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Ontogenic differences in the concentration of domoic acid in the digestive gland of male and female *Octopus vulgaris*

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ABSTRACT: Domoic acid (DA), a neurotoxin produced by the diatom genus *Pseudo-nitzschia*, has been recently found to accumulate in *Octopus vulgaris*. To elucidate inter-animal variation in DA concentration in octopus digestive glands and its relation to biometric (size and body mass) and biological (sex and maturity) parameters, 54 specimens were caught in the same fishing area during a bloom of toxic plankton. Toxin concentration in female octopuses, as determined by liquid chromatography, was significantly inversely correlated with octopus mantle length (r^2 = 0.63), body mass (r^2 = 0.62) and weight of the digestive gland (r^2 = 0.55). A tendency for decreasing DA concentration with maturity stages was also observed in females. No significant correlation was observed between DA concentration and any biometric or biological parameter of male specimens. Negative correlations during periods of toxin uptake suggest that younger female octopuses are greater consumers of toxic prey and represent a higher threat as DA vectors in the marine food web.

KEY WORDS: Amnesic shellfish poisoning · Cephalopods · Sex · Size · Food web

INTRODUCTION

Marine toxins produced by phytoplankton species are traditionally associated with filter-feeding bivalves which are the main vectors causing human poisoning. However, non filter-feeding organisms can also accumulate marine toxins through complex trophic interrelations. Cephalopods play a central role in the marine food web. They are active predators, with high growth and metabolic rates, feeding on a large range of live prey, and are also important in the diets of top predators. Their role as predators of filter-feeding bivalves would suggest that cephalopods might be potential vectors of marine toxins. In fact, domoic acid (DA) has been found in their tissues, with noticeable concentrations detected in the digestive gland (Costa et al. 2004, 2005a,b).

DA, the main toxin responsible for the human illness amnesic shellfish poisoning (ASP), is mostly produced by the diatom species *Pseudo-nitzschia australis* on the Portuguese coast (Costa & Garrido 2004). Their blooms are related to upwelling events occurring predominantly in spring and early summer (Moita 2001). DA levels are recurrently monitored in bivalve molluscs. However, they only occasionally exceed the EU regulatory limit (20 µg DA g^{-1}) stated for safe human consumption. This toxin binds to glutamate receptors in specific regions of the brain (especially the hippocampus), causing destructive neuronal depolarization (Debonnel et al. 1989) and permanent short-term memory loss in mammals (Perl et al. 1990, Todd 1993). Harvesting closures due to DA contamination are not frequent and are characterized by small periods that last no more than 2 wk (Vale et al. 2008). Nevertheless, cephalopods have consistently shown high DA concentrations (>20 µg g^{-1}) accumulated in their tissues. Several routes for DA contamination of cephalopods and benthic communities have been suggested (Costa et al. 2005a, Vigilant & Silver 2007), not unlike those suggested for the high levels of essential and non-essential elements also known to accumulate in cephalopod tissues (e.g. Raimundo et al. 2005).

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The present study assessed the inter-animal variability of DA concentrations in the digestive gland of octopus *Octopus vulgaris*. Knowledge on the variation in toxin levels among individuals is of ecological and physiological relevance and important for environmental management. Several factors contributing to inter-animal variability in DA concentration should be considered: magnitude, persistence and exposure of octopus prey to toxic phytoplankton, geographical location, water depth and currents, body mass/mantle length and differences in octopus feeding rates. To minimise variability, a number of octopuses collected on the same fishing trip and in the same fishing area were used for the quantification of DA concentration in the digestive gland, and its relation to biometric and biological parameters, such as body mass, mantle length, sex and maturity stage was evaluated.

