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Review

Relevance and challenges in monitoring marine biotoxins in non-bivalve vectors

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ABSTRACT

Seafood poisoning outbreaks can be caused by marine biotoxins which are naturally produced by harmful algal blooms. To minimize the risk of acute intoxications due to consumption of contaminated seafood a proper monitoring program must be in place. In recent decades several directives have been laid down by the European Commission to regulate known toxins, reassess their regulatory limits and update their reference detection methods. However, a revision of the seafood organisms that can act as toxin vectors has not been carried out. The control system has been designed based on physiological specificities of live bivalve mollusks. Although the prescribed controls in EC regulation 854/2004 apply to echinoderms, tunicates and marine gastropods, several difficulties are posed to a cost-effective monitoring program for these quite diverse and non-analogous groups of seafood organisms. Echinoderms, tunicates and marine gastropods are frequently secondary target species for toxins surveillance. In this study, the potential of non-bivalve organisms as toxin vectors and their threat for public health is evaluated based on their feeding behavior (i.e. filter-feeders, herbivores, predators), growth and metabolic rates, motile capacity and dynamics of toxin accumulation/elimination. A summary of previous reports on toxin accumulation and human incidents is presented to highlight the seafood species of higher risk to consumers, including crustaceans that are not listed in the EU directives for toxins monitoring and should be strongly considered as potent vectors of biotoxins to humans. Finally, the challenges in terms of sampling efforts and analytical determination for the regular surveillance of biotoxins in non-bivalve vectors are discussed.

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1. Introduction

In Europe, the vast majority of human intoxications attributed to seafood contaminated with marine biotoxins have been related to live bivalve molluscs. These animals are filter feeders, and can concentrate toxins from the recurrent blooms of toxic microalgae becoming vectors to humans.

Along with demographic explosion, bivalve aquaculture started expanding during the XXth century but without the existence of monitoring programmes targeted at marine biotoxins. In the early 1980's, several outbreaks attributed to diarrhetic shellfish poisoning (DSP) occurred, some of them suddenly affecting several thousand people were reported in Spain and France, as well as other countries (FAO, 2004). Due to their characteristic neurological symptoms, outbreaks of paralytic shellfish poisoning (PSP) were also described, starting earlier at the beginning of the XXth century. However, the number of affected people has always been lower compared to DSP outbreaks, generally on the order of tens of people, rarely surpassing a hundred people (FAO, 2004).

Monitoring programmes were often introduced in restricted areas only after these major outbreaks, which mined consumer's confidence on bivalve safety. With the development of the European Economic Community (EEC), common policies were drawn, having in mind the harmonization of national policies to avoid unequal conditions of competition that could directly affect the functioning of the common market. The greatest concern in the 1970's regarding shellfish growing areas was the protection and improvement of the environment, that required concrete measures against pollution (EEC, 1979). Council Directive 79/923/EEC, on the quality required for shellfish waters, focused on anthropogenic contaminants and only required quarterly surveillance of faecal colliforms in bivalves. Saxitoxin, the main PSP toxin known at the time was mentioned but without any sampling frequency, method of analysis or guidance level (EEC, 1979).

It was only a dozen years later that the health conditions for the production and the placing on the market of live bivalve molluscs, and other fishery products were laid down with Council Directive 91/492/EEC (EEC, 1991a) and Council Directive 91/493/EEC (EEC, 1991b), respectively. A quantitative mandatory level was then established for the presence of paralytic shellfish poisoning toxins (PSTs) and a qualitative level for the presence of diarrhetic shellfish poisoning toxins, both to be determined by a biological assay. These rules were updated in subsequent years to include testing for amnesic shellfish poisoning (ASP) toxins with Directive 97/61/CE (EC, 1997), and details for the testing of different lipophilic toxin groups, previously analysed altogether within the 'DSP' biological assay in Commission Decision 2002/225/CE (EC, 2002).

