Gammarus spp. in Aquatic Ecotoxicology and Water Quality Assessment: Toward Integrated Multilevel Tests

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1 Introduction

More than 4500 species belong to the crustacean sub-order Gammaridea (order Amphipoda) (Bousfield 1973). Among Amphipods, the Gammaridea are the most widespread group and are found throughout a range of marine, freshwater, and terrestrial habitats (Bousfield 1973; Lincoln 1979), whereas the three other amphipod sub-orders (Hyperiidea, Ingolfiellidea, and Caprellidea) are highly specialized and ecologically restricted. *Gammarus* is the amphipod genus with the highest number of epigean freshwater species, comprising over 100 species that are distributed throughout the Northern Hemisphere (Karaman and Pinkster 1977). Abiotic factors such as temperature, salinity, oxygen, acidity, and pollution play an important role in the distribution of *Gammarus* species (Whitehurst and Lindsey 1990) and members of this species are often found in great abundance under rocks, in gravel, or in coarse substrates and among living and dead vegetation (Fitter and Manuel 1994). These substrata provide both shelter from predators and a supply of organic detritus and other foodstuffs, with the result that in many riverine communities, amphipod species such as *Gammarus pulex* (Linnaeus) may represent the dominant macroinvertebrate in terms of biomass (Macneil et al. 1997; Shaw 1979).

The species *G. fossarum* and *G. pulex,* for example, are widespread and functionally important in streams throughout much of Europe and Northern Asia (Karaman and Pinkster 1977). They display a wide trophic repertoire, feeding as herbivores, detritivores, and predators. Stream-conditioned leaves, biofilms that grow on them, dead chironomids, live juvenile isopods, and even juvenile and wounded/trapped fish are part of their diet (Fielding et al. 2003; Macneil et al. 1997). Intraguild predation and cannibalism have been observed in many amphipod species, and data from *G. pulex* suggest that this is more common than previously realized (Macneil et al. 1997). This "foraging plasticity" is linked to the success of *Gammarus* spp. as they persist in colonizing and invading disturbance-prone ecosystems (Macneil et al. 1997). Hence, the ecological value of a macroinvertebrate like *G. pulex* in streams may exceed its important role as a shredder in processing leaf material that falls into streams (Welton et al. 1983; Willoughby and Earnshaw 1982; Willoughby and Sutcliffe 1976), and as prey items for many fish species (Maitland 1966; Smyly 1957; Welton 1979).

Gammarus species have a complex life cycle, which is of value in ecotoxicological studies because changes in mating behavior can be observed more easily in the presence of xenoestrogen exposure (Segner et al. 2003; Watts et al. 2001, 2002, 2003). *Gammarus* females are available for mating only during a brief period

directly after the molt. Alternatively, males are available for mating during most of the molt cycle (Sutcliffe 1993), which results in a male-biased operational sex ratio. To address this situation, males engage in precopulatory mate guarding when encountering a female nearing the molt stage (Ridley 1983). Prior to mating, males grab and hold different females before deciding which one is likely to produce the most eggs. Pairing in *Gammarus* is positively size assortive and, unlike other arthropods, males are larger than females (Dick and Elwood 1996; Thomas et al. 1995; Zielinski 1998). When the male has found a suitable female, they form a precopula pair. The male holds the female under and parallel to his body using the first pair of gnathopods (Borowski 1984), and while carrying her, he performs all the necessary swimming movements (Bollache and Cezilly 2004). Pairs can remain in precopula for up to 2 weeks (Hartnoll and Smith 1980). As soon as the female sheds her skin, the male can mate with her. Then, the precopula pair separates and the female carries the developing eggs in her brooding pouch. The young hatch after 1–3 weeks; thereafter, the juveniles remain in the brooding pouch until the next female molt. Then, after 4–6 weeks, the young *Gammarus* swim out of the brooding pouch. Freshly hatched juveniles feed by coprophagy (feces of adults) (McCahon and Pascoe 1988b). The diet thereafter expands to include conditioned leaves, and after 1 month or so, the young feed only on conditioned leaves. The juveniles will, themselves, mate 3–4 months later, after they have reached sexual maturity and completed about 10 molts (McCahon and Pascoe 1988a). Gammarids can reach ages of 1–2 years.

The presence of gammarids in freshwater streams is crucial, because macroinvertebrate feeding is a major rate-limiting step in the processing of stream detritus (Cummins and Klug 1979). Detritus is an integrated decomposition of leaf material in streams and is brought about by a combination of chemical leaching, microbial decomposition (primarily by aquatic hyphomycetes), macroinvertebrate feeding, and physical abrasion (Webster and Benfield 1986). Environmental contaminants can reduce detritus processing by decreasing the microbial conditioning and/or the abundance or the feeding activity of detritivores such as *G. pulex* (Forrow and Maltby 2000; Webster and Benfield 1986). Reductions in gammarid abundance from increased mortality have, for example, been observed after exposing them to acutely toxic landfill leachates that contain environmental contaminants (Bloor et al. 2005). Toxicant-induced reductions in feeding rate can result in reduced growth, size, fecundity, and survival of individuals (Anderson and Cummins 1979; Maltby and Naylor 1990), thereby affecting the stream community structure (Sutcliffe and Hildrew 1989).

Feeding activity is one of many behavioral responses that may be affected by environmental contaminants. For example, changes in locomotory or ventilation behavior are compensatory, reversible adaptive responses to pollutants that may mitigate potential overt effects (e.g., direct behavioral response after perception of stress). Irreversible effects of a toxicant on a behavioral mechanism or expression are also observed in the behavioral response of an organism, after the toxicokinetic and toxicodynamic processes have started [e.g., acetylcholinesterase (AChE) inhibition exerted by neurotoxins; Gerhardt 1995].

Using behavioral parameters in ecotoxicology studies have advantages, to wit short response times (i.e., early warning responses), sensitivity (i.e., for neuromuscular toxins), non-invasiveness, ecological relevance, and the possibility for time-dependent data analysis (Fossi 1998; Gerhardt 2007; Scherer 1992). Changes in behavior may be used as important indicators for ecosystem health, because they rest on biochemical processes, but also reflect the fitness of the individual organism as well as potential effects on the population level, such as altered abundance of the species in the ecosystem. Behavioral responses seem to be of similar sensitivity and efficiency as biochemical and physiological responses and because of their indestructibility, continuous long-term monitoring is possible (Gerhardt 2007; Scherer 1992). To study changes in behavior that result from contaminant exposure is therefore an essential part of behavioral science, which can be called behavioral ecotoxicology (Gerhardt 2007).

In aquatic ecotoxicology, behavioral endpoints have been applied for fish, crayfish, copepods, and gammarids for about 20 years (Atchison et al. 1987; Beitinger 1990). In the case of gammarids, behavioral alteration tests allow one to measure several endpoints related to population structure, population density, and to inter- and intra-specific interactions. Such endpoints may be sensitive indicators of chemical stressors when used for biomonitoring purposes, for instance, biomonitoring with impedance conversion (Gerhardt 1995; Gerhardt et al. 1998), which can be used to quantitatively record behaviors such as ventilation, grazing, filter feeding, net spinning, and locomotion. It is known that *Gammarus* species can locate their food (De Lange et al. 2005) and detect predators through chemical cues from fish and injured conspecifics. Changes in behavior such as hiding in response to predators (Åbjörnsson et al. 2004; Baumgärtner et al. 2002; Gerhardt and Quindt 2000; Williams and Moore 1985; Wisenden et al. 1999; Wisenden et al. 2001; Wudkevich et al. 1997) or avoidance of chemical stress from exposure to pollution pulses (Gerhardt 1995, 1996; Gerhardt et al. 1998; Gerhardt and Quindt 2000) are crucial to optimize the chance of survival. Reproductive behaviors can also be examined; an example is the ability of males and females to detect each other, form precopulatory guarding pairs during the premating period, and display guarding behavior (Watts et al. 2001).

In addition to the vast published literature that addresses the effect of pollutants on *Gammarid* mortality, feeding, and behavior, many publications also address the effects of environmental pollutants on population structure, the endocrine system, stress response, the neural system, and bioenergetics, etc. Of those, only a few address the endocrine disruption in *Gammarids*; key endpoints investigated in these endocrine disruption studies include structure, size, length–frequency distributions, adult sex ratio, number of precopula pairs/ovigerous females, and secondary sex characteristics (Watts et al. 2002). Recently, different biomarkers, e.g., vitellogenin (Vtg) or heat shock proteins (hsp), have been used to investigate endocrine disruption in gammarids (De Coen and Janssen 2003; Gagné et al. 2005; Schirling et al. 2004). Moreover, biomarkers have increasingly been used to assess stress response,

oxidative stress, exoskeleton integrity, and neurotoxicity (Correia et al. 2002; Scheil et al. 2008; Xuereb et al. 2007).

The above-mentioned toxicity endpoints have been utilized to address effects at the individual and population level; however, there have also been investigations on the structure and composition of communities that make important contributions. In this context, neozoa may play an important role in disturbed or polluted aquatic ecosystems. For example, *Dikerogammarus villosus*, a Ponto-Caspian species, is known to be a particularly successful invader and is currently the prevailing invasive gammarid species in large bodies of water in Southern Germany (Kinzler et al. 2008). Field observations suggest that *D. villosus* has replaced the native *G. pulex* and the invasive *D. haemobaphes* in some spans of the German Danube. Such shifts in species composition may help explain the long-term effects of pollutants in those areas and the species sensitivity differences observed for these pollutants.

The widely investigated *gammarids*, in particular species such as *G. pulex* and *G. fossarum,* are important members of freshwater ecosystems. Because they are sensitive to pollutants and other disturbances, *gammarids* may be valuable indicators for ecosystem health in aquatic freshwater ecotoxicology, especially because they have been utilized in manifold exposure scenarios (e.g., in situ, ex situ, via sediment, and to pulsed exposures).

The purpose of this review is to collect available data, methods, and biomarkers cogent to *Gammarus* spp. and investigate the potential of gammarids to serve as an emerging test species for freshwater ecosystems. Gammarids may fill a crucial gap in the assessment of aquatic ecosystem health, which is not yet filled by OECD-proposed test species, because *Gammarus* spp. naturally occur in streams of the Northern Hemisphere. The breadth of ecotoxicological studies published on gammarids suggests that they may be suitable test organisms for a more integrative ecotoxicity testing in situ and ex situ, and consequently the data gained from their use may be more ecologically relevant. In this review, we aim to provide an overview on the status of ecotoxicological testing with gammarids and to suggest avenues for continuing, combining, and integrating future gammarid research in aquatic ecotoxicology.

2 Culturing of Gammarids

To propagate gammarids for broader use as test organisms in aquatic ecotoxicology, the ability to culture them is an essential prerequisite. Few publications exist on how to culture gammarids. Most studies that utilize gammarids address adverse effects of environmental contaminants on them and obtain test specimens by collecting wild animals from uncontaminated, clean sites. Only a limited number of studies have used lab-cultured animals (Bloor et al. 2005; McCahon and Pascoe 1988a), indicating that the culturing and reproduction of gammarids in the lab might

be rather difficult. To our knowledge, only three publications exist that describe in detail how to culture *G. pulex* (McCahon and Pascoe 1988a, 1988b; Bloor 2009). These publications explain that a minimum of 100 precopula pairs and 200 visibly gravid *G. pulex* females from an unpolluted source are needed for culturing purposes. For culturing, *G. pulex* are placed in l-L plastic containers, each with a nylon mesh base through which juveniles can pass after being released from the brood pouch. These containers are suspended in an 8-L rearing tank with a flow-through supply of dechlorinated, aerated tap water. The rearing tank may contain water held under static conditions, if the water is periodically renewed. A 12-hour photoperiod, with a light intensity of 750 lux at the water surface, is used. Adults are fed on conditioned, common leaves (Sutcliffe et al. 1981), which are collected during the autumn, are air-dried, and stored until shortly before use. To incorporate bacteria and fungi into the gammarid diet, leaf material is then conditioned for at least 10 days in organically enriched dechlorinated water to initiate microbial breakdown (Kaushik and Hynes 1971; Willoughby and Sutcliffe 1976). Adults may also be fed on algae (Moore 1975). After 5 days, the breeding container is removed and several newly hatched individuals (500–1000) will be present in the rearing tank. Conditioned leaves are added to the rearing tanks for cover and, eventually, as food. Early hatched juveniles feed upon adult feces, which require that feces be supplied (by pipetting), after removal of the breeding containers; thereafter, after approximately 25 days, the animals can feed entirely on leaves.

McCahon and Pascoe (1988b) monitored growth by removing 40 gammarids from each growing tank at regular intervals, measuring the total body length microscopically (anterior margin of the head to posterior margin of the telson) to the nearest 0.25 mm, and by counting the number of segments on the primary flagella of each antenna. Juveniles that are released from the brood pouch possess five segments on the primary flagellus of each antenna. The number segments increase as growth progresses and a growth curve can be plotted, which allows calculation of the age of the cultured specimens. Approximately 70% of cultured juveniles survive to reach sexual maturity in time periods that are dependent on rearing temperature as follows: 130 days at 13°C (14–16 antennae segments, after 10 molts; McCahon and Pascoe 1988b), 120 days at 15–20°C (Hynes 1955), and 133 days at 15°C (Welton and Clarke 1980). Sexes can then be distinguished by visible identification of genital papillae in males and by fully developed oostegites in females; such oostegites have long fringed bristles, which interlace with one another to form the brood pouch. Females can produce 2–5 broods with a mean of 16 eggs each. By increasing rearing temperature and providing excess food, it is possible to culture animals throughout the year and attain a reduced time to sexual maturity.

For toxicity studies, McCahon and Pascoe (1988b) suggest using a test population that comprises organisms of mixed stages and ages. Therefore, at periodic intervals, the breeding adults in each container are transferred to fresh rearing tanks, and the newly hatched juveniles are gently removed from females by prodding the brood pouch. The authors suggest using the following age groups (in

days) for toxicity testing: 4.5–9.5, 22.5–27.5, 44.5–49.5, 64.5–69.5, 79.5–84.5, and 217.5–222.5. McCahon and Pascoe (1988b) propose using 20 cultured *G. pulex* of each age class as well as 20 field-collected animals of unknown age in toxicity tests.

3 Gammarids in Lethality Testing

Gammarids (e.g., *G. pulex*) are widely used in experimental toxicity tests (McCahon and Pascoe 1988b) because of their well-known sensitivity to a wide range of pollutants and the fact that they are among the most sensitive aquatic invertebrates (Bloor et al. 2005; Cold and Forbes 2004; Mian and Mulla 1992; Van Wijngaarden et al. 2004; Wogram and Liess 2001).

3.1 Pesticides, Metals, and Surfactants

Multiple published studies exist on the acute toxicity of a wide range of chemicals and natural water samples toward gammarids. McCahon and Pascoe (1988b) exposed *G. pulex* to a range of cadmium chloride solutions (0, 10, 30, 50, 100, 300, and 1000 μg/L $CdCl_2 \bullet 2^1/_2H_2O$) and found that the median lethal time to mortality (LT_{50}) for all age classes decreased with increasing cadmium concentration. The slopes of the mortality time curves differed little among age groups or cadmium concentrations. The associated median lethal concentration (LC_{50}) values were very similar for most age classes, except for the oldest (220 days, 48 hour LC_{50} 4.7 mg Cd/L) and youngest (1 day, 48 hour LC_{50} 0.019 mg Cd/L) animals; juveniles were found to be almost 250 times more sensitive than those in the oldest age class. Pantani et al. (1997) investigated the acute toxicity of some common pesticides, metals, and surfactants to the amphipods *G. italicus* and *Echinogammarus tibaldii*. LC_{50} values of 16 insecticides varied from less than 1 μg/L for azinphos-methyl to several milligrams per liter for dimethoate. The sensitivity of *G. italicus* and *E. tibaldii* toward three herbicides and three surfactants was found to be about the same order of magnitude, and not very high (Pantani et al. 1997). Their toxicity findings for atrazine (LC_{50}: *G. italicus* 10.1 mg/L, *E. tibaldii* 3.3 mg/L) are very similar to previously observed acute toxicities in *G. pulex* and in *G. fasciatus* (Macek et al. 1976; Taylor et al. 1991). Metals showed differences in toxicity rankings between *G. italicus (*Zn<Cr<Cd<Hg<Cu) and *E. tibaldii* (Zn<Cd<Cu<Hg<Cr). Comparing these findings to other acute toxicity data for metals, mainly cadmium and copper, *G. fossarum* and *G. pulex* appear to be the most sensitive to cadmium (Musko et al. 1990; Williams and Moore 1985). In another study, several freshwater insects and crustacean species were exposed for 24 hour to the neonicotinoid insecticide thiacloprid. Among the investigated species, an increase in sensitivity, distributed over three orders of magnitude, was found: *Daphnia magna* < *Asellus aquaticus* = *G. pulex* < *Sympetrum striolatum* < *Culex pipiens* = *Notidobia*

ciliaris = *Simulium latigonium*, with median lethal concentrations (LC_{50}s) of 4, 400, 153, 190, 31.2, 6.78, 5.47, and 5.76 μg/L, respectively (post-exposure observation 11–30 days; Beketov and Liess 2008).

3.2 Extracted, Fractionated Sediments

Boxhall and Maltby (1995) assessed the toxicity of sediment contaminated with road runoff by exposing *G. pulex* for 14 days to different extracted fractions of the contaminated sediment. The sediment was extracted with dichloromethane and then fractioned into three portions with increasing polarity using alumina–silica column chromatography. The fractions were analyzed using GC–MS (gas chromatography–mass spectrometry), GC/MS/IR (infrared), and IR spectrophotometry. Results revealed that the first fraction contained aliphatic hydrocarbons, the second fraction 2–5-ring polycyclic aromatic hydrocarbons (PAHs), and the third fraction (FC) substituted phenols and 4- and 5-ring PAHs. The 2–5-ring PAH fraction was toxic to *G. pulex* (mortality > 80%), whereas the other two fractions did not produce signs of toxicity after 14 days of exposure (Boxall and Maltby 1995).

3.3 Coastal Sediment Toxicity

Costa et al. (1998) used the marine amphipod *G. locusta* to assess coastal sediment toxicity in a 10-day static toxicity (mortality) test performed with laboratory-produced juveniles at 15°C and at a salinity of 33–34. *G. locusta* is a common species along European coastal areas and can be raised easily under laboratory conditions. This species is tolerant to a broad range of sediment types and toxins, including heavily contaminated field sediments (Costa et al. 1998), copper (LC_{50} = 56.8 mg Cu/kg dry weight, 0.9% total volatile solids), and to the gamma isomer of hexachlorocyclohexane (lindane) in the sediment (LC_{50} = 560.5 μg lindane/kg dry weight, 2% total volatile solids). The overall assay performance was identical to the American Society for Testing and Materials (ASTM) standard for sediment toxicity tests observed for marine and estuarine amphipods (ASTM 1993), revealing the utility of *G. locusta* in such tests.

