APPLICATION OF POSTEXPOSURE FEEDING DEPRESSION BIOASSAYS WITH DAPHNIA MAGNA FOR ASSESSMENT OF TOXIC EFFLUENTS IN RIVERS

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Abstract—A bioassay that used postexposure feeding depression in Daphnia magna Straus as an endpoint previously had been developed under laboratory conditions. Laboratory studies revealed that this was a sensitive, robust endpoint, which could potentially be used in an in situ bioassay. This study adapted the laboratory bioassay for use in the field and deployed D. magna in situ at four known or suspected contaminated and reference sites. The bioassay was demonstrated to be reliable for use in the field because more than 90% of test organisms were recovered live from the test chambers after exposure allowing feeding rates to be measured after exposure. At each of the contaminated sites, significant depressions in postexposure feeding rates were recorded. Although depressions in postexposure feeding rates were apparent at all contaminated sites, with the exception of Langholm, no impacts were detected on the benthic macroinvertebrate community, when using the Biological Monitoring Working Party scoring system. This demonstrated that during this study, post-exposure feeding depression was a more reliable and sensitive endpoint to use to detect toxicity than were changes in community structure. Therefore, the postexposure feeding depression bioassay can offer a sensitive, robust, ecologically relevant diagnostic endpoint for use in water-quality assessment schemes.

Keywords—in situ bioassay, postexposure feeding depression, Daphnia magna, benthic macroinvertebrates

INTRODUCTION

In situ bioassays with caged, single species have certain advantages over more traditional methods of water-quality assessment, such as whole effluent toxicity tests and biological surveys of the benthic macroinvertebrate community. Key advantages include integration of physical, chemical, and biological processes, which cannot be reproduced in the laboratory, into the test [1]; and elimination of artifacts associated with laboratory testing, such as collection and storage of samples [1]. In situ bioassays also can give a rapid indication of water quality, because effects measured at the individual level often will be manifested more rapidly (hours to days) than resulting changes in community structure (months to years) measured during macroinvertebrate sampling [2].

Many in situ bioassays have been developed that employ fish [3,4], molluscs [5,6], amphipods [1,4,7,8], cladocera [4,9–11], chironomids [8,12], and phytoplankton [13,14] as test organisms. However, most of these use lethality as an endpoint, which may not always be the most sensitive indicator of toxicity. Sublethal, physiological endpoints generally precede lethal responses, and are manifested at lower exposure levels [15].

A recent study by McWilliam and Baird [16] investigated the use of postexposure feeding depression as a novel sublethal endpoint for use in in situ bioassays with Daphnia magna. Feeding rate has already been used as an endpoint in in situ bioassay with Gammarus pulex [7] and could be used as an ecologically relevant endpoint in a D. magna bioassay. Unlike caged G. pulex, measurement of caged daphnid feeding rates is extremely difficult in the field, because microparticles, such as algae, which constitute the primary food source of daphnids, leak from exposure chambers and compromise feeding rate measurements. Although Haney [17] developed a chamber for measuring zooplankton feeding rates in situ, this method would not be suitable for use in a bioassay because it involves the use of radioisotopes and, most importantly, the animals are completely enclosed within the chamber, so they are not continually exposed to the surrounding environment. Exposure to the surrounding environment is essential to gain an accurate assessment of toxicity. To circumvent these problems, the method developed for the postexposure feeding depression bioassay involved a period of contaminant exposure (exposure) followed by immediate transfer to clean medium where feeding rates were measured over a 4-h postexposure period. Laboratory studies demonstrated that postexposure feeding depression, in combination with lethality, was a sensitive, robust, ecologically relevant endpoint that had potential use for application in in situ testing [16].