MATERIALS AND METHODS

Octopus sampling and tissue preparation. A total of 54 specimens of *Octopus vulgaris* were captured when blooms of the diatom *Pseudo-nitzschia* were identified and DA recorded in natural beds of clams by the Portuguese monitoring program for toxic algae and marine toxins (National Institute of Biological Resources–IPIMAR). The specimens were captured at a depth range of 25 to 35 m by a commercial trawler in the fishing area off Peniche (northwest Portuguese coast) on 1 June 2007. All octopus specimens were kept at –20°C for a maximum of 2 wk before dissection, which was carried out under partially defrosted conditions and without rupture of the outer membrane of the organs in order to minimize DA leakage. For each animal the following parameters were recorded: body weight, mantle length and digestive gland weight. Four maturity stages were determined using the scale proposed by Mangold-Wirz (1963). Digestive glands were dissected, homogenized and 5 g aliquots used for analysis.

Toxin extraction and high performance liquid chromatography analysis. Extractions were carried out following the method of Quilliam et al. (1995). High performance liquid chromtography (HPLC) was performed on a Hewlett-Packard (HP) Model 1100 equipped with in-line degasser, quaternary pump, autosampler, oven and diode-array detector. Data collection and treatment of results were performed by the HP Chemstation software. The column used was a Nucleosil 100-5C-18 (125 × 3 mm, 5 µm) with a guard-column Lichrospher 100 RP-18 (4 × 4 mm, 5 µm), both heated to 40°C. The flow rate was set at 0.45 ml min^{-1} of acetonitrile:0.1% formic acid (10:90, v/v) throughout the run. The injection volume was 5 µl and analysis time was set at 10 min. Detection wavelength was set at 242 nm with a 10 nm bandwidth, and reference wavelength was set at 450 nm with a 100 nm bandwidth. Calibration was performed with a full set of calibration standards of DA (0.5, 2, 4 and 10 µg ml^{-1}). Samples over the calibration curve were diluted. Calibration curves were always linear, with correlation coefficients >0.99. A single point calibration, with a working solution of 4 µg ml^{-1} DA in 10% acetonitrile, was performed after 6 consecutive samples. Under these conditions the detection limit was 0.04 µg ml^{-1}, corresponding to 0.2 µg g^{-1} in tissue.

For HPLC analysis Millipore-Q cleaned water and LC grade methanol and acetonitrile supplied by Merck were used. DACS-1D certified DA standard was purchased from the National Research Council of Canada.

Statistical analysis. The Mann-Whitney *U*-test was applied for comparisons of toxin determined in the digestive glands of female and male specimens.

Data on toxins in the digestive gland were analysed as a function of mantle size and body mass with model II linear regressions since these variables are subject to natural variation (Sokal & Rohlf 1995). Toxin concentration data were log-transformed to achieve a normal distribution.

RESULTS

Of the 54 octopus specimens captured, 33 were females and 21 were males, ranging in size, body mass and maturity (Table 1). DA was consistently found in each octopus specimen (Fig. 1). Highly variable concentrations of DA (coefficient of variation of 92%) were determined in the digestive gland of female (1.5 to 124 µg g^{-1}) and male (2.1 to 92 µg g^{-1}) octopuses. No significant differences ($p > 0.05$) in DA concentrations were found between sexes.

Despite the observed elevated variability, a significant relation between DA concentration and both size and body mass of octopus was discovered. DA concentrations determined in the digestive gland decreased significantly ($p < 0.001$) with size (mantle length) and body mass (weight), with these 2 variables explaining 63 and 62%, respectively, of the variability in log-transformed DA concentration for female specimens

Table 1. *Octopus vulgaris*. Biometric (body weight [BW], mantle length [ML]) and biological (sex, maturity) parameters of octopus specimens caught on 1 June 2007 in Peniche (NW Portuguese coast)

Sex	n	BW (g) min–max	ML (mm) min–max	Maturity (% of specimens) Stage 1	Stage 2	Stage 3	Stage 4
Female	33	816–4058	130–250	9	64	24	3
Male	21	799–2150	125–195	0	67	33	0