The above-mentioned acute toxicity studies, as well as those shown in Table 1, indicate that gammarids are among the most sensitive organisms toward metal pollution. They show increased toxicity toward the neonicotinoid insecticide thiacloprid and the 2–5-ring PAHs but are not affected by PAHs with 4–5 rings. In some cases, 1-day-old juveniles were as much as 250 times more sensitive toward acute toxic effects than were 220-day-old adults and therefore should be increasingly used for such assessments. Compared to daphnids, juvenile and adult gammarids were more sensitive to some pesticides (e.g., lindane, thiacloprid) and copper (Table 1).

Table 1 Median lethal concentrations (LC_{50}s) of test chemicals for freshwater invertebrates and fish. For comparability reasons regarding sensitivity of the different species, the LC_{50} of the most sensitive species is indicated (gammarids: bold, daphnids: underlined)

Toxicant	Organism	Life stage	Time (hour)	LC_{50} (mg/L)	Source
Cadmium chloride	*G. pulex*	Juveniles	48	**0.019**	McCahon and Pascoe (1988a)
	G. pulex	Adults	48	**4.700**	McCahon and Pascoe (1988a)
	G. italicus	Adults	96	**9.1**	Pantani et al. (1997)
	E. tibaldii	Adults	96	**1.1**	Pantani et al. (1997)
	G. pulex	7–9 mm long	96	0.0821	Felten et al. (2008)
	G. pulex	7–9 mm long	120	0.0371	Felten et al. (2008)
	G pulex	7–9 mm long	168	0.0216	Felten et al. (2008)
	G. pulex	7–9 mm long	264	0.0105	Felten et al. (2008)
3,4-Dichloroaniline	*D. magna*	Larva, 1 mm	96	0.16	Adema and Vink (1981)
	D. magna	Adult	96	1.0	Adema and Vink (1981)
	Salmo gairdneri	Juvenile	96	2.7	Crossland (1988)
	Pimephales promelas	28–34 days	96	7.0–8.1	Call et al. (1987)
	G. pulex	2–3 molts	240	**5.0**	Taylor et al. (1991)
	Chironomus riparius	2nd instar	96	7.4	Taylor et al. (1991)
	Poecilia reticulata	Young	96	8.7	Adema and Vink (1981)
	G. pulex	2–3 molt	48	**17.4**	Taylor et al. (1991)
	Dreissena polymorpha	Adult, 1 cm	48	22	Adema and Vink (1981)
Atrazine	*C. tentans*	1st instar	48	0.72	Macek et al. (1976)
	G. fasciatus	1st molt	48	**5.7**	Macek et al. (1976)
	Salvelinus fontinalis	–	96	6.3	Macek et al. (1976)
	D. magna	<24-hour old	48	6.9	Macek et al. (1976)
	G. pulex	2–3 molts	96	**14.9**	Taylor et al. (1991)
	P. promelas	–	96	15.0	Macek et al. (1976)
	C. riparius	2nd instar	96	>30	Taylor et al. (1991)
Zinc chloride	*G. italicus*	Adults	96	**8.8**	Pantani et al. (1997)
	E. tibaldii	Adults	96	**25.9**	Pantani et al. (1997)
Copper	*G. pseudolimnaeus*	Adult	96	**0.020**	Arthur and Leonard (1970)
	G. pulex	2–3 molts	96	**0.037**	Girling et al. (2000)
	D. magna	<24-hour old	72	0.09	Winner and Farrell (1976)
	S. gairdneri	–	72	0.4	Brown (1968)
	C. riparius	2nd instar	96	0.7	Taylor et al. (1991)
	C. decorus	4th instar	48	0.74	Kosalwat and Knight (1987)
	P. promelas	–	96	2.2	Brungs et al. (1976)

Table 1 (continued)

Toxicant	Organism	Life stage	Time (hour)	LC_{50} (mg/L)	Source
Lindane	*C. riparius*	2nd instar	96	0.034	Taylor et al. (1991)
	G. pulex	Adult	96	**0.034**	Abel (1980)
	G. fasciatus	3rd molt	48	**0.039**	Macek et al. (1976)
	S. fontinalis	–	48	0.044	Macek et al. (1976)
	B. rhodani	Larva	96	0.054	Green et al. (1986)
	G. pulex	2–3 molt	96	**0.079**	Taylor et al. (1991)
	P. promelas	–	48	>0.100	Macek et al. (1976)
	C. tentans	1st instar	48	0.207	Macek et al. (1976)
	G. pulex	Adult	96	**0.225**	Green et al. (1986)
	C. riparius	4th instar	96	0.235	Green et al. (1986)
	D. magna	<24-hour old	48	0.485	Macek et al. (1976)
Bisphenol A	*G. pulex*	Adult	240	1.49	Watts et al. (2001)
Ethinylestradiol	*G. pulex*	Adult	240	0.84	Watts et al. (2001)
Esfenvalerate	*G. pulex*	Adult	48	0.00014	Cold and Forbes (2004)
	G. pulex	Juvenile	48	0.00013	Cold and Forbes (2004)
	D. magna	Juvenile	48	0.00027	Fairchild et al. (1992)
Fenoxycarb	*G. fossarum*	Adult	96	10	Schmidt (2003)
	G. fossarum	Juveniles	96	4	Schmidt (2003)

4 Feeding Activity

One approach to assess water quality is to use feeding activity/inhibition of macroinvertebrates as a measure for a wide range of stressors. Such tests can be measured ex situ and in situ and have been proposed as good indicators for water quality (Crane and Maltby 1991). Table 2 provides an overview of different feeding activity test types employed with gammarids. Feeding rate is an advantageous endpoint because it is based on sublethal responses of a single species and is therefore more sensitive. Moreover, the use of this endpoint gives a more rapid response, than does community-based measures that require species eradication before an impact is detected (Maltby et al. 2002). However, feeding rate is influenced by intrinsic factors, i.e., parasite load (Pascoe et al. 1995), source population (Crane et al. 1995; Maltby and Crane 1994; Veerasingham and Crane 1992), and body size (Nilsson 1974), and extrinsic factors, i.e., temperature, dissolved oxygen content (Maltby et al. 1990), and pH (Naylor et al. 1989). Because feeding rate is variable, depending on the status of the organism and its surrounding environment, understanding those intrinsic factors and causes for variability is crucial for water quality assessment.

4.1 Time–Response Feeding Assays

Taylor et al. (1993) proposed a feeding assay designed to quantify the feeding activ ity of *G. pulex* by utilizing a time–response analysis of the feeding of amphipods on eggs of *Artemia salina.* In this assay, individual test organisms are transferred

Table 2 Test methods that utilize gammarids to assess toxicant effects on feeding activity

Species	Test substance/media	Type of exposure	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex males > 9 mm	Copper (Cu)	Feeding activity, aqueous static short-term exposures	3, 20, 48, and 96 hour	Median feeding time (MFT) on *A. salina eggs*	Significant increase in MFD: 3 hour: >101 μg/L Cu 20 hour: >72 μg/L Cu 48 hour: >21.5 μg/L Cu 96 hour: >8.3 μg/L Cu		Taylor et al. (1993)
G. pulex juveniles, 5 mm	Cu: Lindane (LD), 3,4-dichloroaniline (DCA)	Feeding activity, static short-term exposures	96, 240 hour	Feeding activity (FT_{50}) on *A. salina* eggs	Significant reduced FT_{50} after 96 hour: >12.1 μg/L Cu >8.4 μg/L LD >918 μg/L DCA	Significantly increased FT_{50} after 240 hour exposure to 0.09 μg/L LD – stimulatory effects at low concentrations	Blockwell et al. (1998)
G. fossarum	Antibiotic mixture: sulfamethoxazole, trimethoprim, erythromycin-H_2O, roxithromycin, clarithromycin	Static food choice experiment with conditioned leaf discs, +/– antibiotics (total conc. 2, 200 μg/L):	48 hour	Food choice	Food choice: 200 μg/L antibacterial conditioned leaves significantly preferred over control leafs, same tendency for 2 μg/L antibiotic leaves	Number of bacteria on antibiotic leaves did not differ from controls, but fungal biomass was significantly higher at 200 μg/L conditioned discs	Bundschuh et al. (2009)
G. fossarum adult males	Highly acidic stream vs. reference stream, with conditioned leaves	In situ: consummation of leaf mass	6 days	Consummation of leaf mass: dry weight, leaf-disk area	Feeding activity: 113 and 46 times lower on exposed and control leaves in acidic vs. control stream	Almost complete stop of the feeding in the acidic stream	Dangles and Guérold (2000)

Table 2 (continued)

Species	Test substance/media	Type of exposure	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex physically pre-disrupted precopula pairs	LD high: (0.5–2.0 mg/L); low: (0.5–5.0 μg/L)	Aqueous exposure, static with conditioned leaf discs	High: 2–20 min + 24 hour post-exposure; low: 48 hour	Feeding rate, precopula re-pairing	Significantly reduced feeding activity: low: 5.0 μg/L LD; high: 1 and 2 mg/L LD	Re-pairing affected by treatments combining higher concentrations and longer exposures	Malbouis-son et al. (1995)
E. toletanus	Unionized ammonia (NH_3-N): 0.06–0.33 mg/L NH_3-N	Static aqueous exposure with conditioned leaf discs	6 days	Egestion rate (mg dry weight feces/mg dry weight amphipod/day)	After 6 days at 0.3 mg/L NH_3-N significantly lower egestion rate. NOEC (no observable effect level): 0.18 mg/L NH_3-N	NOEC confirms the calculated safe concentration of 0.14 mg/L NH_3-N	Alonso and Camargo (2004)
G. pulex, A. aquaticus (2-week-old males)	Water from upstream and downstream of landfill leachate discharge site	Comparison of in situ and ex situ (static renewal) feeding test	6 days	Mortality, feeding rate	Mortalities and feeding rates had similar trend during in situ and ex situ exposure, but responses were amplified in situ.	In situ toxicity tests are a more precise monitoring technique	Bloor and Banks (2006)

Table 2 (continued)

Species	Test substance/media	Type of exposure	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex from two different streams, unpolluted and metal polluted	In situ: metalliferous effluents Laboratory: Iron (Fe): 1, 2, or 3 mg/L Fe Manganese (Mn): 0.1, 0.3, 0.5 mg/L Mn	In situ exposure and lab experiment to validate field data	6 days	Feeding rate, mortality	In situ: significant higher mortality in *G. pulex* from unpolluted stream. Significantly reduced feeding rate in unpolluted *G. pulex* at metal-polluted sites. *Laboratory:* significant reductions in feeding rate at >2.0 mg/L Fe for both populations. Significant mortality increase	Animals from metal-polluted sites might be less sensitive than those from unpolluted sites	Maltby and Crane (1994)
G. pulex adult males > 5 mm	Zinc (Zn), linear alkylbenzene sulfonate (LAS), LD, pirimiphos-methyl (PM), permethrin (P)	Aqueous static renewal	Feeding inhibition: 144 hour Biomarkers: 24 and 48 hour	Feeding inhibition; biomarkers: cholinesterase (ChE), glutathione-*S*-transferase (GST)	ChE: significant reduction; 24 hour: 0.77 μg/L PM 48 hour:1.92 μg/L PM GST: significant increases; 24 hour: 12.3 μg/L LD 48 hour: 6.14 μg/L LD; 0.12 μg/L P	Significant reduction in feeding rate (144 hour): PM: 0.049 μg/L; P: 0.009 μg/L; LD: 3.70 μg/L; Zn: 0.04 mg/L; LAS: 0.062 mg/L	McLoughlin et al. (2000)

Table 2 (continued)

Species	Test substance/media	Type of exposure	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex	4-Nonylphenol (NP) leaf disc: 100 μg/g NP, for aqueous exposure in net	Comparison of aqueous uptake and feeding uptake	48 hour	Feeding rate, uptake depuration	Higher NP body burden from dietary exposure, but uptake from aqueous exposure unexpectedly high: predominant uptake root	No difference in depuration rates after aqueous and dietary exposure	Gross-Sorokin et al. (2003)
G. pulex	Cadmium (Cd) (2.1 and 6 μg/L)	Static aqueous exposure	24-hour exposure + 24-hour post-exposure	Post-exposure feeding depression	Significant decrease in post-exposure feeding rate at 6 μg/L Cd		Brown and Pascoe (1989)
G. pulex parasite-free and parasitized	Cadmium (Cd) (2.1 and 6 μg/L)	Static aqueous exposure	24-hour exposure + 24-hour post-exposure	Post-exposure feeding depression Mortality	Parasitized *G. pulex* significant higher mortality to 2.1 μg/L Cd than uninfected	Parasitized consumed only 17–21% of the food eaten by uninfected *G. pulex* when held in dilution water	Brown and Pascoe (1989)

Table 2 (continued)

Species	Test substance/media	Type of exposure	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex, A. aquaticus	LD, DCA	Species inter actions in toxicant systems	96, 240 hour	Feeding activity (FT_{50}) on *A. salina* eggs	LD: significantly reduced FT_{50} of *G. pulex* coexposed with *A. aquaticus*: 96 hour: >3.8 μg/L 240 hour: > 6.5 μg/L DCA 90 μg/L: 100 and 60% survival for *A. aquaticus* and *G. pulex*, respectively, *G. pulex* no longer dominant species	Exposure to low LD concentrations (0.1 and 0.9 μg/L): significant increase in *gammarid* feeding activity	Blockwell et al. (1998)
G. pulex males 7–9 mm	Cadmium chloride (CdCl) (7.5 and 15 μg/L)	Physiological and behavioral responses, static renewal	168 hour	Feeding rate, hemolymph Ca^{2+} conc., osmolality Na^+/Cl^- conc., Cd accumulation Na^+/K^+-ATPase, locomotion, Ventilation	Significant decrease in osmolality, hemolymph Ca^{2+} conc., mortality, feeding rate, locomotor and ventilatory activities but not hemolymph Na^+/Cl^-	Significant increase in Na^+/K^+-ATPase activity	Felten et al. (2008)

to beakers containing 18 mL of the relevant toxicant or control solution, together with 10 shell-less eggs of *A. salina.* The number of eggs eaten in each beaker is recorded frequently, which allows median feeding times (FT_{50}, the time at which 50% of the eggs have been consumed) to be determined. This nondestructive method provides a rapid indication of the status of groups of individuals (Taylor et al. 1993). Blockwell et al. (1998) used this feeding assay to investigate effects on juvenile *G. pulex* exposed to the freshwater pollutants copper, lindane, and 3,4-dichloroaniline (3,4-DCA). Gammarid feeding was reduced following 96-hour exposure at 12.1 μg/L copper or 8.4 μg/L lindane, and following 240-hour exposure at 918 μg/L of 3,4-DCA. A sustained reduction in feeding rates may cause growth inhibition and impaired reproduction. This has been previously identified as sublethal responses of other freshwater organisms exposed to comparable concentrations of lindane, 3,4-DCA, or copper (Taylor et al. 1991). Interestingly, the feeding rate was higher for *G. pulex* specimens that had been exposed for 240 hour to 0.09 μg/L lindane, when compared to controls; possibly this was caused by stimulatory effects associated with lindane at low exposure concentrations (Blockwell et al. 1998).

4.2 Food Choice Experiments

Feeding experiments may be enhanced by including food choice as an endpoint. Bundschuh et al. (2009) used such an experimental setup to assess whether an antibiotic mixture, consisting of sulfamethoxazole, trimethoprim, erythromycin-H_2O, roxithromycin, and clarithromycin, had an effect on aquatic communities comprising bacterial and fungal decomposers and invertebrate detritivores. Two types of leaf discs were offered to an amphipod shredder, *G. fossarum*, after those discs had been conditioned with and without antibiotics (total concentration of 2 or 200 μg/L) for approximately 20 days. Interestingly, *G. fossarum* preferred the antibiotic-conditioned leaf discs at 200 μg/L over the control discs (pair-wise *t* test; $p = 0.006$). A similar but not significant tendency was observed for leaves conditioned with antibiotics at 2 μg/L. The number of bacteria associated with leaves did not differ between treatments at either antibiotic concentration (*t* test; $p = 0.57$), but the fungal biomass (measured as ergosterol) was significantly higher in the 200 μg/L treatment (*t* test; $p = 0.038$), suggesting that the preference of *Gammarus* may be related to a shift in fungal communities. This may indicate that mixtures of antibiotics may disrupt important ecosystem processes such as organic matter flow in stream ecosystems (Bundschuh et al. 2009).

The importance of fungi in the diet of *G. pulex* and *A. aquaticus* was also stressed by another study (Graça et al. 1993). Herein, in feeding trials with fungal mycelia, either fungally colonized or fungally uncolonized leaves were used to assess feeding preferences. The authors found that *A. aquaticus* scrapes at the leaf surface and selectively consumes fungal mycelia, whereas *G. pulex* nibbles leaf material, apparently only for the quality of the leaf tissue, not for any microorganisms present. This result was reinforced experimentally: *G. pulex* ignored pure

fungal mycelia and preferentially consumed conditioned and, to a lesser extent, unconditioned leaf discs. Food choice of *A. aquaticus* was positively correlated with fungal biomass, whereas for *G. pulex,* fungi appear to be more important as modifiers of leaf material (Graça et al. 1993). Notwithstanding, both gammarids exhibited strong preferences for *Anguillospora longissima* and *Heliscus lugdunensis* (Graça et al. 1994). The relative abundance of the fungi was not important. However, the authors observed intra-population variability in food preferences, both between individuals and for the same individual through time. When looking at different stream categories, microbial breakdown rates of oak and alder leaves did not differ, although aquatic hyphomycete-species richness on leaf litter positively correlated with riparian plant-species richness (Lecerf et al. 2005). Fungal-species richness may enhance the breakdown rate of leaf litter through positive effects on resource quality for shredders; a feeding experiment confirmed this positive relationship between fungal-species richness per se and leaf litter consumption rate by the amphipod shredder *G. fossarum*. Hence, plant-species richness may indirectly govern ecosystem functioning through complex trophic interactions (Lecerf et al. 2005). Integrating microbial diversity and trophic dynamics in in situ exposures of gammarids may improve the understanding and assessment of aquatic pollution.

