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Detection of domoic acid, the amnesic shellfish toxin, in the digestive gland of *Eledone cirrhosa* and *E. moschata* (Cephalopoda, Octopoda) from the Portuguese coast

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Abstract – Domoic acid (DA), the toxin responsible for the illness known as amnesic shellfish poisoning (ASP), is an algal toxin produced naturally by some species of the diatom genus *Pseudo-nitzschia*. The toxin has been detected in a diverse array of marine organisms from copepods to whales. Cephalopods, which are important members of the food chain and active predators of known toxin vectors such as bivalves, crabs and some fishes, have just recently been implicated in DA transfer or accumulation. Here we present data showing detectable values of DA determined by HPLC-UV (high-performance liquid chromatography and ultraviolet detection) and confirmed by HPLC-MS (mass spectrometric detection) in two octopus species collected along the Portuguese continental coast: *Eledone cirrhosa* and *E. moschata*. Domoic acid was frequently detected in the digestive gland of *E. moschata* and occasionally reached concentrations exceeding 100 μg g^{-1}. In contrast, *E. cirrhosa* contained lower concentrations of DA on the few occasions that it was detected. This suggests that *E. moschata* is a potential vector for DA transfer to higher trophic levels in the coastal marine food web, not excluding humans. These data, combined with known aspects of the life history of the species, are a necessary step towards achieving an understanding of the accumulation of phycotoxins in cephalopods.

Key words: Domoic acid / *Eledone moschata* / *Eledone cirrhosa* / Amnesic shellfish poisoning / NE Atlantic

Résumé – Détection de l'acide domoïque (ASP) dans la glande digestive de *Eledone cirrhosa* et *E. moschata* (Céphalopoda, Octopoda) des côtés portugaises. L'acide domoïque (DA), la toxine responsable de la maladie connue sous le nom d'intoxication amnésiante par fruits de mer (IAFM), est une toxine d'algues produite naturellement par quelques espèces de diatomées du genre *Pseudo-nitzschia*. La toxine a été détectée dans une vaste gamme d'organismes marins allant des copépodes aux baleines. Les céphalopodes, qui sont des membres importants de la chaîne alimentaire et des prédateurs actifs de vecteurs connus de toxines, tels que les bivalves, les crabes et quelques poissons, ont été récemment impliqués dans le transfert ou l'accumulation de DA. Nous présentons ici des données montrant des valeurs discernables de DA déterminées par HPLC-UV (chromatographie en phase liquide – détection UV) et confirmées par la HPLC-MS (chromatographie en phase liquide – spectrométrie de masse) chez deux espèces de poulpes récoltées le long des côtes portugaises : *Eledone cirrhosa* et *E. moschata*. L'acide domoïque a été fréquemment détecté dans la glande digestive de *E. moschata* et a occasionnellement atteint des concentrations excédant 100 μg g^{-1}. En revanche, *E. cirrhosa* a présenté des concentrations en DA inférieures dans les quelques occasions où il a été détecté. *E. moschata* serait ainsi un vecteur potentiel dans le transfert de DA vers des niveaux trophiques supérieurs de la chaîne alimentaire marine côtière, n'excluant pas les humains. Ces données, combinées avec des aspects connus du cycle de vie de l'espèce, sont une étape nécessaire pour la compréhension de l'accumulation des phycotoxines chez les céphalopodes.

1 Introduction

Cephalopods constitute a class of marine molluscs that are found in a wide variety of habitats. They are active predators, feeding on a large range of live prey, and their high growth and metabolic rate (Boucaud-Camou et al. 1976; Boucher-Rodoni and Mangold 1977; O'Dor and Weber 1987) make them important in terms of impact on the food web (Mangold 1983a). The diet of *Eledone cirrhosa* and *E. moschata*, two octopod species that occur on the Portuguese coast, consists mainly of crustaceans, fish and occasionally molluscs including bivalves (Sánchez 1981; Boyle 1986; Grisley et al. 1999). These groups of prey may work as domoic acid vectors (Vale and Sampayo 2001; Costa et al. 2003;

