Aquatic Invasions (2010) Volume 5, Issue 1: 49-57
This is an Open Access article; doi: 10.3391/ai.2010.5.1.7

Proceedings of the 16th International Conference on Aquatic Invasive Species (19-23 April 2009, Montreal, Canada)

Research article

Zebra mussels (*Dreissena polymorpha*) are effective sentinels of water quality irrespective of their size

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Received: 4 March 2010 / Accepted: 24 March 2010 / Published online: 27 March 2010

Abstract

Zebra mussels (*Dreissena polymorpha*) are recognised biomonitors in determining the presence and viability of the human waterborne pathogens *Cryptosporidium parvum*, *C. hominis*, *Giardia intestinalis* and microsporidia in surface waters. This study investigated whether the size of zebra mussels is a significant factor in the concentration of protozoan *Cryptosporidium* oocysts, *Giardia* cysts and microsporidian spores. Zebra mussels were collected in Lough Arrow, a small Irish lake, which is utilized for drinking water abstraction and is subject to agricultural and human wastewater pollution drivers, both recognised risk factors for human waterborne pathogens. Zebra mussels were cleaned, divided into size (5 mm) interval classes based on their shell length and made up to 150 g samples (wet weight with shell). Combined fluorescence in situ hybridization (FISH) and immunofluorescent antibody (IFA) techniques were utilized as biomolecular techniques to assess the presence and concentration of the pathogens. PCR analysis provided source-tracking information on human and animal pollution sources. There was no significant relationship between the size of *D. polymorpha* and pathogen loads in similar sized samples, indicating that different sites in the same or different waterbody can be compared in terms of relative concentrations of human waterborne parasites irrespective of the zebra mussels' size. *Cryptosporidium* was the most abundant species, with lower counts of *Giardia* and the microsporidian *Encephalitozoon hellem*, respectively. *Cryptosporidium* oocysts and *Giardia* cysts were detected in zebra mussel samples at all three lake water abstraction points. A lake transect showed a decline in *Cryptosporidium* with increasing distance from a stream discharging sewage. Samples from agricultural sites indicated faecal inputs contaminated with these pathogens. Species identification implicated both human and animal faecal inputs to the lake from treated effluent, septic tanks, and agriculture. The research demonstrates the efficacy of zebra mussels as sentinels of water quality irrespective of their size.

Key words: Lough Arrow, *Cryptosporidium*, *Giardia*, *Encephalitozoon hellem*, *Enterocytozoon bieneusi*, Water Framework Directive

Introduction

Lough Arrow, Co. Sligo, Ireland (54°01′N, 08°19′W) is a small freshwater limestone lake (12.5 km^2), renowned as a fishery for brown trout *Salmo trutta* Linnaeus, 1758. It is also used for drinking water abstraction (three abstraction points) and has two small municipal wastewater treatment plants discharging treated effluent into inflowing streams (Figure 1).

The Irish invasion of zebra mussels *Dreissena polymorpha* (Pallas, 1771) is recent (McCarthy et al. 1998; Lucy 2009), and was first recorded in this lake in 2003 (Lohan 2004). By 2006, zebra mussel populations in the lake had grown exponentially, with a high biomass on stony substrates, native unionid mussels (*Anodonta anatina* Linnaeus, 1758) and aquatic plants. Although there have been no intensive population surveys for Lough Arrow, zebra

mussel numbers are likely to exceed those in a smaller neighboring lake, Lough Key (9 km^2, population estimate, thirty-three billion *Dreissena*) (Lucy et al. 2005; Lucy 2006). While long-term assessments have classified the lake as mesotrophic; this trophic status has now been reassessed as oligotrophic (2004-2006 assessment) due to low chlorophyll levels and increased transparency following the zebra mussel invasion (EPA 2008a).

While increased transparency may be perceived as an improvement in water quality, it is known that this lake in common with many other Irish lakes is subject to organic pollution inputs, namely agricultural wastes and sewage effluent from municipal wastewater treatment plants (WWTPs) and septic tanks, with consequent important implications for human health due to both farm animal and human-derived faecal contamination (Graczyk et al. 2008; Lucy et al. 2008). Human protozoan parasitic enteropathogens include *Cryptosporidium*, *Giardia* and microsporidia, all of which produce a long-lasting and environmentally-resistant infectious stage, i.e., oocysts, cysts, and spores, respectively that can be transmitted via water (Graczyk et al. 2007).

