

# Behavioural ecotoxicology, an “early warning” signal to assess environmental quality

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## Abstract

**Background** In this review, the position of behavioural ecotoxicology within the available means to assess the status of marine environments is described as filling the gap for the needed “early warning” signals. A few examples of studies performed since the 1960s are discussed to highlight the sensitivity of these approaches in investigating the effects of chemicals, including priority pollutants and emerging contaminants, relative to conventional toxicity tests measuring survival.

**Discussion** The advantage of the behavioural response is due to the integration of biochemical and physiological processes that reflect changes at higher levels of organisation with ecological relevance. Avoidance often represents a behavioural symptom easily detected in many animals exposed to contaminants and would be a useful test to explore more widely. This rapid response would reflect a defence mechanism protective against further exposure and the potential development of more pronounced deleterious effects, whilst in some cases, escape could lead to the relocation of a species with negative consequences. An investigation of the avoidance behaviour of mud shrimp, *Corophium volutator*, along with the chemical analyses of

sediments and amphipods to assess the quality of harbour sediments is summarised. The body burden of the amphipods was 1,000 times lower than the one associated with narcosis, emphasizing the sensitivity of this endpoint. The application of this acute toxicity test is briefly compared to additional work that involved intertidal mussels collected in the field.

**Conclusions** Recent research undertaken with mud snails, *Ilyanassa obsoleta*, and harbour sediments confirmed the usefulness of the escape behaviour as an assessment tool. However, the limits of the state of knowledge regarding the fate of contaminants in species with the ability to metabolise contaminants is further discussed along with directions to be pursued to address questions arising from the reviewed literature.

**Keywords** Behaviour · Review · Marine · Toxicity

## 1 Introduction to environmental assessments

Efforts have been ongoing in many countries to develop integrated ecosystem-based assessments using physical–chemical properties, biological abundance and diversity, and/or chemical characterisation to define the ecological quality of aquatic environments. The most recent review by Borja et al. (2008) was produced after the international meeting, EcoSummit 2007—Ecological Complexity and Sustainability, with representation from Africa, Asia, Australia, Europe and North America. This publication discusses the regulations produced in some countries to protect and/or restore marine ecosystems, and the need for “early warning” tools is highlighted. Behavioural endpoints are the subject of the present review and help address this need.

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As pointed out by the above authors, in the USA, the National Oceanic and Atmospheric Administration's (NOAA) National Status and Trends programme responds to the requirements of the Clean Water Act with the Mussel Watch Project, Bioeffects Assessments and the National Estuarine Eutrophication Assessment/Assessment of Estuarine Trophic Status. The ecological condition and impact of humans on estuaries is investigated by the Environmental Protection Agency (EPA) and NOAA. There is also a National Coastal Assessment Program and regional results on Coastal Habitat, Benthic, Fish Tissue Contaminants, Water Quality and Sediment Indices.

In the European Union, several directives were adopted over time to protect estuaries and coasts (Borja et al. 2008). For example, the Water Framework Directive has chemical and ecological objectives. The environmental quality standards rely on the concentrations of contaminants as quality objectives for comparing the state of sites. In some cases, contaminants' concentrations in sediment are considered in conjunction with biomonitoring (Borja et al. 2004, 2006; Crane 2003). The ecological integrity is judged using water or sediment in toxicity tests. In other cases, contaminants' concentrations are used to assess the ecological status of a location (Rodríguez et al. 2006; Bricker et al. 2007; Tueros et al. 2009). Detailed descriptions of analytical approaches, targeted xenobiotics (i.e. polycyclic aromatic hydrocarbons (PAH), polychlorinated biphenyls (PCB), mercury (Hg), cadmium (Cd), lead (Pb) and polybrominated diphenylethers PBDE) and background concentrations expected to be reached by 2020 can be found on web-based Oslo and Paris Convention for the Protection of the Marine Environment of the North East Atlantic (OSPAR) documents published in the past few years (e.g. Roose and Brinkman 2005; Allan et al. 2006; ICES 2008; OSPAR 2002, 2008). Borja and Dauer (2008) describe the four corners of environmental quality assessment as: "(1) assessing ecological integrity, (2) evaluating if significant ecological degradation has occurred, (3) identifying the spatial extent and location of ecological degradation and (4) determining causes of unacceptable degradation in order to guide management actions." The identification of cause represents a major issue that has been addressed in toxicity identification evaluation (TIE) or in effects evaluation analysis studies. Recent publications by Perron et al. (2010), Hecker and Hollert (2009), Schaeffer et al. (2009), Brack et al. (2008) and Gomez-Gutierrez et al. (2007) provide a wealth of references on this aspect of risk assessment and outline the critical role played by chemical analyses. The importance of chemical analyses is also apparent in the work described in a large section of the present manuscript (Section 7).