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Costa and Garrido 2004). Domoic acid (DA), the toxin responsible for the illness known as amnesic shellfish poisoning (ASP), is a non-protein amino acid that binds irreversibly to glutamate receptor sites, causing destructive neuronal depolarisation (Debonnel et al. 1989) and permanent short-term memory loss in mammals (Perl et al. 1990; Todd 1993). The first known ASP episode occurred in 1987 on Prince Edward Island, Canada, when at least 3 people died and more than 100 became ill, suffering neurological effects after consuming blue mussels (*Mytilus edulis*) contaminated with DA (Quilliam and Wright 1989; Todd 1983). After this event, DA production was linked to several species of the pennate chain-forming diatom genus *Pseudo-nitzschia* (Subba Rao et al. 1988; Bates et al. 1989; Garrison et al. 1992; Cusack et al. 2002). Bivalves were the vectors in the first ASP incident and a regulatory limit for DA in shellfish of 20 μg DA g^{-1} soft tissue was established. Subsequent DA poisoning episodes have revealed that many marine organisms other than bivalves can also be vectors of DA. Consequently, DA is considered a food-web-transferred algal toxin that has been detected in several members of the marine food chain from small planktonic organisms such as copepods and krill, to top predators such as seabirds and sea lions (Work et al. 1993; Sierra Beltrán et al. 1997; Lefebvre et al. 1999; Scholin et al. 2000; Lincoln et al. 2001; Tester et al. 2001; Bargu et al. 2002). In Europe there has been extensive research focusing on DA accumulation in bivalves, but studies on toxin transfer along the marine food chain are scarce. Despite the important role that cephalopods play in the food chain (Boyle and Boletzky 1996; Piatkowski et al. 2001) they have only recently been implicated in DA transfer or accumulation with high levels of DA having been found in the digestive gland and the branchial hearts of the common octopus (*Octopus vulgaris*) collected along the Portuguese coast (Costa et al. 2004).

The objective of this work is to evaluate the occurrence of DA in the horned octopus *Eledone cirrhosa* and the musky octopus *E. moschata*. This work characterizes toxin accumulation in the digestive gland and compares DA presence in these two octopus species as related to known aspects of their life histories to better understand the occurrence of phycotoxins in cephalopods.

2 Materials and methods

2.1 Collection and preparation of octopus samples

A total of 40 samples was collected by bottom trawling along the Portuguese coast, from June 2003 to March 2004, during survey cruises onboard R/V *Noruega* and R/V *Capricórnio* of IPIMAR. These comprised a total of 254 individuals of *E. cirrhosa* and 52 *E. moschata*. Samples were numbered and designated with C or M for *E. cirrhosa* or *E. moschata*, respectively. Specimens were kept frozen (−20 °C) and thawed just before analysis. Pooled samples were obtained by homogenizing the digestive glands of several specimens. The digestive gland was chosen as the most appropriated *Eledone* tissue for DA determination in view of the fact that prior studies in other cephalopod species, namely the common octopus (*Octopus vulgaris*) and the common cuttlefish (*Sepia officinalis*), showed the digestive gland as the main organ of DA accumulation (Costa et al. 2004, 2005). A 5g aliquot of the digestive gland homogenate was separately weighed. To evaluate DA variability between individuals, specimens from 10 samples were analysed individually.

2.2 Toxin extraction and HPLC analysis

Extractions were carried out according to the method of Quilliam et al. (1995) with some modifications (Vale and Sampayo 2001). The extraction was performed with aqueous 50% methanol (ratio 1:4) at 20 000 rpm (3360 × g) with a homogeniser probe for 1 min, followed by 10 minutes centrifugation at 4000 rpm (2240 × g). The supernatant was filtered (0.22 μm) and the equivalent of 1.0 mg extract (5 μl) was injected onto the column without any further clean-up.