Cryptosporidium is a life threatening protozoan parasite in immunocompromised patients, and is known to be one of the most serious causes of waterborne-etiology diarrhoea (Graczyk et al. 2008; Tzipori and Ward 2002). *Giardia* and microsporidia are also important in terms of human pathogenicity (Wolfe 1992; Thompson et al. 1993; Didier et al. 2005). These parasites pollute surface waters via wastewater discharges, leaky septic tanks, urban runoff, recreational activities, sewage sludge spreading on fields, and agricultural runoff predominantly from livestock operations (Graczyk et al. 2007, 2008; Lucy et al. 2008; Cheng et al. 2009), and are known to be widespread in Irish aquatic environments (Graczyk et al. 2004, 2007; Lucy et al. 2008). Irish studies have detected *Cryptosporidium* Tyzzer, 1907 (Chalmers et al. 1997; Skerrett and Holland 2000; Lowery et al. 2001), *Giardia intestinalis (*Stiles, 1915) (Graczyk et al. 2004; Lucy et al. 2008), *Encephalitozoon hellem* Didier, 1991*, Encephalitozoon intestinalis* Cali, Kotler, Orenstein, 1993 and *Enterocytozoon bieneusi* Desportes, Le Charpentier, Galian, Bernard, Cochand-Priollet, Lavergne, Ravisse, Modigliani, 1985 (Graczyk et al. 2004; Lucy et al. 2008) in Irish river basins.

Multiple studies have demonstrated that filter-feeding bivalves including zebra mussels can harbor these environmentally derived protozoan parasites as a result of concentrating the recovered particles (Graczyk et al. 1999, 2001, 2003, 2004; Graczyk and Schwab 2000; Lucy et al. 2008). *Cryptosporidium* has already been detected in Lough Arrow, using zebra mussels in 2007 (Lucy et al. 2008).

Because *Cryptosporidium parvum* Tyzzer, 1912, *G. intestinalis*, and microsporidia can infect a variety of non-human hosts, identification of human-specific species represents a challenge. A further demand is to determine the viability of these environmentally-recovered pathogens as they may be non-viable and thus, not of epidemiological importance. Both of these challenges are met by using the fluorescence in situ hybridization (FISH) technique. FISH employs fluorescently labelled oligonucleotide probes that target species-specific sequences of 18S rRNA, leading to identification (Hester et al. 2000; Graczyk et al. 2003, 2004). The FISH technique has been developed for *C. parvum* (Vesey et al. 1998), *G. lamblia* (*intestinalis*) (Dorsch and Veal 2001), *E. hellem* (Hester et al. 2000), *E. intestinalis*, and *E. bieneusi* (Graczyk et al. 2004). Furthermore, FISH has been combined with direct immunofluorescent antibody (IFA) against the wall antigens of *Cryptosporidium* and *Giardia*, and this approach has been successful for detection of *C. parvum* and *C. hominis* and *G. lamblia* (*intestinalis*) in environmental samples (Graczyk et al. 2003, 2004). As *C. hominis* is found only in humans, PCR analysis can be used to differentiate between *C. hominis* and *C. parvum* (Jenkins et al. 2000) identified by FISH and this can be used to implement source-tracking of these pathogens.

In this study, we, for the first time, tested the hypothesis that size of mussels is not an important factor in standard 150 g samples in terms of their efficacy as biotools for human pathogen investigations in various environmental settings. The parallel aim was to investigate the presence of human enteropathogens in Lough Arrow, namely the protozoan parasites *Cryptosporidium*, *Giardia* and the microsporidia *Encephalitozoon intestinalis*, *E. hellem* and *Enterocytozoon bieneusi* and thus to determine any consequent risk to public health, in terms of water abstraction and recreational water use.

Materials and methods

Six sites on Lough Arrow (Figure 1) were selected to research the presence and concentration of *Cryptosporidium*, *Giardia* and microsporidia in zebra mussels. The sampling rationale was to research the pathogens based on lake usage and impacts as follows: (1) at three drinking water plant intake screens (at Sites 1, 2 and 6); (2) two transect sites (at Sites 1 and 3), which have inflowing streams with wastewater effluent discharges (WWTP 1 and WWTP 2); (3) one low-impact site, close to an island shore (Site 4); (4) a site close to sheep/cattle farming activities (Site 5); and (5) a filter located from inside a drinking water plant abstracting water at Site 2.

