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# Anatomical distribution of diarrhetic shellfish poisoning (DSP) toxins in the mussel *Mytilus galloprovincialis* ☆

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**Abstract**

The aim of this work was to shed light on the anatomical distribution of diarrhetic shellfish poisoning (DSP) toxins in the mussel *Mytilus galloprovincialis* and to determine any possible changes undergone during the depuration process. To this end, the distribution of two DSP toxins—okadaic acid and DTX2—and some of their derivatives were studied by means of HPLC/MS at different stages of the depuration process. Mussels were collected from mussel farms located in the Galician Rías and they were collected under three types of circumstances: (a) while ingesting toxic phytoplankton cells; (b) 1 week after the toxic cells had disappeared from the water; and (c) after ca. 2 months of depuration. Additionally, in case (b), the distribution among tissues was checked every week over a depuration period of 35 days in the laboratory. DSP toxins were only detected in non-visceral tissues when the extracts were concentrated 20-fold and, even in these cases, the concentrations found were very low. When the maximum possible contribution of non-visceral tissues was computed, taking into account the technique's detection limits and tissue weight, no relevant contribution to the toxin burden of non-visceral tissues was found at any stage of depuration, with the maximum possible contributions usually below 7%. The concentrated samples analysed showed that the actual contribution in all the cases studied was, in fact, less than 1% of the total toxin burden. These findings suggest that (1) when analytical methods are used to monitor DSP toxic mussels, non-visceral tissues should be assumed to be free of toxins in order to precisely compute the toxin concentration of the whole mass of edible tissues and (2) when studying the accumulation kinetics of DSP toxins, transference from the digestive gland to other tissues should not be taken into account, as the other tissues do not contain relevant amounts of DSP toxins.


*Keywords:* *Mytilus galloprovincialis*; Mussel; Okadaic acid; DTX-2; Diarrhoeic; Diarrhetic shellfish poisoning; Anatomical distibution; Compartmentalization; Accumulation; Depuration; Conjugated forms; Esters; Visceral tissues

## 1. Introduction

The diarrhetic shellfish poisoning (DSP) group of toxins has a huge impact on the exploitation of marine resources. Bans on the marketing of both wild and cultivated shellfish are common all over the world (reviewed in Blanco et al. (2005)).

☆ *Ethical statement*: All the procedures made in this study try to avoid, to the maximum extent possible, using unnecessary animal and any suffering to the animals used.

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Toxins are unevenly distributed among the organs and tissues of bivalves (Bauder et al., 2001; Bricelj and Shumway, 1998; Cembella et al., 1993; Cembella and Shumway, 1995; Braña Magdalena et al., 2003; Pillet et al., 1995; Blanco et al., 2002; Hess et al., 2003, 2005; McCarron and Hess, 2006). This has repercussions on both the analysis and the kinetics of the toxins, since different organs act as different biological matrices (which condition the reliability of the analytical techniques) and they also have different enzymatic and excretory capabilities (which determine their ability to degrade, transform and excrete the toxins).

From an analytical point of view, by choosing to analyse the organ containing most of the toxin in the bivalve (if there is one) the analyst will be able to keep the biological matrix as simple as possible, enabling one to develop analytical techniques that are sensitive and robust. That is why this procedure has been—and still is—used in some techniques not involving mass spectrometry (Quilliam, 2003; Wright and Quilliam, 1995). Nevertheless, one of the drawbacks of this approach is that the results could be incorrect if the proportion of toxin in the tissues that are not analysed differs from those that were established when the method was developed, as could happen if some stages of the uptake/elimination of the toxins were not taken into consideration. From a toxicokinetic point of view, different organs probably accumulate and depurate DSP toxins at different rates, as has been shown in other kinds of toxins (Blanco et al., 2002; Bricelj and Shumway, 1998; Bauder et al., 2001).

Even though the anatomical distribution of toxins is an important issue, the distribution of lipophilic toxins in bivalves has received very little attention in the literature. Early studies on this type of toxins all agree that they are mainly accumulated in the digestive gland (Yasumoto et al., 1978; Murata et al., 1982). This seems to be a common occurrence, as other authors have found a clearly preferential accumulation (in terms of concentration) in the digestive gland of several bivalve species, such as the mussel *Mytilus edulis* (Pillet et al., 1995; Stabell et al., 1992), the bay scallop *Argopecten irradians* (Bauder et al., 2001) and the king scallop *Pecten maximus* (Hess et al., 2003). Pillet et al. (1995) found a constant proportion of 10/1 between the concentration of OA in the digestive gland and in the remaining soft tissues, in *M. edulis*, during the toxin incorporation phase from a culture of the dinoflagellate *Prorocentrum lima*. This proportion between the concentrations made the contribution to the total toxin content of the two body fractions studied approximately equal, taking into account that the ratio between their biomasses was around 1/10. Also bearing in mind that (a) in the scallop *A. irradians* (Bauder et al., 2001), the DSP toxin burden in non-visceral tissues (gonad, gills, mantle, adductor muscle) represents more than 20% of the total body contents and (b) the fact that the anatomical distribution of other toxins varies throughout the accumulation/depuration process (Bricelj and Shumway, 1998), it is clear that a specific study of the anatomical distribution of DSP toxins in the mussel is strongly needed.

Among mussels, *Mytilus galloprovincialis* is one of the most important species, as it makes up the entire production of Spain—which ranks second in mussel production worldwide. It also is the only species farmed by most Mediterranean countries, i.e. Italy, Greece, Turkey, Morocco, and (partially) France.

In this study, we examined the proportion of DSP toxins (okadaic acid (OA), DTX2, and their conjugated forms) in non-visceral tissues of the mussel *M. galloprovincialis* under three different circumstances, in order to quantify the contribution of the digestive gland to the total toxin content and to determine whether or not this contribution may be affected by the depuration process: (a) while ingesting toxic phytoplankton cell, (b) 1 week after the toxic cells had disappeared from the water, and (c) after ca. 2 months of depuration. Additionally, in case (b), the distribution among tissues was checked over 35 days of depuration in the laboratory.

## 2. Materials and methods

Mussels *M. galloprovincialis* were obtained from culture rafts in the Rías of Arousa on September 10, 2006 (case (a)), Pontevedra (case (b)) on June 12, 2004, and Baiona on January 26, 2005 (case (c)), all of which are located in Galicia (NW Spain). In case (a) three 15-mussel samples were collected while *Dinophysis acuminata* was blooming in the area in which the mussels were cultured. The mussels were dissected and their soft tissues were extracted and analysed (as described in detail below). In case (b), mussels collected during the end of the development of a toxic plankton bloom were carefully randomized and six 10-mussel samples were obtained and placed in net bags. The bags were hung, at random