HPLC analysis was performed on a Hewlett-Packard (HP) Model 1100 equipped with an in-line degasser, quaternary pump, autosampler, oven and diode-array detector (DAD); data collection and treatment of results were performed by the HP *Chemstation* software. The column used was a Nucleosil 100-5 C18 (125 × 3 mm, 5 μm), with a guard-column *Lichrospher* 100 RP-18 (4 × 4 mm, 5 μm). Detection wavelength was set at 242 nm with a 10 nm bandwidth, and reference wavelength at 450 nm with a 100 nm bandwidth. A confirmatory wavelength at 262 nm was used. Calibration was carried out using DACS-1D certified DA standard, purchased from the National Research Council of Canada (NRC). Under these conditions, the detection limit was 0.04 μg ml^{-1}, corresponding to 0.2 μg g^{-1} in tissue.

2.3 Mass spectrometry analysis

Analysis was performed as described in Vale and Sampayo (2001). The same chromatograph system as above was used, coupled to an HP model 1100 Series single quadrupole mass spectrometer, through an ionspray LC-MS interface operated in the positive ion mode. High purity nitrogen was used as nebulizing gas and a potential of 5000 V was applied to the interface needle. Selected ion monitoring was used to record the signals from the ($[M+H]^+$) ions at m/z 312 and 266.

Toxins were separated at 40 °C on a *Lichrospher 100* RP-18 (5 μm, 125 × 2 mm) column, protected by the same guard column as above. The mobile phase consisted of acetonitrile: 0.1% formic acid (10:90,v/v).

3 Results

The compound observed from the *Eledone* digestive gland extract eluting at 6.9 min in the HPLC chromatogram corresponded to DA (Fig. 1). Its retention time matched well with the retention time of the calibration standard. The UV-diode array spectra of DA in samples also matched with the spectra of the calibration standard. Other peaks eluting close to DA were identified as their isomers, namely isodomoic D (iso-D) and the C5'-diasteromer of DA (epi-DA). In the UV spectrum, the iso-D and epi-DA peaks had maxima at 244 and 242 nm,

Table 1. Domoic acid concentration ($\mu g\,g^{-1}$) detected in the digestive gland of *Eledone cirrhosa* (C samples) and *Eledone moschata* (M samples) caught along the Portuguese coast (*dash* = pooled samples; *nd* = not detected).

Sampling Location	Sample	Date (dd/mm/yy)	Depth (m)	Octopus weigth (g, mean +SD)	No. of individuals	Domoic acid ($\mu g\,g^{-1}$) mean	[min-max]
S coast	C1	07/06/03	110	601 ± 145	4	0.4	-
	C2	09/06/03	323	323 ± 157	22	2.3	-
	C3	12/06/03	137	328 ± 311	14	8.8	-
	C4	14/06/03	106	378 ± 115	15	nd	-
	C5	07/10/03	118	99 ± 26	3	nd	-
	C6	09/10/03	72	230 ± 65	4	nd	-
	M1	07/06/03	100	287	1	0.8	-
	M2	07/06/03	110	206 ± 52	6	1.8	-
	M3	12/06/03	86	168 ± 63	18	2.9	-
	M4	13/08/03	97	193	1	15.5	-
	M5	07/10/03	110	419	1	22.5	-
	M6	08/10/03	108	182 ± 88	4	5.5	[2.7–9.2]
	M7	09/10/03	72	111 ± 79	15	23.2	[4.1–127.0]
	M8	19/03/04	97	219 ± 104	6	2.8	-
SW coast	C7	22/06/03	382	211 ± 59	18	4.0	-
	C8	26/06/03	257	192 ± 107	22	6.8	-
	C9	14/10/03	379	207 ± 52	3	nd	-
	C10	16/10/03	536	52 ± 15	7	nd	-
	C11	18/10/03	74	281	1	2.1	-
	C12	22/10/03	121	102 ± 18	4	nd	-
	C13	24/10/03	100	172 ± 16	10	2.0	[1.2–3.0]
NW coast	C14	08/09/03	91	40 ± 20	10	nd	-
	C15	09/09/03	95	55 ± 16	10	3.8	-
	C16	09/09/03	106	43 ± 15	10	nd	-
	C17	10/09/03	92	300 ± 53	6	6.3	[0–13.8]
	C18	10/09/03	117	162 ± 95	6	nd	-
	C19	10/09/03	125	284 ± 36	6	nd	-
	C20	10/09/03	82	51 ± 15	10	18.8	-
	C21	11/09/03	144	155 ± 113	3	nd	-
	C22	11/09/03	135	210 ± 108	6	1.9	[0–10]
	C23	13/09/03	127	109 ± 118	5	nd	-
	C24	13/09/03	134	255 ± 40	4	0.6	[0–2.8]
	C25	14/09/03	60	67 ± 67	9	0.4	-
	C26	15/09/03	122	209 ± 13	6	nd	-
	C27	03/10/03	65	119 ± 36	3	7.0	[1.7–14.9]
	C28	14/10/03	81	38 ± 8	9	nd	-
	C29	28/10/03	55	92 ± 57	3	1.4	[0–2.5]
	C30	28/10/03	112	47 ± 17	8	1.9	-
	C31	03/11/03	97	314 ± 155	8	2.2	[0–7.5]
	C32	04/11/03	334	461 ± 163	5	4.9	[2.5–9.5]