This study aimed to adapt the postexposure feeding depression bioassay developed in the laboratory for use in the field and investigate whether it was suitable for field deployment by examining the recovery rate of live animals from in situ chambers after exposure. Sufficient numbers of live animals would have to be obtained after the exposure period to allow the endpoint to be measured after exposure. This study also aimed to investigate the sensitivity of the postexposure feeding depression endpoint in situ by deploying the bioassay at known or suspected contaminated sites and measuring any depressions in postexposure feeding rate. To give insight into how the sensitivity and reliability of the bioassay compared to more traditional methods of in situ bioassessment, results from bioassay deployments were compared to benthic macroinvertebrate community data to observe whether any correlation could be made between data obtained with different methods. In the United Kingdom, the Biological Monitoring Working Party (BMWP) scoring system is used to assess impacts on the benthic macroinvertebrate community [18], and...
is routinely used to assess water quality by environment agencies. The scoring system is based on pollution tolerance of macroinvertebrate families and involves identification of macroinvertebrates to family level and calculation of a BMWP score (additive score of all families sampled) and an average score per taxon (ASPT; the BMWP score divided by number of scoring taxa). Although this is a robust, simple, and rapid method of bioassessment, it does not take into account the effects of physical habitat and habitat stress upon the distribution and abundance of the benthos. This method also may lack precision because identification of macroinvertebrates to family level only could decrease sensitivity and increase failure to detect impacts. Furthermore, community-level effect measures are insensitive to sublethal levels of stress [19], so impacts from low levels of contaminants may be missed. Suitability of the bioassay for field deployment and sensitivity of the postexposure feeding depression endpoint were investigated by deploying D. magna in test chambers in streams in four areas known or suspected to be contaminated, and measuring recovery and survival rates, postexposure feeding rates, and impacts, if any, on the benthic macroinvertebrate community.

**MATERIALS AND METHODS**

**Study areas**

In situ deployments were carried out in four study areas in central and southern Scotland (UK) from 1999 to 2000. Each study area included at least one reference site, and one or more suspected or known contaminated sites, usually situated downstream of the reference site. After consultation with the Scottish Environmental Protection Agency (SEPA, Stirling, Scotland), study areas were selected where previous studies conducted by the SEPA had found evidence of contamination within the benthic community.

**Cumbernauld, August 1999.** Deployment took place at three sites along a stream, all of which were wooded on both banks. The channel width ranged from 110 cm with an average depth of 7 cm at the upstream reference site, to 250 cm with an average depth of 18 cm at the two downstream contaminated sites (designated as site 1 and site 2). The substrate was stony at the reference site and silty at the downstream sites. Suspected contaminants were discharged into the stream through a 1.8-m culvert (~10 m downstream of the reference site), from which water drained in from a nearby road.

**Leadhills, August and September 1999.** The Leadhills study area included three sites. The reference site was situated on an uncontaminated tributary of the River Clyde and was surrounded by open moorland. The channel width was 6 m, with an average depth of 50 cm, and a stony sediment bed. The two contaminated sites were situated along another tributary of the River Clyde, and the catchment area around this stream received several inputs of lead mine drainage. Analytical surveys carried out by the SEPA found that the main metal contaminants were Pb and Zn, with Cd, Cr, Ni, and Cu also present at low levels. The first contaminated site (site 1) was surrounded by open moorland, and the second contaminated site (site 2), located 7.5 km downstream, was surrounded by sheep farming pasture. The channel width ranged from 70 cm, with an average depth of 12 cm at site 1, to 4 m with an average depth of 35 cm at site 2. The stream channel had a stony sediment bed. Two deployments were carried out in this area, although results were only obtained from the reference site during the second deployment.

**Langholm, August 1999.** The study area at Langholm consisted of two sites on the River Esk. The contaminated site (site 1) was situated approximately 500 m downstream of the reference site, and both sites were surrounded by urban area on one bank and farmland on the other. The channel width ranged from approximately 10 m with an average depth of 16 cm at the reference site, to approximately 15 m with an average depth of 18 cm at site 1. The channel had a boulder sediment bed. The suspected contaminant in this study area was permethrin, which was discharged in sewage treatment plant effluent approximately 250 m downstream of the reference site.

**Stewarton, April 2000.** The study area consisted of four sites along the Annick Water, a river running through the town of Stewarton. Two discharges occurred into the river, the first from a wool-processing mill, which contained permethrin, and the second from a sewage treatment plant [20]. Deployment took place at two reference sites upstream of the discharges and two contaminated sites, of which one was downstream of each discharge. However, no results were obtained from the first reference site, so details of only three sites are given. The second reference site was on the outskirts of the town center, 600 m upstream of the wool-processing mill. This site was situated next to a knitwear factory and it was not known if this factory was currently discharging contaminated effluent. The first contaminated site (site 1) was in the center of the town, just below the wool-processing mill. The second contaminated site (site 2) was surrounded by farmland and was located approximately 4 km downstream of the reference site and 1.5 km downstream of the sewage treatment plant discharge. The channel width was approximately 6 m with an average depth of 27 cm and a gravel sediment bed at all sites.