All *D. polymorpha* samples were collected on July 21 of 2008, using a survey boat and divers. Approximately 1 kg of zebra mussels were collected in each sample. Zebra mussel samples from drinking water abstraction screens (Sites 1, 2 and 6) were removed by hand using the diver's glove and placed into bags (Figure 2). Lake transects were undertaken at Sites 1 and 3; zebra mussels were removed from the stony substrate, from the point where the stream entered the lake. Four samples were taken at Site 1 (0 m, 50 m, 75 m and 100 m distance from stream inflow) and three were taken at Site 3 (samples at 0 m, 40 m and 190 m from stream inflow). Zebra mussels were removed from stony substrate at Sites 4 and 5. The drinking water plant at Site 2 provided samples of zebra mussels from a filter used in the treatment process.

All samples were labeled and stored on the boat and at the completion of survey work they were removed to the laboratory and stored at 4°C overnight. The mussel samples were then cleaned and graded into size intervals (5 mm) before analysis. In general, three size ranges were selected (11-15 mm, 16-20 mm and 21-25 mm) based on a size distribution of the zebra mussels from this survey (n = 740, Site 1) (Figure 3) and also from other previous datasets (Minchin et al. 2002; Lucy et al. 2005). Two samples (Site 1, intake and Site 3, transect 190 m) also contained enough zebra mussels for a 6-10 mm size group. Zebra mussels from the filter at Site 2 were not measured as they had been damaged and were considered as a single sample. There was no 11-15 mm size range obtained at Site 4 and no 21-25 mm sample was obtained at the Site 3 transect (40 m). For each of the size ranges at each site, 150 g of zebra mussels were

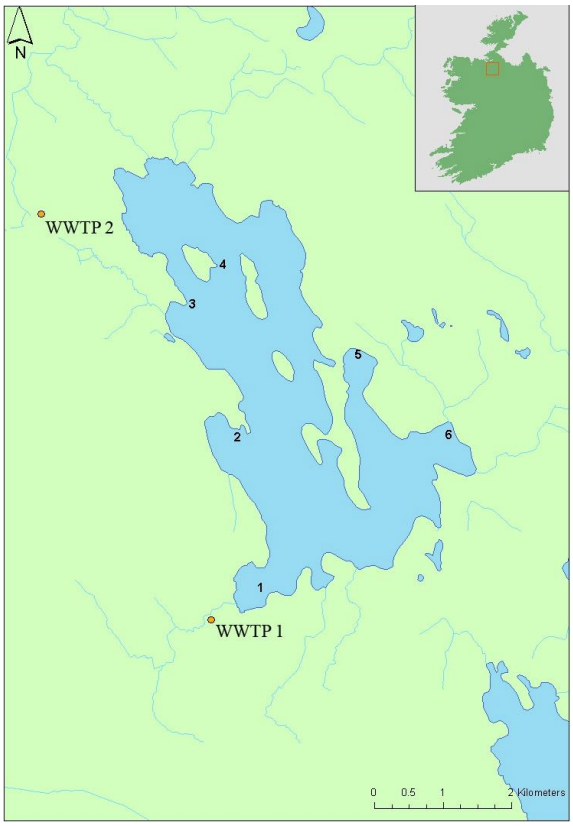

Figure 1. Sampling sites, Lough Arrow human pathogen survey, July 2008.

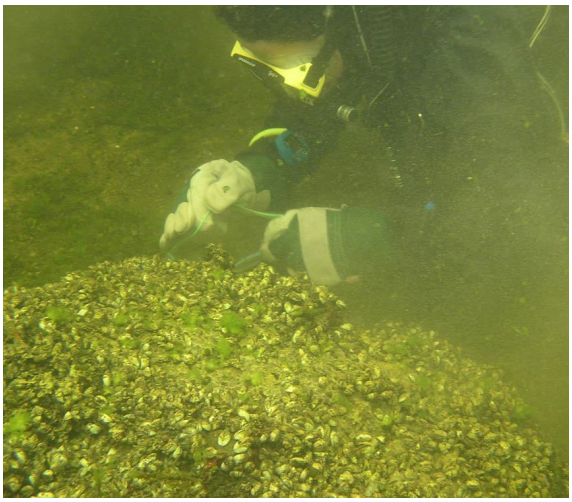

Figure 2. Diver removing *D. polymorpha* from stony substrate at Site 4. Photograph by Moore Marine Services Ltd.

sorted and counted resulting in the selection of 35 zebra mussel samples (Annex 1) for further processing.

