Toxicon 137 (2017) 135–143

Contents lists available at ScienceDirect

Toxicon

journal homepage: www.elsevier.com/locate/toxicon

Presence of azaspiracids in bivalve molluscs from Northern Spain

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ARTICLE INFO

Article history:
Received 20 June 2017
Received in revised form
19 July 2017
Accepted 30 July 2017
Available online 2 August 2017

Keywords:
Azaspiracid
Cantabrian sea
Galicia
Bivalve
Upwelling
Downwelling

ABSTRACT

Low concentrations of azaspiracids have been found in bivalve molluscs all over the Northern and Northwestern coast of the Iberian Peninsula. The detections started in June 2016 and lasted until March 2017. The observed toxin profile was dominated by AZA2, followed by AZA1 and some other AZAs that were detected only in some samples. Some compounds producing fragments characteristics of AZAs but that do not fit with any of the known ones were also found. The causative agent has not been identified but, in sight of the toxin profile in the bivalves, it seems that it should be a new species or strain. The detections of AZAs in bivalves in the Northern Coast was linked to downwelling or upwelling relaxation and, in the Galician Rías, took place (with only a few exceptions) in the outer (more oceanic) part, suggesting that the responsible species develops at the open sea and that the populations are advected to the shore.

1. Introduction

Marine phycotoxins are an important problem for the exploitation of marine living resources in many coastal areas. Their direct impact on human health is considerable as every year close to 2000 cases of human intoxication, through the consumption of fish or shellfish (with approximately 15% mortality), are recorded (Hallegraeff, 2014). This risk of intoxication produces different economic impacts mainly because monitoring systems have to be established and maintained, market must be banned when the toxins are found in concentrations over the regulatory threshold, and market retractions take place when intoxications are reported (Mariño et al., 1998; Hoagland et al., 2002; Fernández et al., 2003; Hoagland and Scatasta, 2006; Dyson and Huppert, 2009; Morgan et al., 2009; Blanco et al., 2013; Reich et al., 2015).

There are different groups of marine phycotoxins, some of them include hydrophilic compounds as Paralytic Shellfish Poisoning (PSP) toxins or Amnesic Shellfish Poisoning (ASP) toxins, but most are constituted by lipophilic or amphiphilic compounds, as the toxins of the okadaic acid group, yessotoxins, pectenotoxins, brevetoxins, ciguatoxins, palytoxins and azaspiracids (Paz et al., 2011). Okadaic acid and its analogues were the first toxins known to be responsible for the toxic syndrome named Diarrethic Shellfish Poisoning (DSP) (Murata et al., 1982; Yasumoto et al., 1984; Hu et al., 1993) and are probably the group with the highest economic impact at a global scale because it is widely distributed through different continents and latitudes (Reguera et al., 2014). Azaspiracids (AZAs) (Fig. 1) produce intoxications with a diarrheic symptomatology similar to that of okadaic acid, and their effects on mice by intraperitoneal injection are also similar. As a consequence, while the DSP toxicity was routinely quantified by mouse bioassay (in many European countries until 2014, when LC-MS/MS replaced mouse bioassay as method (European Commision, 2011)) the joint toxicity of both kinds of toxins were quantified together (EFSA Panel on Contaminants in the Food Chain, 2008), and when they co-occurred the toxicity would probably have been attributed only to okadaic acid, perhaps underestimating the presence and the importance of azaspiracids.

Azaspiracids were discovered in Irish mussels (McMahon and Silke, 1996; Satake et al., 1998) and the only know intoxications reported up to date were due to the consumption of shellfish from that country (Twiner et al., 2014). They are produced by several species of the genera *Azadinium* and *Amphidoma* (Tillmann et al.,

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http://dx.doi.org/10.1016/j.toxicon.2017.07.025
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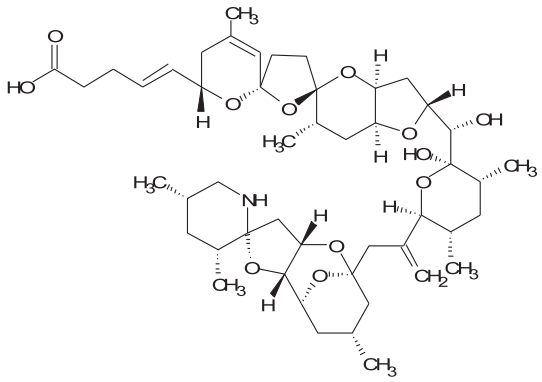

Fig. 1. Structure of Azaspiracid-2.

