Marine Environmental Research 133 (2018) 45–48

Contents lists available at ScienceDirect

Marine Environmental Research

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

Presence and persistence of the amnesic shellfish poisoning toxin, domoic acid, in octopus and cuttlefish brains

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ARTICLE INFO

Keywords:
Domoic acid
Brain
Cephalopod
Octopus
Cuttlefish
Bioaccumulation
Algal toxins
Toxicity

ABSTRACT

Domoic acid (DA) is a neurotoxin that causes degenerative damage to brain cells and induces permanent short-term memory loss in mammals. In cephalopod mollusks, although DA is known to accumulate primarily in the digestive gland, there is no knowledge whether DA reaches their central nervous system. Here we report, for the first time, the presence of DA in brain tissue of the common octopus (*Octopus vulgaris*) and the European cuttlefish (*Sepia officinalis*), and its absence in the brains of several squid species (*Loligo vulgaris*, *L. forbesi* and *Todarodes sagittatus*). We argue that such species-specific differences are related to their different life strategies (benthic/nektobenthic vs pelagic) and feeding ecologies, as squids mainly feed on pelagic fish, which are less prone to accumulate phycotoxins. Additionally, the temporal persistence of DA in octopus' brain reinforces the notion that these invertebrates can selectively retain this phycotoxin. This study shows that two highly-developed invertebrate species, with a complex central nervous system, where glutamatergic transmission is involved in vertebrate-like long-term potentiation (LTP), have the ability of retaining and possibly tolerating chronic exposure to DA, a potent neurotoxin usually acting at AMPA/kainate-like receptors. Here, we filled a gap of information on whether cephalopods accumulated this neurotoxin in brain tissue, however, further studies are needed to determine if these organisms are neurally or behaviourally impaired by DA.

Domoic acid (DA) is a phycotoxin produced by two genera of diatoms, *Pseudo-nitzschia* and *Nitzschia*, and is responsible for the Amnesic Shellfish Poisoning (ASP) in humans (Quilliam and Wright, 1989). DA acts as an analogue of glutamate, an excitatory neurotransmitter, binding to the same receptors as the latter, i.e. ionotropic glutamate receptors in particular AMPA receptor subtypes. This process leaves the receptors permanently open, causing an excessive influx of calcium ions to the cells (Hampson and Manalo, 1998). This leads to neural membrane depolarization and subsequent neurodegeneration, inducing permanent short-term memory loss in vertebrates (Bejarano et al., 2008). This neurotoxin has also been linked to several events of mass mortality in fish, seabirds and marine mammals (see Pulido, 2008 and references therein). In the latter animal group, DA is known to elicit a variety of negative impacts, from behavioural alterations (leading to mass strandings) to premature abortions (Kirkley et al., 2014; Scholin et al., 2000; Silvagni et al., 2005). It is worth noting that although stranded marine mammals (namely sea lions *Zalophus californianus* and Pacific harbor seals *Phoca vitulina richardii*) never presented with DA in their brains, the lesions found in the hippocampus were characteristic of exposure to an neuroexcitatory toxin such as DA (Cook et al., 2015; Gulland et al., 2005; McHuron et al., 2013).

Among the invertebrate DA vectors in coastal food webs, it is known that cephalopod molluscs, in particular the common octopus (*Octopus vulgaris*) and European cuttlefish (*Sepia officinalis*), accumulate high DA concentrations in the digestive gland (Lopes et al., 2013), their main storage site (Rosa et al., 2004). DA is found at much lower levels in other organs and it is undetectable in the muscle (Costa et al., 2004b). Yet, there is no information regarding DA accumulation in the brain of these cognitively skilled invertebrates.