Each 150g sample (wet weight with shell) of zebra mussels was homo-genised, complete with shells, using an industrial blender (Graczyk and Cranfield 1996). The homogenates were gravity sedimented into one-litre settlement cones overnight at 4°C, and 50 ml samples of the top sediment were collected into a plastic tube and centrifuged (3,000 g, 5 min), the supernatant discharged, and the pellet stored in 75% ethanol (Lucy et al. 2008).

Alcohol was washed from the pellets by centrifugation (1,050 g, 10 min) two times using sterile phosphate-buffered saline (PBS), and evenly divided into two aliquots. One aliquot was processed for *C. parvum* and *G. intestinalis* by combined FISH and direct immunofluorescent antibody (IFA), and the other for *E. intestinalis*, *E. hellem* and *E. bieneusi* by FISH (Graczyk et al. 2004). FISH oligonucleotide probes were synthesized by the DNA Analysis Facility of the Johns Hopkins University, Baltimore, MD, in 1.0 µM scale, purified by HPLC, and 5' labeled with a single molecule of a fluorochrome. A FITC-conjugated monoclonal IFA against the cell wall antigens of *Cryptosporidium* and *Giardia* from MERIFLUOR™ *Cryptosporidium*/*Giardia* test kit (Meridian Diagnostic, Inc., Cincinnati, OH) was used. The walls of the pathogen's transmissive stages were permeabilized (Graczyk et al. 2007). All combined FISH and direct IFA reactions were carried out in eppendorf tubes in a total volume of 100 µl of hybridization buffer at 57°C for 1 hr. Concentration of each oligonucleotide probe, i.e., CRY-1, GIAR-4, and GIAR-6 (Graczyk et al. 2007) was 1 mMol l^{-1} and IFA was 1:1 diluted. The FISH reaction for human-infective microsporidia was carried out in eppendorf tubes in a total volume of 100 µl of hybridization buffer at 57°C for 3 hrs. Concentration of each oligonucleotide probe, i.e., HEL 878, INT-1, BIEN-1 (Graczyk et al. 2007), was 1 mMol l^{-1}. Positive and negative controls were as described previously (Graczyk et al. 2007). After hybridization, the tubes were centrifuged twice at 4°C (8,000 g, 5 min) and the pellets were resuspended in 100 µl of sterile PBS. For each sample, a 20 µl aliquot was transferred onto a lysine-coated well (5-mm diameter) on a teflon-coated glass slide (Carlson Scientific, Inc., Peotone, IL, USA) and air-dried. The entire area of each well was examined with the aid of an Olympus BH2-RFL epifluorescent microscope, dry 60× objective, and BP450-490 exciter filter, without knowledge of sample identity; the pathogens were enumerated, and samples uncoded (Lucy et al. 2008).

Zebra mussel samples yielding enough oocysts were analysed by polymerase chain reaction (PCR) to identify *Cryptosporidium* to species level for source tracking. A nested PCR procedure was performed on DNA extracted from *Cryptosporidium* oocysts recovered from zebra mussel homogenates (as previously described). Tris-EDTA (pH 8) was added to the IMS eluants to a final volume of 500 µl. Twenty microlitres of 10% sodium dodecyl sulfate and 5 µl 20-mg/ml proteinase K were added to each tube and then incubated overnight at 45°C. DNA was purified by phenol-chloroform extraction and ethanol precipitation. To determine if PCR inhibitors were preventing detection, genomic DNA was added to the DNA extracted from the zebra mussel sample just prior to PCR amplification. A 434-bp fragment of the 18S rRNA gene was then amplified. Following this, a 1,056-bp fragment was amplified using the forward primer KLJ1 (5′-CCACATCTAAG GAAGGCAGC-3′) and reverse primer KLJ2 (5′-ATGGATGCATCAGTGTAGCG-3′). For the secondary PCR, the forward primer CPB-DIAGF (5′-AAGCTCGTAGTTGGATTTCTG-3′) and the reverse primer CPB-DIAGR (5′-TAAGGTGCTG AAGGAGTAAGG-3′) were used to amplify the 434-bp fragment. The initial amplification reaction was performed with 15 µl of DNA template, and 3 µl of the primary amplification product used as a template for the secondary PCR. Each PCR consisted of 35 cycles of denaturation at 94°C for 45 s, annealing at 55°C for 45 s, and extension at 72°C for 60 s. An initial denaturation step consisting of incubation at 94°C for 4 min and a final extension step consisting of incubation at 72°C for 10 min was also included. PCR products were visualized on a 1% agarose gel stained with ethidium bromide (Jenkins et al. 2000).

