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Research Article

Assessment of human waterborne parasites in Irish river basin districts - use of zebra mussels (*Dreissena polymorpha*) as bioindicators♣

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Abstract

The zebra mussel (*Dreissena polymorpha*) is an abundant and invasive molluscan shellfish species which arrived in Ireland's river basins in the early 1990's. Inland and coastal surface waters can be contaminated by human waterborne zoonotic enteropathogens such as *Cryptosporidium parvum*, *Giardia lamblia*, *Encephalitozoon intestinalis*, *E. hellem* and *Enterocytozoon bieneusi* originating predominantly from wastewater treatment plant effluents and agricultural runoff. Bivalve species, i.e., the invasive zebra mussel, *Mytilus edulis* (blue mussel) and *Anodonta anatina* (duck mussel) were used as sentinels and also as biomonitors of the aforementioned waterborne pathogens at twelve sites located in three Irish river basin districts impacted by pollution related to various water quality pressures. A variety of advanced biomolecular techniques were utilized to assess the presence and concentration of these pathogens in molluscan shellfish. At least one pathogen species was detected in bivalves at each of the twelve sites. *Cryptosporidium*, implicated in several recent Irish gastrointestinal epidemics, was recorded at all sites subjected to agricultural runoff and at one treated wastewater discharge site, linking source-track directly to animal and human fecal wastes. Overall, the results demonstrated a long-term human enteropathogen contamination of Irish waters with consequent public health risk-factors for drinking water abstraction and water-based recreational activities. The study provided further solid evidence that zebra mussels can recover and concentrate environmentally derived human pathogens and therefore can be used for the sanitary assessment of surface water quality.

Key words: biomonitoring, bivalves, *Cryptosporidium*, *Dreissena polymorpha*, environmental contamination, Giardia, Ireland, microsporidia, sentinel organisms, waterborne pathogens, zebra mussels

Introduction

Cryptosporidium parvum, *Giardia lamblia*, and human-virulent microsporidia such as *Encephalitozoon intestinalis*, *E. hellem*, and *Enterocytozoon bieneusi* are human anthropozoonotic pathogens that inflict considerable morbidity on healthy people and can cause mortality (e.g., *Cryptosporidium* and microsporidia) in immunosuppressed population (Wolfe et al. 1992; Graczyk et al. 2004). The tranmissive stages, i.e., oocysts, cysts, and spores, respectively, are

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resistant to environmental stressors and therefore ubiquitous in the environment (Graczyk et al. 2007a; 2007b). *Cryptosporidium* and *Giardia* are very frequently transmitted via water (Wolfe 1992; Graczyk et al. 2004) and considerable evidence gathered to date indicates involvement of water in the epidemiology of microsporidia (Cotte et al. 1999; Fournier et al. 2000).

Because *Cryptosporidium*, *Giardia*, and microsporidia can infect a variety of non-human hosts, identification of human-virulent species represents a challenge. Another challenge is determination of viability of the aforementioned pathogens as they may be non-viable and thus, not of epidemiological importance. Both challenges are met by fluorescence in situ hybridization (FISH) technique. FISH employs fluorescently labeled oligonucleotide probes targeted to species-specific sequences of 18S rRNA, and therefore identification of pathogens is species-specific (Hester et al. 2000; Graczyk et al. 2004; 2007b; 2008a). Also, as rRNA has a short half-life and is only present in numerous copies in viable organisms, FISH allows for differentiation between viable and non-viable pathogens (Vesey et al. 1998; Hester et al. 2000; Dorsch and Veal 2001). The FISH technique has been developed for *C. parvum* (Vesey et al. 1998), *G. lamblia* (Dorsch and Veal 2001), *E. hellem* (Hester et al. 2000) and *E. intestinalis* (Graczyk et al. 2002). Recognized alignment of respective 16S rRNA regions of 22 micro-sporidia species (Hester et al. 2000) allowed for the design of the *E. bieneusi*-specific 19 bp oligonucleotide probe (Graczyk et al. 2004) for the present study. 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 *G. lamblia* in zebra mussels (Graczyk et al. 2004; Lucy et al. 2008).