respectively, as reported by Quilliam and Wright (1989). The most toxic samples were analysed by LC-MS for further confirmation of DA presence in octopus. Retention times of the peaks in the m/*z* 312 and 266 ($[M+H]^+$) ion chromatogram of *Eledone* digestive gland extract matched those of the DA standard (Fig. 2).

Domoic acid was detected in both *Eledone* species. Domoic acid was only detected in some *E. cirrhosa* samples, with the highest DA value registered for samples collected on the NW coast with a maximum concentration of 18.8 $\mu g\,DA\,g^{-1}$ for sample C20, (Table 1). When analysed individually, only in 37% of the *E. cirrhosa specimens* was detected DA. In contrast, DA was detected in all *E. moschata* samples analyzed. Furthermore, DA levels detected in *E. moschata* were higher than those detected in *E. cirrhosa* and ranged from 0.8 to 127 $\mu g\,DA\,g^{-1}$. Samples M7 and C6, are particularly interesting since both *Eledone* species were caught in the same haul yet showed different results: while DA concentrations were recorded for *E. moschata* from 4.1 to 127 $\mu g\,DA\,g^{-1}$ digestive gland, no toxin was detected by HPLC in any of the *E. cirrhosa* individuals.

4 Discussion

Domoic acid was frequently detected in the octopus species examined. This is somewhat surprising since DA is more likely to be depurated than accumulated due to its hydrophilic nature (Wright et al. 1989; Novaczeck et al. 1991). However, DA retention has been reported for other molluscs

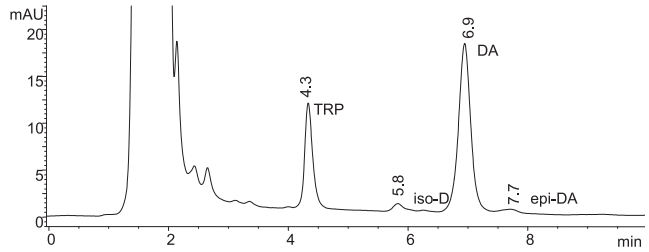

Fig. 1. Chromatogram (wavelength 242 nm) obtained from LC-UV analysis of a digestive gland extract of *Eledone moschata* from sample M7 (DA = 25.4 $\mu g\,g^{-1}$).