The pathogen counts in *D. polymorpha* appeared to have significantly right-skewed non-normal distributions. Thus, nonparametric Kruskal-Wallis ANOVA by ranks was used, with the help of STATISTICA 6.0 software, to test if the counts differed among three of the most commonly occurring pathogens (*Cryptosporidium*, *Giardia* and *E. hellem*) and zebra mussel size classes (n = 32 for each species). The

significance level $\alpha = 0.05$ was accepted as the initial level for type I error. However, since the test was conducted twice on the same dataset, we applied Bonferroni correction to that initial significance level and hence compared the results against the $\alpha = 0.025$ (Rice 1989).

Results

The size range of a sample of zebra mussels taken from Site 1 indicated the presence of young-of-the-year (0+, 2-5 mm), one year old (1+, approx. 6-15 mm), and two year old mussels (2+, approx. 16-29 mm) (Figure 3). One and two year old mussels were the age cohorts used in this study as biomonitors. The pathogen counts (Annex 1) were found to significantly differ among the pathogen species but not with the size or age class of *D. polymorpha* (species: P = 0.003; size class: P = 0.894; Kruskal-Wallis ANOVA, with Bonferroni corrected $\alpha = 0.025$). *Cryptosporidium* was the most abundant species, with lower counts of *Giardia* and *E. hellem*, respectively (Figure 4).

Biofouling was evident at all three water intake screens and was particularly high at Site 2 (Figure 5) and at Site 6. *Cryptosporidium* and *Giardia* were found in *Dreissena* attached to the screens of all three drinking water plants (Table 1), with *E. hellem* (Site 1) and *E. intestinalis* (Sites 2 and 3) also found at different plants. The numbers of potentially viable human pathogens found in zebra mussels attached to drinking water abstraction screens in Lough Arrow did not vary significantly according to pathogen or drinking water plant (pathogen: P = 0.325; site: P=0.818, Kruskal-Wallis ANOVA with Bonferroni corrected $\alpha = 0.025$).

PCR analysis detected the presence of *Cryptosporidium hominis* at the Site 2 drinking plant screen, indicating a human source of contamination. No pathogens were detected however, in the zebra mussels sampled from the filter within this drinking water treatment plant. In the Site 1 lake transect, the numbers of *Cryptosporidium* and *E. hellem* decreased from 128 to 29 oocysts/150 g *Dreissena* sample and from 96 to 21 spores/150 g *Dreissena* sample respectively, between 0 m and 75 m distance along the transect, with a slight increase in *Cryptosporidium* at 100 m distance (Figure 6). Both *Cryptosporidium hominis* and *C. parvum* were detected at the stream outfall (0 m transect), indicating sewage contamination

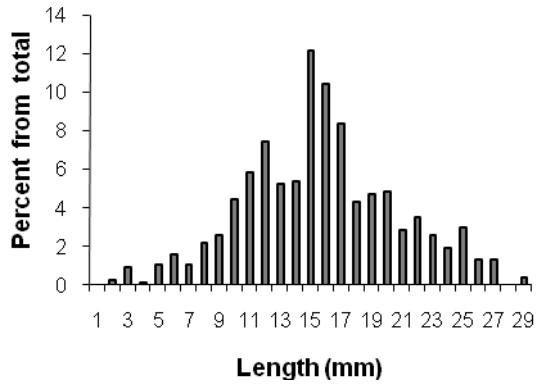

Figure 3. *Dreissena polymorpha* size distribution at Site 1.

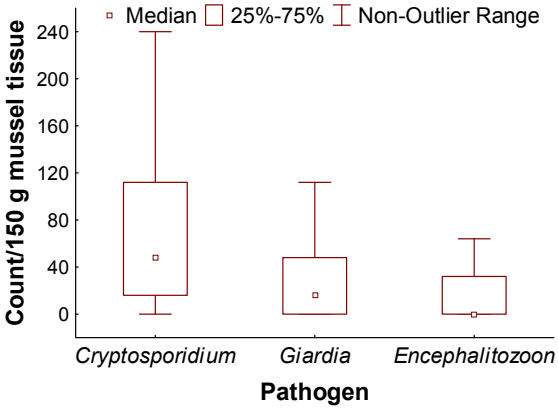

Figure 4. Median counts of the pathogens *Cryptosporidium*, *Giardia intestinalis* and *Encephalitozoon hellem* in *Dreissena polymorpha* pooled samples from Lough Arrow.