Historically, waterborne *C. parvum* oocysts were first identified in the tissue of blue mussels in Ireland, i.e., Sligo Bay, (Chalmers et al. 1997) and this initiated a worldwide investigation of this pathogen in molluscan shellfish (see Graczyk 2003, for review). Since then, multiple studies demonstrated that these filter-feeding organisms can harbor environmentally-derived protozoan parasites as a result of concentrating the recovered particles (Graczyk et al. 2003). This includes another report from Ireland on *C. parvum* oocysts in surface water used for recreation and associated blue mussel beds (Lowery et al. 2001). Oysters, mussels and clams remove and concentrate waterborne pathogens by filtration and can be used for sanitary assessment of water quality (Chalmers et al. 1997; Graczyk et al. 1998; 1999a; 1999b; 2001; 2003; 2004; 2007a; Lucy et al. 2008).

The molluscan shellfish monitored in the present study included the marine blue mussel (*Mytilus edulis*), and two freshwater species; the zebra mussel (*Dreissena polymorpha*), and the duck mussel (*Anodonta anatina*). Of these, the duck mussel is the largest and can attain a shell length of ~11 cm. The blue mussel attains ~6 cm and the zebra mussel 2 to 3 cm (Lucy et al. 2005; Zotin and Ozernyuk 2004). The zebra mussel is an abundant and invasive species, which arrived in Ireland's river basins in the early 1990's (Minchin et al. 2006). It has since spread to many other Irish waterbodies (www.invasive speciesireland.com) and provides a readily accessible biomonitoring tool to detect human pathogens in water catchments (Lucy et al. 2008) characterized under the water framework directive as being 'at risk' from diffuse or point source organic pollution (http://www.wfdireland.ie).

Diffuse or point-source discharges of raw or treated human sewage or agricultural runoff within catchments often contaminate surface waters of river basin districts when followed by precipitation events (http://www.wfdireland.ie), and this cause serious public health risks for drinking water abstraction and production, and for recreational activities (Beach 2008). Several Irish studies have detected *Cryptosporidium* species (Chalmers et al. 1997; Skerrett and Holland 2000; Lowery et al. 2001; Graczyk et al. 2004; Lucy et al. 2008), *G. lamblia* (Graczyk et al. 2004; Lucy et al. 2008) and *E. intestinalis* and *E. bieneusi* (Graczyk et al. 2004; Lucy et al. 2008) in Irish river basins. The purpose of this study was to evaluate the presence, prevalence, and concentration of *C. parvum* oocysts, *G. lamblia* cysts, and spores of *E. intestinalis*, *E. hellem* and *E. bieneusi*, based on FISH and IFA analysis of bivalves over a greater range of Irish sampling sites (Figure 1) with various water quality pressures (Table 1).

Material and methods

Molluscan shellfish were collected from 12 sites (Figure 1) that had various water quality pressures (Table 1). Zebra mussels (Sites 2-12) were collected either from vertical surfaces using a long-handled scraper (Minchin et al. 2002), or

