Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/watres

In situ feeding assay with *Gammarus fossarum* (Crustacea): Modelling the influence of confounding factors to improve water quality biomonitoring

Romain Coulaud [a,b], *Olivier Geffard* [a,*], *Benoît Xuereb* [a,1], *Emilie Lacaze* [a], *Hervé Quéau* [a], *Jeanne Garric* [a], *Sandrine Charles* [b], *Arnaud Chaumot* [a,*]

[a] *Cemagref, UR MALY, 3 bis quai Chauveau-CP 220, F-69336 Lyon, France*

[b] *Université de Lyon, F-69000, Lyon, Université Lyon 1, CNRS, UMR5558, Laboratoire de Biométrie et Biologie Evolutive, F-69622 Villeurbanne, France*

ARTICLE INFO

Article history:
Received 11 March 2011
Received in revised form
31 August 2011
Accepted 15 September 2011
Available online 22 September 2011

Keywords:
In situ assay
Feeding rate
Gammarus
Temperature
Biomonitoring
Modelling

ABSTRACT

In situ feeding assays implemented with transplanted crustacean gammarids have been claimed as promising tools for the diagnostic assessment of water quality. Nevertheless the implementation of such methodologies in biomonitoring programs is still limited. This is explained by the necessity to improve the reliability of these bioassays. The present study illustrates how modelling the influence of confounding factors could allow to improve the interpretation of *in situ* feeding assay with *Gammarus fossarum*. We proceeded in four steps: (i) we quantified the influence of body size, temperature and conductivity on feeding rate in laboratory conditions; (ii) based on these laboratory findings, we computed a feeding inhibition index, which proved to be robust to environmental conditions and allowed us to define a reference statistical distribution of feeding activity values through the data compilation of 24 *in situ* assays among diverse reference stations at different seasons; (iii) we tested the sensitivity of the feeding assay using this statistical framework by performing 41 *in situ* deployments in contaminated stations presenting a large range of contaminant profiles; and (iv) we illustrated in two site-specific studies how the proposed methodology improved the diagnosis of water quality by preventing false-positive and false-negative cases mainly induced by temperature confounding influence. Interestingly, the implementation of the developed protocol could permit to assess water quality without following an upstream/downstream procedure and to compare assays performed at different seasons as part of large-scale biomonitoring programs.

1. Introduction

In aquatic ecosystems, organisms are constantly exposed to different levels of physical and chemical stressors. To estimate and predict their biological effects, the need for relevant tools has increased considerably in the last decades, which is of broad importance in the regulatory framework for the diagnosis of ecological impacts of chemicals (*e.g.* EU Water

Abbreviations: FR, feeding rate; FI, feeding inhibition index.

* *Corresponding authors.* Tel.: +33 4 72208788; fax: +33 4 78477875.
E-mail addresses: olivier.geffard@cemagref.fr (O. Geffard), arnaud.chaumot@cemagref.fr (A. Chaumot).
[1] Present address: Laboratoire d'Ecotoxicologie - Milieux Aquatiques (LEMA: EA 3222), Université du Havre, 76058 Le Havre Cedex, France.

0043-1354/$ – see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.watres.2011.09.035

Framework Directive, 2000/60/EC). Up to now, water quality has been monitored using both chemical and biological measures. Concerning biological measures, several biotic indices have been developed. Because these methods referred to changes in community structure, the established diagnosis of ecosystem quality reflects integrative effects from diverse sources of degradation. That is why the identification of pressure/impacts relationships, is often difficult. To disentangle the role of chemical contaminations in the degradation of environmental quality, a complementary approach consists of methods based on lower levels of biological organization for assessing biological impacts (Chapman, 2007; Dagnino et al., 2008; Damásio et al., 2008), *e.g.* measuring sublethal responses of single species (Maltby et al., 2002). These methods are expected to be more specific and sensitive to the toxic effects of contaminants, and thus to supply early warning indicators of pollution impacts. Nevertheless, the use of individual responses still remains limited because their interpretation under non-controlled environmental conditions often lacks the definition of relevant reference values (Maltby et al., 2002; Hagger et al., 2008).