To fill this knowledge gap, specimens of the common octopus (*Octopus vulgaris*), European cuttlefish (*Sepia officinalis*) and three squid species, namely the European squid (*Loligo vulgaris*), veined squid (*L. forbesi*) and European flying squid (*Todarodes sagittatus*) were collected in the NW (Peniche; 39°21′N 9°22′W) and SE (Olhão; 37°1′30″N 7°50′30″W) Portuguese coast from May to September 2016. The detailed information about cephalopod sample size, sampling period, biometry, sex and maturation stages is provided in the Supplementary Material (Table S1). The digestive gland (DG) and brain (i.e. the supra-, sub-oesophageal masses and optic lobes) were removed, homogenized and 4 and 1 g aliquots of DG and the brain sections combined,

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https://doi.org/10.1016/j.marenvres.2017.12.001
Received 14 July 2017; Received in revised form 30 November 2017; Accepted 1 December 2017
Available online 05 December 2017
0141-1136/

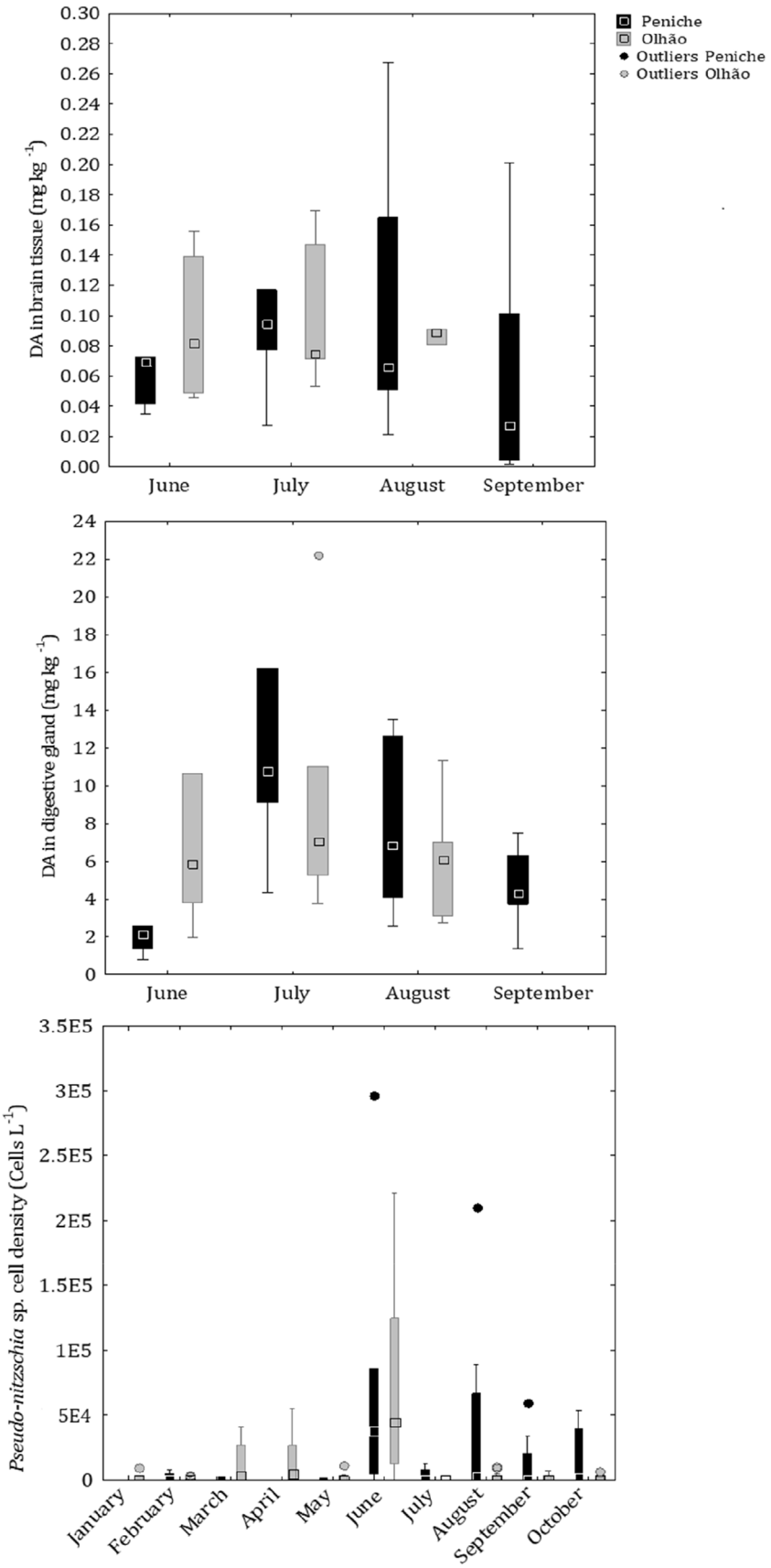

Fig. 1. Box-and-whisker plot showing domoic acid (DA) concentration in mg kg^{-1} in octopus brain tissue (A), digestive gland (B) throughout the sample period, and *Pseudonitzschia* sp. abundance (C) between January and October 2016 in Peniche (NW Portugal) and Olhão (S Portugal).

Table 1
Domoic acid concentrations (mg DA kg^{-1} WW) in the brain and digestive glands (DG) of other cephalopod species. (nd – not detected).

Species	n	Sampling period (2016)	Brain	DG
			(mg DA kg^{-1} WW)	(mg DA kg^{-1} WW)
Sepia officinalis	5	May	0.03–0.29	2.99–75.91
Todarodes sagittatus	5	August	nd	nd
Loligo vulgaris	5	July	nd	nd
Loligo forbesi	6	July	nd	nd

respectively, were used for DA analysis via liquid chromatography with mass spectrometry detection - LC-MS/MS (all details in the Supplementary Material). To compare cephalopod DA brain levels with DA-producing diatom abundance, *Pseudo-nitzschia* sp. data (Jan–Oct 2016) was obtained in the Portuguese Institute for the Sea and Atmosphere online database (IPMA, 2016).