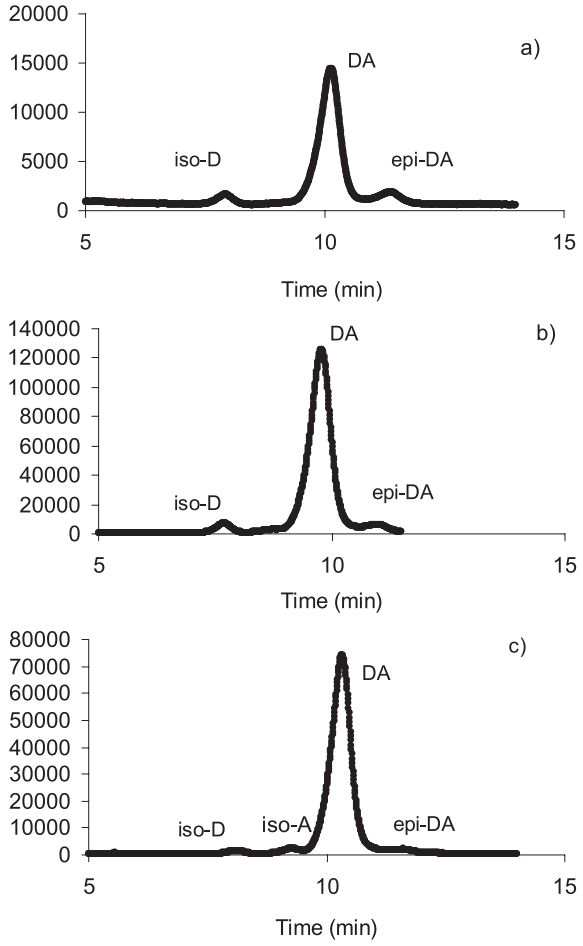

Fig. 2. Comparison of $[M+H]^+$ (m/*z* 312) chromatograms obtained from selected ion-monitoring LC-MS analyses of digestive gland extract of (a) *Eledone cirrhosa*, (b) *Eledone moschata* and (c) certified DA standard (DA domoic acid; iso-D isodomoic acid D; iso-A isodomoic acid A; epi-DA C5'-diasteromer of DA).

such as the king scallop (*Pecten maximus*), where high DA concentrations are found in the hepatopancreas (Arévalo et al. 1998). In cephalopods, the hepatopancreas or digestive gland, similarly to other molluscs, is a site of digestion and digestive absorption and acts as a reservoir of prey material (Boucher-Rodoni et al. 1987; Semmens 2002). Because the physiology of both *Eledone* species is unlikely to be significantly different, the differences in DA concentrations detected in *E. cirrhosa* and *E. moschata* are potentially related to different feeding strategies. Both species are soft bottom dwelling and on the Portuguese coast they partially overlap in geographic and bathymetric distribution. However, the horned octopus *E. cirrhosa* has a wide geographic and bathymetric distribution (in this study from 55 to 536 m) over the shelf regions of the whole coast, while the musky octopus *E. moschata* is primarily a Mediterranean species (Mangold 1983b) limited in the Atlantic Ocean to the southern coast of Portugal (Guerra 1992) and found in shallower waters (in this study from 72 to 110 m).

The diet of *E. cirrhosa*, has been described as consisting mainly of crustaceans, but fish, cephalopods, polychaetes and gastropods have also been reported (Sánchez 1981; Boyle 1983; Grisley et al. 1999) and evidence of bivalve prey was noted once by Boyle (1986). Unfortunately, nothing is known about the prey of *E. moschata* in the field but in the laboratory the species is known to readily feed on crabs (Mangold 1983b). Direct evidence for a vector that may be responsible for DA transfer remains lacking.

Upwelling events are responsible for the occurrence of algal blooms, including DA-producing *Pseudo-nitzschia* species that bloom in high densities in the surface layers of the ocean. Domoic acid associated with these blooms accumulates in planktivorous organisms that filter-feed on the surface waters. Consequently, some octopus prey such as portunid crabs, planktivorous fishes, and bivalves can act as DA vectors in Portuguese marine environments (Vale and Sampayo 2001; Costa et al. 2003; Costa and Garrido 2004), resulting in the uptake of the toxin. Alternatively, towards the end of *Pseudo-nitzschia* blooms, cells in their stationary phases can sink to the seafloor where they can remain alive for a while (Dortch et al. 1997; Parsons and Dortch 2002). This may provide benthic pathways for toxin uptake by the octopus. One toxin source may be filter-feeding invertebrates that have become contaminated with DA when they feed on sinking *Pseudo-nitzschia*. Benthic scavengers that became contaminated with DA after they consume the organic-rich layer containing recently settled material like remnants of toxic *Pseudo-nitzschia* may be another benthic toxin source. Taylor (1993) proposed that Dungeness crabs (*Cancer magister*) from the West coast of the USA, became toxic by either directly or indirectly consuming a *Pseudo-nitzschia* bloom that sank to the bottom.

