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Use of sperm DNA integrity as a marker for exposure to contamination in *Palaemon serratus* (Pennant 1777): Intrinsic variability, baseline level and *in situ* deployment

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ABSTRACT

In a previous study, the Comet assay was optimized for *Palaemon serratus* prawns in order to propose a biomarker for sperm quality in this species. However, better knowledge of its basal level and its natural variability, related to intrinsic biotic and environmental abiotic factors, is required before any relevant use of this biomarker in the field. To fulfill this goal, the present study proceeded in three steps: (i) the temporal variability of DNA integrity was followed monthly in a reference population over a 2-year period, (ii) the correlation between the main intrinsic biotic (i.e. size, weight and molting stage) and abiotic factors (i.e. water temperature) were recorded in the field, and the basal DNA integrity was assessed in order to scrutinize any confounding influence of factors unrelated to toxic response, (iii) the baseline level was used to discriminate biomarker response among different stations displaying contrasting contamination levels. The results of the two-year monitoring in the reference population revealed no correlation between the levels of spermatozoa DNA damage and temperature, body size, weight or molting stage. Only a slight variability between monthly samplings was detected. On the basis of these field-collected data, we defined a reference distribution (i.e. 52.6 ± 5.6 A.U) with a threshold value (i.e. 61.7 A.U). Finally, this threshold value proved its relevance to discriminate among stations with contrasting pollution levels around the Seine Bay. Indeed, the results suggest significant DNA damage in populations nearest the Seine estuary, a major source of contaminants in the Bay, and a lower effect in populations further away from the estuary. The overall conclusion was that the Comet assay on *P. serratus* spermatozoa could be a useful tool for the monitoring of the toxicological print within sperm and main globally the contamination exposure of crustaceans in marine waters.

1. Introduction

Pollution released from industrial wastewater, farming, and urban sources is a major threat to organisms living in the aquatic environment. Nowadays, estuarine and marine areas are one of the main preoccupations of aquatic ecotoxicologists; due to their ultimate destination position for most anthropogenic compounds, they are subjected to contamination from point and diffuse sources (Tappin and Millward, 2015). From a toxicological point of view, biocenotic approaches and chemical analyses (e.g. sediment and biota), which are recommended by the Water Framework Directive 2000/60/EC, appear to be limited in their capacity to identify contamination effects on aquatic populations. To address this constraint, the UE Marine Strategy Framework Directive 2008/56/EC introduced the development of biomarkers with the view to their deployment in transitional and coastal waters. Indeed, biomarkers are early-warning tools assessing a causal relationship between the exposure to chemicals and the impacts on organisms, including factors such as bioavailability and mixture effects of known and unknown chemicals (Hanson et al., 2010). Biomarkers should be reliable, robust, easily applicable, only modulated by contaminants, and predictors of adverse impacts on populations

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and communities. In reality, almost all biomarkers are influenced by abiotic factors such as water temperature, salinity, pH and dissolved oxygen (Buschini et al., 2003; Bolognesi et al., 2004; Leinio and Lehtonen, 2005; Pfeifer et al., 2005) or intrinsic variables linked to age, sex or reproductive stage (Jha, 2008; Lacaze et al., 2011a; Sanchez et al., 2008; Sheehan and Power, 1999; Wiklund and Sundelin, 2004; Xuereb et al., 2009). Because of these confounding factors, biomarkers are still not often routinely employed in biomonitoring surveys, particularly in studies on a spatial or temporal large scale. The major part of *in situ* application with biomarkers is generally based on the comparison between a reference site and an impacted site (e.g. upstream and downstream comparisons) (Flammarion et al., 2002; Flammarion and Garric, 1997). This (i) imposes similar physicochemical conditions between stations in absence of contamination (Underwood, 2000) and (ii) consequently limits the assessment in punctual studies and local areas. Alternatively, some solutions have been proposed to control the influence of both biotic and environmental factors on the response of biomarkers, and make possible their deployment within field surveys on a larger scale. Among them, the assessment of natural variability to propose a basal reference while taking into account a spatiotemporal change has proved its effectiveness (Barrick et al., 2016; Hagger et al., 2008; Hanson, 2011; Jubeaux et al., 2012; Lacaze et al., 2011a; Xuereb et al., 2009). This methodology makes it possible to (i) compare sites without a reference site, (ii) reveal the exposure of organisms to one or several contaminants in low contrasted or contaminated situations and (iii) prevent false negatives. For the estimation of such ranges, a monitoring of several reference sites in different seasons is recommended. The OSPAR convention in 2013 has recommended that organisms should be collected from reference sites for at least two seasonal cycles to assess the influence of confounding factors (Amiard-Triquet et al., 2015).