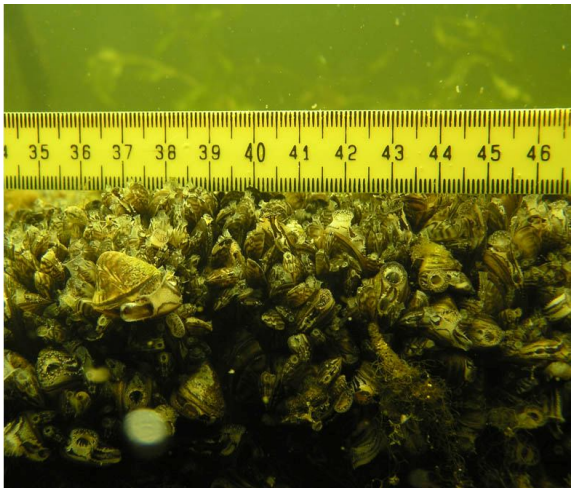

Figure 5. *Dreissena polymorpha* with open filters attached to drinking water abstraction screen at Site 2. Photograph by Moore Marine Services Ltd.

Table 1. Potentially viable human pathogens (median numbers followed by min and max) found in zebra mussels (150 g) attached to screens at three Lough Arrow's drinking water plants.

Site/Pathogen	*Cryptosporidium*	*Giardia*	*E. hellem*	*E. intestinalis*
Site 1*	8 (0 – 48)	32 (16 – 48)	48 (0 – 96)	0
Site 2*	112 (112 – 112)	24 (0 – 48)	0	16 (0 – 32)
Site 6*	48 (0 – 48)	32 (16 – 64)	0	96 (0 – 96)

* n = 3 for each site

Table 2. Human enteropathogens in *Dreissena polymorpha* from the transect at Site 3.

Site 3 Transect: Distance (m)	*Cryptosporidium*	*Giardia*	*E. hellem*	*E. intestinalis*
0 m (n = 2)	96 (16 – 176)	8 (0 – 16)	112 (0 – 224)	0
40 m (n = 2)	40 (32 – 48)	8 (0 – 16)	0	0
190 m (n = 4)	56 (0 – 240)	24 (0 – 112)	0 (0 – 192)	0 (0 – 32)

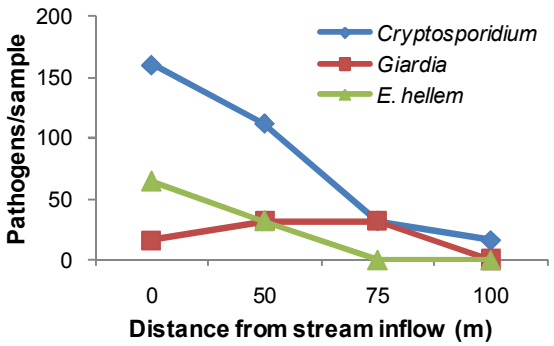

Figure 6. Numbers of potentially viable pathogens in *Dreissena polymorpha* along Site 1 transect.

entering from the stream. *Giardia* numbers were relatively low along this transect and showed no consistent decline with distance. No *E. hellem* spores or *Giardia* cysts were detected 100 m distant from the stream outfall. This transect was located within 100 m of the water abstraction point at Site 1, with the same pathogens identified from both transect and the water screen, i.e. *Cryptosporidium*, *Giardia* and *E. hellem*. Neither *E. intestinalis* nor *E. bieneusi* were recorded in these samples. In the other transect, *Cryptosporidium* and *Giardia* were present at all distances (Table 2); *E. hellem* and *E. intestinalis* were both present at 0 m point and in the latter case at 190 m. In terms of species identification, *Cryptosporidium parvum* and *Cryptosporidium meleagridis* (Slavin, 1955) were both identified in zebra mussel samples from this transect.