**Physicochemical parameters**

The following physicochemical parameters were measured for each site at the start and finish of the exposure period: flow rate, temperature, dissolved oxygen, pH, conductivity, alkalinity, total hardness, and total suspended solids. Postexposure feeding temperatures were measured by recording ambient air temperature. Differences in postexposure feeding temperatures could affect baseline feeding rates. Flow rate was measured with an Ott current meter (Ott Hydrometrie, Kempten, Germany). Dissolved oxygen was measured with a Yellow Springs Instrument model S7 oxygen meter (Yellow Springs Instruments, Yellow Springs, OH, USA). The pH was measured with a Philips PW 9409 digital pH meter (Philips, Cambridge, UK), and conductivity was measured with a Hach conductivity meter (Hach, Loveland, CO, USA). Duplicate 1-L water samples were collected in 1-L polyethylene sample bottles for later alkalinity, total hardness, and total suspended solids analysis in the laboratory. Alkalinity was measured with a colorimetric method given in Golterman et al. [21], and total hardness was measured with a colorimetric method and kit supplied by Merck (method sheet 1009/MP/1-0/1177, Poole, UK). Total suspended solids were measured by filtering 1 L of sample water by using a suction pump fitted with a 200-µm, 47-mm-diameter cellulose nitrate membrane filter (Whatman, Maidstone, UK). Total suspended solids were weighed to the nearest 0.1 mg.

**Test organisms: Culture conditions and acclimation**

Daphnia magna (clone F [22]) were maintained in bulk culture as described in McWilliam and Baird [16]. Animals
used for bioassay deployments were acclimated as described in McWilliam and Baird [16], until 4 d old.

**Deployment of in situ postexposure feeding rate bioassay**

Four-day-old *D. magna* were transported to field sites in groups of 10 in 175-ml screw-capped glass jars containing medium from the acclimation tanks. All field sites were less than a 3-h drive from the laboratory. The in situ chambers used to deploy *D. magna* (see Fig. 1) were constructed from clear polyvinyl chloride cylindrical piping (13 cm long, 5.0-cm external diameter). Each chamber had two rectangular windows (7 × 3.5 cm) cut into either side of the cage, covered with 150-µm nylon mesh. Pipe ends were sealed with polypropylene caps [9].

At each site, five chambers, each containing 10 *D. magna*, were placed inside a 13-mm² wire-mesh cylinder that was positioned in the stream perpendicular to flow. After 24 h, animals were retrieved and five surviving animals were placed into 60-ml screw-capped glass jars containing 60-ml of American Society for Testing and Materials hard water [23], with *Chlorella vulgaris* (Beijerinck, strain CCAP C211/12) at a concentration of 5 × 10⁵ cells/ml. Three jars contained no animals and were used to establish initial algal densities. Animals were allowed to feed for 4 h in darkness, because this has been found to produce more uniform feeding rates [24], after which they were removed from the jars. Preliminary experiments demonstrated that mortality-induced, reduced-crowding–increased feeding-rate artifacts were not a problem because the stocking rate was fixed at five *D. magna* per 60 ml of medium (one animal per 12 ml of medium) and no mortalities occurred during the postexposure period [25].

The mean percentage of animals (dead and alive) recovered from the chambers after the exposure period was calculated to observe whether any animals were lost during exposure or through the process of transferring into and out of the cages. The mean percentage of recovered animals surviving after the exposure period also was calculated. Algal density was measured with a Coulter Multisizer, fitted with a 70-µm orifice tube (Coulter Electronics, Luton, UK), and postexposure feeding rates were calculated as in McWilliam and Baird [16]. Within each study area, postexposure feeding rates at contaminated sites were compared to feeding rates at reference sites by Student’s one-way two-sample *t* test [26].

**Benthic macroinvertebrate community sampling**

The benthic macroinvertebrate community was sampled three times at each study site with a standard pond net made of 0.5-mm mesh. Each kick sample was taken over a 3-min period and all microhabitats were sampled. Invertebrates sampled were preserved in 90% (v/v) ethanol and were identified to family level and counted. Data were then used to compute BMWP and ASPT scores with Species Diversity and Richness software (Ver 2, Pisces Conservation, Lymington, UK).