Individual responses can supply ecologically relevant endpoints because some of them constitute or can at least be related to fitness traits (survival, reproduction, growth). In the diagnostic context, they are rarely used because the measurement of such physiological or demographic rates necessitates the adaptation of laboratory bioassay protocols to field exposure. Hence, protocols for post-exposure measurements with either indigenous or transplanted organisms (Soares et al., 2005; Galloway et al., 2006; Barata et al., 2007; Krell et al., 2011), and protocols for *in situ* measurements with caged organisms (Maltby and Crane, 1994; Dedourge-Geffard et al., 2009) are developed for physiological rate and life-history trait measurements. Among the individual responses which can be monitored, feeding inhibition is of great interest for multi-scale assessment of water quality. On one hand, it is an ecological concern because it can be related to alteration in life-history traits (Maltby, 1999; Baird et al., 2007; Barata et al., 2007) and because it can be correlated with ecosystem processes (Forrow and Maltby, 2000; Maltby et al., 2002). On the other hand, its interpretation can be linked with the modulation of molecular biomarkers of specific modes of action (Barata et al., 2007; Xuereb et al., 2009b). In aquatic invertebrates, feeding inhibition is in most cases one of the first observed responses to environmental pollution (Gerhardt, 1995; Macedo-Sousa et al., 2007; Alonso et al., 2009; Mouneyrac et al., 2010). Since the 1990s, several laboratory studies have shown that the feeding rate (*FR*) of amphipods (in particular freshwater gammarids) can be inhibited by a large range of chemical stressors (metals, insecticides, fungicides, herbicides, drugs, organic compounds… see Suppl. Table 1A). *Gammarus pulex* (Linnaeus) and *Gammarus fossarum* (Koch) are highly relevant as sentinel species to study feeding inhibition in streams. They are widespread in European ecosystems, where they play a key role in nutrient cycles as decomposers of coarse organic matter. By performing a short review of the literature since 1990, we noted that several studies showed *in situ* feeding inhibitions in gammarids in various contamination profiles (industrial wastes, acid mine drainage, agricultural catchments… see Suppl. Table 1B). Consequently, *FR* assessment that can be easily measured *in situ* with caged gammarids (mainly by leaf-mass feeding assays), has been proposed as an ecologically relevant *in situ* indicator of water quality (Maltby et al., 2002).

The main limitation for the use of individual responses in monitoring programs is the difficulty to define baseline values due to spatial and seasonal variability related to the effects of biotic and abiotic factors (Hagger et al., 2008; Hanson et al., 2010). Such biotic and non-toxic environmental influences could lead to the misinterpretation of individual markers in water chemical quality assessment during *in situ* or post-exposure assays with caged organisms (Maltby et al., 2002; Moreira et al., 2006; Kater et al., 2001; Krell et al., 2011). Indeed, the inflated variability of responses in controls results in a decreased statistical power explaining a low sensitivity of bioassays (*i.e.* high rate of false negatives). In addition, confounding effects could give rise to false-positive cases, when deviation from controls is caused by a difference in the level of a non-toxic influential factor (*i.e.* low specificity). *FR* measurement in gammarids can be affected by many biotic and abiotic factors. Biotic factors include source population (Maltby and Crane, 1994; Veerasingham and Crane, 1992; Crane et al., 1995), parasite load (McCahon et al., 1988; Pascoe et al., 1995; Fielding et al., 2003; Lettini and Sukhdeo, 2010), or body size (Nilsson, 1974). With the aim to reduce the variability related to these biotic factors, the use of transplanted standard organisms is proposed for water quality assessment (Liber et al., 2007) because it allows to play down the impact of biotic factors (one population source, same physiological parameters such as size, sex, reproductive and energetic status).

The confounding effect of abiotic factors, which can not be controlled during *in situ* exposure, has limited the application of bioassays with transplanted organisms to paired comparisons between stations upstream/downstream from identified point-source pollutions. In this specific context, the assessment of chemical water quality strongly relies on a questionable experimental design which assumes that physicochemical conditions are similar between stations, excepted for levels of bioavailable toxic compounds (Liber et al., 2007). As an alternative, modelling the influence of confounding factors can make measurements comparable in space and time (Maltby et al., 2002; Moreira et al., 2006; Krell et al., 2011). This could allow to benefit from robust reference conditions defined at larger scales of space and time. For instance, through an empirical analysis of the influence of environmental conditions (temperature, alkalinity,…) on *FR*s in *Gammarus*, Maltby et al. (1990b, 2002) underlined that taking into consideration the most influential environmental conditions in order to define reference values of biological activities could improve the *in situ* approach for site-specific studies. Furthermore, such a methodological advance could permit the application of *FR in situ* bioassays to large scale and long-term biomonitoring programs.

The present study illustrates how modelling the influence of confounding factors allows to improve the interpretation of *in situ* feeding assays with the widespread keystone species *G. fossarum* as an indicator of water quality. We proceeded in four steps: (i) we quantified the influence of important