Collectively, these data suggest that octopods, such as the species examined here, could act as vectors of DA to predators, such as marine mammals (Blanco 2001; Salman 2001). Despite of DA has not been detected in the flesh (edible part) of cephalopods such as the *Octopus vulgaris* and *Sepia officinalis* (Costa et al. 2004, 2005), the *Eledone* juveniles may represent a risk to humans, particularly in the case of *E. moschata*, since their whole body is consumed without evisceration, as what happens in some Mediterranean regions such as in Catalonia and Tuscany, where these *Eledones* are known locally as "popets" and "moscardini", and represent important fishery targets (Sánchez and Martín 1993; Belcari and Sartor 1999; Sánchez et al. 2004). This study is the first report of DA detected in these cephalopod species, and provides a better understanding of the presence of DA in cephalopods and the potential movement of the toxin through the marine food web.

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References

Arévalo F.F., Bermúdez M., Salgado C., 1998, ASP toxicity in scallops: individual variability and tissue distribution. In: Reguera B., Blanco J., Fernández M.L., Wyatt T. (Eds.) Harmful algae. Xunta de Galicia and the IOC of UNESCO, Paris, pp. 499-502.

Bargu S., Powell C.L., Coale S.L., Busman M., Doucette G.J., Silver M.W., 2002, Krill: a potential vector for domoic acid in marine food webs. Mar. Ecol. Prog. Ser. 237, 209-216.

Bates S.S., Bird C.J., de Freitas A.S.W., Foxall R., Gilgan M., Hanic L.A., Johnson G.R., McCulloch A.W., Odense P., Pocklington R., Quilliam M.A., Sim P.G., Smith J.C., Subba Rao D.V., Todd E.C.D., Walter J.A., Wright J.L.C., 1989, Pennate diatom *Nitzschia pungens* as the primary source of domoic acid, a toxin in shellfish from eastern Prince Edward Island, Canada. Can. J. Fish. Aquat. Sci. 46, 1203-1215.

Belcari P., Sartor P., 1999, *Eledone cirrhosa*. In: Relini G., Bertran J.A., Zambroni A. (Eds.) Synthesis of the knowledge on bottom fishery resources in central Mediterranean (Italy and Corsica). Biol. Mar. Medit. 6, 737-746.

Blanco C., Salomón O., Raga J.A., 2001, Diet of the bottlenose dolphin (*Tursiops truncatus*) in the western Mediterranean Sea. J. Mar. Biol. Assoc. UK 81, 1053-1058.

Boucaud-Camou E., Boucher-Rodoni R., Mangold K., 1976, Digestive absorption in *Octopus vulgaris* (Cephalopoda, Octopoda). J. Zool. 179, 261-271.

Boucher-Rodoni R., Mangold K., 1977, Experimental study of digestion in *Octopus vulgaris* (Cephalopoda: Octopoda). J. Zool. 183, 505-515

Boucher-Rodoni R., Boucaud-Camou E., Mangold K., 1987, Feeding and digestion. In: Boyle P.R. (Eds.) Cephalopod Life Cycles. Comparative Reviews. London, Academic Press, Vol. 2, pp. 85-108.

Boyle P.R., 1983, *Eledone cirrhosa*. In: Boyle P.R. (Ed.) Cephalopod life cycles. London, Academic Press, Vol 1., pp. 365-386.

Boyle P.R., 1986, A descriptive ecology of *Eledone cirrhosa* (Mollusca: Cephalopoda) in Scottish waters. J. Mar. Biol. Assoc. UK 66, 855-865.

Boyle P., Boletzky S., 1996, Cephalopod populations: definition and dynamics. Phil. Trans. R. Soc. London B 351, 985-1002.

Costa P.R., Garrido S., 2004, Domoic acid accumulation in the sardine (*Sardina pilchardus*) and its relationship to *Pseudo-nitzschia* diatom ingestion. Mar. Ecol. Prog. Ser. 284, 261-268.