4.3 Leaf-Mass Feeding Assays Linked to Food Consumption

Dangles and Guérold (2000) conducted a *G. fossarum* feeding assay with emphasis on consumption of leaf mass, a parameter that could be used to assess potential changes in ecosystem processes. This approach allows one to measure if, for example, acidification or another external impact on a stream leads to alteration in leaf breakdown, which may affect accumulation of leaf litter. Such an effect can be assessed by comparing leaf dry weight and area between undisturbed/unexposed and disturbed/exposed sampling areas. Dangles and Guérold (2000) used beech leaves and conditioned them for 136 days, either in a highly acidic stream (mean pH 4.68; mean alkalinity –19 μEq/L; mean total Al 801 μg/L) or in a reference stream (mean pH 7.36; mean alkalinity 539 μEq/L; mean total Al 36 μg/L). The two diets were tested in situ with adult males in feeding trial units that were then placed in tanks anchored to stream bottoms. After 6 days, the organisms were removed, gammarids and leaf disks were dried, weighed, and the consumed leaf mass determined (Dangles and Guérold 2000). No mortalities were observed, but *G. fossarum* almost completely ceased feeding activity in the acidic stream, and after 6 days, feeding activities on both exposed and control leaves were 113 and 46 times lower than in the reference stream. This experimental setup nicely shows that the water quality of the stream has an impact on the feeding activity of gammarids. Under acidic-stress conditions, gammarids are apparently unable to increase or even maintain their energy uptake from available food resources. In addition, the quality of the leaves had an impact on the feeding activity of gammarids (Dangles and Guérold 2000).

4.3.1 Feeding Activity and Survival Related to Toxicity or Abiotic Parameters (Ex Situ)

The effects of toxicants on gammarid feeding activity and survival were examined in several studies (Alonso and Camargo 2004; Bloor and Banks 2006; Maltby and Crane 1994), and in one study McLoughlin et al. (2000) investigated biochemical biomarkers in addition to feeding activity and survival.

Alonso and Camargo (2004) investigated the toxicity of unionized ammonia (NH_3) to determine if the presence of this more toxic form of ammonia has a negative effect on the feeding activity of amphipods. Instead of leaf-disk-measurements, the authors measured egestion rate (milligram dry weight of feces/milligram dry weight of amphipod/day) as an endpoint for feeding activity. Feeding activity was investigated during a 6-day exposure of the amphipod *Eulimnogammarus toletanus* to 0.06–0.33 mg/L NH_3-N, by measuring the egestion rate on days 2 and 6 of exposure. After 2 days of exposure, the egestion rate was slightly higher in exposed compared to control animals, but the difference was not significant. After 6 days of exposure, however, the egestion rate of *E. toletanus* exposed to 0.3 mg/L NH_3-N was significantly lower than for the control. The NOEC (no observable effect concentration), based on the second assay, was 0.18 mg/L NH_3-N, confirming the calculated safe concentration of 0.14 mg/L NH_3-N (Alonso and Camargo 2004).

Bloor and Banks (2006) recently evaluated mixed species feeding assays with the pollution-sensitive *G. pulex,* and the pollution-tolerant *A. aquaticus*, to determine if test animals' response was comparable during in situ vs. ex situ toxicity tests. Seven sampling points were chosen along a stream, which received leachate discharge from an unlined, unused UK landfill site. Points A and B were upstream of the contamination, C was adjacent to the influx, and D–G were downstream of the leachate discharge. For the in situ and ex situ tests, 2-week-old male laboratory-bred *A. aquaticus* and *G. pulex* were used as test animals and were either (1) transplanted to the seven sampling points for the duration of the in situ tests or (2) exposed in the laboratory (in situ) to water samples taken from each sampling site. The authors found that mortalities and feeding rates followed similar trends during the in situ and ex situ tests, but that the response of test animals was amplified during in situ testing. The authors also observed that the effects were greater in April than in August, possibly because of a higher rainfall frequency during the spring, which may have resulted in greater portions of flushed contaminant load from the landfill site and thus higher levels of pollution leaching into the stream. The conclusion, therefore, was that in situ toxicity tests are a more precise monitoring technique, when compared to ex situ assays (Bloor and Banks 2006).

A similar study was performed a few years earlier to evaluate whether an in situ bioassay was useful for evaluating the impact of complex effluents on freshwaters and to identify toxic components (Maltby and Crane 1994). In this study, *G. pulex* was exposed to metalliferous effluents that reduced the feeding rate, which was a sensitive indicator. Chemical analysis showed that the effluents contained a mixture of five potentially toxic metals. Based on feeding rate and bioaccumulation data, two metals (iron and manganese) were identified as the probable toxic agents.

Laboratory experiments were performed to validate the results of the field conclusions. Results confirmed that iron was a major toxicant. During both the field and laboratory studies, the sensitivities of *G. pulex* from a metal-contaminated and a clean site were compared. Interpopulation response differences to the toxicants for *G. pulex* were found in the field study, but not in the laboratory experiments; this indicates that animals from metal-polluted sites may be less sensitive than those from unpolluted sites.

4.3.2 Feeding Rate, Uptake, and Depuration

In general, the major exposure and uptake route for soluble toxins by aquatic organisms is regarded to be through the water column. For hydrophobic chemicals, however, exposure and uptake through the diet is often of greater importance, because the chemicals adsorb onto organic sediments and food. To date, the relative importance of water and food in the uptake and accumulation of a toxin by a benthic detritivore has not been assessed. Gross-Sorokin et al. (2003) investigated the importance of feeding rate on uptake and depuration of the hydrophobic endocrine disruptor 4-nonylphenol (NP) by *G. pulex.* These authors compared this result with NP uptake from leaves into the water. To this end, dried horse chestnut leaf disks were soaked in stream water and then dosed with NP for 24 hour to achieve a nominal, environmentally relevant leaf concentration of 100 μg/g NP. NP concentrations were measured in the gammarids, water, and leaf disks at 8, 12, 24, and 48 hour, respectively. In addition, the amount of leaf material ingested relative to the total weight of the gammarids was measured. After 48 hour of exposure, gammarids were transferred to clean water to determine the degree of depuration. By using a bootstrap nonlinear regression technique, the authors showed that a higher body burden of NP resulted from dietary exposure, partly because of the higher source concentration in leaf disks. If leaf-disk concentration is taken into account, uptake from water is unexpectedly high and confirms the assumption that it is the predominant uptake route and derives from the large volumes transferred across gills. Depuration rates following aqueous and dietary exposures were rapid and did not differ significantly among different treatments (Gross-Sorokin et al. 2003).

4.4 Modeling of Feeding Activity and Rate

4.4.1 Field Estimates of Feeding Rates – Modeling, Ingestion, and Egestion Rates

Attempts were made to understand and estimate field-feeding rates by using exponential regression models (Marchant and Hynes 1981). The feeding rate of *G. pseudolimnaeus* was measured monthly for 7 months in the field by monitoring the weight decline of gut contents, when the amphipod was starved. This weight decline was then modeled by an exponential regression of weight on time. With the assumption that amphipods are continuous feeders, feeding rate was calculated by

multiplying the dry weight of a full gut by the specific rate of gut emptying, i.e., the slope of the exponential regression. The authors found that the specific rate of emptying was independent of animal size but increased with temperature, resulting in longer digestion times at lower temperatures. This may lead to an increase in assimilation efficiency at lower temperatures. In the laboratory, however, the assimilation efficiency of amphipods that fed on decaying maple leaves was only 10% and did not vary with temperature. The turnover time of the contents of a full gut, however, was often similar to the turnover time measured in the field, i.e., the reciprocal of the specific rate of emptying, thus confirming the use of an exponential regression model (Marchant and Hynes 1981).

4.4.2 Carnivorous Feeding Activity

Werner et al. (2002) used a similar approach to estimate the potential predation impact of the under-ice arctic amphipod *G. wilkitzkii* by combining information on ingestion rates with population densities. The carnivorous feeding activity and the energy budget were studied by estimating a maximum potential ingestion rate (I_{max}). This I_{max} value was 2.1±0.4% of body carbon/day and was calculated from an allometric literature function and from body mass. In addition, respiration measurements were integrated to assess the lower specific ingestion rates (1.4±0.4%) of body carbon/day, required to meet metabolic demands. Feeding experiments, with co-occurring pelagic calanoid (*Calanus* spp.) or sympagic harpacticoid (*Halectinosoma* sp.) copepods as prey, were conducted. From these prey species, actual ingestion rates of 8.0±5.6% of body carbon/day and 0.1±0.1% of body carbon/day, respectively, were determined. The values indicate that predatory feeding on pelagic copepods may constitute an important food source for *G. wilkitzkii*. Estimates on the potential predation impact of *G. wilkitzkii* were made by combining information on ingestion rates with population densities. Predation impact was very high on *Calanus* spp. in the under-ice water layer (61.5% of under-ice standing stock/day), but comparatively low on *Halectinosoma* sp. in the bottom of the ice (3.8% of standing stock/day) (Werner et al. 2002). The observation that *G. wilkitzkii* prey on pelagic copepods is obviously significant and potential predation impact should be taken into account when studying freshwater amphipods.

4.5 Post-exposure Feeding Depression Assay

Recently, a bioassay that uses post-exposure feeding depression (PFD) in *D. magna* as an endpoint has been developed to assess toxic effluent in rivers (McWilliam and Baird 2002b). Previous studies have suggested that *D. magna,* exposed to toxic substances, may exhibit delayed feeding behavior recovery, suggesting that PFD is a sensitive and robust endpoint (McWilliam and Baird 2002b) that could be included in toxicity assessments with *G. pulex*.

McWilliam and Braid (2002a) investigated whether this endpoint could be used in the field as an in situ bioassay with *D. magna;* in this study, *D. magna* were

exposed in test chambers to four known or suspected contaminated and reference sites. The authors found that the bioassay was reliable for use in the field, because more than 90% post-exposure survival of test organisms was observed and allowed post-exposure feeding rates to be measured. At each contaminated site, significant depression in post-exposure feeding rates was recorded (McWilliam and Baird 2002a). Feeding depression was also observed in a similar study conducted with *G. pulex*, wherein 6 μg/L of cadmium produced a decreased feeding rate after a 24-hour exposure (Brown and Pascoe 1989). Although depression in post-exposure feeding rates was apparent at all but one contaminated site in the McWilliam and Braid study, no impact was detected on the benthic macroinvertebrate community when using the Biological Monitoring Working Party scoring system (McWilliam and Baird 2002b). This indicates that post-exposure feeding depression was a more reliable and sensitive endpoint for detecting toxicity than were changes in community structure and therefore offers a sensitive, robust, and ecologically relevant diagnostic endpoint for use in water quality assessment schemes.

4.6 Effects of Parasites on Gammarid Feeding Ecology

The amphipod *G. pulex* is an intermediate host to the acanthocephalan fish parasite *Echinorhynchus truttae* (Fielding et al. 2003). It is not clear if feeding activity (herbivore, detritivore, or predatory) of *G. pulex* is affected when it is parasitized by *E. truttae.* Fielding et al. (2003) investigated the effects of *E. truttae* parasitism on the following components of the *G. pulex* diet: stream-conditioned leaves, dead chironomids, and the live juvenile isopod *A. aquaticus*. After 21 days of exposure, parasitism had no effect on daily feeding rates or wet weights of *G. pulex* that fed on leaves or chironomids. However, parasitism significantly affected the number of *A. aquaticus* killed by *G. pulex*, with parasitized individuals killing significantly fewer and smaller *A. aquaticus* than did their unparasitized counterparts.

The above findings on *G. pulex* raise the question if parasite-related behavioral changes also negatively affect the response of *G. pulex* toward toxicant exposure (Brown and Pascoe 1989). To test this question a total of 600 *G. pulex* were collected from the River Teme and 52% of the collected population were infected with *Pomphorhynchus laevis*; 20% contained larvae, and 80% contained cystacanths, the stage that is infective to fish, the definitive host. After 80 days of exposure to dilution water, 14% of uninfected and 94% of infected *G. pulex* were dead. When infected *G. pulex* were exposed to a nominal cadmium concentration of 2.1 μg/L, they were significantly more sensitive (LT_{50} = 17.5 days) to the toxicant than were uninfected conspecifics (LT_{50} = 42 days), while at 6 μg/L, differences in mortality rates between infected and uninfected individuals were insignificant (Brown and Pascoe 1989). In general, the authors found that infected *G. pulex* consumed only 17–21% of the food eaten by uninfected *G. pulex,* when held in dilution water. A further disadvantage for *G. pulex* was that *P. laevis* was insensitive to cadmium (Brown and Pascoe 1989).

5 Behavior

Behavioral assays are used to detect several individual and inter-individual behaviors, i.e., avoidance or preference reactions of macroinvertebrates to toxic pollutants. Table 3 gives an overview of different behavioral tests conducted with gammarids. These tests, or their endpoints, originate from antipredator behaviors such as drift, reduction of activity, habitat choice, and diel pattern of movement.

5.1 Antipredator Behavior

5.1.1 Drift Behavior Linked to Predators

It is known that drift of *Gammarus* individuals was reduced by chemical clues remaining from the presence of predatory fish (Andersson et al. 1986; Friberg et al. 1994; McIntosh et al. 1999). Predation on amphipods in leaf litter was found to be significantly lower than in other microhabitats, showing the importance of habitat choice as an antipredator behavior (Holomuzki and Hoyle 1990). To respond to the fluctuating risk of the presence of predator, prey need information on predation risk, and the quality of this information is reflected in prey behavioral time lags. For example, prey benefit from remaining in a refuge long after the predator has left. Experimental investigation showed that prey behavior time lags were longer when predator density was higher and prey were less hungry, and had lower escape ability (Sih 1992).

Wisenden et al. (1999) examined whether *G. minus* exhibit antipredator behavior in response to injury-released chemicals from conspecifics or heterospecifics (Crustacea and Isopoda). Relative to the control group, *G. minus* exposed to conspecific cues showed decreased activity and moved to the substratum after detecting the cue. Alternatively, *G. minus* moved up into the water column and increased activity after being exposed to the heterospecific cue. In addition, the time to first attack increased when *G. minus* was exposed to a predator (green sunfish) if *G. minus* was also exposed to conspecific cues; in contrast, the time to first attack decreased when *G. minus* were exposed to heterospecific cues. Thus, *G. minus* seems to benefit from its antipredator response to conspecific cues (Wisenden et al. 1999).

5.1.2 Drift Behavior Resulting from Pollution

Antipredator behavior may be induced not only by a predator but also by chemical threads. Previous studies on pulsed exposures to pyrethroid insecticides showed that many stream macroinvertebrates respond to such exposures by demonstrating catastrophic drift (Breneman and Pontasch 1994; Sibley et al. 1991). Lauridsen and Friberg (2005) investigated the behavioral changes of stream macroinvertebrates exposed to a pulse of the pyrethroid insecticide lambda-cyhalothrin in outdoor experimental channels. The number of macroinvertebrates drifting, as well as the

Table 3 Test methods with *gammarids* to assess toxicant effects on behavior

Species	Test substance/ media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex, B. rhodani, L. fusca	Pyrethroid insecticide lambda-cyhalothrin (LC): 0.001, 0.01, 0.1, 1.0 µg/L	Pulsed exposure in outdoor experimental channels	2 hour + 60 min pulse + 24 hour	Drift response	*G. pulex*: > 0.001 µg/L LC, significant catastrophic drift. *B. rhodani*, *L. fusca*: > 0.01 µg/L LC, significant catastrophic drift	Drift behavior of insect species (*B. rhodani*, *L. fusca*) is less sensitive than that of *G. pulex*	Lauridsen and Friberg (2005)
G. pulex	Unpolluted stream, copper (Cu)-polluted stream (peak concentrations: 69, 58, 30 µg/L Cu-tot)	In situ: one reference + one local population. One simulated pulsed Cu exposure: 70 µg/L Cu^{2+}	5–6 days: 24-second recordings every 30 min	Locomotion and ventilation activity with the MFB®	Reference population was significantly less active than local population. Cu-pollution pulses increased number of active organisms and time spent in locomotion	No effects on natural drift behavior	Gerhardt et al. (1998)

Table 3 (continued)

Species	Test substance/ media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex (8–10 mm)	*Pharmaceuticals*: fluoxetine: 0.1–100,000 ng/L; ibuprofen: 1–100,000 ng/L; carbamazepine: 1–100,000 ng/L *Surfactant*: cetyltrimethylammonium bromide (CTAB): 0.0001–100 mg/L	Short-term exposure with static renewal	1.5 hour: 240-second recordings every 10 min	Activity pattern: locomotion and resting activity with the MFB®	10–100 ng/L fluoxetine and ibuprofen: significant activity reduction. 10–100 ng/L CTAB: acute immobilization	Observed behavioral effect concentrations for all three chemicals were 104–107 times lower than previously reported LOECs (lowest observable effect concentrations), in the range of environmentally concentrations	De Lange et al. (2006a)
G. pulex (5–8 mm)	Rivers: Aller (clean water) Meuse (simulated pulses of metals and organic xenobiotics) Rhine (frequent natural pulsed pollutions)	Aller: in situ biomonitoring; Meuse: water flow-through, ex situ, +/– simulated pulsed exposures; Rhine: in situ	4-min recordings every 10 min Aller: 14 days; Meuse: 5 days; Rhine: 1.5 months	Locomotory activity with the MFB®	Aller: no effect; Meuse: 20% decrease in activity (1st pulse) and increased mortality (2nd, 3rd pulse); Rhine: 20% activity decrease during biomonitoring	*G. pulex* in the MFB is a suitable alert system for water quality monitoring at sensitive sites and sites with accidental pollution	Gerhardt et al. (2007)

Table 3 (continued)

Species	Test substance/ media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
E. meridionalis; H. pellucidula; C. picteti	Acid mine drainage (AMD)	Simulated short-term acid pulses	40 hour, with a 1-hour pulse of AMD after approx. 12 hour	Locomotory and ventilatory activity with the MFB ®	*E. meridionalis*: increased locomotion, then increased ventilation. *C. picteti*: increased locomotion *H. pellucidula*: no effect	Sensitivity: *E. meridionalis* > *C. picteti* > *H. pellucidula*	Macedo-Sousa et al. (2008)
G. duebeni (15–21 mm)	Cu Pentachlorophenol (PCP), benzo[a]pyrene (B[a]P)	48 hour static renewal	7 days	Pleopod beat frequency, swimming endurance	Significantly impaired swimming endurance at 45 µg/L Cu, 20 µg/L PCP, 8 µg/L B[a]P. Pleopod beat frequency: 50 µg/L Cu	Swimming endurance is the more sensitive and consistent endpoint	Lawrence and Poulter (1998)
C. marinus (15–21 mm)	Cu PCP B[a]P	Flow-through	180 hour	Swimming endurance	Significantly impaired swimming endurance at 15 µg/L Cu, 40 µg/L PCP, 20 µg/L B[a]P		Lawrence and Poulter (2001)

mobility of macroinvertebrates caught in the drift, was assessed. In small replicated subsections of the experimental channels, two insect species (*Baetis rhodani, Leuctra fusca/L. digitata*) and an amphipod (*G. pulex*) were allowed to acclimatize for 26 hour before 60-min pulsed exposures to lambda-cyhalothrin (0.001, 0.01, 0.1, and 1.0 μg/L). Measurement started 2 hour before the insecticide was applied and continued for the following 24 hour. All three species responded to the insecticide pulse by demonstrating catastrophic drift, starting at 0.001 μg/L for *Gammarus*, whereas the drift response threshold was 0.01 μg/L for the two insect species (Lauridsen and Friberg 2005). The higher the pulse concentration, the more the individuals of the three species exhibited drifting behavior. Drift response onset was directly linked to the applied pulse concentration, with the highest concentrations resulting in more individuals of all species entering drift at an early stage. In addition, both insect species were in the process of being immobilized at the two highest exposure concentration; in *Gammarus,* this was only the case for a few individuals. The clear species-specific responses indicate that sublethal doses have the potential to change macroinvertebrate community structure.