Directive 91/492/EEC specified the requirements to be laid down for all stages during harvesting, handling, storage, transport and distribution of live bivalve molluscs shall apply equally to echinoderms, tunicates and marine gastropods (EEC, 1991a). A few years later, a major review of the rules for the hygiene of foodstuffs was published comprising Regulations (EC) nº 852/2004 (EC, 2004a), (EC) nº 853/2004 (EC, 2004b), (EC) nº 854/2004 (EC, 2004c) and (EC) nº 882/2004 (EC, 2004d). In Regulation (EC) nº 853/2004, live bivalve molluscs, live echinoderms, live tunicates and live marine gastropods were clearly separated from the remaining "fishery products" (EC, 2004b).

The number of marine biotoxins to be tested has been updated along the years. However, a revision of the animal groups acting as toxin vectors was not carried out, remaining unchanged since directives laid down in 2004 (EC, 2004b,c). Testing methods were also updated to include analytical methods as alternatives or replacements to mouse bioassays (EC, 2005, 2006, 2007, 2011). The move to analytical methods meant not only further sensitivity, but also further selectivity and detail on toxin profiles present. With technological advancements, new toxin groups were identified, while others were designated as 'emerging toxins', often due to the apparently absence in a recent past in European waters and novel appearance somewhat related to climate change, affecting distribution of phytoplankton species (EFSA, 2009, 2010a, 2010b). These toxin groups 'emerged' not only in bivalves, but in several animal groups included in the fishery products (*e.g.* puffer fish and ciguatoxic fish) (Vale, 2011). Other animal groups, often maintained alive even in restaurants – *e.g.* crustaceans such as *Cancer pagurus* or *Nephrops norvegicus* – were never grouped so far with the live bivalve molluscs for monitoring purposes. Nevertheless, an outbreak of DSP involving more than 200 persons who ate brown crabs was reported in Norway in 2002 (Castberg, Torgersen, Aasen, Aune, & Naustvoll, 2004), and a smaller outbreak was also suspected to have happened in Portugal in 2001 (Vale & Sampayo, 2002).

In Directive 91/492/EEC, sampling plans were required to detect changes in the composition of the plankton containing toxins and their geographical distribution (EEC, 1991a). Upon suspicion of the presence of toxic plankton, intensive sampling had to be triggered by increasing the number of sampling points and samples, accompanied by toxicity tests. With Regulation (EC) nr 853/2004, sampling frequency for toxin analysis in the molluscs was established, as a general rule, to be weekly during the periods at which harvesting is allowed (EC, 2004b). However, this frequency can be reduced depending on risk assessment of toxins or phytoplankton occurrence suggesting a very low risk of toxic episodes.

Monitoring plans and toxin testing have associated costs. Obviously a higher frequency implicates higher costs. For non-bivalve vectors no specific details on sampling plans were ever established. In addition, animals from these groups include filter-feeders (that can directly acquire toxins from microalgae), others are grazers, while others predate bivalves among other prey. The situation has remained quite vague so far. Despite this omission, not testing for gastropods can be subject for non-compliance reported in the country audits performed to member states by the Food and Veterinary Office of the European Commission (*e.g.*: FVO, 2012).

To maintain an effective monitoring program for assessing the risk of toxins occurring in bivalve mollusks, echinoderms, tunicates and marine gastropods, it is necessary to establish a sampling plan taking into account the different feeding strategies of these organisms. However nothing is stated (*e.g.* sampling frequency) for the remaining seafood organisms that should be considered for toxins analysis (EC 2004 b,c).

This review is aimed at compiling known information about human outbreaks associated with non-bivalve vectors contaminated with toxins, in order to, infer their relevance or potential threat for public health, and examine challenges that are posed for effective monitoring.

2. Feeding behavior of bivalve mollusks, echinoderms, tunicates and marine gastropods

Understanding the feeding behavior of bivalve mollusks, echinoderms, tunicates and marine gastropods is essential to assess their potential as toxin vectors and their dynamics of toxin accumulation. A diverse range of feeding behaviors, from simple passive filter-feeders to active predators, can be found among these seafood species.

Filter-feeding organisms are directly exposed to toxic algae cells, being their toxin levels often well correlated with the abundance of toxic phytoplankton in seawater, and their toxins profile a resemblance of the toxigenic algae. While bivalve mollusks and tunicates