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Development of histopathological indices in the digestive gland and gonad of mussels: Integration with contamination levels and effects of confounding factors

Nagore Cuevas[a,*], Izaskun Zorita[b], Pedro M. Costa[c], Javier Franco[b], Joana Larreta[b]

[a] AZTI – Txatxarramendi ugartea z/g, 48395 Sukarrieta, Spain
[b] AZTI – Herrera Kaia, Portualdea z/g, 20110 Pasaia, Spain
[c] MARE – Marine and Evironmental Sciences Centre, Departamento de Ciências e Engenharia, Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

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ABSTRACT

Bivalve histopathology has become an important tool in aquatic toxicology, having been implemented in many biomonitoring programmes worldwide. However, there are various gaps in the knowledge of many sentinel organisms and the interference of confounding factors. This work aimed (i) to develop a detailed semi-quantitative histopathological index of the digestive gland and gonad of the *Mytilus galloprovincialis* mussel collected from five sites contaminated with distinct patterns of organic and inorganic toxicants along the Basque coast (SE Bay of Biscay) and (ii) to investigate whether seasonal variability and parasitosis act as confounding factors. A total of twenty-three histopathological alterations were analysed in the digestive gland and gonad following a weighed condition index approach. The alterations were integrated into a single value for a better understanding of the mussels' health status. The digestive gland was consistently more damaged than the gonad. Mussels from the most impacted sites endured the most significant deleterious effects showing inflammation-related alterations together with digestive tubule atrophy and necrosis. Neoplastic diseases were scarce, with only a few cases of fibromas (benign neoplasia). In contrast, in moderately or little impacted sites, contamination levels did not cause significant tissue damage. However, parasites contributed to overestimating the values of histopathological indices (*i.e.* more severe tissue damage) in mussels from little impacted sites, whilst the opposite occurred in mussels from highly polluted sites. Accordingly, inter-site differences were more pronounced in autumn when natural physiological responses of advanced maturation stages did not interfere in the histological response. In conclusion, although seasonal variability and parasitosis mask the response of histopathological indices, this biomonitoring approach may provide good sensitivity for assessing the health status of mussels if fluctuations of these confounding factors are considered.

1. Introduction

In the European Union (EU), the Marine Strategy Framework Directive, MSFD (Directive 2008/56/EC), introduced a series of criteria ("descriptors") that must be addressed in order to achieve "Good Environmental Status" (GES) of marine habitats. As such, the MSFD stipulates that the monitoring of marine ecosystems is to be endeavoured regionally, in face of the broad biogeographical diversity of the European coastline, albeit the need for some degree of standardisation of biomonitoring procedures (see the reviews by Borja et al., 2010; Lyons et al., 2010).

The effects of anthropogenic contaminants on the biota are included in Descriptor 8 of the MSFD. However, the choice for adequate biological endpoints and sentinel organisms is challenging on its own and relies on solid knowledge of the aetiology of stressor-induced adverse effects and the specific life-history traits that modulate them (Ruiz et al., 2011; Van der Oost et al., 2003). For instance, the expert scientific groups of International Council for Exploration of the Seas (ICES), such as the Working Group on Biological Effects of Contaminants (WGBEC) and the Study Group

* Corresponding author. Tel.: +34 94 657 40 00; fax: +34 94 657 25 55.
E-mail address: nagore.cuevas@mail.es (N. Cuevas).

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on Integrated Monitoring of Contaminants and Biological Effects (SGIMC), assist in the development of assessment tools under Descriptor 8 premises within the North East Atlantic maritime region (Davies and Vethaak, 2012). Since the establishment of the Mussel Watch Program in the mid-1970s (Goldberg, 1975), mussels have been extensively used as a sentinel organism of pollution in marine coastal biomonitoring programmes (Bignell et al., 2011; Brenner et al., 2012; Garmendia et al., 2010; Zorita et al., 2007). Mussels (Bivalvia: Mytiloidea) hold many advantageous characteristics since they have a wide geographic distribution (with potential implications for standardised analyses); they are sedentary filter feeders (thus prone to be affected by their immediate surroundings); have a relatively simple anatomy and physiology; and are acknowledged as being sensitive to a range of pollutants (Bignell et al., 2011; Brenner et al., 2012; Cajaraville et al., 2000; Widdows et al., 2002).

Defining histopathological lesions and alterations in wild organisms has been considered as one of the most important approaches to assess pollution-driven adverse effects to organisms in biomonitoring studies. Histopathological biomarkers are considered to be high-value endpoints since they may provide direct information on the organisms' health status (Adams and Sonne, 2013; Bignell et al., 2008; Feist et al., 2004). Advances in semi-quantitative approaches (or even fully quantitative, in a few cases) have been equipping histopathology with an important statistical power that is critical in biomonitoring (Bignell et al., 2011; Costa et al., 2013; Marigómez et al., 2013). Histopathological indices that integrate different histopathological alterations of a target organ into a single value have already been developed in different fish species (Bernet et al., 1999; Costa et al., 2011, 2009; Van Dyk et al., 2007) and, to a lesser extent, in invertebrates, such as clams (Costa et al., 2013). However, to our knowledge, there is no detailed histopathological index that integrates and unifies all the lesions recorded in mussels, one of the proposed sentinel species for the biomonitoring of EU coastal waters within the scope of the MSFD.