## 2 Ecotoxicology

Toxicology studies aim to determine whether harmful substances are affecting the health of organisms and to assess the state of an ecosystem. Ideally, these assessment tools should promote the sustainability of ecosystems and pinpoint early symptoms of exposure in order to stop the progression of environmental degradation whilst conditions are still reversible. This protection can be accomplished if causes associated with effects are both quantifiable and can be used to generate preventive guidelines. The guidelines should be protective of as many species as possible and should be flexible to ensure the generation of new data that would provide maximum protection (OSPAR 2002).

The steps needed to investigate and validate an approach used in toxicity assessment have been outlined by Atema et al. (1982). The authors identified a sequence of research efforts when describing their studies regarding the behaviour of lobsters exposed to oil. The four aspects of the required knowledge were outlined as: (1) an ecological background on the studied animals, (2) investigations of effective test methodologies, (3) defining the detailed experimental conditions that will lead to obtaining the best response data and (4) field measurements to confirm the laboratory results. The choice of species also requires consideration, though in that study lobster was selected because of its economic importance at the contaminated site (Section 6.2).

## 3 Historical perspective on toxicity tests

When toxicity tests are viewed within a legal context as needed to implement regulations, they are also accepted based on the ease and expense of performing them, the acquisition of irrefutable proof of harm and financial implications of the lost or threatened resource. Perhaps for those reasons, environmental risk assessment initially focused on a simple and straightforward endpoint, lethality or survival ( $LC_{50}$  representing the lethal concentration to 50% of a population) and alternatively narcosis. This measurement represents a baseline for toxicity, as discussed in Ankley et al. (2010). Narcosis induced by non-ionic lipophilic organic contaminants correlates with a body burden of 2–8  $\mu\text{mol/g}$ , with some variability due to the lipid content of the studied species (McCarty and Mackay 1993). Over time, additional population level effects, growth and reproduction have become accepted in toxicity tests and adopted in the regulatory process. Lethality, growth and reproduction are meant to reflect the outcome of chronic field exposure at the population level of complexity.

#### 4 Behavioural toxicology

Behaviour is slowly gaining more recognition due to its 10–1,000 times higher sensitivity than the conventional LC<sub>50</sub> (Hellou et al. 2008; Robinson 2009). Behaviour is an organism-level effect defined as the action, reaction or functioning of a system under a set of specific circumstances. It results from the integration of conditions to which organisms are exposed and represents an acute cumulative effect. Behavioural endpoints can consist of a variety of activities (Table 1; Clotfelter et al. 2004) that could be potentially ranked according to the time leading to the response or the relativity of the “early warning”.

- E1: rapid response that would be expected as immediately protective
- E2: a sign of an impact that is less immediate than E1 and can progress further
- E3: behaviour after longer exposure with worse expected consequences

Table 1 outlines a variety of available behavioural endpoints and provides a ranking of the behavioural response observed in snails, *Ilyanassa obsoleta* (Section 7.4). Ranking can change relative to the species under consideration. For example, in the case of fiddler crabs exposed to tributyltin (Weis and Perlmutter 1987), escape was not displayed and therefore burrowing would represent E1 and can lead to changes in mating (E2) and therefore on the reproductive success (E3, Section 6.1). The faster effect, E1, such as avoidance or escape can be viewed as a sign displayed readily by organisms as a defence mechanism that reflects a first signal before succumbing to less rapid E2 symptoms and to potentially an E3 response after longer

duration with more detrimental effects (Table 1). This ranking or time relationship between different behavioural symptoms is further described in Section 6. Many studies propose what the laboratory observations indicate about long-term effects in the field. The importance of relating acute toxic effects to those detected in chronic exposures is well recognised and emphasised in a recent publication (Solomon et al. 2009). Interrelating short-term and long-term effects is a goal that should be widely supported. Kuntz et al. (2010) propose the development of a comprehensive multilevel toxicity testing approach using the amphipods *Gammarus* spp. and discuss behavioural studies in that context.