5.1.3 The Effect of Parasitism on Antipredator Behavior

Baldauf et al. (2007) examined whether a parasite infection (*P. laevis*) influenced an amphipod's reaction to a fish predator's odor. A series of choice experiments, with infected and uninfected *G. pulex*, were performed to distinguish between the effects of visual and olfactory predator cues on parasite-induced changes in host behavior. The authors reported that uninfected individuals significantly avoided predator odors, while infected individuals significantly preferred the side with predator odors (Baldauf et al. 2007). However, when only visual contact with a predator was allowed, infected and uninfected gammarids behaved similarly and had no significant preference, indicating that the parasite *P. laevis* increases its chance of reaching a final host by olfactory-triggered manipulation of the antipredator behavior of its intermediate host.

A similar study was conducted on parasite-induced behavior and color changes in *G. pulex* and its relevance to the risk of predation by fish (Bakker et al. 1997). In infected *G. pulex,* the conspicuous orange yellow parasite *P. laevis* is visible through the transparent cuticle of *G. pulex.* Because it was previously reported that infected gammarids are significantly less photophobic than uninfected ones (Cezilly et al. 2000), Bakker et al. (1997) tested whether parasite color and parasite-induced changes in host behavior affected the predation rate of *G. pulex.* Therefore, hungry three-spined sticklebacks (*Gasterosteus aculeatus*) were offered uninfected *G. pulex* that had a painted orange spot on their cuticle (to simulate infection) and infected *G. pulex* with brown paint on the parasite infection spot. Sticklebacks consumed significantly more infected *G. pulex* than uninfected ones. Experimental exclusion of behavior or color effects of the parasite on its intermediate host showed that both parasite color and parasite-induced changes in *G. pulex* behavior significantly increased their vulnerability to predation by sticklebacks.

The joint influence of two acanthocephalan parasites on the behavior of *Gammarus* has also been investigated (Cezilly et al. 2000). These authors studied the effects of simultaneous infection by a fish acanthocephalan parasite (*P. laevis*) and a bird acanthocephalan parasite (*Polymorphus minutus*) on the behavior of their common intermediate host, *G. pulex*. In this study, the reaction to light and vertical distribution of infected (by one or both parasites) and uninfected individuals was investigated. Generally, uninfected gammarids tended to be at the bottom of the water column and were photophobic, but *P. laevis*-infected gammarids were attracted to light; *P. minutus*-infected individuals showed a modified vertical distribution and swam closer to the water surface (Cezilly et al. 2000). The observed behavioral changes of the organisms infected with both parasites seemed to be dependent on only the presence but not the intensity of the parasite infection. Gammarids harboring both parasites were vertically half-way between those of *P. laevis*- and *P. minutus*-infected individuals, whereas *P. laevis* was able to induce altered reaction to light even in the presence of *P. minutus*.

5.2 Multispecies Freshwater Biomonitor® (MFB)

5.2.1 Method Description

Gerhardt et al. (1994) developed an online biomonitoring system capable of quantitatively assessing several aspects of behavior in situ and ex situ, and doing so in real time. The system is based on a quadrupole impedance conversion technique that simultaneously records several behavioral parameters of a wide range of aquatic organisms, such as *D. magna, G. pulex, Sialis lutaria, Leptophlebia vespertina, B. niger,* Simuliidae, *Dinocras cephalotes, Hydropsyche siltalai,* and tadpoles of *Rana temporaria.* During exposure, the organisms move freely between two pairs of electrodes on each sidewall of a test chamber, which receives unfiltered stream water or exposure water (Gerhardt et al. 1994). The organism's behavior is expressed as movements that lead to changes in an electrical field and these are measured as changes in the impedance of the system. For example: (1) locomotion: swimming and crawling result in irregular amplitudes and frequencies, (2) resting: small signals that cannot be separated from background noise, (3) ventilation: regular, high-frequency movements with, e.g., pleopods to establish a constant water flow across the gills, (4) feeding: species-specific patterns for grazing, filtering, and hunting. The impedance converter proved to be a sensitive and quantitative tool for use in behavioral, ecological, and ecotoxicological studies, which makes it a promising tool for continuous biomonitoring purposes (Gerhardt 1999).

5.2.2 Behavioral Changes in the MFB Related to Toxic Effects

The biomonitoring system was used to compare the behavior of two different *G. pulex* populations, one originated from an anthropogenically unpolluted stream

and the other from a copper-polluted study site, where the biomonitor was placed (Gerhardt et al. 1998). *G. pulex* were exposed to simulated copper pollution peaks of 70 μg Cu^{2+}/L and their drift behavior was compared to natural drift. A nocturnal drift peak was observed for both *G. pulex* populations. The exposure resulted in significantly less activity (number of active organisms per day and time spent in locomotion and ventilation) among members of the reference population compared to the local population. Copper pollution pulses provoked increased activity in a number of organisms that were active in both populations and the time these organisms spent on locomotion. However, no significant changes in the natural drift were registered, probably from dilution downstream of the pulse (Gerhardt et al. 1998).

DeLange et al. (2006a) also used the MFB® to investigate whether a prolonged exposure to low concentrations of anthropogenic chemicals may lead to sublethal effects, including changes in behavior. In their study, *G. pulex* were exposed to three pharmaceuticals, the antidepressant fluoxetine, the analgesic ibuprofen, and the anti-epileptic carbamazepine, and one cationic surfactant, cetyltrimethylammonium bromide (CTAB). Low concentrations (10–100 ng/L) of fluoxetine and ibuprofen resulted in a significant decrease in activity, and the response to carbamazepine showed a similar pattern; however, differences were not significant. The surfactant CTAB led to a dose-dependent decrease in activity with increasing concentrations. Surprisingly, the observed behavioral effect concentrations for all three chemicals were 104–107 times lower than previously reported LOECs (lowest observable effect concentrations) and were in the range of actual environmental concentrations (De Lange et al. 2006a).

Another in situ biomonitoring study was conducted along the rivers Meuse (NL), Aller (GER), and Rhine (F) for the purpose of validating the MFB® for an in situ application approach with *G. pulex* as the new indicator species (Gerhardt et al. 2007). Three field sites were selected for characteristics adequate to answer the following research questions: (1) Is *G. pulex* able to survive in clean unfiltered surface water (Aller River), with detritus as the food source in the MFB? (2) Is *G. pulex* able to react to a cocktail of metals or organic xenobiotics that are applied as pulse pollution in concentrations relevant to those occurring under accidental circumstances (Meuse River)? (3) Is the MFB with *G. pulex* ready to be used for a long-term evaluation at a location (Rhine River), with frequent pulse pollution and changes in water quality? *G. pulex* used in the reference stream Aller did not show any negative effects and had a 100% survival. Alternatively, *G. pulex* responded to a pulsed exposure of a mixture either of trace metals or of several organic xenobiotics, with up to a 20% decrease in locomotory activity (at the 1st pulse) and increased mortality (at 2nd or 3rd pulse only). Deployment at the monitoring station on the Rhine River demonstrated that *G. pulex* were able to detect chemical irregularities by displaying up to a 20% decrease in locomotory activity, confirming the suitability of *G. pulex* in the MFB as an alert system for water quality monitoring at sensitive or accidentally polluted sites (Gerhardt et al. 2007).

5.2.3 Behavioral Early Warning Responses After Pulsed Exposures

The effects of draining abandoned mines were investigated by exposing *E. meridionalis, H. pellucidula,* and *Choroterpes picteti* to short-term pulses of drained acid mine waste (Macedo-Sousa et al. 2008). Possible negative effects of such pulses, resulting from acidity and heavy metal contamination, were assessed by using the MFB® behavioral early warning responses (locomotion and ventilation). *E. meridionalis* was the most sensitive species in terms of mortality and behavioral endpoints, followed by *C. picteti* and *H. pellucidula*; this demonstrates the suitability of using benthic invertebrates' behavioral early warning responses for detecting spikes of pollutants. Exposed *E. meridionalis* showed increased locomotion, with a subsequent increase in ventilation, whereas *C. picteti* reacted with increased locomotion; *H. pellucidula* was unaffected.

5.3 A Sublethal Pollution Bioassay with Pleopod Beat Frequency and Swimming Endurance

Lawrence and Poulter (1998) developed a bioassay with *G. duebeni* for sublethal ecotoxicological studies, based on the monitoring of pleopod beat frequency (ventilation) and swimming endurance against a head flow of water. Pleopod beat frequency showed a complex dose- and time-dependent response to copper, whereas swimming endurance showed a clear dose–response relationship and was identified as the more reproducible technique (Lawrence and Poulter 1998).

By using swimming efficiency again, Lawrence and Poulter (2001) investigated the effect of copper, pentachlorophenol (PCP), and benzo[a]pyrene (B[a]P) on the amphipod *Chaetogammarus marinus.* Moreover, swimming endurance was determined by following the protocol developed by Lawrence and Poulter (1998). Swimming endurance was significantly impaired at concentrations of 15 μg/L copper, 40 μg/L PCP, and 20 μg/L B[a]P.

5.4 Behavior in Combination with Other Endpoints

5.4.1 Drift and Foraging Activity

Behavioral aspects, combined with feeding activity, were examined by Allan and Malmqvist (1989). These authors studied the relationship between drift and foraging activity in *G. pulex* by comparing catches of *G. pulex* from the benthos, drift, and small traps baited with cheese. They investigated two field sites, one with both sculpins and trout, and one without the fish. Within 15 min, many G. *pulex* were captured in the baited traps, and a sculpin, caught in an adjacent cage, had no counteracting influence, which demonstrated the effectiveness of chemical attractants.

These authors reported that trap collections appeared to be useful for detecting small-scale spatial patterns and was an indication of a highly aggregated distribution. Traps exclusively captured organisms from the two largest size classes of *G. pulex.* In contrast, drift collections consisted almost exclusively of individuals <4 mm during the day and the larger *G. pulex* in the night drift. When analyzing stomach contents of trout and sculpins, the authors found that they selectively captured larger prey that were proportional to their size. Allan and Malmqvist (1989) attributed the rarity of larger *G. pulex* in the daytime drift to a greater daytime risk of predation, but not to the absence of foraging activity in the amphipod, because baited traps and direct observation indicated that *G. pulex* is continuously active.

5.4.2 Species Interaction and Feeding Activity in a Toxic System

Feeding bioassays may also be used to investigate species interaction in toxicant systems; in such systems, the stresses of toxicant and competition are integrated. In one such system, *G. pulex* was coexposed with *A. aquaticus* to different concentrations of lindane or 3,4-DCA, and the feeding response of *G. pulex* was recorded (Blockwell et al. 1998). A 96-hour exposure to 3.8 and 6.0 μg/L lindane led to a reduced *G. pulex* feeding rate; coexposure of *G. pulex* with *A. aquaticus* produced the same result, but at a higher rate. After 240 hour of exposure, only gammarids exposed to 6.5 μg/L lindane showed a reduced feeding rate, but exposure to very low concentrations of lindane (0.1 and 0.9 μg/L) resulted in a significant increase in gammarid feeding activity. In the 3,4-DCA coexposure of gammarids with *A. aquaticus* (96 and 240 hour), the calculation of the gammarid median feeding times (FT_{50}) could not be performed, because, in most groups, less than 50% of the *A. salina* eggs were eaten. However, a comparison to controls showed that a substantial reduction in gammarid feeding activity had occurred in the majority of the 3,4-DCA treatment groups. Interestingly, exposure to 3,4-DCA at 90 μg/L apparently reversed the direction of the species interaction, with 100 and 60% survivorship recorded for *A. aquaticus* and *G. pulex,* respectively (i.e., *G. pulex* was no longer the dominant species). The different modes of action of 3,4-DCA and lindane may be responsible for the recorded results (Blockwell et al. 1998).

5.4.3 Combined Assessment of Locomotory, Ventilatory, and Feeding Activity

In a very recent study (Felten et al. 2008), the combination of behavioral endpoints and feeding activity was used to investigate the effects of cadmium (7.5 and 15 μg/L) on physiological and behavioral responses of *G. pulex*. Mortality and whole-body cadmium concentration of exposed gammarids were found to be significantly higher than were in controls. Cadmium exposure exerted a significant decrease in osmolality and hemolymph Ca^{2+} concentration, but not in hemolymph Na^+ and Cl^- concentrations, whereas the Na^+/K^+-ATPase (adenosine triphosphatase) activity was significantly increased to maintain homeostasis. Cadmium exposure resulted in a significant reduction of behavioral responses, such

as feeding rate, locomotor (number of moving animals), and ventilatory activities (pleopod beating frequency), possibly to limit energy loss and redirect it to osmoregulation and detoxification (Felten et al. 2008). The results of this study indicate that osmolality and locomotor activity in *G. pulex* could be effective ecophysiological/behavioral markers to monitor freshwater ecosystems and to assess the health of organisms and associated implications on population levels.

5.4.4 Combined Assessment of Re-pairing of Precopula Pairs and Feeding Rate

Malbouisson et al. (1995) used feeding rate in combination with re-pairing of precopulatory *G. pulex* to assess the toxicity of lindane. In this study, precopulatory pairs were physically pre-disrupted, fed with alder leaves (previously conditioned by *Cladosporium* sp.), and exposed to either high concentrations (0.5–2.0 mg/L) for 2–20 min or low concentrations (0.5–5.0 μg/L) for 48 hour at 15°C. The 48-hour exposure to 5.0 μg/L lindane led to significantly reduced feeding activity but did not disrupt re-pairing of precopula pairs. The brief exposures to 1.0 (for 20 min) and 2.0 mg/L of lindane (2–20 min) resulted in significantly reduced feeding rates during the first 24 hour post-exposure, and re-pairing was affected by treatments that combined higher concentrations and longer exposures. The median survival time for briefly exposed animals varied with concentration and exposure period (Malbouisson et al. 1995).

The investigation of combinations of behavioral endpoints, such as locomotory and ventilatory activities, avoidance and drift away from predators, and/or pollutants and/or parasites, may be a very promising direction for future behavioral ecotoxicology studies. It is anticipated that such studies may provide a more realistic assessment of the consequences of pollution.

6 Mode-of-Action Studies and Biomarkers

Some recently published studies with gammarids and related species suggest that *Gammarus* spp. may not only be suitable for nonspecific chronic toxicity testing for pollution-induced impairment of feeding activity, behavior, or development and sometimes mortality, but may also be useful for the assessment of more subtle, mode-of-action-driven chronic toxicity patterns. Such patterns can be investigated by using specific, mode-of-action-related endpoints or biomarkers at several organization levels.

At the cellular level, pollutant exposure may inhibit energy production (oxidative phosphorylation) or enzyme activity, or cause gene toxicity, carcinogenic activity, and oxidative stress. Some cellular responses can be measured by using biomarkers. Examples of potential biomarkers are metallothionein (MT) that protects the organism against metal-induced toxicity, stress proteins (heat shock proteins) that protect cells, and acetylcholinesterase, Na^+/K^+-ATPase, and Ca^{2+}-ATPase that protect against certain types of neurotoxicity.

At the individual and population levels, nonspecific chronic effects, such as those mentioned above (behavior and feeding activity), may occur. Effects with specific known modes of action may also occur. By using biomarkers for a set of specific endpoints, it is possible to evaluate a pollutant for its potential to induce several effects, e.g., developmental toxicity, chronic toxicity, immunotoxicity, and endocrine disruption. Table 4 provides a list of methods used to assess bioenergetic responses and effects on reproduction at the population level, whereas Table 5 summarizes biomarkers available for use in detecting different specific endpoints, including endocrine disruption.

6.1 Bioenergetic Responses, Excretion Rate and Respiration Rate

6.1.1 O:N Ratio, Respiration, and Ammonia Excretion

Gammarids have been used to assess the impact of oil and oil dispersants on a model littoral ecosystem in the Baltic Sea (Carr and Linden 1984). Bioenergetic (O:N ratio) measurements were made for *G. salinus;* ammonia excretion and respiration rates were also measured. No effects on ammonia excretion rates, respiration rates, or O:N ratios were observed after 1 day of exposure. However, after 10 days, highly significant differences were recorded between experimental and control groups for all three parameters, showing that both oil and oil/dispersant treatments produced subtle physiological alterations. Interestingly, the use of a chemical dispersant apparently resulted in a more rapid recovery of *G. salinus* than would have occurred if the oil had not been chemically dispersed (Carr and Linden 1984). Olsen et al. (2008) measured cellular energy allocation (CEA) in the sea ice amphipod *G. wilkitzkii* after this organism was exposed for 1 month to the water-soluble fraction (WSF) of oil. With the CEA biomarker, one is able to measure the energy budget of organisms by biochemically assessing changes in carbohydrates, protein and lipid content, as well as changes in electron transport system activity. The authors observed a significantly higher protein content at the medium dose compared to controls, but no effects were observed on the total energy budget, indicating that parts of the energy budget of *G. wilkitzkii* were affected by a WSF component of oil (Olsen et al. 2008).