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**Table 1. Mean percentage (%) recovery of *Daphnia magna* from in situ chambers after exposure and mean % survival of recovered *D. magna* after exposure at each study site in Scotland (UK)**

<table>
<thead>
<tr>
<th>Study area</th>
<th>Site</th>
<th>% D. magna recovered from cages (mean ± SE)a</th>
<th>% Survival of recovered D. magna (mean ± SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumbernauld</td>
<td>Reference</td>
<td>96 ± 3</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Site 1</td>
<td>98 ± 0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Site 2</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Leadhills</td>
<td>Reference</td>
<td>92 ± 8</td>
<td>98 ± 2</td>
</tr>
<tr>
<td></td>
<td>Site 1a</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Site 1b</td>
<td>88 ± 12</td>
<td>76 ± 15</td>
</tr>
<tr>
<td></td>
<td>Site 2a</td>
<td>78 ± 12</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Site 2b</td>
<td>100</td>
<td>98 ± 2</td>
</tr>
<tr>
<td>Langholm</td>
<td>Reference</td>
<td>98 ± 5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Site 1</td>
<td>92 ± 11</td>
<td>88 ± 11</td>
</tr>
<tr>
<td>Stewarton</td>
<td>Reference</td>
<td>90 ± 10</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Site 1</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Site 2</td>
<td>80 ± 12</td>
<td>53 ± 17</td>
</tr>
</tbody>
</table>

a SE = standard error.
b First deployment.
c Second deployment.

Pearsons product–moment correlation analyses [26] were performed on benthos and postexposure feeding rate data (one with BMWP scores versus feeding rate, and one with ASPT vs feeding rate) to examine the strength of the relationship between these indicators of toxicity.

**RESULTS**

**In situ recovery and survival rates**

Mean recovery of *D. magna* (dead and alive) from the in situ chambers was 90% or higher at most sites after the 24-h exposure period (Table 1). Lower recovery rates were found at Leadhills site 1 after the second deployment (78 ± 12%), and at Stewarton site 2 (80 ± 12%). Mean survival rates of *D. magna* recovered from the in situ chambers were also high at most study sites (≥90%; Table 1). Lower survival rates were recorded at Leadhills site 2 (76 ± 15%) after the first deployment and at Stewarton site 2 (53 ± 17%).

**In situ postexposure feeding rates**

Figure 2 shows postexposure feeding rates of *D. magna* after deployment at field sites. Animals deployed at Cumbernauld (Fig. 2a) and Langholm (Fig. 2c) both exhibited significantly lower feeding rates at contaminated sites when compared to reference sites (*p* = 0.02–0.04). At Cumbernauld, feeding rates decreased from 5.66 ± 1.1 × 10⁵ cells/individual/h at the reference site to 3.89 ± 0.93 and 3.87 ± 1.83 × 10⁵ cells/individual/h at contaminated sites 1 and 2, respectively. At Langholm, feeding rates decreased from 4.85 ± 0.61 × 10⁵ cells/individual/h at the reference site to 3.83 ± 0.52 × 10⁵ cells/individual/h at site 1.

Although data were only obtained from the Leadhills reference site for one deployment, they were used to compare to all feeding rate data from contaminated sites in this area (Fig. 2b). During the first deployment, feeding rates at both contaminated sites were significantly lower than those at the reference site (*p* = 0.003–0.007). Feeding rates decreased from 5.48 ± 1.0 × 10⁵ cells/individual/h at the reference site to 2.06 ± 0.74 and 2.54 ± 0.65 × 10⁵ cells/individual/h at site 1 and site 2, respectively. In the second deployment, feeding
rates at the contaminated site did not differ significantly from those at the reference site \( (p = 0.08–0.15) \).

Figure 2d shows postexposure feeding rates of animals deployed at Stewarton. Only animals exposed at site 2 had feeding rates that were significantly lower than those at the reference site \( (p = 0.02) \). Here, feeding rates had decreased from \( 3.17 \pm 0.08 \) \( \times 10^5 \) cells/individual/h at the reference site to \( 0.81 \pm 0.17 \) \( \times 10^5 \) cells/individual/h at site 2. Chambers exposed to air at the end of the exposure period were excluded from the bioassay; therefore, feeding rates at the reference site were a mean of four replicates and feeding rates at site 2 were a mean of three replicates.