If toxicity research is based on ecological studies and chemical data, then the measured changes obtained from laboratory exposures could be applied more readily to interpret field studies. The latter approaches have adopted the concept of sediment quality (Long and Chapman 1985) in which the abundance and diversity of a benthic community, priority pollutants in sediments and lab-based toxicity tests are measured to assess the quality of contaminated sites. Results of triad studies can be challenging to interpret, and numerous recommendations have been made to improve this approach as could be needed (Chapman and Hollert 2006). For example, difficulties can be related to the choice of an appropriate reference site mimicking the habitat of the contaminated one, to making a link between the presence and bioavailability of contaminants to associate a cause to toxic effects, or to questioning the ecological relevance of the commonly used survival tests. Investigating the body burden of an impacted organism could offer an additional tier to add or consider relative to the original triad studies (Section 7).

**Table 1** Potential behavioural responses elicited by the exposure of a species to contaminants

Response	Example of ranking <sup>a</sup>
Avoidance/escape	E1
Balance, righting ability	E2
Burrowing	
Fear response	
Feeding	
Locomotion	E3
Mating, courtship response	
Memory learning	
Nesting, offspring protection	
Respiration	E3
Risk taking	

<sup>a</sup> Ranking illustrates the response of *I. obsoleta* to harbour sediments (Section 7), where E1 precedes E2 which in turn precedes E3

#### 5 Reviews on behavioural toxicology

Early studies involving the response of fish to the presence of metals, surfactants, pesticides as well as other chemicals such as pH or chlorine were reviewed by Hara (1973, 1982, 1994, 2006). The discussion was directed to chemoreception with an emphasis on the senses of smell and taste. A phenomenal amount of studies on the behaviour of fish and the physiological aspects of the response of olfactory and gustatory cells using natural and anthropogenic chemicals were used in this groups’ work.

The behavioural toxicity of metals was reviewed by Atchinson et al. (1987), placing levels inducing avoidance side by side with those associated with conventional toxicity tests. Although that review is more than two decades old, it was already pointed out that avoidance was not predicted by, and more sensitive than survival, growth

or reproduction endpoints but that further lab tests could help predict observations in the field. For example, Sprague (1964) and Sprague et al. (1965) demonstrated that levels associated with escape were lower in the lab than in the field (2.3 vs 17–21  $\mu\text{g/L}$ ) when testing the response of salmon to copper (Cu), but the small difference was understandable in view of the wider context of the environmental variables.

A book co-authored by many specialists covered numerous topics within behavioural ecotoxicology (Dell’Omo 2002), including aquatic and terrestrial species, a variety of chemicals, modes of action, as well as case studies. The importance of using behaviour in site-specific environmental assessments as applied under various US regulations, such as the Clean Water Act, Natural Resource Damage Assessments and Oil Pollution Act was the topic of a chapter by Little (2002) who suggested a role for behavioural ecotoxicology in determining the restoration of sites with a focus on resident species. Little (2002) encouraged the development of telemetry for field assessments and the production of well-defined methodologies such as those outlined by the American Society of Testing and Materials. These additional tools would help in the adoption of behavioural toxicology in investigations.

Pinpointing the mechanism of action of a toxic response would help in understanding, diagnosing, quantifying and perhaps predicting effects. In their review of behaviour relative to exposure to endocrine disruptors (EDR), including the effect of legacy chemicals such as the well-known DDT and PCB, Zala (2004) described the link between the central nervous system, symptoms of behavioural modifications and endocrine disruption. The mechanism of action for different chemicals and animals including birds, mice, monkeys and rats was discussed when known, such as being associated with an oestrogen receptor, androgen receptor or altered hormone metabolism. Defining the mechanism of action to understand toxicity has been recommended by many work groups, including one from an EPA workshop on EDR (Kavlock et al. 1997), and it is now well recognised that the sensitivity of behavioural responses varies with species and age along with the intensity, frequency, duration and timing of the exposure. The literature is in fairly good agreement that behavioural ecotoxicology is developing sensitive tools to investigate toxic effects and that behaviour results from the integration of complex biochemical and physiological processes. As well, that these studies can be especially useful when encompassing a range of toxic endpoints, since results are not always predictable when trying to apply knowledge gained with one species to another one or between chemicals.