6.1.2 Energy Input and Output with "Scope-for-Growth" Assays

Another approach for assessing stress was introduced by Naylor et al. (1989). The "scope-for-growth" (SfG) approach (Bayne et al. 1979) uses the difference between the energy input to an organism from its food and the output from respiratory metabolism to provide a good physiological measure of stress; in principle, this approach is directly related to population and community processes (Naylor et al. 1989). The rationale behind this approach is that physiological processes can often be assessed more easily and precisely than population and community ones, and

Table 4 Test methods for mode-of-action-related endpoints with gammarids

Species	Test substance/media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
Bioenergetic responses, excretion rate, and respiration rate							
G. salinus immature males	Baltic sea water + crude oil 20 mg/L + crude oil 20 mg/L and oil dispersants (15.4 mL)	Continuous seawater flow into circular pools	12 days	Ammonia (NH4), excretion rate and respiration rate, O:N ratio	Crude oil +/– dispersant: significant NH_4 increase, significant O_2 increase, significant O:N decrease	The use of a chemical dispersant resulted in a more rapid recovery	Carr and Linden (1984)
G. wilkitzkii ovigerous females	Water-soluble fraction (WSF) of oil: high: PAH 55–8 ppm; medium: PAH 10–2 ppm; low: PAH 5–1 ppm	Continuous flow-through	1 month	Cellular energy allocation (CEA)	10–2 ppm PAH: significantly higher protein content	No effects on the total energy budget	Olsen et al. (2008)
G. pulex males	Zinc (Zn): 0.3, 0.5, 0.7 mg/L Low pH: pH 5	Scope of growth (SfG)	5 days	SfG: Food absorption (A) – energy output (respiration, R)	A and SfG significantly reduced by 0.5 and 0.7 mg/L Zn and pH 5	Most sensitive endpoint: A	Naylor et al. (1989)
G. pulex males	Zn: 0.3, 0.5, 0.7 mg/L 3,4-Dichloroaniline (DCA): 0.125, 0.25, 0.5, 1 mg/L Oxygen: 100, 75, 50% saturation Ammonia (NH_3): 0.07 mg/L	Scope of growth (SfG)	6 days	SfG: Food absorption (A) – energy output (respiration, R)	A and SfG significantly reduced for Zn (>0.5 mg/L), DCA (>0.5 mg/L), oxygen (>50%), ammonia (0.07 mg/L)	Most sensitive endpoint: A	Maltby and Naylor (1990)

Table 4 (continued)

Species	Test substance/media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex males	In situ: control upstream vs. polluted downstream site	SfG – in situ	6 days	SfG: Food absorption (A) – energy output (respiration, R)	In situ: A and SfG significantly reduced at downstream-polluted site	Most sensitive endpoint: A	Maltby et al. (1990)
G. pulex brooding females	Zn: 0.1, 0.3, 0.5 mg/L	SfG and reproduction	7 days	SfG: Food absorption (A) – energy output (respiration, R), Reproduction	>0.3 mg/L Zn: A and SfG significantly reduced, significant decrease in the size of released offspring from subsequent brood, increased no. of aborted broods	No effect on the number or the size of released offspring from current brood	Maltby and Naylor (1990)
G. pulex	Copper (Cu): 11.2, 14.6, 18.2, 23.1 µg/L	Flow-through	100 days	Growth, population density (PD), age composition (AC), number of adults (NoA)	Increase in Cu, decrease in density and number of juveniles. LOEC: PD: 14.6 µg/L Cu, AC: 14.6 µg/L Cu, NoA: 18.2 µg/L Cu	Control and 11.0 µg/L Cu, the initial density doubled and mainly juveniles were present	Maund et al. (1992)

Table 4 (continued)

Species	Test substance/media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex copulatory pairs and newly released juveniles	Esfenvalerate (ESV): 0.05, 0.1, 0.3 μg/L	Reproduction test	1 hour pulse + 2 weeks post-exposure	Survival, pairing behavior, reproductive output	0.1–0.6 μg/L ESV: decreased survival, pairing behavior, reproductive output. 0.05 μg/L ESV: immediate disrupted reproducing pairs, egg or offspring release from brood pouch, delays in pair formation and reproduction following transfer to clean water		Cold and Forbes (2004)
G. locusta juveniles (2–4 mm)	Cu-spiked sediments: 1.4, 2.8 and 4.9 mg/kg dry weight	Static renewal, seawater and spiked sediment	28 days	Cu body burden, metallothionein (MT), growth, reproduction	4.9 mg/kg Cu: stimulation of growth and reproduction, bioaccumulation of 95 μg Cu/g dry weight, significant synthesis of MT (1.7 mg/g dry weight) in males	Cu contamination seems to lead to an unexpected condition improvement	Correia et al. (2001)

Table 4 (continued)

Species	Test substance/media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. wilkitzkii females with embryos in brood pouch	Water-soluble fraction (WSF) of oil, PAH concentrations: high dose: 55–8 ppm; medium dose: 10–2 ppm; low dose: 5–1 ppm	Embryogenesis	30 days	Reproductive stage, eggs per female, weight, developmental malformations	High dose: frequency of embryo aberrations significantly higher compared to controls	No significant differences in reproductive stage, eggs per female or weight	Camus and Olsen (2008)
C. marinus freshly fertilized embryos	Cu, pentachlorophenol (PCP), benzo[a]pyrene (B[a]P)	In vitro embryo culture, aqueous exposure	Until embryos hatched or development ceased	Embryo development length, width, stage	Significantly impaired embryo development at 20 µg/L Cu, 20 µg/L PCP, 20 µg/L B[a]P Cu. PCP: extended embryogenesis by 4–8 days. B[a]P: hatched at the same time as controls but were significantly smaller	Stages 2–4 were all prolonged by Cu, PCP, B[a]P and the time to complete stage 5 was reduced	Lawrence and Poulter (2001)
G. fossarum	Sewage treatment plant (STP) discharging effluent containing xenoestrogens: locations: Lu, Ku above: control Ld, Kd below: exposure	Population structure investigation by field sampling	1.5 years, field sampling every 2 (Lu, Ld) and 4 (Ku, Kd) weeks	Sex ratio, intersexuality, population structure	No effect on sex ratio and intersexuality. Proportion of breeding female gammarids downstream (Kd, Ld) was tentatively lower than upstream (Ku, Lu).	Kd, Ld: tendency toward a decreased proportion of smallest juvenile gammarids in the population compared to Ku, Lu	Ladewig et al. (2006)

Table 4 (continued)

Species	Test substance/media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	Reference
G. pulex, *A. aquaticus* (2-week-old juveniles)	Landfill leachate [1200 mg/L chemical oxygen demand (COD) and 600 mg/L biological oxygen demand$_5$ (BOD_f)]	Reproduction experiment, static renewal	4 months	Reproduction	A dilution as high as 1:66 influences the fecundity of the *Gammarus* population. A dilution of 1:20 affects the size of the *Asellus* breeding colony		Bloor et al. (2005)

Table 5 Biomarkers for multiple stressors in *Gammarus* spp.

Abbreviation	Name	Species	Function	Toxicant: activity/effect	Reference
Molting process and exoskeleton integrity					
Chitin	Chitin, glycosaminoglycans	*Gammarus* sp.	Addition of chitin during the endocuticle production between the cuticle and the epiderm, ecdysone-mediated chitin formation process, or glucosamine formation	Xenoestrogens: ↑ chitin Polluted marina: ↑ chitin	Gagné et al. (2005)
Arthropodin	Neutral arthropodin	*Gammarus* sp.	Post-molt phase, arthropodin inclusion in the cuticle supports the exoskeleton-hardening process	Polluted marina: ↑ arthropodin	Gagné et al. (2005)
Sclerotin	Alkali-extractable sclerotin	*Gammarus* sp.	Post-molt phase, sclerotin inclusion in the cuticle to support exoskeleton-hardening	Polluted marina: ↑ sclerotin	Gagné et al. (2005)
ASP	Acid-soluble proteins	*Gammarus* sp.	Post-molt phase, ASP production assists calcareous layer formation	Polluted marina: ↑ ASP	Gagné et al. (2005)
ALP	Alkali-labile phosphates	*Gammarus* sp.	alkali-labile phosphates in proteins, important for phosphate mobilization	Polluted marina: ↑ ALP	Gagné et al. (2005)
Endocrine disruption					
Vtg	Vitellogenin-like proteins	*Gammarus* sp.	Egg-yolk protein precursor, oocyte-maturation stage	Polluted marina: ↑ Vtg	Gagné et al. (2005)
hsp90	Heat-shock protein 90	*G. fossarum*	Steroid receptor interactions and the modulation of sex hormone signal transduction level of stress protein reflects the maturity stage of the oocytes	Bisphenol-A: ↑hsp90	Schirling et al. (2006)

Table 5 (continued)

Abbreviation	Name	Species	Function	Toxicant: activity/effect	Reference
Energy demand/glucose metabolism/lipid anabolism					
G6PD	Glucose-6-phosphate dehydrogenase	*Gammarus* sp.	Intermediary metabolism of glucose, lipogenic metabolism enzyme	Polluted marina: ↑ G6PD + ↑ ME ↑ energy demand, lipid anabolism	Gagné et al. (2005)
ME	NADH-generating malic enzyme	*Gammarus* sp.	Intermediary metabolism of glucose, lipogenic metabolism enzyme	Polluted marina: ↑ G6PD + ↑ ME + ↑ Vtg: delay in gametogenesis	Gagné et al. (2005)
ICD	Isocitrate dehydrogenase	*Gammarus* sp.	Aerobic metabolism of glucose, lipogenic metabolism enzyme	Polluted marina: ↑ G6PD + ↑ ME + ↑ ICD Increased glucose metabolism	Gagné et al. (2005)
General stress response					
hsp70	Heat shock protein 70	*G. fossarum*	Sensitive biomarker induced by various proteotoxic stressors	Cd^{2+} and 3BC: ↑ hsp70, low + intermediate concentrations ↑ hsp70, high concentrations, pathological damage	Scheil et al. (2008) Schill et al. (2003)
Metal exposure/cellular stress					
MT	Metallothionein	*G. locusta*	Homeostasis and detoxication, indicator for cellular stress	Water-borne Cu: ↑ MT	Correia et al. (2002)
Oxidative stress					
LP	Lipid hydroperoxides	*G. locusta*	Oxidative damage: peroxidation of unsaturated lipids to cytotoxic lipid hydroperoxides from reactive oxidative species (ROS) (i.e., metals)	Water-borne Cu: ↑ LP 1–4 d), ↑ LP (4–6 d) Sediment Cu: ↑ LP, after 4 days of exposure	Correia et al. (2002)

Table 5 (continued)

Abbreviation	Name	Species	Function	Toxicant: activity/effect	Reference
Neurotoxicity/pesticide exposure					
Che	Cholinesterase	*G. pulex*	Che and ACHE degrade the neurotransmitter acetylcholine in cholinergic synapses	Pirimiphos-methyl: ↑ ChE	McLoughlin et al. (2000)
AChE	Acetylcholinesterase	*G. pulex* *G. pulex* *G. pulex* *G. pulex*		Fenitrothion: ↑ AChE Parathion: ↑ AChE Malathion 60: ↑ AChE Chlorpyrifos: ↑ ChE Chlorpyrifos: ↑ ChE Cypermethrin: ↑ ChE	Streit and Kuhn (1994) Crane et al. (1995) Xuereb et al. (2007) Maltby and Hills (2008)
Detoxification					
GST	Glutathione-*S*-transferase	*G. pulex*	Detoxification enzyme	Lindane: ↑ GST Permethrin: ↑ GST	McLoughlin et al. (2000)

although toxic substances affect the physiological processes of individual organisms, their ecological impacts occur at the population and community levels. The SfG was measured in *G. pulex* organisms which were exposed to zinc (0.3, 0.5, and 0.7 mg/L) and low pH (pH 5); reported results were that both treatments significantly reduced the SfG of individuals, and the most sensitive component of the energy budget was food absorption (Naylor et al. 1989).

Another group also investigated the use of SfG for freshwater systems (Maltby and Naylor 1990). The authors exposed *G. pulex* to conditions, often associated with pollution, by assessing the effects of four specific substances: a metal (zinc), an organic compound (3,4-dichloroaniline), and two dissolved gases (oxygen and ammonia). In all cases, SfG was reduced by the stress, primarily from a depression of energy intake. The energy output (respiration) was significantly affected only by ammonia (Maltby and Naylor 1990). Maltby et al. (1990) then deployed the SfG system to examine whether it would be an equivalent sensitive indicator of stress for *G. pulex* in the field, as it had under laboratory conditions. In every case, SfG was reduced at downstream-polluted sites compared to upstream reference sites. This reduction in SfG was the result of a decrease in energy intake (absorption) rather than an increase in energy expenditure (respiration). Maltby and Naylor (1990) used *G. pulex* brooding females to compare the effect of Zn on SfG and on reproduction. At 0.3 mg/L of zinc (and higher), SfG was significantly reduced, which resulted from significant decreases in energy absorption. The offsprings released from the subsequent brood were smaller, but there was no effect on size or number of offspring released; this result was true for both current and subsequent broods. However, both present and past zinc stress caused an increase in the number of broods aborted (Maltby and Naylor 1990).

6.2 Population Experiments, and Development and Reproduction Modeling

6.2.1 Growth, Density, and Age Composition

Gammarids were used to investigate the chronic toxicity of metals, e.g., copper. *G. pulex* populations were exposed for 100 days to copper concentrations below the 240-hour LC_{50} for juveniles (Maund et al. 1992). Copper significantly affected growth, density, and age composition of the populations and the effects were dose dependent. In the control and the lowest treatment (11.0 μg/L) groups, the initial population density doubled and mainly juveniles were present. With increasing copper concentration, a decrease in both density and number of juveniles present was observed. The density was lower than that of the initial population. At the highest concentration (23.1 μg/L Cu), the number of adults was significantly reduced. The LOEC values for population density, age composition, and number of adults were 14.6, 14.6, and 18.2 μg/L Cu, respectively.

6.2.2 Life-History Traits

Different life stages of *G. pulex* were used to examine effects on key life-history traits following short and environmentally realistic pulse exposures of the pyrethroid insecticide esfenvalerate (Cold and Forbes 2004). Concentrations in the range of 0.1–0.6 μg/L for as little as 1 hour affected *G. pulex* survival, pairing behavior, and reproductive output that still could be detected at least 2 weeks following the pulse. Exposure to 0.05 μg/L for 1 hour induced immediate disruption of reproducing pairs, release of eggs or offspring from the brood pouch, and substantial delays in pair formation and subsequent reproduction, following transfer to clean water (Cold and Forbes 2004).

6.2.3 Population Endpoints Combined with Body-Burden and Metallothionein Induction

In another study, the effect of copper-spiked sediments on *G. locusta* was evaluated during 28 days of exposure (Correia et al. 2001); key measures included copper body-burden and metallothionein (MT) induction and an integration of these with organism and population-level endpoints. The most relevant sublethal effects detected in this study were stimulation of growth and reproduction at the highest treatment level (4.9 mg Cu/kg dry weight), bioaccumulation of Cu (95 μg Cu/g dry weight), and increased synthesis of MT (1.7 mg/g dry weight in males, $p < 0.001$), suggesting that the observed effects were associated with Cu contamination. The observed higher offspring production was regarded to be a direct consequence of faster growth rates. The authors suggest that hormesis is responsible for faster growth rates, which was an unexpected improvement induced by Cu contamination, because crustaceans need Cu in their blood pigment (hemocyanin; Gerhardt 1995, 1996).

6.2.4 Embryogenesis

Camus and Olsen (2008) studied malformations in embryos of the Arctic sea ice amphipod *G. wilkitzkii* exposed to the water-soluble fraction of oil. The female growth stages ranged from development stage three to nine, and no differences in reproductive stage were observed among the different treatments after 30 days of exposure. However, the frequency of embryo aberrations was significantly higher in the high-dose group compared to controls; this indicated that the embryos of *G. wilkitzkiii* were affected by exposure to the oil (Camus and Olsen 2008).

Embryogenesis was also used as an endpoint by Lawrence and Poulter (2001) to investigate the developmental toxicity of copper, PCP, and B[a]P toward the amphipod *C. marinus*. To conduct this study, the authors used *C. marinus* in an adjusted in vitro embryo culture method (Morritt and Spicer 1996). Maximal width and length of freshly fertilized and exposed embryos were measured daily and the developmental stage was determined for each individual until either development ceased due to disruption or the first juveniles hatched. Development of in vitro cultured embryos was significantly impaired by 20 μg Cu/L, 20 μg PCP/L, and 20 μg B[a]P/L. Cu and

PCP extended the period of embryogenesis by 4–8 days, whereas embryos cultured with B[a]P hatched at the same time as controls but were significantly smaller. Each pollutant affected specific stages, from Stage 2 onward. Stages 2–4, in which the embryo undergoes development of the germinal disc, dorsal organ rudiments, cordal furrows, appendage rudiments and segments, eye, and heart, were all prolonged in toxicant-exposed treatments. Generally, the time to complete Stage 5 was reduced in pollutant-exposed embryos. The results indicate that both swimming stamina and embryogenesis may be used in amphipods as sensitive bioassays for toxic effects (Lawrence and Poulter 2001).

6.2.5 Population Structure and Dynamics

Population structure and population dynamics of *G. fossarum* (Ladewig et al. 2006) were investigated in a field experiment. Gammarids were sampled at two streams in Germany, each with two sampling sites above and below a sewage treatment plant (STP) that discharged effluents known or assumed to have endocrine-disrupting potential. Changes in the sex ratio of *G. fossarum* or occurrence of intersexuality was not observed in either stream, but differences in the structure and dynamics of *G. fossarum* populations were found, and these were more pronounced in one stream. This result agrees with findings on *G. pulex* populations, which significantly differed in population density, standing crop biomass, individual size, and sex ratio in streams with different lotic conditions, suggesting that some of those dissimilarities were caused by pollutants (Crane 1994).

Interestingly, in a study conducted by Ford et al. (2006), with *E. marinus* populations from polluted and reference sites, consistently higher level of intersexuality was found throughout the year at sites receiving industrial contaminants when compared with reference sites. Infection of *E. marinus* by microsporidian parasites appeared to be more prevalent at impacted sites, indicating that parasitism may partly be responsible for intersexuality but may not be its only cause. Other environmental factors probably exist. Whether pollution can cause intersex directly remains to be confirmed, although, due to the apparent fragility of sex determination in the Amphipoda, it cannot be ruled out (Ford et al. 2006).

Possible negative effects on *G. pulex* and *A. aquaticus* populations were investigated to determine the potential ecological implications of leached contaminants reaching the water table (Bloor et al. 2005). A specific landfill leachate [1200 mg/L chemical oxygen demand (COD) and 600 mg/L 5-day biological oxygen demand ($BOD_{(5)}$] was used to develop a standardized long-term sublethal ex situ toxicity-testing program with juveniles. The authors found that a dilution even as high as 1:66 influenced the fecundity of a *Gammarus* population, while a dilution of 1:20 affected the size of an *Asellus* breeding colony (Bloor et al. 2005).

