality of salted dry fish is affected by the quality of the raw fish prior to any processing, maintenance of fish quality is important, which requires precise evaluation. In this study, we investigated the change in the bacterial count and K-value, an indicator of the rate of production of ATP-related compounds, over time as metrics to evaluate the quality of these fishes. All parameters were monitored daily for 8 days from the day the samples arrived at our laboratory (day 0). Flesh and seawater bacterial counts were obtained by the plate count method. The flesh was blended with 0.9% NaCl in sterilized water, and the diluted supernatant was smeared on a standard agar plate with or without 1% NaCl and incubated at 35°C for 48 h or 20°C for 168 h. The K-value was measured by high-performance liquid chromatography after extracting ATP-related compounds from the fish flesh in 10% perchloric acid. The flesh bacterial count in pointhead flounder was  $<10^4$  colony forming units (CFU)/g until day 2 and then increased to  $10^5$  CFU/g on day 4. The seawater bacterial count in pointhead flounder was  $<10^5$  CFU/g until day 2 and increased to  $10^7$ – $10^8$  CFU/g on day 4. The flesh bacterial count in Japanese sandfish was  $10^3$  CFU/g until day 2 and increased to  $10^4$ – $10^5$  CFU/g on day 4. The seawater bacterial count in Japanese sandfish was  $<10^5$  CFU/g until day 2 and then increased to  $10^6$  CFU/g on day 4. The K-values in both pointhead flounder and Japanese sandfish increased rapidly, from 30% to 89% and from 73% to 89%, respectively. The results suggest that these fishes should be subject to processing as soon as possible after they are caught.

**P.18. Pyrolysis-GC/MS: a powerful tool for microplastics identification.**

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Recently, plastic pollution has gained a large interest among the scientific community. Indeed plastic are found to be the major debris found in marine litter. Moreover, ingestion of microplastics particles (<5 mm) by numerous seafood products including multiples species of fish and bivalve, has gained in importance. Therefore, microplastics can represent an emerging hazard for human consumption of seafood products. Only a few microplastics studies performed the identification of plastic polymer from inorganic debris with reliable methods using spectroscopy, spectrometry and chromatography. Fourier Transform infrared (FTIR) and Raman spectrometry are the most employed techniques for polymer identification. For a couple of years, Pyrolysis-GC/MS (Pyr-GC/MS) have been used to get more information on the composition of plastic polymers with some applications on microplastics. This technique has the advantage to be relevant to specifically identify polymers and plastic additives. The purpose of this work was to develop a method using Pyrolysis-GC/MS to characterize microplastics in seafood products. Microplastics from fifteen polymer plastics families were analyses by Pyr-GC/MS and successfully identify. Nonetheless, for some polymers, as polyethylene (PE) or polystyrene (PS), this method is not able for now to discriminate the type of polymerization. As a final approach in this work, Pyr-GC/MS was applied to different samples including different plastics from the marine environment to assess the performance of the method.



## **P.19. Study on selected contaminants in seafood from the Black Sea, Bulgaria**

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Different groups of chemical substances pollute the marine environment. They pose a potential risk to seafood safety and human health. Among them are polychlorinated biphenyl congeners and organochlorine pesticides and marine biotoxins. They build up in the food chain, causing a wide range of possible adverse effects to humans.

Seafood is becoming a substantial part of the Bulgarians' diet as the benefits are already well known.

The aim of the study was to determine concentrations of selected contaminants - polychlorinated biphenyl congeners (PCBs) and organochlorine pesticides (DDT and its metabolites) in bluefish (*Pomatomus saltatrix*), garfish (*Belone belone*), sprat (*Engraulis encrasicolus ponticus*) and mussels (*Mytilus galloprovincialis*) and marine biotoxins (PSP, ASP and DSP) in mussels (*Mytilus galloprovincialis*) collected from the Black Sea, Bulgaria.