## 6 Studies of behavioural endpoints in the literature

### 6.1 Exposure to metals

Copper can be produced from mining, antifouling paints, as a fungicide, wood preservative and in vehicle brake pads. In crabs, exposure to Cu can cause a series of deleterious outcomes starting with escape (Hebel et al. 1997) and progresses to a reduction in feeding and possibly sex hormones, then changes in cardiac function and respiration followed by cellular damage leading to death. When examined together, this succession of toxic endpoints would demonstrate the importance of escape behaviour as an “early warning” signal and the link to population-level effects.

In fish, escape was most sensitive and associated with 0.1–6.3  $\mu\text{g/L}$  of Cu that varied with pH (7.3–8.4; Atchinson et al. 1987). Ventilation and coughing were detected at higher exposure levels (9–48  $\mu\text{g/L}$ ) of Cu. In chronic exposures, the lowest observed effects concentration associated with Cu and different fish species was in the same range of concentrations as the latter symptoms (17–40  $\mu\text{g/L}$ ). As would be expected in shorter 96-h exposures, higher doses of Cu were detrimental (75–1,000  $\mu\text{g/L}$ ).

A study with field and lab components was performed on two species of trout in relation to a Superfund site associated with an acid mine system with Cu, Cd, Pb and Zn (Hansen et al. 1999). These four metals were present at a consistent ratio in the river, and exposures were performed to examine the behaviour of fish since the abundance of four species, including rainbow trout, had decreased between 1979 and 1983 from 1,200 to 25 trout per kilometre downstream from the mine. Brown trout were still found in the river and less rainbow trout further away. Rainbow trout avoided the four metals mixture at 10–1,000% of concentrations detected in the river, with complete avoidance at the 50% level. In comparison, brown trout were less affected at the same tested concentrations and displayed a U-shaped response, indicating less susceptibility to avoiding exposure. This study demonstrated that rainbow trout were more sensitive to the presence of these metals than brown trout and that the escape response provided a powerful explanation for the species distribution in the river. The influence of the range of pH associated with precipitation and of fish acclimation times were considered in the experiments and did not affect the interpretation of results. As well, the authors pointed out that damage to the chemoreception neurons was not observed and could not explain the avoidance.

In contrast to Cu, mercury at 0.2  $\mu\text{g/L}$  induced attraction in fish, whereas avoidance of iron was initiated at 4,250  $\mu\text{g/L}$  (Atchinson et al. 1987). This portrays the complexity of a

field situation in which it would be impossible to make a conclusion regarding cause, if only behaviour is examined as an endpoint. Measures of chemicals in the field and as residues in species of concern would be decisive in interpreting effects, with a laboratory component needed to validate the observed response (Hecker and Hollert 2009). The latter aspect of body burden would provide support for bioavailability (Section 7).

Tributyltin is an organometallic compound commonly used in the past as an antifouling in paints. It is persistent and recognised for its role in the masculinisation of snails. Weis and Perlmutter (1987) investigated its effects on the escape, burrowing and righting ability of crabs exposed for 1, 2 and 3 weeks to sand spiked at three concentrations. Escape from contamination was not observed for any treatment, but burrowing behaviour increased in males and decreased in females. Females righting ability also increased relative to control in all exposures and at each period in time. Males did not display this behavioural change, but their limb regeneration was reported to be affected, with more deformities reported in a different study (Weis et al. 1987). Fiddler crab burrowing was also impacted by exposure to other contaminants, and this behaviour is important in hiding from predators, for mating and moulting (Weis and Perlmutter 1987).

## 6.2 Toxic effects due to oil

Studies were performed in the 1970s on the sublethal effects of no. 2 fuel oil. They were undertaken because of a spill that took place in Narragansett Bay, Massachusetts, where lobster fisheries have been of paramount importance. The work investigated changes in behaviour and chemoreception in animals exposed to the water-soluble fraction of the oil. The introduction section of the EPA project summary (Atema et al. 1982) outlined the behavioural endpoints that were shown to be caused by oil exposure. They included changes in feeding, growth, the search for a mate, loss of equilibrium and coordination, reproduction and reaction towards predators, each obviously leading to population decline.

