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Biomarker responses in wild three-spined stickleback (*Gasterosteus aculeatus* L.) as a useful tool for freshwater biomonitoring: A multiparametric approach

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Abstract

The biochemical response of wild sticklebacks collected in Autumn 2005 at seven stations in the North of France was studied using a set of complementary biomarkers. Here, data on biotransformation of xenobiotics, oxidative stress exposure and damages, neurotoxicity and endocrine disruption are provided. All the sites are characterized by a specific response pattern that allows distinguishing sampling locations. Moreover, these responses are in accordance with data on existing environmental pressures and the chemical analysis of metals performed in surface water. The assessment of individual responses is completed by fish population disturbance monitoring. Based on these measurements, the investigated sites are characterized by different levels of disturbance. This study argues for a multi-parametric approach of aquatic ecosystem contamination based on association between chemical, biochemical and ecological endpoints and provides a testimony of the usefulness of stickleback for this purpose.

Keywords: Three-spined stickleback; Biomarkers; Fish; Biotransformation enzymes; Oxidative stress; Neurotoxicity; Endocrine disruption, Field study

1. Introduction

Water quality of aquatic ecosystems is assessed using a combination of chemical and ecological methods. Chemical monitoring is based on quantification of pollutant groups in water, sediments and biota. This targeted approach provides a valuable information on media contamination by a limited number of chemicals but it is not integrative since the effects of contamination on aquatic organisms are not considered (Amiard et al., 1998). Ecological monitoring is based on community structure assessment in various taxonomic assemblages including diatoms (Prygiel, 2002), oligochaetes (Prygiel et al., 1999) and fish (Oberdorff et al., 2002). These methods allow to detect adverse ecological effects but they respond to various disturbances acting at the community level and they are no reliable indicators of ecological impairment caused by specific contaminants (Amiard et al., 1998).

To assess the effects of contamination at low organisation levels, several studies have focused on biomarker utilisation as indicators of chemical exposure and early responses of aquatic organisms. Biomarkers are defined as observable or measurable modifications at the molecular, biochemical, cellular, physiological or behavioural levels revealing the exposure of an organism to xenobiotics (Lagadic et al., 1997). To evaluate the various responses to pollutant mixtures in organisms, the usefulness of a set of complementary biomarkers has been demonstrated and is now applied in environmental biomonitoring programs (Flammarion et al., 2002b; Schmitt et al., 2005; Triebskorn et al., 2001).

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In this study, we have selected the three-spined stickleback (*Gasterosteus aculeatus* L.) as sentinel fish species. This is a small size fish widely distributed in European waters including fresh, estuarine and coastal waters, and characterised by a relatively sedentary behaviour. The stickleback is a well-studied model for biologists since their behaviour, ecology and evolution have been extensively characterised. Moreover, this is the only species with a quantifiable androgen and anti-androgen biomarker: the spiggin (Katsiadaki et al., 2002a). This androgen-induced glue protein is synthetised by the kidney (Jakobsson et al., 1999). In environmental toxicology, stickleback is also considered as a valuable model species that enable the measurement of both biochemical and physiological responses to pollutants such as metals (Sanchez et al., 2005), pesticides (Katsiadaki et al., 2006; Sanchez et al., 2006; Wogram et al., 2001), halogenated organic compounds (Holm et al., 1994) and steroids (Pottinger et al., 2002; Sebire et al., 2007). Stickleback biomarker responses can also be used for the biomonitoring of freshwater ecosystem contamination as previously described for cholinesterase activities (Sturm et al., 2000) and liver histology (Handy et al., 2002). More recently, we have demonstrated the potential of using the stickleback as a sentinel fish species to assess sublethal stress in multipollution context using a set of biochemical biomarkers (Sanchez et al., 2007).