**Benthic macroinvertebrate community sampling**

The Appendix lists benthic macroinvertebrates sampled at reference and contaminated field sites. Many taxa were present at both reference and contaminated sites. Taxa present only at reference sites consisted of a mixture of high- and low-scoring individuals in each study area. This also was observed for families present only at contaminated sites in all study areas. Overall, no marked difference was found between reference site and contaminated site BMWP scores (Table 2). For both Cumbernauld and Langholm, BMWP scores from reference sites were lower than BMWP scores from the contaminated sites. The ASPT values for Cumbernauld were similar to those for the reference site and site 2 (4.67, 4.57, respectively) but were higher (5.25) at site 1. At Langholm, the reference site ASPT was higher than at site 1. The number of taxa sampled was similar for reference and contaminated sites at both Cumbernauld and Langholm. At Leadhills, BMWP scores were higher at the reference site (site 1) than at site 2 (72), than at site 1 (59). The ASPT was highest at site 1 (6.56). The reference site had the highest number of taxa sampled. The BMWP scores and ASPT were similar for the reference site (38 and 5.43, respectively) and site 1 (34 and 5.67, respectively) at Stewarton. Site 2 had the highest BMWP score (64), the lowest ASPT (5.33), and the highest number of taxa sampled.

Pearsons product–moment correlation analyses performed on benthos and postexposure feeding rate data did not reveal a significant relationship between feeding rate and BMWP score \( \left(r = -0.26, \ p = 0.39, n = 13\right) \), or between feeding rate and ASPT \( \left(r = -0.13, \ p = 0.68, n = 13\right) \).

**DISCUSSION**

Results of this study demonstrated that *D. magna* can be successfully deployed in situ. At most sites, recovery and survival rates of recovered animals were high \( (\geq 90\%); \) see Table 1). Both of these parameters were important in determining the ability of the bioassay to be conducted under field conditions, because sufficient live animals were required after the exposure period to complete the bioassay.

Lowest recovery rates of animals from the test chambers were found at Leadhills site 1 (second deployment, 78%) and at Stewarton site 2 (80%). Animals that were not recovered after the exposure period most likely were lost during transfer into or out of the chambers. Lowest survival rates were recorded at Stewarton site 2 (53%). Daphnid survival at other sites in this area was 100%. Stewarton site 2 was downstream of the wool-processing mill and sewage treatment plant effluents, which might account for the low survival at this site. However, during the exposure period, the cage containing the chambers had been removed from the water (and then returned), which may have exposed the daphnids to air for some time. This could have contributed to mortalities.

Recovery and survival rates were high at most sites \( (\geq 90\%); \) so the bioassay was suitable for use in situ deployments. Ten animals was an adequate number to place in each chamber to compensate for loss of animals during the exposure period, because only five were needed to complete the postexposure feeding test.

The bioassay also was effective in detecting depressions in postexposure feeding rates. At both Cumbernauld (Fig. 2a) and Langholm (Fig. 2c), postexposure feeding rates at contaminated sites were significantly lower than at reference sites \( (p = 0.02–0.04) \).

Results from the reference site in the Leadhills study area were obtained only during the second deployment, although they were still used to compare to data from contaminated sites for both deployments. Feeding rates at reference sites were consistent and always between 4.5 \( \times 10^5 \) and 6 \( \times 10^5 \) cells/individual/h (Fig. 2a to c). For this reason, the assumption was made that feeding rates for the reference site during the first deployment would have been similar to that of the second deployment. By using the assumed feeding rate, a comparison...
was made between reference and contaminated sites, even though deployments took place at different times. (During development of this bioassay, the observation was made that baseline feeding rates [control feeding rates] consistently were between \(4 \times 10^3\) and \(6 \times 10^3\) cells/individual/h whether measured in the laboratory [16] or in the field.) During the first deployment, postexposure feeding rates were significantly lower (see Fig. 2b; \(p = 0.003–0.007\)) at both contaminated sites, compared to feeding rates at the reference site.

Postexposure feeding rates at contaminated sites during the second deployment were not significantly lower than those at the reference site (\(p = 0.08–0.15\)). The most likely explanation for the lack of reduction in postexposure feeding rates during the second deployment was dilution of the metal contaminants from mine drainage. Stream discharge rates were greater during the second deployment and increased from 0.13 to 0.55 m/s at site 1 and from 38.4 to 42.0 m/s at site 2, from the first to second deployments, respectively.