In a study by Lee et al. (1981), nesting behaviour and lipid content of a marine amphipod, *Amphitoe valida*, were tested during a 6-day exposure and over variable depuration times. Nesting ability was examined every day relative to exposure dose and decreased over time and with higher exposure to water-soluble oil (5–25%). Nesting was still affected after 7 days of depuration. Lipid content of the animals did not vary at the end of 6 days of exposure, except for the higher dose, but decreases were apparent after 4 and 5 weeks of recovery. During the uptake period, survival was similar between control and exposed amphipods. However, survival decreased steadily with exposure

level during the additional day of recovery. The above demonstrated the importance of investigating effects during and post-exposure, along with the sensitivity of behaviour relative to the more common survival endpoint.

## 6.3 Emerging contaminants

Concern with emerging contaminants (EC) such as pharmaceuticals and personal care products arose from field and then lab studies that associated endocrine disruption (ED) with the presence of a female hormone, 17 $\beta$ -estradiol and the active ingredient in the birth control pill, 17 $\alpha$ -ethynylestradiol in UK estuaries (Ternes and Siegriest 2004). A number of toxicology studies on ED demonstrated the difference between conventionally expected dose–response monotonic curves and the inverted U-shaped curves associated with reproductive effects. Very few behavioural studies with aquatic species have used pharmaceuticals or other EC, a class of chemicals with diverse structures that have been analysed most often in influents and effluents of sewage treatment plants, with detected levels of most of these compounds commonly in the nanogram per litre range, but with some exceptions up to 1  $\mu$ g/L (Fent et al. 2006; Kidd et al. 2007).

Endpoints of feeding, ventilation and locomotion were studied using three pharmaceuticals, an anti-epileptic carbamazepine, a non-steroidal anti-inflammatory, ibuprofen and an antidepressant acting as a serotonin reuptake inhibitor, fluoxetine (de Lange et al. 2009) tested at 0.1–10<sup>6</sup> ng/L. Changes in feeding were detected in freshwater amphipods at low exposure, whilst increased ventilation was apparent in some cases at 1–100 ng/L and increased locomotion at doses reaching up to 10<sup>6</sup> ng/L (De Lange et al. 2005, 2009). The progression of endpoints was not similar for the three chemicals.

Painter et al. (2009) studied predator avoidance behaviour of larval fish exposed to four antidepressants including fluoxetine. These compounds target brain neurotransmitters, and a close relationship exists between mammals and teleosts. There was reduced avoidance displayed by larval fish exposed to the mixture of four compounds regardless of a variety of combinations of exposure levels. A lack of escape would limit survival and future reproductive success, leading to diminished fish populations.

Another study involving the active ingredient of the birth control pill spiked at an environmentally relevant concentration of 15 ng/L examined the nesting behaviour of three-spined stickleback (Bell 2001). Exposed males were reported to display reduced aggressiveness towards conspecifics with time. This behaviour contrasted with control that increased aggressiveness with time, and the latter was previously associated with reproductive success and the fitness of males. The level of male and female hormones in

plasma of fish was also measured, and a discussion of the effect of the hormone-mimicking chemical on the endogenous natural products was proposed as leading to changes in behaviour.

The above examples on EC are representative of the available data. The environmentally realistic level of exposure tested with the most potent ED indicated toxic effects in a small fish used in biomonitoring (Bell 2001). The sensitivity of early stages of development was indicated when testing antidepressants (Painter et al. 2009). More studies need to be pursued using EC at the concentrations expected in the field and to determine if E1 type symptoms would be protective of species important to a site receiving sewage effluents or a remediated site. TIE type approaches would be valuable in providing a better understanding of the role of EC and EDR present in complex environmental mixtures, as might be needed for better specific remediation.

## 7 Research integrating body residue and behaviour

The importance of choosing experimental species relevant to the food web and that can be used in field investigations is an important consideration in designing studies. The mud shrimp, *Corophium volutator*, and mud snail, *I. obsoleta*, play an important role in the Bay of Fundy, Nova Scotia, Canada, an area within the Gulf of Maine with relatively pristine intertidal beaches away from urbanisation. These amphipods have been the subject of ecological and toxicological investigations, whilst the snails are abundant at the same locations, therefore worthy of intercomparison for application in studies of the abundance of a benthic community. These two invertebrates are preyed on by sand pipers, grey whales, tomcod, winter flounder, snow crab and other fish. These two species were used in our behavioural research interrelating toxicity and chemistry (Escher and Hermens 2002; Barron et al. 2002; Solomon et al. 2009).