2009, 2012a, 2016, 2017; Rossi et al., 2017;) from different oceans (Ueoka et al., 2009; Álvarez et al., 2010; Lopez-Rivera et al., 2010; Trainer et al., 2013; Turner and Goya, 2015). In the Iberian peninsula its presence was reported in 2003 (Braña Magdalena et al., 2003) after analysing a single mussel sample obtained from the Ría de Vigo (Galicia, NW Spain). In that sample, the toxin profile was very close to that found previously in mussels from Ireland and Norway (James et al., 2002) and the concentration, 240 μg of total AZA·kg^{-1}, would be well over the EU regulatory limit for AZA toxicity (160 μg AZA1-eq·kg^{-1}) if the current toxicity equivalence factors (TEFs) are used in the computation. Up to the end of 2016 (data in this study) no Azaspiracid was detected in bivalves or seawater (using passive samplers) (pers. observation and pers. com. by Arévalo, Franco and Riobó) even when the methodology used allowed us to find those compounds in other areas (Álvarez et al., 2010). In 2006, after being monitored since 2002, AZA1-3 were found in bivalves from the Portuguese coast with a toxin profile different from the typical one found in Irish mussels but similar to that from the Moroccan coast (Taleb et al., 2006), dominated by AZA2, instead of AZA1 and with only a very small proportion of AZA3 (Vale et al., 2008). The AZA concentrations attained by the bivalves were very low, ranging from 1.6 to 6.1 μg kg^{-1}, so, well below the European regulatory limit (160 μg AZA1-eq·kg^{-1}). Also in 2006, azaspiracids were found in several bivalves from the coast of Huelva (SW Spain), again with a profile characterized by the dominance of AZA2 but, in this case, attaining levels that surpassed the European regulatory limit (Tillmann et al., 2017). *Amphidoma languida* was found to be the AZA-producer species in that episode and a new azaspiracid, AZA 43, which is isobaric with AZA3 and has a very similar chromatographic retention time, was found.

In this work, a number of detections of azaspiracids in samples from the official monitoring programmes of bivalve production areas (areas with natural beds or sites for the cultivation) of the Atlantic and Cantabrian coast of Spain, from Portugal to France are described.

2. Material and methods

2.1. Sampling

In Galicia, cultured mussels from 52 locations in the Rías of Sada, Corme, Muros, Arousa, Pontevedra and Vigo were obtained and analysed on a weekly basis by HPLC-MS/MS since January 2016 using a Waters Xevo TQ-S mass spectrometer. Additionally, 37 infaunal mollusc (clams, cockles, etc) production areas all over the coast were also sampled but with slightly lower frequency (depending on the time-course of the toxic episodes). Previously, part of the samples monitored by mouse bioassay were also analysed with a Thermo Deca XP plus ion trap mass spectrometer, a Thermo TSQ Quantum Access MAX and a Waters Xevo TQ-MS.

In Asturias, Cantabria and the Basque Country, one, three and three locations, respectively, were monitored, in all cases fortnightly (Fig. 2).

2.2. Reagents and reference materials

AZA1-3 reference certificate solutions were obtained from NRC (Canada) and from Laboratorio CIFGA, (Spain). Acetonitrile (MeCN) was obtained from Rathburn (Walkeburn, Scotland). Methanol (HPLC quality) was obtained from VWR (Barcelona, Spain) and ammonium hydroxide (for analysis) from Merck (Barcelona, Spain). Ultrapure water was obtained from a Milli-Q A-10 (Millipore Ibérica, Madrid, Spain).

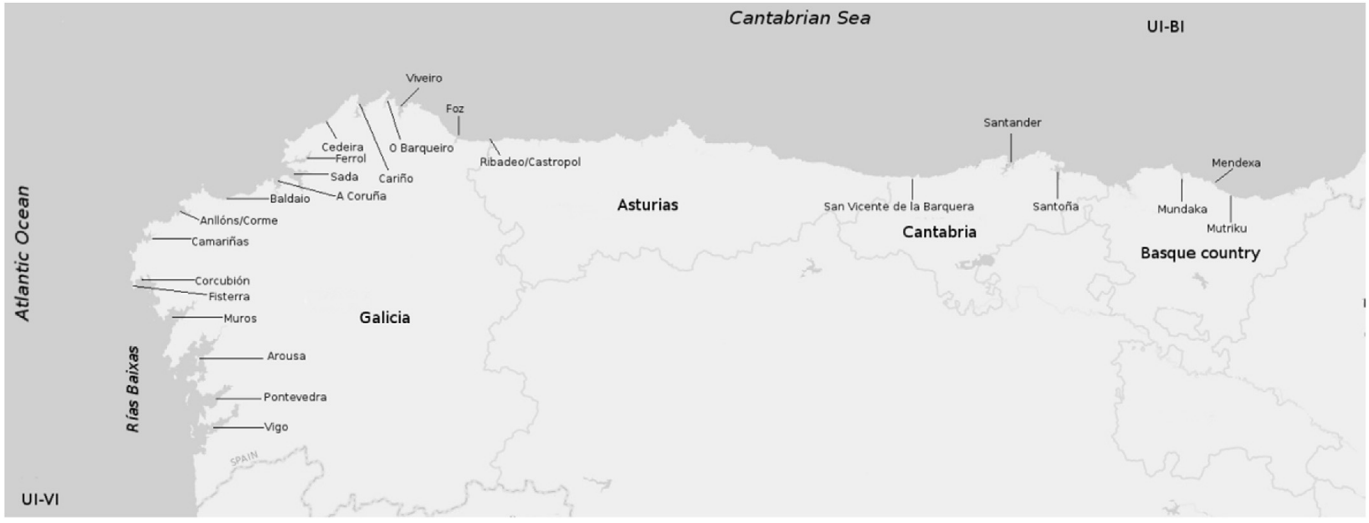

Fig. 2. Area of study indicating the main sampling Rías and stations. UI-VI and UI-BI = locations used to compute the upwelling indices for the Rías Baixas (Vigo Area) and Cantabrian sea (Bilbao Area), respectively. (Details of the sampling areas in Galicia are given in the Supplementary material).