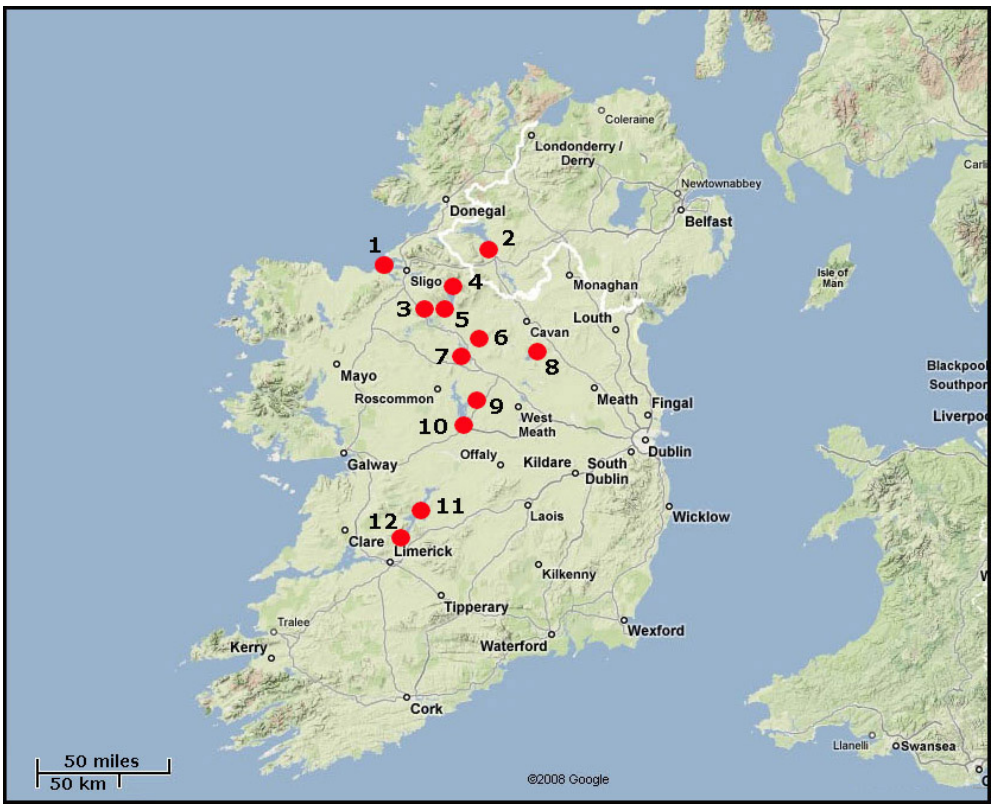

Figure 1. Map of Ireland showing sites where blue mussels (*Mytilus edulis*) (1), zebra mussels (*Dreissena polymorpha*) (2-12) and duck mussels (*Anodonta anatina*) (4) were collected: (1) Sligo Bay; (2) Lough Erne; (3) Lough Arrow; (4) Lough Meelagh; (5) Lough Key; (6) Shannon River A; (7) Lough Forbes; (8) Lough Sheelin; (9) Lough Ree; (10) Shannon River B;(11) Lough Derg and (12) Ardnacrusha Headrace.

from bottom substrate by diving (Lucy et al. 2005). Blue mussels (Site 1) were hand-collected during a low equinoxial tide, and duck mussels (Site 4) were collected by diving (Lucy et al 2005). Zebra mussels (n = 350 per site) were measured to the nearest mm, weighed, and homogenized using an industrial blender (Graczyk and Cranfield 1996). Blue mussels (n = 105) and duck mussels (n = 15) were measured, weighed, and shucked from shells; all liquor and flesh was pooled and homogenized. The homogenates were gravity sedimented (Graczyk et al. 2007b) overnight at 40°C, and 50 ml samples of the top sediment were collected into a plastic tube and centrifuged (10,000g, 10 min), supernatant discharged, and the pellet stored in 75% ethanol. Ethanol was washed from the pellets by centrifugation (10,000g, 10 min) two times using sterile phosphate-buffered saline (PBS) pH 7.4, and evenly divided into two aliqouts. One aliquot was processed for *C. parvum* and *G. lamblia* by combined FISH and direct immunofluorescent antibody (IFA), and the other for *E. intestinalis*, *E. hellem* and *E. bieneusi* by FISH (Graczyk et al., 2007b). 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 (Graczyk et al. 2007b). A FITC-conjugated monoclonal IFA against the cell wall antigens of *Cryptosporidium* and *Giardia* from MERIFLUORTM *Cryptosporidium*/*Giardia* test kit (Meridian Diagnostic, Inc., Cincinnati, OH) was used (Graczyk et al. 2007b). The walls of the pathogen's transmissive stages were permeabilized (Graczyk et al. 2007b). All combined FISH and direct IFA reactions were carried out in eppendorf tubes in a total volume of 100 µl of hybridization buffer

Table 1. Geographical location, name, and characteristics of 12 molluscan shellfish collection sites (as shown in Figure 1) with associated water quality pressures.