Feeding rate data obtained from the Stewarton study area were inconclusive because data were not obtained from the first reference site. Although data were obtained from the second reference site, feeding rates should be interpreted with caution because this site was downstream of a knitwear factory, which may have contributed contaminated effluent. Problems occurred while deploying in this study area because of a drop in water level (from an average of 32 cm to 21 cm) over the 24-h exposure period. This decline left some of the cages partially exposed to air and 100% mortality occurred at the first reference site (no data are shown for this site). The cage deployed at site 2 had also been removed from the river for an unknown period of time, then replaced. This situation illustrated that vandalism can be a limitation to some types of in situ assays.

Postexposure feeding rates at all sites in the Stewarton study area were low (Fig. 2d), and feeding rates at the reference site were low compared to feeding rates at other reference sites in this study. Lower feeding rates were not due to lower ambient temperatures during the postexposure feeding period because temperatures measured at Stewarton (15.5–16°C) were within the range of temperatures measured during the postexposure feeding period at other field sites (13–17°C). The lower feeding rates could have been due to contaminant-induced feeding depression (from possible contaminated effluent at the knitwear factory), the combination of high flow rates and the presence of suspended solids, or both. Studies by the authors on the effect of various environmental parameters on postexposure feeding rates (which included temperature, water hardness, and pH) found that only high flow rates in the presence of suspended solids had negative effects on postexposure feeding rates [25]. The combination of high flow rates and the presence of suspended solids could have accounted for the low feeding rates at all sites in the Stewarton area. The average flow rate in the Stewarton area was 26 m/s and average levels of total suspended solids were 29 mg/L. Feeding rates and survival at site 2 were the lowest measured (0.8 \(\times 10^3\) cells/individual/h and 53%, respectively). These values were significantly lower than those for the reference site (\(p = 0.02\)). This could have been due to effluent toxicity or high flow rates and the presence of suspended solids. However, the cage from this site had been out of the water for an unknown period of time, which may have contributed to the low feeding rates as well.