### 7.1 Exposure of amphipods to harbour sediments

A publication by Kravitz et al. (1999) provided the background for the approach adopted testing harbour sediments. When the amphipods *Eohaustorius estuaries* were offered two choices of sediments, the one containing PAH was avoided over a 2- or 3-day exposure period. A study by Long et al. (2001) examining the link between acute tests measuring LC<sub>50</sub> and the composition of marine benthic communities reported no decrease in the abundance of polychaetes and molluscs to concord with a decrease in survival of amphipods. However, the abundance of arthropods, crustaceans and amphipods were in agreement with

the toxicity tests. Halifax harbour sediments were devoid of amphipods, with the odd worm detected in grabs, but mussels were abundant and few snails were seen at the water line. This raised our interest in the bioavailability and toxicity of sediment-bound contaminants to amphipods.

The first investigations with mud shrimp pursued lethality; however, no reduction in survival was observed over 10-day exposures. This was also reported by Kravitz et al. (1999). Amphipods always survived (>80%) if fed 2 days before being placed on contaminated harbour sediments for 10 days (Hellou et al. 2005). It was also discovered fortuitously that if the feeding of the animals was omitted the Friday prior to the Monday exposure, the animals would perish. In that case, the transparent animals had dark particles visible in their stomach, and the fingerprint of the amphipods' extract was identical to that of the sediments, confirming that consumption of particles took place. Effects similar to the ones being used at the time with field mussels collected in the harbour (Hellou et al. 2002, 2003, 2006, Yeats et al. 2008), i.e. lipid content, growth and reproduction, were observed in amphipods. Amphipods were exposed to sediments collected in the vicinity of the sites where mussels were exposed to soluble and particle-bound contaminants in the water column.

Changes in behaviour are somewhat logical to accept from a human perspective when faced with sewage. Behavioural effects appeared promising to pursue with amphipods, relatively easy to explain and understand. Field sediments were mixed in various proportions with those collected along with the amphipods from a non-urbanised beach. Animals were examined for their preference between two choices, the pristine or harbour-derived mixed sediment. Avoidance was detected above a narrow range of PAH in sediments (Hellou et al. 2008). In the case of five out of seven sediments collected near numerous raw sewage discharges, amphipods avoided sediment containing PAH at concentrations labelled as "probable effects levels" (>50% probability of toxicity) by the Canadian Council of Ministers of the Environment (Hellou et al. 2002, 2005) sediment quality guidelines. Two samples further away from sewage effluents did not display the concentration-escape link. The same trends in ranking the quality of harbour sites were obtained with lab amphipod and field-collected mussel.

Since our expertise was to analyse the fates of PAH, i.e. bioaccumulation and biotransformation, the bioavailability of the priority pollutants to amphipods was pursued. The ability of this species to transform reactive chemicals was assessed by exposing them to several single PAH and the presence of transformation products examined in tissue extracts; however, only bioaccumulation was detected. The lowest per cent of harbour sediment mixed with the amphipods' native sediments and generating an escape response were then used in exposures and the animals

analysed. The sum of detected PAH in tissue extracts were of 0.3–1.1 nmol/g or about 1,000 times lower than those associated with  $LC_{50}$  (Hellou et al. 2008, 2009b). This difference in body burden explained why animals did not perish when exposed to harbour sediments and demonstrated that avoidance is 1,000 times more sensitive than survival. It can easily be called an E1 signal and would possibly prevent chronic exposure and more signs of toxicity.

In a follow-up study, sediments were spiked with a mixture containing the seven abundant PAH detected in harbour sediments. An avoidance response was apparent when each PAH was >100 ng/g. Amphipods exposed to the avoided PAH-spiked sediments were also analysed. Their body burden was two to seven times lower than observed with the avoided harbour sediments. This difference would likely mean that there are more chemicals available from the sediments that are associated with the escape (Hellou et al. 2008, 2009b). Numerous other non-ionic hydrophobic or lipophilic EC are discharged in sewage effluents, such as phthalate esters, non-ylphenol ethoxylates, musks, polybrominated diphenyl ethers, other fluorinated and chlorinated chemicals, as well as EDR. Questions regarding the identity and level of additional xenobiotics would need attention if behaviour is to be used in risk assessment of harbour sediments and the connection to body residue validated.