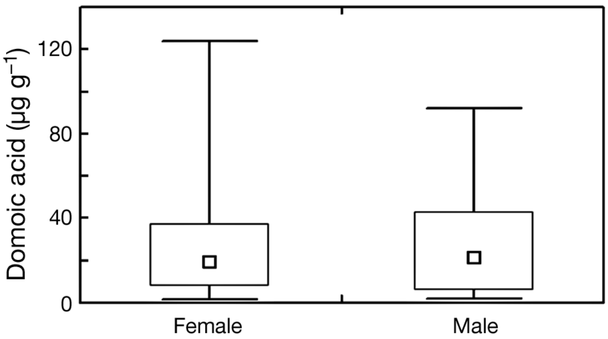

Fig. 1. *Octopus vulgaris*. Domoic acid concentration (median, 25 and 75 quartiles, minimum and maximum) determined in the digestive gland of female (n = 33) and male (n = 21) octopuses caught in Peniche (NW Portuguese coast) on 1 June 2007

(Fig. 2a,c). The weight of the digestive gland, which is positively correlated to octopus size ($r^2 = 0.61$, data not shown) and body mass ($r^2 = 0.66$, data not shown), explained 55% of the variability in log-transformed DA concentration for females (Fig. 2e). The same was not shown for males (mantle length, $r^2 = 0.05$; body mass, $r^2 = 0.07$; weight of digestive gland, $r^2 = 0.02$; Fig. 2b, d, f).

Although not as significant as the changes in DA concentrations, a negative correlation between log-transformed toxin burden (absolute amounts) and animal size was found for female specimens (Fig. 3).

From the application of the maturity scale developed by Mangold-Wirz (1963), the great majority of octopuses were found to be in Stages II and III. There were a few immature (Stage I) females and 1 mature Stage IV female. Males were found in maturity Stages II and III. Maturity was shown to generally increase with mantle length, in spite of the characteristic cephalopod overlap of mantle lengths between maturity stages (Fig. 4). Therefore, a significant relation between growth and maturity was not apparent and thus only a non-significant tendency was observed for the relation between maturity stages and the log-transformed DA concentration in the digestive gland of female specimens (Fig. 5).

DISCUSSION

DA was detected in each octopus specimen subjected to the same environmental conditions. Due to natural variability of DA in octopus prey and octopus