Determination of six Indicator PCB congeners, DDT and its metabolites DDE and DDD was carried out by gas chromatography coupled to mass spectrometry after clean-up of the fatty extracts. The levels of organochlorine pollutants in seafood were found in the order DDTs > PCBs. The sum of DDT and its metabolites was determined from 2.01 to 56.81 ng/g wet weight (in mussels and bluefish, respectively) and PCBs concentrations were found below 17.58 ng/g ww (bluefish).

The results for sum of I-PCBs in all seafood studied did not exceed the EU maximum level of 75 ng/g ww.

The first combined determination of both hydrophilic and lipophilic marine biotoxins in mussel samples from Bulgarian Black Sea coast was initiated. Investigation of PSP was performed by HPLC with postchromatographic oxidation and of ASP and DSP on liquid chromatography coupled to mass spectrometry. The concentrations of the analyzed marine biotoxins were below the limit of quantification. Further analyses are planned.

Key words: fish, mussels, polychlorinated biphenyls; organochlorine pesticides; marine biotoxins, Bulgaria

## Emerging approaches for future seafood safety

### P.20. Coping with MeHg in a climate change context: the case study of the benthic fish species *Solea senegalensis*

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Methylmercury (MeHg) is a ubiquitous pollutant with negative health effects (e.g. neurotoxicity). Benthic predatory fish species like Senegal sole (*Solea senegalensis*) are susceptible to accumulate high levels of this contaminant. Since elemental availability in the marine environment is largely dependent on seawater physical-chemical properties, the expected effects of climate change (warming and acidification) may alter the bioaccumulation and detoxification mechanisms of MeHg. This study aimed to evaluate the effect of simulated warming and acidification scenarios on MeHg bioaccumulation in juvenile *Solea senegalensis*. Juvenile sole specimens were maintained in recirculation tanks. Five independent treatments were conducted for a period of 28 days: a) control (average seawater conditions used in sole rearing, 19 °C, pH=8.0), with fish fed a non-contaminated diet; and b) four treatments with fish fed a MeHg enriched diet (cross-factorial design combining the current culture conditions with simulated warming and acidification, i.e. +4°C and -0.4 pH). Samples (brain, liver and muscle) were collected from 6 specimens per treatment at days 0, 14 and 28 for MeHg quantification. An increase of MeHg concentration was observed in all tissues of contaminated fish. Specimens subjected to seawater warming accumulated higher levels of MeHg compared to the other scenarios, whereas, seawater acidification resulted in lower MeHg accumulation. Brain revealed the highest MeHg levels regardless of treatment, emphasizing the neurotoxic effects of MeHg. Warming seems to promote MeHg accumulation in sole, whereas acidification hampers MeHg accumulation. This study highlights the need to implement integrated mitigation strategies to decrease the environmental levels of this pollutant and to ensure that consumers exposure will be limited in the future.

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## **P.21. Climate change effects on the bioaccumulation and elimination of pharmaceuticals and endocrine disrupting compounds in bivalves**

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The occurrence of pharmaceuticals and endocrine disrupting compounds (EDCs) in seawater has been widely reported in coastal areas. Bivalves are susceptible to bioaccumulate these contaminants as they are filter feeding organisms. Since they are widely consumed, such accumulation may be of great concern from a human health perspective. Besides, the expected climate change effects on seawater (warming and acidification), may have an impact on the accumulation and elimination of these emerging contaminants in marine organisms.

In this way, a mesocosm experiment was performed, where mussels (*Mytilus galloprovincialis*) were exposed to a mix of pharmaceuticals and EDCs. The experiment lasted 40 days. In the first 20 days, bivalves were exposed via water to a mixture of venlafaxine, citalopram and carbamazepine at 10 ppb, and sotalol, sulfamethoxazole, methylparaben and triclosan at 20 ppb. In the last 20 days no contaminants were added. The organisms were distributed in five different tanks: control (T1pH1 without spiking) and spiked tanks: T1pH1, T1pH2, T2pH1, and T2pH2 (T1: 18°C; T2: 22°C; pH1: 7.6; pH2: 8.0.). Bivalves and water were sampled at days 0, 2, 10, 20, 22, 30 and 40.

