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Variability in biochemical components of the mussel (*Mytilus galloprovincialis*) cultured after *Prestige* oil spill

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Abstract

The biochemical composition (proteins, carbohydrates, glycogen, total lipids and lipid classes) of the mussel *Mytilus galloprovincialis* was investigated during an experimental culture using mussel seed from areas with different degree of exposure to the *Prestige* oil spill. The aim of the study was to identify alterations in the biochemical composition of mussel seed from natural populations commonly used in Galicia for mussel raft culture that might be linked to previous oil exposure. We have selected three mussel seed populations from Pindo, Miranda and Redes, that were characterised in a previous study according to the oil exposure three months after the spill. These populations were transplanted to a raft culture system in the Ría de Ares-Betanzos where our experimental culture followed standard commercial techniques from March 2003 to February 2004. Mussels from Pindo (characterised as the most affected area by the oil spill) showed marked differences in lipid composition with regard to other populations in the content of triacylglycerols, ($P<0.001$), free fatty acids ($P<0.001$) and phospholipids ($P<0.05$) at the onset of the culture. Although these differences in lipid composition might reflect their previous exposition to hydrocarbons, this pattern did not last longer most likely due to depuration of hydrocarbons stored in the tissues or by the development of certain tolerance to PAHs. These significant differences were not detected between Miranda (designed as hardly affected area) and Redes (designed as reference area) which may reflect that Miranda mussels were not affected or only hardly affected by the spill. With the exception of the onset of the culture, biochemical composition showed similar patterns in all mussel populations. Then, the fact of being cultured in a common environment seemed to be more responsible for the long-term variability in the energetic reserve than the origin of the populations or their previous biochemical status.

Keywords: Biochemical composition; Lipid classes; *Mytilus galloprovincialis*; *Prestige* oil spill; Raft culture; Sublethal effect

1. Introduction

After the *Prestige* oil tanker sank near the Finisterre Cape in November 2002, successive black waves affected a wide area of the Galician Coastline (NW Spain). Although significant amounts of spilled oil did not enter the inner part of the estuaries in which commercial raft culture of mussels is carried out, there was a significant effect on an extensive area where mussel seed is gathered for culture purposes.

Bivalves, particularly mussels, are commonly used as sentinel organisms that may help to identify the status and trends of chemical exposure and to establish pollution gradients in a variety of marine coastal environments. The latter fact occurs because mussels are sessile, filter feeders, widely distributed and may react to changes in the environment through physiological responses that are easily measurable (Salazar and Salazar, 1995; Dame, 1996). Ecological and physiological disturbances have been reported in different species of molluscs due to their exposure to hydrocarbons (Widdows et al., 1982; Strömgren et al., 1986; Larratxea and Pérez Camacho, 1996; Modassir and Ansari, 2000; Porte et al., 2000; De Luca-Abbot, 2001; Le Floch et al., 2003; Amiard et al., 2004; Duquesne et al., 2004; Olsson et al., 2004; Pérez-Cadahía et al., 2004). These disturbances might be manifested at different levels of biological organization, although biochemical and cellular levels are the first in which the effects are perceptible (Livingston, 1985; Smolders et al., 2004). Alterations in the biochemical composition and specifically in those fractions that involve changes in the cycle of accumulation and use of energetic reserve are commonly used to identify pollution exposure (Bayne et al., 1978; Patel and Eapen, 1989; Leavitt

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et al., 1990; Capuzzo, 1996; Modassir and Ansari, 2000; Smolders et al., 2004). Several surveys have reported a decrease in the glycogen concentration (Patel and Eapen, 1989; De Luca-Abbot, 2001; Duquesne et al., 2004; Smolders et al., 2004) that is considered one of the most important energetic reserves in bivalves, although alterations in total carbohydrates or proteins have also been documented (Patel and Eapen, 1989; Modassir and Ansari, 2000; Olsson et al., 2004; Smolders et al., 2004). Nevertheless, studies about lipophilic organic contaminants exposure such as PAHs or PCBs are primarily focused in the impairment of lipid metabolism, mainly in the distribution and concentration of polar and neutral lipids (Capuzzo and Leavitt, 1988; Lowe, 1988; Leavitt et al., 1990; Capuzzo, 1996; Ferreira and Vale, 1998; McDowell et al., 1999; Bergen et al., 2001; Chu et al., 2003; Petrovic et al., 2004).

The aim of the present study was to observe the seasonal trend of the biochemical composition (proteins (prot), total carbohydrates (CH), glycogen, total lipids and lipid classes) of mussel seed that were exposed to the spill in their early life stage (settlers of late summer or early autumn).