6.2.6 Population Experiments Combined with Modeling on Reproductive Output

The author of a dissertation at the Technical University of Dresden (Germany) investigated the activity of environmental chemicals toward *G. fossarum,* focusing on

population experiments and on an individual-based reproduction model (Schmidt 2003). Artificial indoor streams, containing *G. fossarum* and other aquatic invertebrates to simulate a community, were used to investigate the herbicide terbutryn and the insecticide fenoxycarb. No statistically significant effects on population-related parameters were found for these chemical, but at the highest exposure concentrations of each (289 μg/L terbutryn and 50 μg/L fenoxycarb), chronic toxic effects were reported, although they were not statistically significant. The nature of this chronic effect was decreased formation of precopula pairs and a reduced number of offspring. Levels of terbutryn in excess of 2 μg/L decreased growth in a dose-dependent manner, probably because of a dose-dependent decreasing algae availability, which comprised a food source. A reproduction model (GamMod) was also developed that incorporated the following fenoxycarb exposure parameters: the number of juvenile offspring/female (control, 10.5; 0.05 μg/L, 10.0; 0.5 μg/L, 7.6; 5 μg/L, 8.0; and 50 μg/L, 10.3), the duration of brood development (28–29 days), and juvenile mortality (control, 6 days; 0.05 μg/L, 12 days). By comparing the experimental and modeling data it was possible to show that the GamMod model gave a plausible description of the population dynamics of *G. fossarum*. GamMod was able to describe the measured data well, with a geometric performance index between 1.6 for controls and 2.7 for the highest exposure concentration. A sensitivity analysis showed that juvenile mortality is the most sensitive parameter in this experimental setup. By evaluating several different parameters (e.g., juvenile mortality, sex ratio, and number of juveniles/female), it was possible to show how the model could be useful for calculating outcomes of possible scenarios, such as reducing the number of females in a population.

6.3 Endpoints and Biomarkers for Endocrine Disruption in Gammarids

Until the present, investigations involving endocrine-disrupting compounds (EDCs) in aquatic ecosystems have generally been performed on fish and mollusks. Some research, however, has been dedicated to other organisms, including arthropods. During certain periods of somatic growth, arthropods periodically go through molting (ecdysis). During such periods of growth, gametogenesis, production of a new cuticle, and shedding of the old exoskeleton are physiologically regulated and are especially vulnerable to endocrine disruption (Schirling et al. 2004). For example, during the growth phase of adult female crustaceans, hormonally controlled vitellogenesis occurs after ecdysis (Subramoniam 2000). Molting is controlled by a steroid hormone 20-hydroxyecdysone (Baldaia et al. 1984), but its involvement in vitellogenesis is controversial. During oocyte growth, various arthropods were found to have elevated levels of vertebrate-type steroids such as progesterone, testosterone, and 17β-estradiol (Cardoso et al. 1997; Fairs et al. 1990). However, treating *D. magna* with the estrogenic diethyl phthalate inhibited molting but did not disrupt vitellogenin (Vtg). This indicates that estrogenic vertebrate EDCs also interfere with

the arthropod endocrine system by acting through the ecdysteroid receptor (Zou and Fingerman 1997). To complicate the issue further, ecdysone not only is important for molting but also plays a major role during embryogenesis, where it is bound to Vtg in oocytes (Subramoniam et al. 1999). Chitin synthesis, which plays an important role during molting, was also found to be controlled by the ecdysone receptor pathway (Gagou et al. 2002; Nakagawa et al. 1995).

Mechanistic studies on the interaction of potential xenohormones with the endocrine system are still lacking for gammarids. There are only a limited number of publications for EDC in crustaceans, but those provide early ideas on what may become suitable endpoints, biomarkers, exposure scenarios, and ways to assess endocrine disruption in this genus.

6.3.1 Vitellogenin-Like Proteins and Lipogenic Enzymes (ICD, ME, G6PD)

Vtg, the energy-rich egg-yolk protein precursor, has been proposed as a biomarker to characterize the maturation stage of oocytes in crustaceans (Chang and Jeng 1995; Oberdörster et al. 2000). In addition, it was proposed that the increase in energy demand (glucose metabolism) leading to lipid anabolism during gametogenesis could also be followed by measuring intermediary lipogenic metabolism enzymes such as glucose-6-phosphate dehydrogenase (G6PD), isocitrate dehydrogenase (ICD), and the NADH-generating malic enzyme (ME) (Mori 1967; Sunny et al. 2002). Gagné et al. (2005) investigated the suitability of those biomarkers to assess changes in gametogenesis by collecting gammarids at polluted sites. They found that females from polluted sites had increased levels of Vtg-like proteins, indicating a delay in gonad maturation from the presence of environmental contaminants. In addition, increased lipogenic enzyme activities (ME and G6PD) were observed in females, supporting the delay-in-gamete-maturation hypothesis (Gagné et al. 2005). However, increases in aerobic metabolism of glucose (ICD) and intermediary metabolism of glucose (G6PD and ME) indicated an increased glucose metabolism, which is often observed in organisms exposed to pollutants (De Coen and Janssen 2003).

6.3.2 Heat Shock Proteins (hsp90) as Biomarkers for Endocrine Disruption in Gammarids

In vertebrates, it is known that the heat shock protein hsp90 is of crucial importance for steroid receptor interactions and modulation of sex hormone signal transduction (Pratt and Toft 1997). For invertebrates, the understanding of an equally complex system is still limited. The discovery of estrogen receptors in invertebrate taxa (De Waal et al. 1982) indicates that steroid-binding proteins and, therefore, mechanisms associated with the signal transduction process are phylogenetically very old (Thornton et al. 2003). Schirling et al. (2004) selected hsp90 as a potential biomarker for endocrine disruption, although it is well known that hsp90, like all stress proteins, also responds to stressors that do not target the endocrine system. To control for this, the authors also measured the well-established general stress

marker hsp70, as a "nonspecific stress effect control" for hsp90. For 12 weeks, animals were removed every 14 days to histologically determine the stage of the reproductive cycle and to measure variations in levels of hsp70 and hsp90 by using an immunoblotting assay. The maturation stage of the female gonad was identified from the structure of the oocytes, confirming that an almost complete reproductive cycle occurred within 12 weeks. Hsp70 and hsp90 levels were found to be inversely correlated over the course of the reproductive cycle. The authors reported that the hsp90 level at the beginning of the reproductive cycle was low, whereas the hsp70 level was at its peak. At the end of the cycle, when mature oocytes were present, the opposite was true. The finding that levels of stress proteins reflect the maturity stage of the oocytes provides prerequisite baseline information that may become quite useful. Such information enhances the ability to interpret biomarker studies on endocrine effects of chemicals in gammarids.

6.3.3 Chitin as a Biomarker for EDC Effects on Molting

Parts of the molting process, e.g., chitin synthesis and characteristics of exoskeleton protein and carbohydrate, are at least partially controlled by the molting steroid hormone ecdysone (Gagné and Blaise 2002; Gagou et al. 2002; Nakagawa et al. 1995). Indeed, incorporation of glucosamine into the integument was shown to be enhanced by ecdysone (Nakagawa et al. 1995). During the post-molt phase, the cuticle precursor is mainly composed of a flexible and transparent organic matrix and is usually devoid of chitin (glycosaminoglycans; Nation 2002). To support the exoskeleton-hardening process, neutral (arthropodin) and alkali-extractable (sclerotin) proteins are incorporated into the cuticle. At the inter-molt and post-molt stages, the endocuticle, between the cuticle and the epiderm, is produced, wherein the addition of chitin, sulfur, and calcium phosphate takes place (Nation 2002; Vigh and Dendinger 1982). During crustacean ecdysis, the cuticle is gradually dissolved so that the relative proportion of chitin increases as salt and protein content decreases. Thus, Gagné and Blaise (2002) propose that the integrity of ecdysis and the maturation state of exoskeletons could be followed by measuring the relative proportions of the acid-extractable proteins sclerotin, arthropodin, and chitin in exoskeletons, and phosphate level in proteins of the arthropod epidermis (Gagné and Blaise 2002).

The notion that xenoestrogens can affect molting in invertebrates was supported in fiddler crabs, where synthetic estrogens (i.e., diethyl phthalate, 4-(*tert*)-octylphenol, and 2,4,5-trichlorobiphenyl) were found to reduce molting-relevant chitobiose activity in the epidermis and hepatopancreas, and increase the proportion of chitin in the exoskeleton (Zou and Fingerman 1999).

A contrary result was observed by Gagné et al. (2005) when they collected *Gammarus* sp. individuals at four intertidal sites subjected to direct sources of pollution (marinas, ferry traffic, and harbors) and at one site with no direct source of pollution. Gammarids from polluted sites had significantly less chitin in exoskeletons, suggesting disruption in the ecdysone-mediated chitin formation process or decreased formation of glucosamine from glucose (Gagné et al. 2005).

6.3.4 Sex Ratio and Precopula Pairs

Watts et al. (2002) investigated how an artificial estrogen 17α-ethinyl estradiol (EE2) affects the sex ratio in cultures of freshwater crustaceans of *G. pulex*. Mixed populations of 90 individuals were exposed to 0.1, 1, and 10 μg/L EE2 for 100 days in a flow-through system. In all treatment groups, population size dramatically increased due to recruitment, with neonate and juvenile gammarids being the most abundant. Mean population sizes in the solvent control (257) and 0.1 μg/L EE2 treatment groups (267) did not differ from standard controls, but at the 1 and 10 μg/L EE2 exposures groups (385 and 411, respectively), population numbers were significantly greater than in the control population (169). The sex ratio of adults for all EE2 treatments was greater than 2:1 (female:male), with significantly more females than in the controls. The number of male adults, precopula guarding pairs, and ovigerous females did not differ among treatments. Secondary antennal and gnathopod length in males was consistently greater than in females, but no other differences were found between groups (Watts et al. 2002).

Effects on sex ratio were also detected in *G. pulex* exposed to estrogenic substances released by a sewage treatment plant, where an increased proportion of females was observed, along with an abnormal oocyte structure during vitellogenesis (Gross et al. 2001).

In another study, Watts et al. (2001) investigated the effects of EE2 and bisphenol-A (BPA) on survival and reproductive behavior of *G. pulex*. Reproductive behavior, like ability of males and females to detect each other to form precopulatory guarding pairs and to continue guarding behavior, was disrupted only at relatively high concentrations (3.7 mg/L EE2 and 8.4 mg/L BPA). This was probably caused by general toxicity rather than an endocrine-mediated process, indicating that precopulatory guarding in acute exposures is not a suitable endpoint for detecting EDCs (Watts et al. 2001).

6.3.5 Gonad Histology and hsp90

BPA was investigated by Schirling et al. (2006) regarding how it affects stress protein levels (hsp70 and hsp90) and gonad histology of *G. fossarum* in artificial indoor streams. Exposure to 50 and 500 μg/L of BPA resulted in accelerated maturation of oocytes in females and in a decline in the number and size of early vitellogenic oocytes. The level of hsp90, which plays a pivotal role in vertebrate sex steroid signal transduction, was significantly reduced by BPA at those concentrations (Schirling et al. 2006). This result is in line with the strong co-variation of hsp90 level with the reproductive cycle (Schirling et al. 2004). The authors reported that in early stages of oocyte development, the hsp90 level of individuals was three times lower than in specimens with mature eggs. BPA seems to disrupt this correlation and leads to reduced hsp90 levels and accelerated maturation of oocytes.

In a field study, again using gonad histology and hsp90, individuals of autochthonous populations of *G. fossarum* were examined for their maturity status, oocyte development, and biochemical parameters associated with their reproductive cycle (Schirling et al. 2005). Despite the isolated investigation of different size

classes of *G. fossarum* individuals to reduce data variability, a high variability was recorded, which prevented observation of significant differences for most of the measured parameters. Nevertheless, effects on gonad development and hsp90 level, both parameters related to the endocrine system, were found in the Kçrsch river. Downstream from the discharge of treated sewage, larger late vitellogenic oocytes, increasing atresia, and decreasing hsp90 levels were observed. This corresponds well with a higher estrogenic potential introduced by those effluents (Jungmann et al. 2004), compared to the other site, Lockwitzbach, wherein no effects were found.

6.3.6 Gametogenesis Activity and Gonad Maturation

In yet another field study, *Gammarus* sp. individuals were collected at four intertidal sites that were subjected to direct sources of pollution and at one site with no direct source of pollution. Subsequently, levels of Vtg-like proteins, MT, alkali-labile phosphates (ALPs) in proteins, and lipogenic enzyme activities (i.e., glucose-6-dehydrogenase, isocitrate dehydrogenase, and malate enzyme) were measured in whole soft tissues (Gagné et al. 2005). In addition, levels of pH-dependent extractable protein and chitin were determined in the exoskeleton to assess potential impacts of pollution on exoskeleton integrity and the molting process. The authors found that whole-body weights of both sexes were significantly lower at polluted sites and that females displayed either induced or decreased Vtg-like proteins at polluted sites, indicating significant changes in gametogenesis activity (Gagné et al. 2005). MT levels were not sex dependent and tended to be induced at all affected sites. At some impacted sites, females had a tendency toward higher ALP levels, indicating altered phosphate mobilization at those sites. In addition, lipogenic enzyme activities were increased at impacted sites for both sexes, suggesting a delay in gonad maturation rates. Principal component analysis revealed that gammarids collected at affected sites displayed substantial changes in the proportion of chitin, arthropodin, sclerotin, MTs, and intermediary glucose metabolism (glucose-6-phosphate dehydrogenase and isocitrate dehydrogenase in soft tissues) and thus suffered from disturbed gametogenesis and exoskeleton integrity (Gagné et al. 2005).

6.4 Other Specific Biomarkers for Detecting Multiple Stressors in Gammarids

6.4.1 Heat Shock Proteins (hsc/hsp70) as Biomarkers for Stress Response in Gammarids

Cells from virtually all organisms respond to a variety of stresses by the rapid synthesis of a highly conserved set of polypeptides called heat shock proteins (hsp), suggesting that hsp play an important role in both normal cellular homeostasis and stress response (Kregel 2002). Production of high hsp levels can be triggered by exposure to different kinds of environmental stress conditions, such as

inflammation, exposure of the cell to toxins like trace metals, starvation, or hypoxia (oxygen deprivation). In most organisms, hsp70 is among the most prominent proteins induced as a stress response (Li and Werb 1982). Hsp70 is known to be a sensitive biomarker induced by various proteotoxic stressors (Nadeau et al. 2001; Triebskorn et al. 2002) and there has been a growing interest in tracing contaminant effects by tracking hsp70 levels in crustaceans (Arts et al. 2004; Köhler et al. 2000) and amphipods (Schill et al. 2003).

Scheil et al. (2008) examined the responses of hsp70 and of hepatopancreatic cells and cells of gut appendices in the freshwater amphipod *G. fossarum* following a short-term exposure (4 days) to five different concentrations of the chemical UV filter 3-benzylidene camphor (3-BC). Male and female gammarids showed increased hsp70 levels after exposure to low concentrations of 3-BC (0.033, 0.33, and 3.3 μg/L), with a maximum response at 3.3 μg/L, indicating physiological stress. Higher concentrations (33 and 330 μg/L) resulted in lower hsp70 levels, indicating an overwhelming stress response. This effect probably resulted from a cessation of hsp70 synthesis following pathological impact, as indicated by strong cellular responses and cellular damage obtained in epithelia of the hepatopancreas and the gut appendices after treatment with 330 μg/L 3-BC (Scheil et al. 2008).

By simulating a mining accident, Schill et al. (2003) investigated immediate stress responses to toxicants, by measuring two forms of the 70-kDa hsp hsc/hsp70 and assessing the recovery time an organism needs after the end of the exposure. During a 20-day experiment, adult *G. fossarum,* separated by sex, were exposed to nine cadmium concentrations for 5 days to simulate a short-term pulse of xenobiotics, followed by a recovery period of 15 days. Females were much more sensitive to cadmium than were males, and 4.28 ± 2.45 μg Cd^{2+}/L resulted in strong effects on survival rate of females, but not males. At the cellular level, cadmium induced an hsc/hsp70 response, with the lower Cd^{2+} concentrations leading to an induction of stress proteins, whereas higher Cd^{2+} concentrations resulted in a proportionately reduced hsc/hsp70 response, probably from pathological damage. Surviving individuals retained their capacity to induce stress protein production in the recovery period, even if the stress protein response system was overwhelmed by cadmium during the exposure period (Schill et al. 2003).

6.4.2 Metallothioneins and Lipid Peroxidation as a Biomarker for Metal Exposure and Oxidative Stress

Inducible MT has a key role in maintaining homeostasis and in detoxifying certain substances in aquatic invertebrates and other animals (Schlenk et al. 2000). MT is not only a very sensitive biomarker of exposure to certain metals but also an indicator of cellular stress through its role as a scavenger of organic free radicals and reactive oxygen species (ROS; Viarengo et al. 2000). There have been recent advances in facilitated research on MT in crustacean amphipods (Correia et al. 2001; Correia et al. 2002; Gagné et al. 2005). For example, Cu is needed for normal metabolic function but may become toxic if intracellular concentrations exceed the organism's requirements or detoxication capacity (Dos Santos Carvalho

et al. 2004; Viarengo et al. 2000). The toxicity caused by Cu and other metals may derive from multiple mechanisms, including generation of ROS and induction of various types of oxidative damage, such as peroxidation of unsaturated lipids to cytotoxic lipid hydroperoxides (lipid peroxidation, LP)(Livingstone 2001).

Correira et al. (2002) exposed the marine amphipod *G. locusta* to sublethal concentrations of copper in water (4 days of exposure to 3, 5, and 10 mg/L Cu) or sediment (28 days of exposure to 1, 3, and 6 mg/kg Cu dry weight) and investigated the effects on putative MT and LP levels. In addition, they carried out a time-course exposure study (over 10 days) to a single water-borne concentration of Cu (4 mg/L). MT and LP were quantified by differential pulse polarography and as thiobarbituric acid-reactive malondialdehyde equivalents, respectively (Correia et al. 2002). MT was significantly induced by all water-borne Cu concentrations, but no increase in LP was observed in these animals. In contrast, LP levels increased in the time-course experiment within 1 day of exposure, peaked at 4 days, and returned to control value levels by day 6. Paralleling the decrease in LP, higher levels of MT were observed at days 6 and 10. In *G. locusta* exposed to Cu-contaminated sediments, no increase in MT levels was recorded, but significantly higher levels of LP were seen compared with controls. The observed inverse relationship between putative MT induction and the occurrence of LP indicates that MT may protect against the pro-oxidant effects of Cu. MT and LP may be suitable biomarkers for metal exposure and oxidative stress in gammarids.