The present study shows, for the first time, the presence of DA in brain tissue of *O. vulgaris* (Fig. 1A; n = 54) and *S. officinalis* (Table 1; n = 5), revealing that it permeates into cephalopod's central nervous system. Even though these species possess a tight blood-brain interface (Abbott et al., 1985; Bundgaard and Abbott, 1992), the presence of DA in brain tissue of *O. vulgaris* and *S. officinalis* was consistently observed, as it was present in every single octopus and cuttlefish brain, reaching concentrations up to 2.14 and 0.29 mg DA kg^{-1} wet weight (WW), respectively. Regarding other tissues, and as already observed in our previous studies (Costa et al., 2004b, 2005), DA was found at higher concentrations in the DG of *O. vulgaris* (1.38–22.19 mg DA kg^{-1} WW; Fig. 1B) and *S. officinalis* (Table 1). In contrast, there was no detectable DA content in the brain or DG of the myopsid and ommastrephid squids (Table 1). Accumulation of a toxin depends on the dynamic between accumulation and elimination, and the organism's feeding ecology. There are no studies regarding DA accumulation and elimination kinetics in squids. On the other hand, small pelagics are known to accumulate DA exclusively during bloom periods (Costa and Garrido, 2004; Lefebvre et al., 2001). Thus, we may argue that squids may be less prone to feed on contaminated fish, and accumulating this toxin.

In higher vertebrates, such as sea lions, DA toxicosis has been associated with brain lesions, seizures and memory deficits with implications for strandings (Silvagni et al., 2005; Cook et al., 2015). In cephalopods, which have a complex and vertebrate-like central nervous system, the effects of DA are not known. The learning and memory area in octopus brain have been pointed out as similar to vertebrates with glutamate as a central neurotransmitter (Hochner et al., 2003). Moreover, the excitatory effect of glutamate and kainic acid has already been demonstrated in octopus (Andrews et al., 1983, 1981). In fact, administration of these neurotransmitters, structurally related to DA, into the blood stream of an octopus through an aortic perfusion technique, revealed strong but transient chromatic and motor effects in the arms and mantle. Upon injection with L-glutamate, orange and black chromatophores expanded and muscle tone increased in the injected area and mantle. Concomitantly, the presence and abundance of L-glutamate in cephalopod's brain, peripheral nervous system and muscle (Messenger, 1996), suggest the presence of glutamate receptors in cephalopods central nervous system. The apparent lack of DA effects on octopus' brain suggests that cephalopods may possess some protective factors or defence mechanisms against DA neurotoxicity.