Costa P.R., Rodrigues S., Botelho M.J., Sampayo M.A.M., 2003, A potential vector of domic acid: the swimming crab *Polybius henslowii* Leach (Decapoda-Brachyura). Toxicon 42, 135-141.

Costa P.R., Rosa R, Sampayo M.A.M., 2004, Tissue distribution of the amnesic shellfish toxin, domoic acid, in *Octopus vulgaris* from the Portuguese coast. Mar. Biol. 144, 971-976.

Costa P.R., Rosa R, Duarte-Silva A., Brotas V., Sampayo M.A.M., 2005, Accumulation, transformation and tissue distribution of domoic acid, the amnesic shellfish poisoning toxin, in the common cuttlefish, *Sepia officinalis.* Aquat. Toxicol. 74, 82-91

Cusack C.K., Bates S.S., Quilliam M.A., Patching J.W., Raine R., 2002, Confirmation of domoic acid production by *Pseudo-nitzschia australis* (Bacillariophyceae) isolated from Irish waters. J. Phycol. 38, 1106-1112

Dortch Q., Robichaux R., Pool S., Milsted D., Mire G., Rabalais N.N., Soniat T.M., Fryxell G.A., Turner R.E., Parsons M.L., 1997, Abundance and vertical flux of *Pseudo-nitzschia* in the northern Gulf of Mexico. Mar. Ecol. Prog. Ser. 146, 249-264.

Garrison D.L., Conrad SM., Eilers P.P., Waldron E.M., 1992, Confirmation of domoic acid production by *Pseudonitzschia australis* (Bacillariophyceae) cultures. J. Phycol. 28, 604-607.

Grisley M.S., Boyle P.R., Pierce G.J., Key L.N., 1999, Factors affecting prey handling in lesser octopus (*Eledone cirrhosa*) feeding on crabs (*Carcinus maenas*). J. Mar. Biol. Assoc. UK 79, 1085-1090.

Guerra A., 1992, Mollusca Cephalopoda. Fauna Iberica. Vol. 1. Museo Nacional de Ciencias Naturales – CSIC, Madrid.

Lefebvre K.A., Powell C.L., Busman M., Doucette G.J., Moeller P.D.R., Silver J.B., Miller P.E., Hughes M.P., Singaram S., Silver M.W., Tjeerdema R.S., 1999, Detection of domoic acid in northern anchovies and California sea lions associated with an unusual mortality event. Nat. Toxins 7, 85-92.

Lincoln J.A., Turner J.T., Bates S.S., Léger C., Gauthier D.A., 2001, Feeding, egg production and egg hatching success of the copepods *Acartia tonsa* and *Temora longicornis* on diets of the toxic diatom *Pseudo-nitzschia multiseries* and the non-toxic diatom *Pseudo-nitzschia pungens*. Hydrobiologia 453, 107-120.

Mangold K., 1983a, Food, feeding and growth in cephalopods. Mem. Nat. Mus. Victoria 44, 81-93.

Mangold K., 1983b, *Eledone moschata*. In: Boyle P.R. (Ed.) Cephalopod life cycles. London, Academic Press, Vol. 1, pp. 387-400.

Novaczek I., Madhyastha M.S., Ablett R.F., Johnson G., Nijjar M.S., Sims D.E., 1991, Uptake, disposition and depuration of domoic acid by blue mussels (*Mytilus edulis*). Aquat. Toxicol. 21, 103-118.

O'Dor R.K., Weber M.J., 1987, Energy and nutrient flow. In: Boyle P.R. (Ed.) Cephalopod life cycles. Comparative reviews. London, Academic Press, Vol. 2, pp. 109-133.

Parsons M.L., Dortch Q., Turner R.E., 2002, Sedimentological evidence of an increase in *Pseudo-nitzschia* (Bacillariophyceae) abundance in response to coastal eutrophication. Limnol. Oceanogr. 47, 551-558.

Piatkowski U., Pierce G.J., Cunha M.M., 2001, Impact of cephalopods in the food chain and their interaction with the environment and fisheries: an overview. Fish. Res. 52, 2-10.

Quilliam M.A., Wright J.L.C., 1989, The amnesic shellfish poisoning mystery. Anal. Chem. 61, 1053A-1060A.