2. Materials and methods

2.1. Experimental design

In March 2003, mussel seed *Mytilus galloprovincialis* was gathered from three locations along the Galician Coastline: Pindo, Miranda and Redes (Fig. 1). All sources of mussel seed corresponded to the first collected after the *Prestige* oil spill for commercial cultivation. We have selected three mussel seed populations Miranda, Pindo and Redes, firstly because these corresponded to locations commonly used for gathering mussel seed in Ría de Ares-Betanzos and secondly, because these populations were characterised according to their exposure to hydrocarbons three months after the spill by Labarta et al. (2005) according to several biochemical and physiological stress indicators. Pindo is located in the central area of the spill (Labarta et al., 2005; González et al., 2006), Miranda is located far from the most exposed area (Labarta et al., 2005; González et al., 2006) and Redes is the location that we have considered as reference because it is far from the central area of the spill and is inside the Ría de Ares-Betanzos where oil did not enter (Labarta et al., 2005; Laffon et al., 2006). These mussel populations were transplanted from their original locations to the Lorbé area in the Ría de Ares-Betanzos. A number of two ropes were used for each mussel population, with a density of approximately 15 kg of mussel seed per rope, an average density commonly used in the Galician raft culture (Pérez Camacho and Labarta, 2004). Experimental culture was carried out until February 2004 using a raft system (500 m^2) usually employed for the mussel culture in Galicia with commercial protocols and techniques described by Peteiro et al. (2006).

2.2. Mussel sampling

Individuals of *M. galloprovincialis* were collected monthly from each rope at 3–4 m depth, using two replicates of 200–300 individuals. Individual mussel lengths were recorded as the

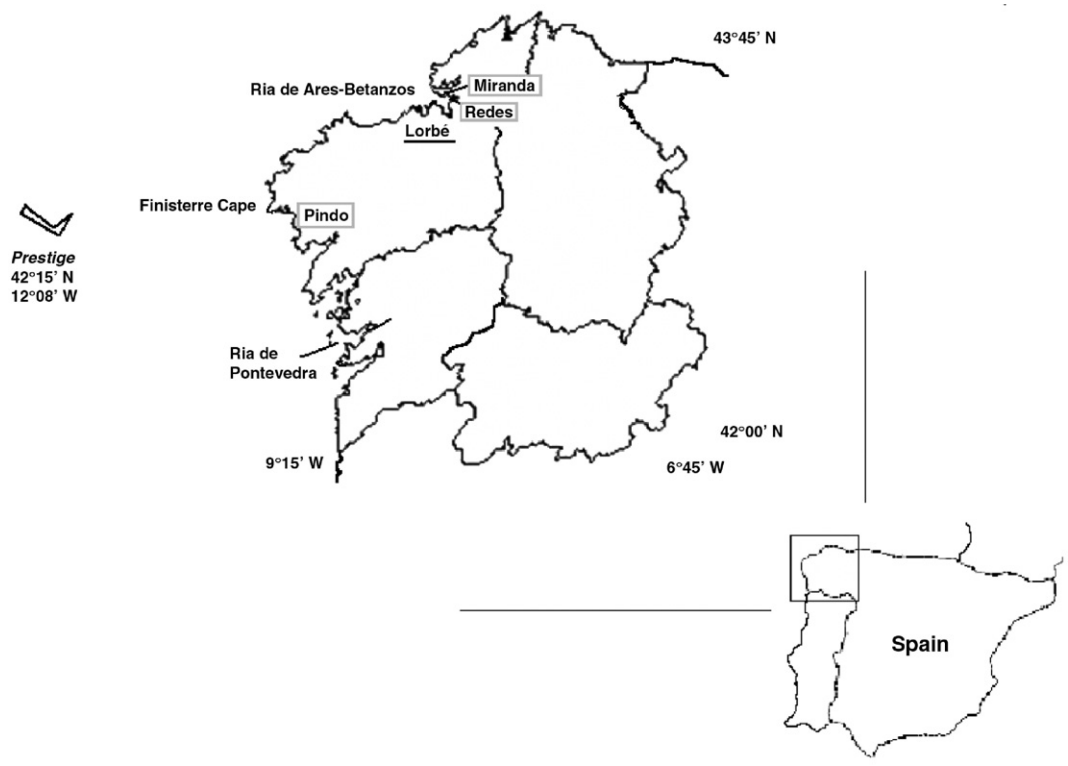

Fig. 1. Map of Galicia (NW Spain) showing the three mussel seed origins (in squares), the location of the experimental culture (underlined), the place where the *Prestige* oil tanker sank, and the area designed by Labarta et al. (2005) as the most affected by the oil spill (between Finisterre Cape and Ría de Pontevedra).