Among biomarkers for damage, genotoxicity biomarkers are considered as integrated tools, able to provide complementary information to chemical and ecological analyses for field monitoring (Lacaze et al., 2011a). Indeed, contaminants described as potentially genotoxic would represent one-third of the anthropogenic compounds released into the marine environment (Claxton et al., 1998). Interaction of these compounds on the reproduction process may have an even greater impact at the population level than carcinogenic effects for organisms (Aitken and De Iuliis, 2007). In germ line cells, spermatozoa proved to be sensitive to water contamination because of their inability to prevent oxidative stress and to repair DNA damage. These particularities make them a more integrated cellular model of chemical exposure than oocytes (Aitken et al., 2004; Aitken and Baker, 2006; Lacaze et al., 2010; Lee and Steinert, 2003; Lewis and Galloway, 2009; Santos et al., 2014). Moreover, several authors have already demonstrated the relationship between genotoxicity in sperm and reproductive impairment, in invertebrates (Lacaze et al., 2011b; Lewis and Galloway, 2009) as well as in fish species (Devaux et al., 2011; Santos et al., 2013a,b). These studies highlighted the main interest of including a biomarker of genotoxicity measured in sperm for environmental monitoring and ecological risk assessment. The Comet assay or Single-cell gel electrophoresis assay (SCGE) has become one of the most widely used for detecting DNA strand breaks in aquatic animals (Cotelle and Fe, 1999; Frenzilli et al., 2009; Jha, 2008; Lacaze et al., 2011a,b; Lee and Steinert, 2003; Martins and Costa, 2015; Mitchelmore and Chipman, 1998). Furthermore, Comet assay on spermatozoa for environmental monitoring has been recommended in many reviews (Jha, 2008; Speit et al., 2009; Villani et al., 2010).

As a member of the crustacean family, Palaemonid prawns have been commonly used as a relevant sentinel species for assessing the health conditions of estuaries and coastal systems (Bocquene et al., 1995; Frasco et al., 2008; Key et al., 2006). These species are relatively easy to identify, manipulate and maintain in the laboratory or to use for *in situ* biosurveys. *Palaemon* genus is widespread and common in the coastal and estuarine waters of Western Europe, where they are often found in high density (Campillo, 1975). Moreover, these prawns are an important trophic component for many fish and other crustacean species, including commercially valuable ones, and play a major part in the detritus breakdown process, being primary and secondary consumers (Anderson, 1985). A particular interest has recently been attributed to the *Palaemon serratus* species, which is a coastal species with a large distribution around the European coast (North Sea, English Channel, Atlantic and Mediterranean coast; Campillo, 1975).

Our work aims to develop the Comet assay on Palaemonid spermatozoa and propose it as a reliable and robust biomarker for the surveys of the European coastal and estuarine water bodies. As recommended by Azqueta and Collins (2013), a preliminary study related to the methodological optimization of the Comet assay protocol was performed in *P. serratus* (Erraud et al., 2017). This step, which is unavoidable before any deployment in field studies, led us to propose a procedure for collecting a homogeneous population of mature spermatozoa and measuring their DNA integrity. Palaemonid sperm turned out to be sensitive to different genotoxic pathways. Henceforth, the aim of the present study was (i) to develop a procedure to obtain the lowest variability of biomarker response related to intrinsic biotic factors, and (ii) to define reference values of our genotoxicity biomarker taking into account the effect of environmental confounding factors. For that, the inter-individual and seasonal variability of the basal level of sperm DNA damage was investigated during a two-year period within a wild population of prawns in a reference site (i.e. Yport), in order to propose a reference distribution and a threshold value for this biological measurement. In the second step, sperm DNA damage was measured during two campaigns of biomonitoring within populations in different stations of the Seine bay displaying contrasting degrees of contamination, in order to assess the relevance of this reference distribution to discriminate toxic effect.

2. Materials and methods

2.1. Localization of studied area

The mouth of the Seine (Normandy, France) is marked by the discharge of a wide diversity of chemical contaminants (i.e. drained by the Seine River), most of which are associated to the particles of the sedimentary plume. The contamination is transported by the longshore drift along the coastline mainly in a northerly direction (Augris, 2004), up to the seawall of Antifer Harbor. This 3-km long transverse structure deflects the contaminated plume offshore, creating a sedimentation area upstream of the seawall (i.e. south of Antifer Harbor) (Brivois et al., 2015).

The contamination gradients from the Seine estuary toward both the north and the south of the bay are well illustrated by the ROCCH data records (Chemical Contamination Observation Network; http://www.ifremer.fr/envlit/) synthetized in Table 1. This table indicates the annual mean (i.e. 2015 and 2016) of cumulated concentrations of polychlorobiphenyl congeners (i.e. CB138; CB180; CB156; CB28; CB52; CB105; CB153; et le CB101), polycyclic aromatic hydrocarbons (i.e. Benzo(b)fluoranthene; Chrysene; Dibenzo(a,h)anthracene; Fluoranthene; Indeno(1,2,3-cd) pyrene; Anthracene; Phenanthrene; Pyrene; Benzo(g,h,i)perylene; Benzo(k)fluoranthene; Benzo(a)pyrene; Benzo(a)anthracene) and seven metals (namely. Zinc, Cadmium, Copper, Mercury, Silver, Nickel and Lead) measured in soft tissues of mussels sampled from