Quilliam M.A., Xie M., Hardstaff W.R., 1995, Rapid extraction and cleanup for liquid chromatographic determination of domoic acid in unsalted seafood. J. AOAC Int. 78, 543-554.

Salman A., Bilecenoglu M., Güçlüsoy H., 2001, Stomach content of two Mediterranean monk seals (*Monachus monachus*) from the Aegean Sea, Turkey. J. Mar. Biol. Assoc. UK 81, 719-720.

Sánchez P., 1981, Régime alimentaire d'*Eledone cirrosa* (Lamarck, 1798) (Mollusca, Cephalopoda) dans la mer Catalane. Rapp. Comm. Int. Mer. Médit. 27, 209-212.

Sánchez P., Martín P., 1993, Population dynamics of the exploited cephalopod species of the Catalan Sea (NW Mediterranean). Sci. Mar. 57, 153-159.

Sánchez P., Maynou F., Demestre M., 2004, Modelling catch, effort and price in a juvenile *Eledone cirrhosa* fishery over a 10-year period. Fish. Res. 68, 319-327.

Semmens J.M., 2002, Changes in the digestive gland of the loliginid squid *Sepioteuthis lessoniana* (Lesson, 1830) associated with feeding. J. Exp. Mar. Biol. Ecol. 274, 19-39.

Scholin C.A., Gulland F., Doucette G.J., Benson S., Busman M., Chavez F.P., Cordaro J., DeLong R., De Vogelaere A., Harvey J., Haulena M., Lefebvre K., Lipscomb T., Loscutoff S., Lowenstine L.J., Martin R.I., Miller P.E., McLellan W.A., Moeller P.D.R., Powell C.L., Rowles T., Silvagnl P., Silver M., Spraker T., Trainer V., Van Dolah F.M., 2000, Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403, 80-84.

Sierra Beltrán A., Palafox-Uribe M., Grajales-Montiel J., Cruz-Villacorta A., Ochoa J.L., 1997, Sea bird mortality at Cabo San Lucas, Mexico: evidence that toxic diatom blooms are spreading. Toxicon 35, 447-453.

Subba Rao D.V., Quilliam M.A., Pocklington R., 1988, Domoic acid - a neurotoxic amino acid produced by the marine diatom *Nitzschia pungens* in culture. Can. J. Fish. Aquat. Sci. 45, 2076-2079.

Taylor F.J.R., 1993, British Columbia: implications of the North American west coast experience. Harmful Algae News 6, 2-3.

Tester P.A., Pan Y., Doucette G.J., Scholin C.A., 2001, Accumulation of domoic acid activity in copepods. In: Hallegraeff G.M., Blackburn S.I., Bolch C.J., Lewis R.J. (Eds.) Harmful Algal Blooms 2000. Paris, Intergovernmental Oceanographic Commission of UNESCO, pp 418-421.

Todd E.C.D., 1993, Domoic acid and amnesic shellfish poisoning- a review. J. Food. Protect. 56, 69-83.

Vale P., Sampayo M.A.M., 2001, Domoic acid in Portuguese shellfish and fish. Toxicon 39, 893-904.

Work T.M., Beale A.M., Fritz L., Quilliam M.A., Silver M., Buck K., Wright J.L.C., 1993, Domoic acid intoxication of brown pelicans and cormorants in Santa Cruz, California. In: Smayda T.J., Shimizu Y., (Eds.) Toxic phytoplankton blooms in the sea. Amsterdam, Elsevier Sci. Publ. BV, pp. 643-650.

Wright J.L.C., Boyd R.K., de Freitas A.S.W., Falk M., Foxall R.A., Jamieson W.D., Laycock M.V., McCulloch A.W., McInnes A.G., Odense P., Pathak V., Quilliam M.A., Ragan M.A., Sim P.G., Thibault P., Walter J.A., Gilgan M., Richard D.J.A., Dewar D., 1989, Identification of domoic acid, a neuroexcitatory amino acid, in toxic mussels from eastern Prince Edward Island. Can. J. Chem. 67, 481-490.