Nevertheless, histopathological studies may be impaired by several factors that call for in-depth knowledge of the sentinel organisms' specific biological features, from season-related changes (including gonad development) and parasites to the identification of specific microanatomical traits (Bignell et al., 2008; Garmendia et al., 2010; Kim et al., 2008). In fact, the latter is regarded as a major drawback in histopathological appraisals of invertebrates since these are a long way off benefitting from the level of histological knowledge as their vertebrate counterparts (see Au, 2004, for a review).

This work is aimed at (*i*) developing and validating a species-specific semi-quantitative approach based on histopathological indices for the digestive gland and the gonad of mussels (*Mytilus galloprovincialis*) collected from five sites contaminated with distinct patterns of organic and inorganic toxicants along the Basque coast; and (*ii*) investigating the effects of two critical confounding factors, seasonality and parasites, on mussel health status and how these may hamper biomonitoring procedures.

2. Material and methods

2.1. Sampling area and sample collection

The Basque coast (SE Bay of Biscay), in particular its estuaries, has been receiving special attention in marine environmental assessment due to its historical concentration of heavy industry and dense human settlement (Borja et al., 2006). For many years, the ecological status of the Basque estuaries deteriorated, especially because of the direct dumping of untreated domestic and industrial wastewaters (Cearreta et al., 2004). However, it is generally acknowledged that recent environmental strategies regarding the treatment and discharge of effluents has led to a significant recovery of the environmental status along the Basque coast (Borja et al., 2009). Yet the full extent of this recovery remains to be determined. Hence, this study focused on five different sites along the Basque coast: Getxo, Gorliz, Mundaka, Pasaia and Hondarribia (Fig. 1). These estuarine environments are classified into two major groups, according to their geomorphological and hydrological characteristics (Borja et al., 2004): Gorliz and Mundaka are estuaries dominated by marine influence with a high percentage (>75%) of intertidal area; while Getxo, Pasaia and Hondarribia are sub-tidal estuaries. Getxo and Pasaia boast important and industrialised harbours on the North Iberian coast, presenting a deteriorated ecological status characterised by low oxygen levels, disappearance of fauna and significant levels of sediment pollution (Borja et al., 2006). Conversely, Mundaka is located in the Urdaibai Biosphere Reserve, which is considered one of the best preserved estuaries of the Basque coast (Marigómez et al., 2013; Orbea et al., 2002). This estuary is mainly important for leisure and tourism, although some industrial activities, such as metallurgic and shipyard facilities, can be found further upstream. Gorliz lacks significant contamination hotspots, being an area exposed to moderate waves (Orbea et al., 2002; Solaun et al., 2013). Last, Hondarribia is located off the mouth of Bidasoa estuary, a highly populated area that has been recovering its ecological status due to the recent implementation of a sanitation plan that included a treatment plant with undersurface outflow pipe (Solaun et al., 2013).

In each town, two sediment samples were collected by hand in intertidal areas and with Van Veen or Day grabs in sub-tidal areas every winter between 2009 and 2012. Then, one sediment sample was stored at 4 °C for sediment characterisation, whereas the other sample was frozen at −20 °C for contaminant determination. Additionally, adult mussels (3.5–5.5 cm in length) were sampled at each sampling site ($n = 20$) during low tide in the four seasons of a full year, from spring 2012 to winter 2013. The specimens were transported in sea water to the laboratory until subsequent histological analyses.

2.2. Sediment contamination levels

Granulometric analyses were conducted on dried sediments (60 °C, 24 h) by running samples through a column of sieves (gravel, >2 mm; sand, 2–63 mm and mud, <63 mm) according to Holme and McIntyre (1971). Metals (Cd, Cr, Cu, Hg, Ni, Pb and Zn) were measured following acid digestion of dry sediment samples in a microwave (CEM MARS 5 Xpress) by Atomic Absorption Spectrometry (PerkinElmer AAS AAnalyst 800). For organic toxicants [polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs)], sediment sample extracts were pre-concentrated by accelerated solvent extraction (Dionex ASE 200 system) following gas-chromatography–mass-spectrometry (GC–MS) determination in Selective Ion Monitoring mode (Agilent 6890 GC coupled with an Agilent 5973 MSD instrument).

Contaminant levels were contrasted to the threshold effects level (TEL) and probable effects level (PEL) proposed by MacDonald et al. (1996). The potential risk to cause adverse effects to biota for each sediment (per class of contaminant or total contaminants) was determined through sediment quality guideline quotients (SQG-Qs) described by Long and MacDonald (1998). Furthermore, the classification of potential impact to cause adverse biological effects was calculated according to MacDonald et al. (2004), as follows: no impact <0.1, moderate impact 0.1–1 and strong impact >1.

Medians of granulometry and SQG-Qs of sediments collected between 2009 and 2012 were calculated in order to characterise the sediment matrix of each estuarine area.