The unimpacted Site 4 was negative for *Cryptosporidium* and *Giardia* but was positive for the microsporidian species *E. hellem*. The zebra mussels at the agricultural site (Site 5) yielded a median of 48 (min = 16, max = 112) *Cryptosporidium*/150 g *Dreissena* sample, 32 (min = 16, max = 48) *Giardia*/150 g *Dreissena* sample, but no microsporidia. The species identified were *Cryptosporidium parvum* and *C. muris*; indicative of animal faecal inputs from agricultural and possibly wild animal sources and in fact an invasive mink *Mustela lutreola* (Linnaeus 1761) was observed at this site during sampling.

Discussion

The results indicate that based on the same sample mass, the size of the zebra mussels did not influence the pathogen loadings. This is an important finding because zebra mussels have been found to be effective biomonitors of *Cryptosporidium*, *Giardia* and microsporidia in a number of studies at different river and lake sites (Graczyk et al. 2001, 2003, 2004; Lucy et al. 2008). Zebra mussels are known to vary in size due to a number of age-cohort, density and environmental factors (Bij de Vaate 1991; Minchin et al. 2002; Lucy et al. 2005) and these may be site specific. It is therefore important to know that different sites in the same or different waterbody can be compared in terms of relative concentrations of human waterborne parasites irrespective of the mussels' size.

The Lough Arrow catchment, in common with all Irish and EU waters is subject to the river basin district management plans laid out in the EU Water Framework Directive (European Community Directive 2000/60/EC). As part of the Irish Western River Basin District, the identified pressures to the water quality of this lake include on-site wastewater treatment, discharge of treated effluents from wastewater treatment plants and agriculture in the catchment (WRBD 2006). While these activities are all well-known contributors of phosphorus, and hence implicated as being eutrophication factors, they are also potential sources of *Cryptosporidium* and other waterborne human pathogens, which are a public health threat particularly, as in the case of Lough Arrow, when drinking water abstraction takes place. Health risks to other Lough Arrow users also apply, i.e., private raw-water abstraction and fishing.

While risk assessment models have become increasingly popular in terms of water management (EPA 2008b; *Cryptosporidium* Scottish Waters Directions 2003), this survey provides actual results for *Cryptosporidium* and other pathogens present in the lake by using zebra mussels as biomonitors in a range of lake sites to determine the presence of viable pathogens in the lake. In addition to determining the actual presence and relative concentration of *Cryptosporidium* and other pathogens, this Lough Arrow survey provides data, which can be used to assist to source track pathogens in this catchment.

The results for the water abstraction points (Sites 1, 2 and 6) indicate that both *Cryptosporidium* and *Giardia* were present at all three sites in zebra mussel samples. The identification of *Cryptosporidium hominis* (Sites 1 and 2) and *C. parvum* (Sites 1 and 6) indicates faecal contamination from human and animal/human origin respectively. *Cryptosporidium*, *Giardia* and the microsporidian species detected in this survey have also previously been recorded in four Co. Sligo WWTPs (Cheng et al. 2009).

The results of the lake transect sampling at Site 1 indicates contamination from WWTP1 as there were higher concentrations of *Cryptosporidium* at the stream (sewage effluent source) inflow, which decreased with distance into the lake. In terms of WTTP2 and inputs of treated effluent, the results of the transect survey at Site 3 were less conclusive than at Site 1, but nevertheless determined the presence of *Cryptosporidium* and *Giardia* in zebra mussels at all distances indicating faecal contamination.

Other pollution sources resulting in the presence of pathogens at the three drinking water abstraction points (Sites 1, 2 and 6) could include septic tanks and also agricultural sources, since *C. parvum* is anthropozoonotic and may enter the lake via non-point source agricultural pollution. *Cryptosporidiosis* is one of the chief causes of diarrhea in neonatal ruminants (Zintl et al. 2006); as Lough Arrow is surrounded on three sides by hills, the geography of the catchment would facilitate runoff from grazing sheep and cattle or from slurry spreading in the catchment.

No *Cryptosporidium* or *Giardia* were detected in any zebra mussel samples from Site 4, the low-impact site. This was located away from all known animal or human faecal sources on the windward side of an island. As zebra mussels remain relatively stationary during their life-cycle (Ackerman 1994; Tuchman et al. 2004) this research indicates the difference in relative concentrations of pathogens at different sites in waterbodies, which can aid in source determination and subsequent water quality management.