The present study was designed to evaluate the use of a suite of biomarkers in stickleback as an early warning system for aquatic contamination. For this purpose, we used a set of parameters including biochemical biomarkers and biometrical measurements to characterise the various contaminated study sites. The investigated endpoints were selected in order to represent several biological processes. These included xenobiotic biotransformation activities such as 7-ethoxyresorufin-*O*-deethylase (EROD) and glutathione-*S*-transferase (GST), and neurotoxicity of pollutants with acetylcholinesterase (AChE) activity in the muscle. Antioxidants such as glutathione peroxidase (GPx), total glutathione content (GSH) and oxidative damages with thiobarbituric acid reactive substances (TBARS) were also studied. Vitellogenin (VTG) and spiggin (SPG) concentrations in males and females respectively were measured to evaluate exposure to endocrine disrupter compounds. Physiological parameters such as liver and gonad somatic index (HSI and GSI respectively) and condition factor (CF) were also determined. The biomarker measurements were complemented with (1) assessment of water quality of sampled sites based on physico-chemical characterisation and metal quantification; and (2) analysis of fish assemblage disturbances.

2. Materials and methods

2.1. Site descriptions

Fish were collected from 7 sites (Table 1) located in the North of France and characterised by the presence of wild stickleback populations. To reduce variations due to geographical factors, sampling sites were selected within the same hydro-ecoregion (Wasson et al., 2002). These sites were selected in order to assess the effects of various qualitative and quantitative impacts on stickleback biochemical responses as well as on fish population and community assemblages. Three sampling sites including Lézarde River (lez), Vallon du Vivier (vdv) and Val des Fontaines (vdf) were considered as having a low level of contamination by anthropogenic activities. The other four sites were considered as heavily contaminated by different activities. Among them, the stations located in the Réveillon River (rev) and the Rhonelle River (rho) were selected because of their position within an urbanised and cultivated area. The Vilpion River station (vil) was chosen because of its position within an intensive agricultural area, and the Escaut River station (esc) because of its location within an heavily industrialised and urbanised area.

Since sticklebacks are able to inhabit in various hydrosystems, all investigated stations were characterised by position according to Huet's (1949) zonation and Strahler's (1957) rank determination (Table 1). Moreover, water temperature, pH, dissolved oxygen concentration, conductivity and hardness were measured prior to fish sampling.

2.2. Metal analysis in water

In the beginning of the study, all water samples were analysed by ICP/AES (Inductively Coupled Plasma /Atomic Emission Spectrometry) according to the standard NF EN ISO 11885. These analyses were carried out in a semi-quantitative way (screening). A total of 33 inorganic elements were studied over a 0–10 mg/L concentration range. The limit of quantification, for all of them, was equal to 50 μg/L. Subsequently to these results, the same samples were analysed by ICP/MS (Inductively Coupled Plasma / Mass Spectrometry) according to the standard NF EN ISO 17294. Samples were filtered (porosity 0.45 μm) prior to the analysis. Cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) were analysed quantitatively on the dissolved fraction. The concentration range was between 0 and 50 μg/L. The limit of quantification was equal to 1 μg/L except for Cd that was equal to 0.09 μg/L.

2.3. Fish collection and tissue sampling

Three-spined sticklebacks were collected during autumn 2005, between 16th September and 5th October, by electrofishing according to the standards "Sampling of fish with electricity" (EN 14011). The station of rev, lez, rho, vil

Table 1
Summary of some general characteristics of the investigated sites

Sites	Réveillon (rev)	Lézarde (lez)	Escaut (esc)	Rhonelle (rho)	Vilpion (vil)	Vallon du Vivier (vdv)	Val des Fontaines (vdf)
GPS coordinates	N 48°43′00″ E 2°32′09″	N 49°34′09″ E 0°13′20″	N 53°23′40″ E 3°32′56″	N 50°17′49″ E 3°32′41″	N 49°43′23″ E 3°42′45″	N 49°43′23″ E 0°27′42″	N 49°29′33″ E 0°24′37″
Watershed area (km^2)	145	62	1650	73	377	25	10
Strahler's rank [a]	2	1	4	2	3	1	1
Huet's zonation [b]	Barbel	Trout	Bream	Barbel	Barbel	Trout	Trout
Pressure [c]	Urban dense	Mixed	Urban dense	Urban dense	Intensive agriculture	Mixed	Mixed
Water Quality [d]	Very bad	Good	Bad	Bad	Very bad	Very good	n.d.

[a] Strahler's rank based on structure and density of hydrographic network (Strahler, 1957).
[b] Huet's zonation was determined according to Huet, (1949).
[c] Data obtained from the Eurowaternet database of European Environment Agency (EEA, 2001).
[d] Data obtained from French water agencies.