6.4.3 Biomarkers to Assess Exoskeleton Integrity and the Molting Process

Exoskeleton integrity and molting can be assessed by measuring the levels of arthropodin, sclerotin, acid-extractable proteins, and chitin in high molecular weight proteins from whole tissues (Gagné et al. 2005). *Gammarus* sp. individuals were collected at four intertidal sites subjected to direct sources of pollution (marinas, ferry traffic, and harbors) and at one site with no direct source of pollution. The levels of pH-dependent extractable protein and chitin in the exoskeletons were used to assess the possible impacts of pollution on exoskeleton integrity and the molting process. Gammarids from contaminated sites had significantly higher levels of extractable proteins in their exoskeletons (i.e., arthropodin, sclerotin, and acid-soluble proteins) and a lower proportion of chitin in their exoskeletons at most impacted sites. It is possible that a disruption of chitin and pH-dependent protein mobilization led to disturbed exoskeleton integrity.

6.4.4 (Acetyl)-Cholinesterase Activity as a Biomarker for Neurotoxicity

Recently, the suitability of acetylcholinesterase (AChE) (Crane et al. 1995) and cholinesterase (ChE) (McLoughlin et al. 2000; Xuereb et al. 2007) activities as biomarkers of neurotoxic stress was investigated in the freshwater amphipod *G. pulex*. For decades, the inhibition of AChE and ChE activities has widely been used as biomarkers for the presence of certain pesticides (organophosphorus and carbamate) in aquatic species (Fulton and Key 2001). Such pesticides elicit their toxicity by inhibiting the activity of AChE or ChE enzymes, which are necessary

to degrade the neurotransmitter acetylcholine in cholinergic synapses. This enzyme inhibition leads to accumulation of acetylcholine and thereby interferes with nerve function, inducing deleterious effects and eventually respiratory failure and death (WHO 1986). ChE activities have long been investigated in fish (Fulton and Key 2001). However, in aquatic invertebrates, classification of ChE isoforms has been investigated much less frequently than in vertebrates (Forget et al. 2002; Garcia-de la Parra et al. 2006; Varo et al. 2002). Moreover, several studies have pointed out the difficulty of using the ChE vertebrate classification scheme (AChE and BChE) for invertebrates, since invertebrate ChE retains characteristics of vertebrate forms and cannot be clearly distinguished from it (Varo et al. 2002).

McLoughlin et al. (2000) evaluated the usefulness of ChE as a biomarker in combination with feeding inhibition and mortality in *G. pulex* after exposing this species to zinc, linear alkylbenzene sulfonate (LAS; surfactant), lindane (organochlorine insecticide), pirimiphos-methyl (organophosphorus insecticide), and permethrin (pyrethroid insecticide). Lethality was the least sensitive endpoint. ChE inhibition was found to be a specific indicator of organophosphate exposure, although it was a considerably less sensitive biomarker (than 13-fold lower) than was feeding rate (McLoughlin et al. 2000). Exposure to 1.92 μg/L of the organophosphate pirimiphos-methyl led to a significant inhibition of enzyme activity after 24 hour, and the same was observed for 0.077 μg/L after a 48-hour exposure. Significant reductions in AChE activity have also been observed after exposing *G. pulex* to 1 μg/L each of the insecticides fenitrothion, parathion, and malathion for 24 hour (Crane et al. 1995; Streit and Kuhn 1994). However, no clear effects on *G. pulex* feeding rates or significant detrimental effects could be observed for the insecticide malathion (Crane et al. 1995). The inhibition of AChE activity by fenitrothion and parathion indicates that tolerance in various *Gammarus* species toward organophosphorus insecticides differs widely; the introduced species *G. tigrinus* showed a higher tolerance compared to the autochthonous species *G. pulex* and *G. fossarum*. This may help to explain recent changes in species composition (Streit and Kuhn 1994).

Xuereb et al. (2007) recently characterized ChE activity in *G. pulex* by using different substrates (acetylthiocholine iodide, propionylthiocholine iodide, and butyrylthiocholine iodide) and selective inhibitors (eserine sulfate, BW284c51, and *iso*-OMPA). The effect of chlorpyrifos, the widely used organophosphorus insecticide, on ChE activity was investigated. The results suggest that *G. pulex* possess only one ChE, which displays the typical properties of an acetylcholinesterase: it hydrolyses the substrate acetylthiocholine at a higher rate than all other tested substrates, and it is highly sensitive to eserine sulfate and BW284c51, but not to *iso*-OMPA (Xuereb et al. 2007). When *G. pulex* was exposed to realistic environmental concentrations of chlorpyrifos, significant AChE inhibition was observed; lethal effects appeared at inhibitions higher than 50%.

To understand impoverished stream communities in agricultural landscapes, Maltby and Hills (2008) used an experimental approach to investigate the effects of the insecticides cypermethrin and chlorpyrifos, which are possible candidates to contribute to such impoverishments. In this study, *G. pulex* were deployed during

the application of the pesticides to the stream edge. The pesticides inhibited ChE enzyme activity, depressed feeding rate, and reduced survival. The authors found no clear insecticide-related effects on macroinvertebrate community structure or on the population densities of individual species. However, the adaptation of a no-spray buffer zone mitigated the individual-level effects (Maltby and Hills 2008).

The results of the above studies show the value of *G. pulex* as a sentinel organism for environmental assessment of sublethal neurotoxicity.

6.4.5 Glutathione-*S*-Transferase Activity as a Biomarker for Detoxification

The glutathione-*S*-transferases (GSTs) represent a major group of detoxification enzymes, and all eukaryotic species possess multiple cytosolic and membrane-bound GST isoenzymes (Hayes and Pulford 1995). Exposure to organochlorine compounds, such as the insecticides aldrin, endosulfan, or lindane, led to the induction of GST (Hans et al. 1993); other inducers are the pyrethroid insecticides such as cypermethrin (Gowland et al. 2002).

McLoughlin et al. (2000) evaluated the usefulness of the GST biomarker response in *G. pulex* after exposing this species to zinc, linear alkylbenzene sulfonate, lindane, pirimiphos-methyl, and permethrin. In addition, the authors evaluated feeding inhibition and mortality. A significant increase in GST enzyme activity occurred after 48 hour for both lindane (6.14 mg/L) and permethrin (0.12 mg/L). Thus, the GST biomarker performed with greater sensitivity but lower specificity, when compared with the ChE biomarker that was investigated in the same study. However, the more sensitive feeding rate was only marginally outperformed by the GST biomarker. The GST biomarker in *G. pulex* may be used as a rapid and sensitive indicator for toxicant exposure, but it has limited use as a diagnostic tool and provides only limited improvement in sensitivity over more ecologically relevant sublethal endpoints (e.g., feeding rate and growth rate) (McLoughlin et al. 2000).

When investigating impoverished stream communities in agricultural landscapes, Maltby and Hills (2008) evaluated how GST activity was affected by exposing *G. pulex* to the insecticides cypermethrin and chlorpyrifos. Contrary to the results on ChE inhibition, there was no significant difference on the GST activity in gammarids exposed to a combination of cypermethrin, isoproturon, and simazine (Maltby and Hills 2008). The reason for this may be that GST induction is less specific (responding to pyrethroid and organochlorine insecticides) and appears to have a longer response time (48 hour of permethrin exposure are needed for a significant induction; McLoughlin et al. 2000). The slow response time may explain why cypermethrin did not induce GST.

6.4.6 ATP Content as a Biomarker for Mycelium Species Composition on Gammarid Diet

The composition of the assemblage of fungi colonizing leaf material may be important in determining its quality as food for shredders like *G. pulex* (Barlocher and Kendrick 1975; Rossi 1985). Determining the species composition of mycelium patches on leaf material may enhance the understanding of fungus–invertebrate

interactions, which are crucial to detritus processing in many freshwater bodies (Arsuffi and Suberkropp 1989; Maltby 1992). Bermingham et al. (1995) developed a monoclonal antibody-based (MAb) immunoassay for the detection and quantification of *A. longissima* that colonize leaf material, which allows for the determination of species-specific mycelium colonization. By using a co-immunization program, MAbs (to *A. longissima)* were raised in mice, then a cell line that produces a MAb of the immunoglobulin M class was cultured. This MAb was specific for *A. longissima,* both in an enzyme-linked immunosorbent assay (ELISA) and by immunofluorescence, but the immunoassay did not recognize other members of the aquatic hyphomycetes (Bermingham et al. 1995). This MAb (AL-HH8c) was then used to develop a quantitative ELISA in vitro. The antigen recognized by AL-HH8c is produced throughout the mycelium, irrespective of mycelial age and culture conditions. By using this MAb, mycelium of *A. longissima* colonizing leaf material can be detected (Bermingham et al. 1995).

7 Exposure Types

Our literature search showed that gammarids are used in different exposure scenarios. Often, similar experimental setups were used in the lab and in situ, with pollutants exposed via the aqueous, dietary, or sediment routes, either continuously or in pulsed exposure regimes (see Table 6).

7.1 Pulsed Exposure Assays and Models

Pulsed exposures of toxicants are often used in ecotoxicological studies because of their ability to describe the dynamics of toxicant exposure, as aquatic organisms experience them, in more detail. In addition, pulsed exposure has the advantage, depending on the test design, to show acute (during the pulse) as well as delayed (during a recovery phase between the pulses and at the end of the exposure) toxicity patterns.

7.1.1 Pulsed Exposure, Uptake, and Elimination of Pesticides in Lab and In Situ

Gammarids are often used in pulsed exposure experiments and seem to be useful organisms to determine uptake and elimination rates, and bioconcentration factors. Such studies were undertaken for chlorpyrifos and PCP by measuring internal concentrations of the two pesticides in *G. pulex* over a 3-day exposure phase and a subsequent 3-day elimination phase (Ashauer et al. 2006). Rate constants were obtained by fitting measured internal concentrations to a one-compartment, single first-order model. The uptake rate constants were 747±61 L/kg/day for chlorpyrifos and 89±7 L/kg/day for PCP. The elimination rate constants were 0.45±0.05 L/kg/day for chlorpyrifos and 1.76±0.14 L/kg/day for PCP. The resulting bioconcentration factors at steady state were 1660 and 51 for chlorpyrifos and PCP, respectively.

Table 6 Different exposure modes used to assess toxicant effects in gammarids

Species	Test substance/ media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	References
Pulsed exposure assays							
G. pulex males and females	Chlorpyrifos (CPF), pentachlorophenol (PCP)	Pulsed exposure, static renewal	3-day exposure and 3-day elimination phase	Uptake rate, elimination rate, bioconcentration factor	*Uptake rate constants*: CPF: 747±61 L/kg/day PCP: 89±7 L/kg/day *Elimination rate const*ants: CPF: 0.45±0.05 L/kg/day PCP: 1.76±0.14 L/kg/day *Bioconcentration factors*: CPF: 1660 PCP: 51	Uptake and elimination rate constants were estimated using ModelMaker	Ashauer et al. (2006)
Sediment toxicity assays							
G. locusta juveniles (2–4 mm)	Estuarine sediment toxicity Control, Muddy: T, P, D, sandy: S1, S2	Static renewal with seawater and sediment samples	28 days	Growth, reproduction, metallothionein (MT), DNA strand breakage	T, P: higher growth rates, improved reproductive traits. D, S1: DNA strand breakage, D: MT induction, S2: loss of DNA integrity, enhanced growth	S1 acutely toxic at 50% dilution, stimulated growth at 75% dilution	Costa et al. (2005) Neuparth et al. (2005)

Table 6 (continued)

Species	Test substance/ media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	References
G. pulex, A. aquaticus (4–7 mm)	PAH-spiked sediment (fluoranthene, pyrene, chrysene, benzofluoranthene) with 30 mg PAH/kg dry weight	Choice experiment. Spiked vs. clean sediment	72 hour	Habitat choice	*G. pulex* and *A. aquaticus* avoided PAH-spiked sediment, the origin of the population (clean reference site or polluted site) did not affect habitat choice	Animals move away from the most polluted spots.	De Lange et al. (2006b)
In situ tests							
G. pulex, A. aquaticus	20 natural streams with differences in pH (4.3–7.5) and humic substances (color range: 8–280 mg Pt/L).	In situ with caged animals	25 days	Animal interactions on survival and physiological status	*G. pulex:* pH < 6.0: increased mortality. lower physiological status *A. aquaticus:* physiological status correlated with pH and significantly affected by humus	Under optimal conditions of high pH and low humus concentrations, species interactions seem asymmetric, where *Gammarus* decreases the survival and physiological status of *Asellus*	Hargeby (1993)

Table 6 (continued)

Species	Test substance/ media	Exposure type	Exposure period	Endpoints/ biomarkers	Effect/effect concentration	Remarks	References
G. pulex adult males	24 reference sites, 15 contaminated sites downstream of point-source discharges	In situ water quality biomonitoring with caged *G. pulex*	6 days	In situ feeding rate, ability of the assay to detect impacts of point-source discharges	Feeding rate inhibition between 27 and 99.6% downstream of point-source discharges	Reference sites: feeding rate strongly influenced by water temperature (76% of the variation) with a 30% feeding inhibition in summer (>90% power)	Maltby et al. (2002)

7.1.2 Pulsed Exposure Models

Ashauer et al. (2006) reviewed, evaluated, and compared two models that may be used to simulate effects, including survival, resulting from pulsed or fluctuating exposures of nontarget organisms to pesticides. The threshold damage model (TDM) and the time-weighted average (TWA) model were capable of simulating the observed survival of *G. pulex* (mean errors 15% or less, r^2 between 0.77 and 0.96) when exposed to two pesticides having contrasting modes of action (PCP and chlorpyrifos). The TDM proved to be particularly useful, because its parameters can be used to calculate recovery times, separate toxicokinetics from toxicodynamics, and parameter values reflect the mode of action (Ashauer et al. 2007b). When predicting the outcome of an exposure of *G. pulex* to carbaryl and using all exposure data (carbaryl, PCP, and chlorpyrifos) for fitting the models, the TDM outperformed the TWA model by facilitating an understanding of the underlying ecotoxicological processes and permitting calculation of recovery times (3, 15, and 25 days for PCP, carbaryl, and chlorpyrifos, respectively). In addition, the TDM enabled the prediction of long-term exposure effects after sequential pulses or fluctuating concentrations. This was also the case for the predicted outcomes of sequential pulsed exposure to carbaryl and chlorpyrifos (Ashauer et al. 2007a). The TDM predicted that recovery of damage to *G. pulex* from exposure to chlorpyrifos takes longer than that from exposure to carbaryl; therefore, the sequence of exposure matters and provides a process-based ecotoxicological explanation for the observed effects (Ashauer et al. 2007a).

7.2 Sediment Toxicity Assays

Gammarids and other benthic macroinvertebrates are often used in sediment toxicity assessment. The use of this species is detailed in guidelines issued by the American Society for Testing and Materials (ASTM 1993) and by SETAC in Europe (Hill et al. 1993). Gammarids are effective and sensitive indicators of ecosystem pollution (Munawar et al. 1989) and a substantial database on the responses of these macroinvertebrates to xenobiotics, nutrients, and other physicochemical perturbations exists (Burton et al. 1992). During their life cycle, gammarids spend extended periods of time in close contact with bottom sediments and in the water column above such sediments, which makes them susceptible to adverse effects lurking in contaminated sediments (Burton et al. 1992).

7.2.1 Sediment Tests with Marine Amphipods

A study was performed to evaluate the performance of *G. locusta* in chronic sediment toxicity tests (Costa et al. 2005). A multilevel assessment of chronic toxicity of estuarine sediments was used to integrate organismal- and population-level endpoints with biochemical marker responses. *G. locusta* were exposed for 28 days to five moderately contaminated sediments designated as follows: muddy: T, P, D;

sandy: S1, S2. The endpoints measured were survival, individual growth, and reproductive traits. Two of the muddy sediments (T, P) induced higher growth rates and improved reproductive traits, possibly because of the amount of organic matter in the sediment, which was nutritionally beneficial to the amphipods, while concurrently decreasing contaminant bioavailability. Biomarker responses did not reveal toxicant-induced stress in amphipods exposed to these sediments. One of the sandy sediments (S1) was acutely toxic at 50% dilution but stimulated amphipod growth at a dilution of 75% (Costa et al. 2005). The two sediments (D, S1) showed pronounced chronic toxicity, which affected survival and reproduction (female-based sex ratio and severely impaired offspring production). The biological marker responses of *G. locusta* showed the following (Neuparth et al. 2005): Two of the muddy sediments (T, P) did not cause chronic toxicity and were consistent with higher growth rates and improved reproduction at the population and organism level. Two other sediments (D, S1) exhibited pronounced chronic toxicity, affected DNA strand breakage, metallothionein induction (D), survival, and reproduction. The last sandy sediment (S2) exhibited some loss of DNA integrity, but growth was enhanced (Neuparth et al. 2005). Potential toxicants that might be responsible for those effects were identified by a weight of evidence method: Observed toxicity in sediment appeared to result from high copper levels. S2 seemed to be less toxic than S1, because it was further away from an industrial effluent at the sampling site and had a lower PAH concentration.

7.2.2 Sediment Assays Combined with Behavior

De Lange et al. (2006b) investigated the avoidance of PAH-contaminated sediments by two freshwater invertebrates, *G. pulex* and *A. aquaticus*. The aim of the study was to assess the effect of PAHs on habitat choice of the amphipod *G. pulex* and the isopod *A. aquaticus*. Therefore, clean field sediment was spiked with a mixture of four PAHs, fluoranthene, pyrene, chrysene, and benzo[*k*]fluoranthene, to a total concentration of 30 mg PAH/kg dry weight each. In laboratory experiments, both species were then offered a choice between PAH-spiked sediments and clean sediments. Results showed that both species avoided PAH-spiked sediment, whereas the origin of the population, either from a clean reference site or from a polluted site, did not affect habitat choice of either species.