## 7.2 Exposure of amphipod to pesticides

In studies performed by our group to investigate the toxicity of the pesticides atrazine, azinphos methyl, carbofuran and endosulfan, variably weak behavioural responses were detected in amphipods (Hellou et al. 2009c). In the case of endosulfan, >20% of the animals died before a preference for uncontaminated sediments was apparent. Using the fungicide chlorothalonil, a U-shaped response demonstrated an attraction for contaminated sediments, especially when amphipods were started on spiked sediments at mid concentrations (2.5, 12.5 and 125 ng/g) relative to the wider range of 0.01- to 10,000-ng/g exposure (Hellou et al. 2009a). The reported properties of these biocides had revealed a short half-life (days). This research reinforced the importance of analysing the media when examining toxicity, as well as the differences in the response of a species exposed to different types of contaminants.

## 7.3 Comparing the response of amphipods and snails exposed to freshwater

According to experiments performed by changing the salinity of seawater, amphipods are less tolerant than snails

(Hellou et al. 2009a). The survival of amphipods determined that 1–2‰ corresponded to  $LC_{50}$  in 1-day exposures. In contrast, snails survived readily; however, there was an increase in retracted snails. Retraction reached 60% when salinity was 6‰ over a 2-day exposure. The tolerance of snails is interpreted to be due to their ability to retract within the shell, a defence mechanism that is absent in amphipods. These gastropods would therefore survive more than crustaceans with a freshwater input due to rain or perhaps snow melt or river overflow.

## 7.4 Exposure of snails to harbour sediments

The behavioural response of mud snails relative to harbour sediments was also studied (Marklevitz et al. 2008a, b). Experiments were modified with time going from using two sediments overlaid with seawater to only one sediment overlaid with variable amounts of seawater or to using only seawater (Hellou et al. 2009a; Erskine et al. 2010). Unlike the mud shrimp which spend all of their time in sediments, the snails reside on diverse substrates in contact with one or more media: air, water or sediments. They offered the opportunity to perform more types of exposures. The behaviour of snails reported in the literature had more visually informative endpoints, required less manipulations and observations could be noted frequently in time. Three stages of stress response starting with escape could be observed with snails, progressing to animals being flipped over on their shell with soft tissue protruding, then retracted within their shell. Moreover, unlike the amphipods that could only be used in the summer and early fall, and became dormant and fragile when manipulated during the colder months, the snails could be handled in fall and winter.

Snails offered two choices of sediments avoided harbour sediments, and the response was statistically more pronounced between the 48- and 72-h period (Marklevitz et al. 2008a, b). The snails also moved away from sediments containing solvent extracts obtained from harbour sediments or containing a mixture of the seven abundant PAH tested with amphipods. Three natural products present in harbour sediments were also tested for their effect on behaviour. A general lack of preference from a dose–response perspective was witnessed when offering a fatty acid methyl ester, cholesterol and coprostanol between 0.0007 and 1.2 mg/g. Cholesterol was only avoided at 0.2 µg/g. Coprostanol, a sewage marker deriving from the degradation of cholesterol in the digestive system of mammals, was avoided at 0.05 mg/g. C-18 fatty acid methyl ester was only avoided at the highest concentration. These chemicals are present at varying levels in harbour sediments along with many other non-polar anthropogenic chemicals and would be detected in the tested solvent extracts.

### 7.5 Exposure of snails to PAH

In order to pursue the fate of PAH in snails and a potential link to behaviour, it was important to determine if biotransformation took place along with bioaccumulation. It was discovered that three species of gastropods, a large commercial one, *Neptunea lyrata*, a large but poisonous species, *Buccinum undatum*, plus the small intertidal snails, *I. obsoleta*, produced up to eight metabolites (Beach et al. 2009, 2010; Beach and Hellou 2010; Erskine et al. 2010; Hellou et al. 2010). The most commonly used combustion-derived PAH, pyrene (PY), ubiquitous in environmental compartments and abundant in harbour sediments, was used in these investigations along with its more toxic oxidation product produced by sunlight as well as by microorganisms, 1-hydroxypyrene, PYOH. Two modes of exposure were attempted over a range of concentrations and behaviour examined alongside. In a series of experiment, animals were provided with one portion of PY-spiked fish, whilst in another, snails were placed on PY-spiked sediments containing the innate detritus material, bacteria and algae, representing the food that would be available in the field.