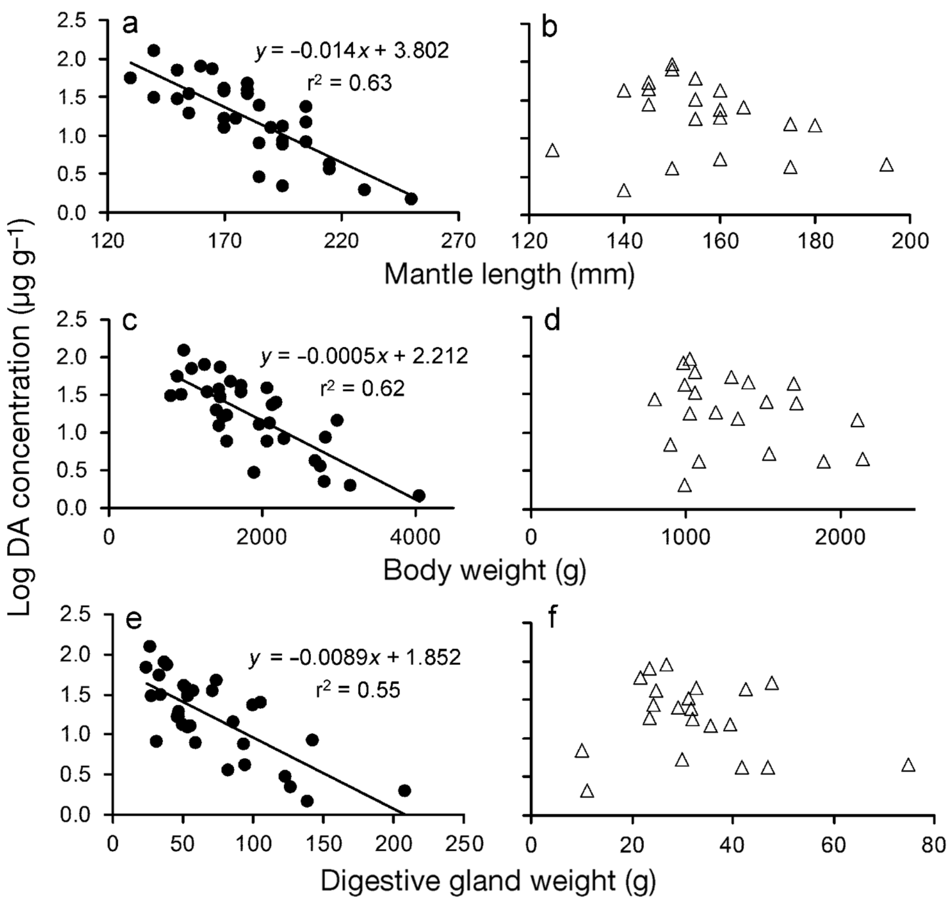

Fig. 2. *Octopus vulgaris*. Log-transformed DA concentration in the digestive gland of female (●, n = 33) and male (△, n = 21) octopuses as a function of (a, b) mantle length, (c, d) body weight and (e, f) digestive gland weight

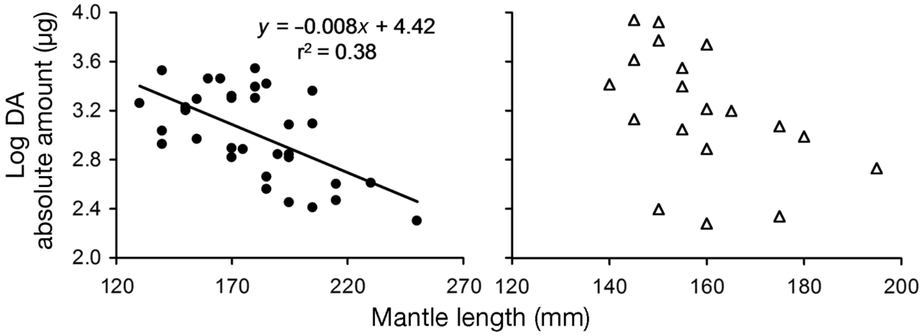

Fig. 3. *Octopus vulgaris*. Log-transformed DA absolute amount (µg) per digestive gland of female (●, n = 33) and male (△, n = 21) octopuses as a function of mantle length

feeding rates, a high heterogeneity of DA concentrations determined in the octopus digestive gland was expected and confirmed by the coefficient of variation of 92%. Nevertheless, after specimens were grouped by sex, DA concentrations were found to decrease with increasing body size (mantle length) in females. Body weight and digestive gland weight were also negatively correlated to DA, as they are not independent of size. Assessment of maturation in cephalopods usually involves the qualitative evaluation of several aspects of the reproductive development of the species, and is most frequently described with the support of numerical scales made up of recognizable quantitative stages (Boyle & Rodhouse 2006). Although these data in the present study were provided by qualitative morphological observations, determination of quantitative relationships were possible, which show a trend for decreasing DA concentrations with increasing maturity stages.

Octopus feeding rates are known to decrease with size, which leads to younger octopuses growing proportionally faster than older ones (García & Giménez 2002). A study on the relationship between the length of prey selected in the field and octopus size has shown smaller octopuses preying on smaller mussel size classes (Smale & Buchan 1981). Since higher toxin concentrations have been found in smaller mussels than in large ones (Novaczek et al. 1992, Morono et al. 2001), octopus feeding behaviour will also contribute to the accumulation of higher DA values in smaller specimens. In terms of the toxin burden, higher amounts of DA were also found in smaller octopus specimens. Smaller octopuses that have higher feeding rates should therefore consume a higher number of mussels of smaller size which are more contaminated.