The presence of *E. hellem*, detected at Site 4, is normally associated with birds including waterfowl (Graczyk et al. 2008). Lough Arrow is a designated Special Protection Area (Birds Directive 79/409/EEC) for bird life with good populations of breeding species and overwintering waterfowl, so this result is not unexpected. The presence of another *Cryptosporidium* species in some samples, i.e. *C. muris*, is normally associated with wildlife (Fayer 2008), for example the mink (a mammal invasive to Ireland) noted during survey work.

The widespread contamination of Irish river basins with *Cryptosporidium* and other human enteropathogens has already been reported in earlier studies (Graczyk et al. 2004; Lucy et al. 2008). It is believed that ingestion of only ten oocysts can cause a human cryptosporidiosis infection (Okhuysen et al. 1999). This study indicates that the current utilisation of Lough Arrow for drinking water and recreational uses poses public health risks. In 2007 *Cryptosporidium* in another Irish lake, Lough Corrib, utilised for drinking water, lead to over 182 notified cases of cryptosporidiosis (Pelly et al. 2007). Species identification implicates both human and animal faecal inputs to the lake from treated effluent, septic tanks and agriculture,

vindicating the assessment of this lake as 'at risk' in terms of lake characterisation (1a, WFD).

Moreover, the research provides evidence that, irrespective of their size distributions, zebra mussels are very effective for the long-term monitoring of animal and human faecal water pollutant inputs to surface waters.

Acknowledgements

This research was funded by a small-scale STRIVE EPA grant. The study was also supported by the Fulbright Senior Specialist Fellowship (grant no. 2225 to T. K. Graczyk), and the School of Science, Institute of Technology, Sligo, Ireland. Thanks to Eoghan Kieran and Moore Marine for diving, camera work and navigation. We would like to acknowledge Ciaran Jennings and Aisling Donegan respectively, of the North Western Regional Fisheries Board for assistance in site selection and for producing the GIS map of Lough Arrow. The participation of S.E. Mastitsky in the 16th ICAIS to contribute to the presentation of this paper was supported by the Research Foundation of SUNY at Buffalo State College. We acknowledge the input of two anonymous reviewers who made helpful suggestions for this paper.

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Annex 1. Human enteropathogens in Lough Arrow in 150 g zebra mussel samples.

Site (zebra mussel size,mm)	*Cryptosporidium* oocysts	*Giardia* cysts	*E. hellem* spores	*E. intestinalis* spores	*E. bieneusi* spores
Site 1 Intake (5-10)	48	16	0	0	0
Site 1 Intake (11-15)	16	48	64	0	0
Site 1 Intake (16-20)	0	32	96	0	0
Site 1 Intake (21-25)	0	0	32	0	0
Site 1 0m, transect (11-15)	48	0	224	0	0
Site 1 0m, transect (16-20)	176	16	64	0	0
Site 1 0m, transect (21-25)	160	48	0	0	0
Site 1 50m, transect (11-15)	80	0	32	0	0
Site 1 50m, transect (16-20)	112	48	0	0	0
Site 1 50m, transect (21-25)	112	32	32	0	0
Site 1 75m, transect (11-15)	38	51	0	0	0
Site 1 75m, transect (16-20)	32	32	64	0	0
Site 1 75m, transect (21-25)	16	32	0	0	0
Site 1 100m, transect (11-15)	16	0	0	0	0
Site 1 100m, transect (16-20)	0	0	0	0	0
Site 1 100m, transect (21-25)	96	0	0	0	0
Site 2 (16-20)	112	0	0	32	0
Site 2 (21-25)	112	48	0	0	0
Site 2 Filter (not measured)	0	0	0	0	0
Site 3 0m, transect (11-15)	176	0	0	0	0
Site 3 0m, transect (16-20)	16	16	224	0	0
Site 3 40m, transect (11-15)	48	0	0	0	0
Site 3 40m, transect (16-20)	32	16	0	0	0
Site 3 190m, transect (5-10)	32	0	192	0	0
Site 3 190m, transect (11-15)	240	112	0	32	0
Site 3 190m, transect (16-20)	80	48	0	0	0
Site 3 190m, transect (21-25)	0	0	0	0	0
Site 4 (11-15)	0	0	128	0	0
Site 4 (16-20)	0	0	0	0	0
Site 5 (11-15)	16	32	0	0	0
Site 5 (16-20)	48	48	0	0	0
Site 5 (21-25)	112	16	0	0	0
Site 6 (11-15)	48	64	0	96	0
Site 6 (16-20)	0	16	0	0	0
Site 6 (21-25)	48	32	0	96	0