Results showed clearly bioaccumulation for all compounds. The compound which presented the highest bioconcentration factor (mean of all spiking treatments) was citalopram (1648 L/Kg) followed by venlafaxine, triclosan, methylparaben, sotalol, carbamazepine and sulfamethoxazole. The bioaccumulation patterns varied mostly due to the physico-chemical properties of each compound. However, results showed that bioaccumulation is affected by both warming and acidification. Warming resulted in higher bioconcentration of sotalol, carbamazepine and triclosan, while acidification promoted the bioconcentration of methylparaben, but decreased venlafaxine, citalopram and triclosan levels. In addition, results clearly showed that after 20 days of clearance most contaminants were still present in the organisms. This experiment confirmed that emerging contaminants bioaccumulation may be altered by the expected climate change effects.

## **P.22. Phycoremediation potential of brown macroalgae species *Saccharina latissimi* and *Laminaria digitata* towards inorganic arsenic in a multitrophic pilot-scale experiment**

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The presence of organic pollutants and toxic elements in aquatic ecosystems can cause serious problems to the environment and marine organisms and subsequently lead to adverse effects to human health following consumption of contaminated seafood. Hence, technological solutions for the reduction and mitigation of contaminants in the aquatic food production chain are called upon. The phycoremediation technology is a cost-effective algae-based approach that utilizes the ability of macroalgae to concentrate elements and compounds from the environment and to metabolize various molecules in their tissues.

Arsenic (As) is a ubiquitous metalloid found in soils, groundwater, surface water, air, and consequently also in various food items. Arsenic is bioaccumulated in the marine food chain and total arsenic concentrations in the mg/kg range is usually found in marine organisms. The toxicity of arsenic depends on the chemical species, where inorganic arsenic is considered to be the most toxic form of arsenic.

The aim of the present study was to evaluate the phycoremediation capacity of the two brown seaweed species Sugar kelp (*Saccharina latissima*) and Oarweed (*Laminaria digitata*) in a controlled multitrophic (water, algae, mussels) pilot experiment with exposure to inorganic arsenic. The results of the experiments indicated that of the two algae species used in the experiment, *Laminaria digitata* was more efficient for removal of arsenic from seawater and hence a better choice for phycoremediation practises towards this parameter.

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### **P.23. Phycoremediation as mitigation strategy for removing venlafaxine from the aquatic environment**

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Phycoremediation has emerged as a promising technology for removing undesirable substances from the aquatic environment. While there is extensive information on the capacity of macroalgae to take up and concentrate heavy metals little is known about its ability to accumulate organic pollutants. Venlafaxine is a psychiatric drug usually found in the aquatic environment (Gros et al., 2012) and also previously detected in seafood (Alvarez-Muñoz et al., 2015). Therefore, the goal of this work was to study if phycoremediation can be used as mitigation strategy for venlafaxine.

Two experiments were performed (phase A and B). In phase A, *Saccharina latissima* and *Laminaria digitata* were exposed to 0.5, 1, 5 and 10 µg/L of venlafaxine during two weeks. Once demonstrated that macroalgae had the capacity to accumulate venlafaxine, the impact of the improved strategy was evaluated combining seaweeds with farmed bivalves (phase B). This experiment was undertaken using *L. digitata* at one exposure concentration (10 µg/L) during 5 days.

In phase A, it was observed that *S. latissima* accumulated more venlafaxine than *L. digitata* (6.9 mg/kg dry weight (dw) in 24 h versus 3.6 mg/kg dw in 48h). A strong decrease in water concentration of venlafaxine was also observed after 12h of experiment. In phase B, the results showed that bioconcentration of venlafaxine in mussel was higher than in algae (1.5 mg/kg dw in mussel versus 1.1 mg/kg dw in algae). Regarding the concentration of venlafaxine in seawater, it was shown that in the absence of any organism the levels were constant. A similar trend was observed when only mussels were present in the experiment. However, when algae were included either alone or together with mussels a great drop in venlafaxine concentration was measured after 24h of exposure. This result highlights the potential of phycoremediation for removing venlafaxine from the aquatic environment.