7.3 In Situ Tests

7.3.1 Ecological Relevance of In Situ Data

Baird et al. (2007), in a review, examined how the choice of test species and study design employed in the use of in situ approaches in ecological risk assessment can maximize the ecological relevance of data. In situ effect measurement is a rapidly evolving field in ecotoxicology, with a variety of techniques employed to assess responses of ecosystems to toxic substances under field situations. Regarding "ecological relevance," Baird et al. (2007) and others (Crane et al. 2002) have stressed

the value of in situ effect measures as a new and important line of evidence in ecological risk assessment. They provide a framework to define and assess ecological relevance and suggest that the following points should be taken into account for in situ study design:

1. *The test species*: If the in situ assay is used to generate information about effects on a resident biota within a specified area, a species relevant for the area of concern should be chosen (Pereira et al. 2000); moreover, the selected species should be abundant and play a key role in the local food web (Baird et al. 2007). However, when the aim of a study is to evaluate if the site in question is contaminated, a standard test organism may serve equally well. A standard organism may have a background data advantage. The disadvantage of a standard organism is that direct extrapolations to predict effects, within the local community, are not possible. Thus, ideally an "ecologically relevant test organism" should be used (Baird et al. 2007).
2. *The endpoints*: Baird et al. (2007) provide a working definition of an ecologically relevant effect: "It is a direct or an indirect response to exposure, resulting in changes in life-history characteristics that impair or diminish the growth potential of a population such that long-term maintenance of structural or functional qualities of that population, the community to which it belongs, or the ecosystem within which it exists is compromised or threatened".
3. *Relevance of effects*: Effects measured at one level of biological organization (i.e., feeding and metabolic rate) should be translatable into consequences for higher levels of organization (i.e., growth and reproduction), with ideally quantitative and mechanistic linkages to ensure robust extrapolations.
4. *Endpoints vs. protection goals*: Suborganism-level responses, i.e., biomarkers for stress, should be used to assess individual health and provide a useful early warning. Individual-level responses such as growth, behavior, reproduction, and survival should be used to assess individual performance. Population-level responses, for example, the growth of algal populations, should be used to assess population viability. Unfortunately, no animal-based population-level in situ bioassays exist to date. Finally, community-/ecosystem-level responses should be used to assess risks to biodiversity and ecosystem support services.
5. *Extrapolations*: To extrapolate from suborganism-level effects to higher-order consequences, the biomarkers utilized must be essential to normal function and be mechanistically linked to individual health status. To extrapolate from individual-level effects to population-level effects, information on stress-induced effects on vitality rates, measured in situ, can be combined with population models to predict population growth rate and abundance. Extrapolations from community-level effects with attributes of community structure have proven to be of value and can be assessed in situ (e.g., enclosures, recolonization, and transplantation). Such factors include species richness, organism abundance, biomass, and food web (trophic) composition. Similarly, community-level functional responses that are ecologically important can be assessed and include estimates of net primary productivity, carbon sequestration, and nutrient cycling.

6. *Using models*: To maximize the ecological relevance of data obtained from the use of in situ approaches (Baird et al. 2007).

7.3.2 In Situ Survival and Physiological Status

In situ exposures have also been used to assess effects of pH, humic substances, and animal interactions on survival and physiological status of *A. aquaticus* and *G. pulex* (Hargeby 1993). Caged *A. aquaticus* (Isopoda) and *G. pulex* (Amphipoda) were exposed for 25 days in 20 natural streams; these streams had a pH range of 4.3–7.5 and a color range of 8–280 mg Pt/L. In streams with a pH lower than 6.0, *G. pulex* responded with increased mortality and lower physiological status of surviving individuals. In *Asellus* the physiological status was correlated with pH and significantly affected by humus, whereas the mortality was not pH dependent (Hargeby 1993). Under optimal conditions of high pH and low humus concentrations, the interactions between the species appeared to be asymmetric, wherein the presence of *Gammarus* decreased the survival and physiological status of *Asellus.* The presence of *Asellus* did not increase the mortality or decrease the physiological status of *Gammarus,* because *Asellus* feed only on *Gammarus* that solely died from physiological stress. This mechanism suggests that food quality, and thus effects of diffuse competition, may be important in the context of withstanding acid stress. The results, though, give no support for the hypothesis that competition from *Asellus* is important for the disappearance of *Gammarus* as a consequence of stream acidification (Hargeby 1993).

7.3.3 In Situ Feeding Activity and Litter Breakdown

Feeding activity. Maltby et al. (2002) evaluated whether the *G. pulex* in situ feeding assay was useful for water quality biomonitoring. Uncontaminated reference sites were used to quantify background variability in feeding rates of aged *G. pulex* and to elucidate sources of variation. The ability of the assay to detect the impact of point-source discharges was assessed and the ecological relevance of the assay was determined by comparing assay responses to aspects of community structure and function. At the reference sites, feeding rate was strongly influenced by water temperature (76% of the variation), with a 30% feeding inhibition during the summer (>90% power). Downstream of point-source discharges, the inhibition of *G. pulex* feeding rates ranged between 27 and 99.6% (Maltby et al. 2002). These authors also found a strong positive correlation between in situ feeding rate, measured over 6 days, and leaf decomposition rate, measured over 28 days, as well as between in situ feeding and macroinvertebrate diversity and a biotic index. This underscored the importance of *G. pulex* as a detritivore in stream communities. Maltby et al. (2002) concluded that the *G. pulex* in situ feeding assay is a short-term sublethal biomonitor of water quality that is indicative of community- and ecosystem-level responses that occur over longer time periods. It is robust, responsive, and relevant.

Litter breakdown. Litter breakdown is a very suitable endpoint for assessing ecosystem conditions and the influence of multiple anthropogenic stresses imposed

on them (Gessner and Chauvet 2002). Dangles et al. (2004) investigated the impact of stream acidification on litter breakdown and its consequences for assessing ecosystem function. Breakdown rates of the European Beech *Fagus sylvatica* varied more than 20-fold between the most acidified and the circumneutral sites, with stream water alkalinity and total Al concentration accounting for 88% of the variation in litter breakdown rates among streams. Interestingly, the abundance and biomass of the amphipod *G. fossarum*, an acid-sensitive and particularly efficient leaf shredder, showed a strong positive relationship with leaf breakdown rate. A variation of 85% in litter breakdown rates among streams could be accounted for by the combination of *G. fossarum* presence and microbial respiration (Dangles et al. 2004; Dangles and Guérold 2001).

The above results agree with results from Niyogi et al. (2001), wherein increased concentrations of zinc and increased deposition rates of metal oxides from mine drainage were closely related to a reduction in litter breakdown rates. The biomass of shredders was also found to decrease with decreased litter breakdown rates, whereby shredder biomass and microbial respiration together accounted for 76% of the variation in breakdown rates (Niyogi et al. 2001).

Microbial colonization and decomposition of leaves in a stream are also modified by coal ash effluent (Forbes and Magnuson 1981). Leaf surface area and disc weight were greater at the effluent-exposed site than at the reference site, after 96 days. Intercellular enzyme activity (measured by ATP content) of leaves from the reference stream quadrupled between 27 and 96 days, whereas ATP content of effluent-exposed leaves remained low. Macroinvertebrates colonized the leaf packs in the reference site but were not found on or in effluent-exposed packs, possibly as a consequence of reduced colonization and decomposition by fungi.

7.3.4 In Situ Tests as Part of a Whole Effluent Toxicity Study

A complete effluent toxicity (WET) study was conducted to investigate the toxicity and biological impact of a point-source discharge and to identify the in situ exposures to major toxicants of indigenous (*G. pulex*) and standard (*D. magna*). WETs are increasingly used to monitor compliance of consented discharges, but few studies have attempted to relate toxicity, measured using WET tests, to receiving water impacts. Maltby et al. (2000) adopted a four-stage procedure, in which the first stage standard WET tests were employed to determine the toxicity of the effluent, followed by the in situ deployment of *G. pulex* and *D. magna*. Then, biological survey techniques were used in the third stage to assess the impact of the discharge on the structure and functioning of the benthic macroinvertebrate community. Finally, in stage 4, toxicity identification evaluations (TIEs) were used to identify toxic components in the effluent. Maltby et al. (2000) found that receiving water toxicity and the ecological impact detected downstream of the discharge were consistent with the results of WET tests performed on the effluent. *D. magna* survival was reduced downstream of the discharge, as were survival and feeding rate of *G. pulex.* In addition, reductions in detritus processing and biotic indices, based on macroinvertebrate community structure, were found downstream of the discharge,

most probably because of chlorine, which was determined by TIE studies to be the principal toxicant in the effluent (Maltby et al. 2000). With this approach, single species toxicity tests, community-level responses, and TIEs may be appropriately used to investigate effluent impacts.

7.3.5 In Situ Drift Behavior Resulting from Parasites

There is a question as to whether drift behavior of *G. pulex* is negatively affected by parasitism (McCahon et al. 1991). In a *G. pulex* population having approximately 20% *P. laevis*-infected adults, drift was monitored at margin and mid-river sites over a 24-h period. The authors observed a diurnal pattern of drift densities with a large increase at night, independent of parasite burden, and site location did not influence the proportion of parasitized and unparasitized *G. pulex* found in the drift or in the benthos. The drift of parasitized *G. pulex* was significantly greater than unparasitized animals and individuals harboring only one parasite were found in significantly higher proportions in the drift than were those with two or more parasites (McCahon et al. 1991). At both sites, significantly more unparasitized individuals were present in the benthos than in the drift, indicating that *P. laevis* infection alters drift behavior of *G. pulex* (McCahon et al. 1991).

8 Discussion

8.1 Evaluation of Existing Methods

The purpose of this review is to investigate the potential of gammarids as emerging test species for freshwater ecosystem effects (particularly in streams), by collecting available data, methods, and biomarkers on *Gammarus* spp. and then providing an overview of the currently used tests. We reviewed more than 200 publications that address the ecotoxicological effects on gammarids, biological background information, and related topics, and were surprised that so many aspects of gammarid ecotoxicology have already been investigated. The largest portion of the ecotoxicological investigations that have already been performed address acute toxicity testing (21 publications, but there are probably others not listed in Table 1). Lethal effect concentrations (LC_{50}s) were assessed for a diversity of environmental contaminants such as the metals cadmium, copper, zinc; the insecticides fenoxycarb and lindane; the pesticides esfenvalerate, terbutryn, and atrazine; and a herbicide (3,4-dichloroaniline). In all of the studies that were conducted either with juveniles or with adults for periods of 48–264 hour of exposure, juveniles appear to be more sensitive than adults; moreover, LC_{50} concentrations generally decrease with increasing exposure duration. Data on other invertebrates and fish species were also included in some of these. Generally, it appears that gammarids are among the most sensitive of exposed organisms. Compared with EC_{50} values found for dahpnids,

gammarids were more sensitive toward esfenvalerate, lindane, atrazine, and copper. Thus, gammarids not only may be suitable as an additional invertebrate species for use in aquatic ecotoxicology testing, but also, in addition, are both a sensitive species and a stream-dwelling organism. Therefore, they cover a specific niche (streams of the Northern Hemisphere), which is not covered by planktonic daphnia found in lakes and ponds.

The second largest group of studies we reviewed addressed feeding activities of gammarids exposed to metals, antibiotics, insecticides, herbicides, and polluted water samples. Feeding activity proves to be a very sensitive endpoint for assessing general subacute toxicity, particularly for studies employing in situ exposures. One disadvantage may be the need for rather long exposure times when investigating low dose effects of contaminants. In addition, there may be difficulties in distinguishing between acute and chronic effects, and the nonspecific nature of some observed responses. Food choice and post-exposure feeding depression experiments may be good alternatives/extensions for feeding activity tests, because the former provides additional information on possible avoidance behaviors, whereas the latter gives an idea about reversible, sublethal effects after pulsed exposures.

Besides feeding, gammarid behavioral responses, such as locomotory and ventilatory activity, swimming endurance, pleopod beat frequency, and drift response, were also extensively studied. Behavioral responses are also rather nonspecific, when not linked with specific biomarkers, but proved to be very useful in in situ biomonitoring as a sensitive early warning endpoint. This is also the case for studies on population structure, which were employed in in situ comparisons of non-polluted and polluted sites.

In recent years, new studies have emerged on xenobiotics that address mode of action and identify new specific endpoints and biomarkers. For example, the effects of several known vertebrate xenoestrogens were investigated for possible endocrine activity in gammarids. The results indicate that some xenoestrogens possess endocrine potential in gammarids but do not necessarily target the same endpoints. Molting appears to be a central process that is at least partially controlled by the steroid hormone ecdysone, and it can be disrupted by ecdysone-like steroid hormones, as well as vitellogenin-inducing xenohormones. Other promising endpoints for xenoestrogens are sex ratio and gonad histology. Heat shock proteins or ecdysone have been investigated for their usefulness as biomarkers for crustacean endocrine disruption, but more data are needed to understand their roles. Moreover, other specific biomarkers such as stress response, metal exposure, oxidative stress, neurotoxicity, and detoxification have been used successfully for some time now.

In this review, we have summarized a diversity of test strategies with gammarids, ranging from acute to chronic in situ exposures and have covered a multitude of endpoints/effects, such as bioenergetic responses, metabolism, behavioral response, feeding activity, reproduction and population parameters, and in addition numerous specific biomarkers. In the future, the aim will be to combine and extend knowledge already gained in such areas to yield more integrative testing approaches with gammarids.

8.2 Perspectives on a Multimetric *Gammarus* spp. Test System

To date, few studies have incorporated sensitive, multi-stress test systems that use native invertebrate species for assessing freshwater ecosystem health in an integrated manner covering several biological organization levels and different levels of complexity and ecological relevance. Moreover, this gap is not yet filled by test systems proposed by international validation and standardization bodies.

We propose that *Gammarus* as an emerging test species for use in ecologically relevant and integrative aquatic ecotoxicity testing. *Gammarus* can be used in both in situ and ex situ testing, which allows one to link ecotoxicity test results to water quality bioassessment data for key species in European streams. Gammarid species hold a central position in the food web of streams, because they are structurally and functionally important keystone species; gammarids are also present in a variety of other ecosystems, ranging from freshwaters to marine waters. Such breadth of distribution provides opportunity for using them in different ecosystems as test organisms. Next to *D. magna/D. pulex* as representative invertebrate species for lakes, *Gammarus* is accepted in ecotoxicology for toxicity assessment in streams. *Gammarus* and *Daphnia* display similar sensitivities to toxic insult. Gammarids have frequently been used as test organisms in a suite of test methods for investigating different types of specific (MOA-based) and non-specific toxicity endpoints at different organizational levels, both in situ and in the lab.

Use of gammarids in integrative, multilevel elements of aquatic ecotoxicology studies requires that current methods be combined into more complex and sophisticated testing approaches; if properly done, such testing would be capable of assessing several different endpoints in one experiment. For example, the in vitro embryo culture method could be extended by incorporating biomarkers for endocrine disruption. To use *Gammarus* spp. as a standard test organism, it is necessary to improve the culturing methods for gammarids, which have recently been described by Bloor (2009). A steady supply of cultured, healthy embryos, juveniles, and adults is crucial for the development of new biomarkers. A key element in creating new biomarkers, e.g., for endocrine disruption, is to learn more of the sensitivity windows in the gammarid life cycle. With such insight, one can better distinguish between normal and pollutant-induced increases or decreases of selected biomarkers. The ability to maintain a gammarid culture is crucial if they are to become a widely accepted test species that can be used in other experimental setups (e.g., bioaccumulation, long-term, and multigenerational exposures to environmental micro-pollutants), thereby producing data with increased environmental relevance. Gammarids may also be useful for investigating possible relationships between molecular and biochemical biomarkers and endpoints on the physiological and organism level, for example, effects on behavior, histopathology, and morphology. Such operant biomarkers could become cost-effective tools to predict chronic long-term effects at the individual and population level.

9 Summary

The amphipod genus *Gammarus* is widespread and is structurally and functionally important in epigean freshwaters of the Northern Hemisphere. Its presence is crucial, because macroinvertebrate feeding is a major rate-limiting step in the processing of stream detritus. In addition, *Gammarus* interacts with multiple trophic levels by functioning as prey, predator, herbivore, detritivore, and shredder. Such a broad span of ecosystem participation underlines the importance of *Gammarus* spp. in freshwater ecosystems. The sensitivity of *Gammarus* to pollutants and other disturbances may render it a valuable indicator for ecosystem health.

This review summarizes the vast number of studies conducted with *Gammarus* spp. for evaluating aquatic ecotoxicology endpoints and examines the suitability of this native invertebrate species for the assessment of stream ecosystem health in the Northern Hemisphere. Numerous papers have been published on how pollutants affect gammarid behavior (i.e., mating, predator avoidance), reproduction, development, feeding activity, population structure, as well as the consequences of pollution on host–parasite, predator–prey, or native–invasive species interactions. Some biochemical and molecular biomarkers have already been established, such as the measurement of vitellogenin-like proteins, metallothioneins, alkali-labile phosphates (in proteins), and lipogenic enzyme activities for assessing endocrine disruption and detoxification mechanisms.

Despite the range and diversity of studies performed thus far on gammarids, we propose that future gammarid research should address the following aspects to enhance the integrative and multilevel approach outcomes in aquatic ecotoxicology testing:

1. Routine laboratory culturing and reproduction of gammarids seems to be difficult and is, to our knowledge, at the moment only done in one laboratory (Bloor 2009). As a pre-requisite for general use of gammarids in freshwater ecotoxicology, a solution is needed to this problem. Such a solution would allow testing of lab-cultured gammarids with different gender and age classes.
2. Up to the present, most chronic toxicity studies have been limited to short-term exposures. We propose that chronic in situ, ex situ, and pulsed long-term exposures be performed to investigate, for example, bioaccumulation, reproduction, and multigenerational effects of micro-pollutants. Such data are needed for incorporation into ecotoxicological risk assessment databases.
3. More information on the sensitivity windows is needed for existing and new gammarid biomarkers.
4. In future gammarid studies, it would be most useful to intensify the efforts to link biochemical, physiological, and molecular biomarkers to effects on behavior, histopathology, and morphology. Such a linkage would increase the ecotoxicological relevance of the data.
5. Efforts should be made to develop additional biomarkers. For example, in the field of endocrine disrupters, biomarkers are needed to enhance identification of

different EDC effects, such as effects on sexual development, reproduction, and molting. Such biomarkers would be useful in understanding the differences and similarities of endocrine disruption in invertebrates and vertebrates.

For future integrative ecotoxicology testing we suggest that established test procedures, for endpoints like feeding activity, behavior, development, and reproduction, be combined with new state-of-the-art, mode-of-action-based endpoints and biomarkers (i.e., for endocrine disruption and oxidative stress). Such a combination would produce an integrated, modular test system with *gammarids* for use in aquatic ecotoxicity testing. If available, this test system would fill a crucial gap in ecotoxicological assessments (e.g., as an ecotoxicological test system for addition to the water quality assessment within the EU-WaterFramworkDirective). It could become a sensitive, multilevel test system for use with a native invertebrate species for assessing in situ and ex situ freshwater ecosystem health in the Northern Hemisphere.

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