Exposures were performed in the summer and fall. In all cases, the animals were analysed 3 days after beginning the exposures, and bioaccumulation plus transformation occurred according to a specific balance in proportions of PY and derivatives (Erskine et al. 2010; Hellou et al. 2010). A statistically significant linear regression was drawn between the amount of PY and the sum of the two major transformation products detected in extracts of fed snails. More transformation was detected at low exposure levels and more bioaccumulation at higher doses. This trend of changing proportions demonstrates a limited capacity within the animals to handle anthropogenic chemicals and was observed with larger snails (Beach and Hellou 2010). Biotransformation represents a biochemical defence mechanism aiming to eliminate chemicals. It is used by animals to produce more water-soluble and more easily eliminated derivatives of the initial lipophilic compounds. Stressed animals with retracted soft tissue were detected when the balance of proportions of products was not observed. Stressed snails compared to healthy animals displayed different relative amounts of transformed and initial PY. Under conditions with a fast feeding uptake lasting minutes, the production of five times more transformation products was associated with stressed snails. When uptake was over a longer period of time as in the sediment exposure, the presence of twice the level of transformation products in tissue extract was apparent in stressed relative to healthy animals. These experiments associated a visually simple-to-track toxic effect with the complex fate of a PAH. They raise

questions about the toxic mode of action of other aromatic molecules present in harbour sediments and in other animals that biotransform molecules. Stress was only seen in animals that were exposed in the fall and not in the summer. The biotransformation capacity of the snails was also higher in the fall relative to summer.

The interpretation of the mode of action of the behavioural toxicity detected in snails is lacking the detail that would be provided by using more sensitive instrumentation where the eight produced derivatives, rather than just three abundant ones, could be quantified, to potentially link structure with toxicity, in order to compare and possibly predict toxicity between species and make an informed risk assessment at the ecosystem level.

### 7.6 Lessons learned

The studies presented in this section relate to working on relevant mixtures of contaminants present in harbours where the analysis of sediments for priority pollutants indicated a high probability of toxicity. The escape behaviour of amphipods reflected E1 “early warning” of toxicity in acute exposures. This was only associated with a subset of sediments, five out of seven, that ranked above sediment quality guidelines according to the analysis of PAH priority pollutants. The amphipods’ body burden of abundant PAH proved bioavailability and perhaps a mode of action. Exposure to PAH-spiked sediments indicated that they represent a fraction of the cause of escape in the five samples and perhaps a larger per cent in other matrices. Unidentified bioavailable chemicals carry the balance of toxicity and justify investment in multidisciplinary research. Experiments with mud snails amplified the complexity of pursuing the mode of action in behavioural ecotoxicology and the need to invest resources in questions that affect ecosystem health. The challenges are numerous and definitely worthwhile.

## 8 Conclusion

It seems obvious from the above examples that behaviour can change relative to an acute exposure to contaminants and that it is more sensitive than survival. Information combining results of toxicity tests and the body burden of animals could offer the ability to identify the chemicals that should be reduced in amount to improve the state of a site. Age and reproductive stage can affect behavioural results; when energy is placed in reproduction, less energy can be dedicated to behaviour. Seasonal variations are especially pronounced with cold-water marine organisms and hinder the repetitive use of the above species at any time of the year. Comparison between model freshwater and marine



organisms is needed. Behaviour can be specific to a species and endpoints other than escape help to interpret effects. Behavioural endpoints are good candidates as “early warning” signals and are needed with chemical support for risk assessment.

Behavioural tests are relatively fast, simple to perform, noninvasive, cheap and, as described in many studies, with a high ecological relevance. These characteristics are essential when designing toxicity tests and the development of integrated behavioural tools with chemical and toxicological aspects should be supported and expanded for the management of sustainable ecosystems.

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