The influence of size on DA concentration has been studied for other molluscs such as the king scallop *Pecten maximus* (Bogan et al. 2007), where it was shown to negatively affect DA concentration only during blooms of toxic plankton (period of toxin uptake). Our observations suggest that during the algal bloom, the accumulation of DA in the predator *Octopus vulgaris* is similar to that of the filter feeder *P. maximus*.

In the present study, a negative correlation between DA concentrations and animal size and body weight was only exhibited by females. A possible explanation is the fact that the smaller number of male specimens, as well as their smaller range of size, body weight and maturity stage, may not allow similar significant correlations. However, several authors have observed higher growth and feeding rates and food conversion for octopus females than males, due to higher energy demands of the female development (Smale & Buchan 1981, Domain et al. 2000, García & Giménez 2002,

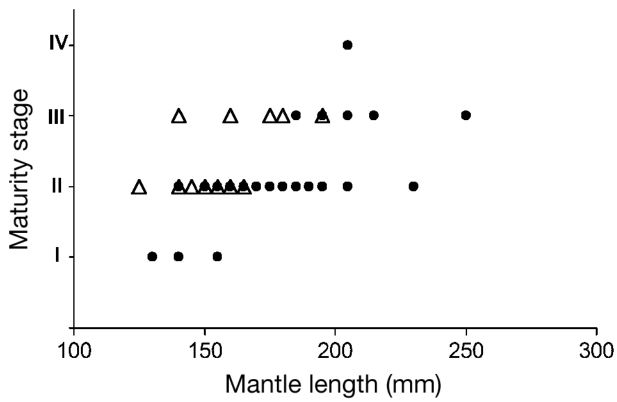

Fig. 4. *Octopus vulgaris*. Relation between mantle length and maturity stages in female (●) and male (△) octopuses

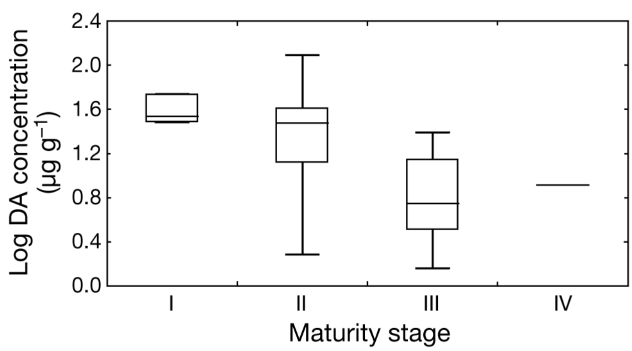

Fig. 5. *Octopus vulgaris*. Log-transformed DA concentration determined in the digestive gland of female octopuses in different maturity stages (median, 25 and 75 quartiles, minimum and maximum, total n = 33)

Giménez & García 2002). Thus feeding dynamics of females should be more regular and intense. Previous research has shown that the biochemical composition of the digestive gland seems not to be influenced by sexual maturation but rather by biotic factors, namely feeding activity and food availability (Rosa et al. 2004), which further supports the hypothesis that differences in the feeding regime of males and females and between younger and older specimens may be responsible for the different patterns of DA concentration found in the species.

The digestive gland is the largest octopus organ (only surpassed by the female gonads at the peak of maturity), representing on average 4 ± 1% of the total body mass of specimens caught in the present study. The concentration of toxin in the whole octopus body can be estimated applying a 25-fold dilution to the DA concentration determined in the digestive gland. Nevertheless, this value will be underestimated due to the concomitant accumulation of DA in other organs such as branchial hearts, kidney, stomach and intestine (Costa et al. 2004). The total amount of DA in the whole octopus body can be a valuable parameter for evaluation of the potential risk of this seafood for human consumption. Although human consumption of this seafood is usually limited to larger and eviscerated animals, human health hazards may be expected from the consumption of whole small octopuses as a delicacy (a custom in southwestern European and Mediterranean countries).

We conclude that after toxic blooms and contamination of the benthic communities, younger octopuses (and notably female specimens) are expected to accumulate the highest levels of DA and therefore represent potential toxin vectors in the marine food web.

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