Another relevant result is the ability of octopus and cuttlefish to retain DA, which is a water-soluble compound usually found in marine organisms only during the toxic algae bloom or shortly after the bloom died out (Lefebvre et al., 2007). One exception to this rule are scallops, namely the king scallop, *Pecten maximus*, which is known to accumulate DA for long periods of time (Bresnan et al., 2017; Costa et al., 2004a). Similarly, *O. vulgaris* accumulates DA for longer periods of time in the brain (and DG) implying the octopus' ability to selectively retain DA in these tissues. As we previously showed (Lage et al., 2012), DA is mostly present in the soluble fraction of the digestive gland, the cytosol. Thus, although the pathways for DA uptake are not known, we may argue that upon ingestion, DA is dissolved in the cytosol and is carried to the brain cells through haemolymph penetrating de neural cell membranes.

During the sampling period, the abundance of *Pseudo-nitzschia* sp. was higher in June in both sampling locations (and also in August in Peniche; Fig. 1C). However, DA concentrations in shellfish, according to the official control for marine biotoxins carried out by IPMA (IPMA, 2016), was only found between April and June in bivalves. The presence of DA in shellfish from the Portuguese coast occurs typically during late spring-early summer, as described in Vale et al. (2008). In bivalves, DA is accumulated sporadically, and it is rapidly eliminated from most shellfish species. Scallops, as previously mentioned, are known to accumulate DA for long periods of time, and could act as a consistent source of DA for cephalopods. Yet, these organisms are not abundant along the Portuguese coast. Thus, one can argue that the higher concentration of DA in the brain of octopuses from Olhão in June/July is likely due to the higher abundance of DA-producing diatoms, and its rapid transfer through the food web. Once DA is accumulated in octopus and cuttlefish, it is retained and not easily eliminated, contrarily as what happens in most bivalve species. Moreover, detection of DA in cephalopod tissues was consistently found, but not in bivalve molluscs that may have acted as DA vectors.

As previously observed in octopus's DG (Costa and Pereira, 2010), a negative correlation between DA accumulation in octopus brain tissue and total weight was also found (Table 2). Such finding could be due to the fact that smaller octopuses feed on smaller bivalves, which contain higher toxin concentrations, since their DG accounts for a greater percentage of their body weight than in larger bivalves (Moroño et al., 2001; Novaczek et al., 1992).

Although important findings are here reported from field observations, further studies are needed to investigate whether brain-accumulated DA has any neurodegenerative effects or elicit behaviour impairments in these skilful invertebrates.

Conflicts of interest

None.

Authors' contributions

PRC conceived the study, RR and PRC designed the experiment, VML and PRC performed the sample collection and toxin analysis, VML,

Table 2
Spearman's rank order correlations between total weight (g), mantle length (ML, mm), gender, maturity stage (MS), domoic acid concentrations (mg DA kg^{-1} WW) in the brain and digestive glands (DG) of *Octopus vulgaris*. Marked correlations in bold with asterisks are significant at $p < 0.05$.

	Weight (g)	ML (mm)	Sex	MS	DG (mg DA kg^{-1} WW)	Brain (mg DA kg^{-1} WW)
Weight (g)	1.00					
ML (mm)	**0.40***	1.00				
Sex	−0.01	0.05	1.00			
MS	**0.48***	**0.31***	**−0.30***	1.00		
DG (mg DA kg^{-1} WW)	0.11	−0.07	−0.14	0.11	1.00	
Brain (mg DA kg^{-1} WW)	**−0.30***	−0.23	−0.04	−0.17	**0.62***	1.00

RR and PRC interpreted the data, statistical analysis and wrote the manuscript. All authors reviewed the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The research leading to these results has received funding from Portuguese TOXMASS project (Operational Fisheries Program - Promar 31-03-01-FEP-194). This work contributes to project UID/Multi/04326/2013 from the Portuguese Foundation for Science and Technology (FCT). The authors would like to thank the Portuguese Foundation for Science for the "Investigador FCT" grants to RR and PRC and the Ph.D. scholarship to V.M. Lopes (SFRH/BD/97633/2013). We would also like to thank Pedro da Conceição for his technical assistance.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2017.12.001.

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