The Appendix shows the families of benthic macroinvertebrates collected at each site, and Table 2 shows the respective BMWP scores and ASPT. The ASPT was more appropriate than the BMWP score for site assessments because it is less sensitive to sampling effort and can be predicted with greater reliability [27]. From Table 2, it can be seen that ASPT were, with the exception of Langholm, similar for all sites within each study area; therefore, depressions measured in Daphnia feeding rates at these contaminated sites did not correlate with impacts on macroinvertebrate communities (\(r = -0.13, p = 0.68\)). This outcome was due to both reference and contaminated sites containing a mixture of high-scoring families, such as Leuctridae, and low-scoring families, such as Chironomidae (Appendix). Only at Langholm did the reference site have a higher ASPT than the contaminated site, which allowed a depression in postexposure feeding rate to be linked to macroinvertebrate community impacts. Previous SEPA studies in the Leadhills area noted little discernible impact on benthic macroinvertebrate community structure (although elevated metal concentrations were found in Gammarus sp.; R. Doughty, SEPA, personal communication), which was similar to results obtained in this study. This may have indicated adaptation of the benthos to contaminants in this area. Studies conducted in 1994 at Stewarton recorded impacts on the benthic macroinvertebrate community at contaminated sites by using the BMWP scoring system [20]. No impact was measured in this study based on the BMWP score and ASPT, which could indicate recovery in this system due to a reduction in toxicity (postexposure feeding depression measured in this area may then not be due to toxicity), or adaptation of the benthos to contaminants. The lower BMWP score and ASPT (28 and 4.67, respectively) obtained in this study, compared to a SEPA study score (99 and 6.6, respectively) measured in 1991 at the Cumbernauld reference site, may indicate recent water-quality deterioration. However, differences in scores also might have been due to different sampling procedures being used. The BMWP score (and to a lesser extent, the ASPT) can be increased by increasing sampling effort. Additionally, BMWP scores depend on the type of river sampled, the method of sampling, type of marginal area, and the individual carrying the bioassay operator error, allowing a more reliable assessment of toxicity. A lack of correlation between postexposure feeding depression and macroinvertebrate community structure at most sites demonstrated key advantages that this bioassay has over biological survey techniques. The standardized method should rule out inconsistencies in deployment methods and operator error, allowing a more reliable assessment of toxicity to be obtained. This is a distinct advantage over the use of biological survey techniques. The standardized method used should rule out inconsistencies in deployment methods and operator error, allowing a more reliable assessment of toxicity to be obtained. This is a distinct advantage over the use of biological survey techniques. The standardized method should rule out inconsistencies in deployment methods and operator error, allowing a more reliable assessment of toxicity to be obtained. This is a distinct advantage over the use of biological survey techniques.
community structure because of adaptation to contaminated conditions. The use of a sublethal endpoint allows toxicity to be detected at lower levels than would be measured by mortality alone. ABiological survey relies on the disappearance of individuals to induce changes in community structure to detect impacts. Biological indices often are insensitive to lower levels of toxicity, where changes in functional processes such as energy flow may not be apparent from species occurrence data. A further advantage of the postexposure bioassay was sensitivity to natural stressors such as flow rate and suspended solids, as demonstrated by postexposure feeding rates obtained from the Stewarton study area. Therefore, the bioassay could be of use in more comprehensive assessments of water quality where both natural and anthropogenic stressors are present. An in situ bioassay employing feeding as an endpoint in Daphnia sp. could provide ecologically important information in water-quality assessments. Daphnia sp. provide an important link between ecosystem trophic levels [28,29] and toxic impairment of daphnid feeding rate has been well documented to result in direct changes in growth and reproduction of Daphnia sp. [30,31] and indirect effects on community structure, leading to great increases in phytoplankton biomass in ponds, lakes, and reservoirs [32,33]. Therefore, information gained from short-term bioassay deployments may provide insight into the mechanisms of long-term community structure alterations, or give warning of potential contaminant-induced impacts at the community level. This bioassay may be usefully applied in water-quality assessment schemes, where it could be employed alongside more traditional methods of water-quality assessment to provide a set of diagnostic tools. The in situ bioassay could complement whole effluent toxicity tests by reducing the uncertainty of laboratory to field extrapolations, and could increase the reliability of in situ bioassessment data by offering a robust and sensitive tool for detecting toxicity in the field [34]. Therefore, results obtained from bioassay deployments could provide invaluable data when integrated into toxicity assessment schemes offering a weight-of-evidence approach to water-quality assessment.

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REFERENCES
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**APPENDIX**

Benthic macroinvertebrates present at reference and contaminated sites in Scotland for each study area sampled

<table>
<thead>
<tr>
<th>Study area</th>
<th>Present at reference site, absent at contaminated sites</th>
<th>Present at both reference and contaminated sites</th>
<th>Absent at reference site, present at contaminated sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumbernauld</td>
<td>Baetidae, Hydropsychidae</td>
<td>Gammaridae, Leuctridae, Oligochaeta, Chironomidae</td>
<td>Planorbidae, Sericostomatidae, Limnephilidae, Glossiphoniidae, Pisidium</td>
</tr>
<tr>
<td>Leadhills</td>
<td>Oligochaeta, Dytiscidae, Hydrobiidae, Neritidae, Ancyliidae</td>
<td>Beraeidae, Limnephilidae, Sericostomatidae, Gammaridae, Leuctridae, Baetidae, Chironomidae, Elminthidae, Polycentropidae, Tipulidae</td>
<td>Goeridae, Hydropsychidae, Rhyacophilidae, Halilidae, Perlodidae</td>
</tr>
<tr>
<td>Langholm</td>
<td>Baetidae, Ancyliidae, Elminthidae</td>
<td>Leuctridae, Perlodidae, Chironomidae, Gammaridae, Oligochaeta, Limnephilidae, Hydropsychidae</td>
<td>Erpobdellidae, Glossiphoniidae</td>
</tr>
<tr>
<td>Stewarton</td>
<td>Ecdyonuridae, Elminthidae, Chaoboridae</td>
<td>Leptophlebiidae, Leuctridae, Perlodidae, Chironomidae, Gammaridae, Baetidae, Oligochaeta, Ancyliidae</td>
<td>Hydrobiidae, Limnephilidae, Erpobdellidae, Rhyacophilidae, Hydropsychidae, Philopotamidae, Glossiphoniidae</td>
</tr>
</tbody>
</table>