Site	Geographic coordinates		Location name	Description and Water Quality Pressures
	Latitude, N	Longitude, W		
1	54º17'	08º31'	Sligo Bay	Sheltered bay: routine discharge of untreated wastewater
2	54º20'	07º39'	Lower Lough Erne	Urban lakeside park: leisure craft, abundant waterfowl, intense surface runoff
3	54º01'	08º19'	Lough Arrow	Rural lake: wastewater discharges, agricultural runoff
4	54º03'	08º09'	Lough Meelagh	Rural lake: wastewater discharges
5	53º59'	08º14'	Lough Key	Rural lake: leisure craft, abundant waterfowl, waterside park, wastewater discharges, agricultural runoff
6	53º56'	08º06'	River Shannon (A) at Carrick on Shannon	Urban river boat mooring: leisure craft
7	53º46'	07º52'	Lough Forbes	Rural lake: agricultural runoff
8	53º48'	07º19'	Lough Sheelin	Rural lake: agricultural runoff
9	53º28'	07º56'	Lough Ree	Rural lake: leisure craft
10	53º19'	07º59'	River Shannon (B) at Clonmacnoise	River: agricultural runoff, managed wetland area
11	52º53'	08º23'	Castlelough, Lough Derg	Rural lake: human bathing area
12	52º42'	08º35'	Ardnacrusha Headrace	Canal, sheep grazing on sloping banks

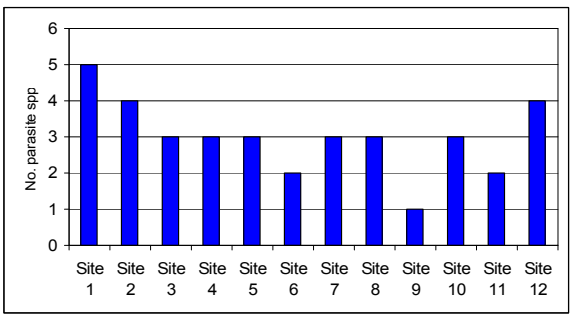

Figure 2. Cumulative number of species of human waterborne parasites identified in molluscan shellfish at each of 12 collection sites as shown in Figure 1.

at 48°C for 1 hr (Graczyk et al. 2007b). Concentration of each oligonucleotide probe, i.e., CRY-1, GIAR-4, and GIAR-6, (Graczyk et al. 2007b) was 1 mMol l^{-1} and IFA was 1:1 diluted. The FISH reaction for human-virulent microsporidia was carried out in eppendorf tubes in a total volume of 100 µl of hybridization buffer at 57°C for 3 hrs (Graczyk et al. 2007b). Concentration of each oligonucleotide probe, i.e., HEL 878, INT-1, BIEN-1, (Graczyk et al. 2007b), was 1 mMol l^{-1}. Positive and negative controls were as described previously (Graczyk et al. 2007b). After hybridization, the tubes were centrifuged twice at 4°C (2,000g, 5 min) and the pellets were resuspended in 100 µl of sterile PBS. Five, 20 µl samples were transferred onto lysine-coated wells (5-mm-diameter) on a teflon-coated glass slide, and air-dried. The entire area of a well was examined with the aid of an Olympus BH2-RFL epifluorescent microscope, dry 60X objective, and BP450-490 exciter filter without knowledge of sample identity, the pathogens were enumerated, and samples uncoded.

Results

The mean values of shell length and wet weight for zebra mussels, blue mussels and duck mussels and were: 1.5 cm and 1 g; 3.2 cm and 7 g and 7.4 cm and 38 g, respectively.

Of the five enteric parasite species tested, there was at least one species detected in molluscan shellfish at each of the 12 sites (Figure 2). Site 1, Sligo Bay, had the highest cumulative number of all five pathogen species in marine blue mussels (Figure 2). Sligo Bay was a site to which raw sewage and secondary-treated wastewater were routinely discharged (Table 1).

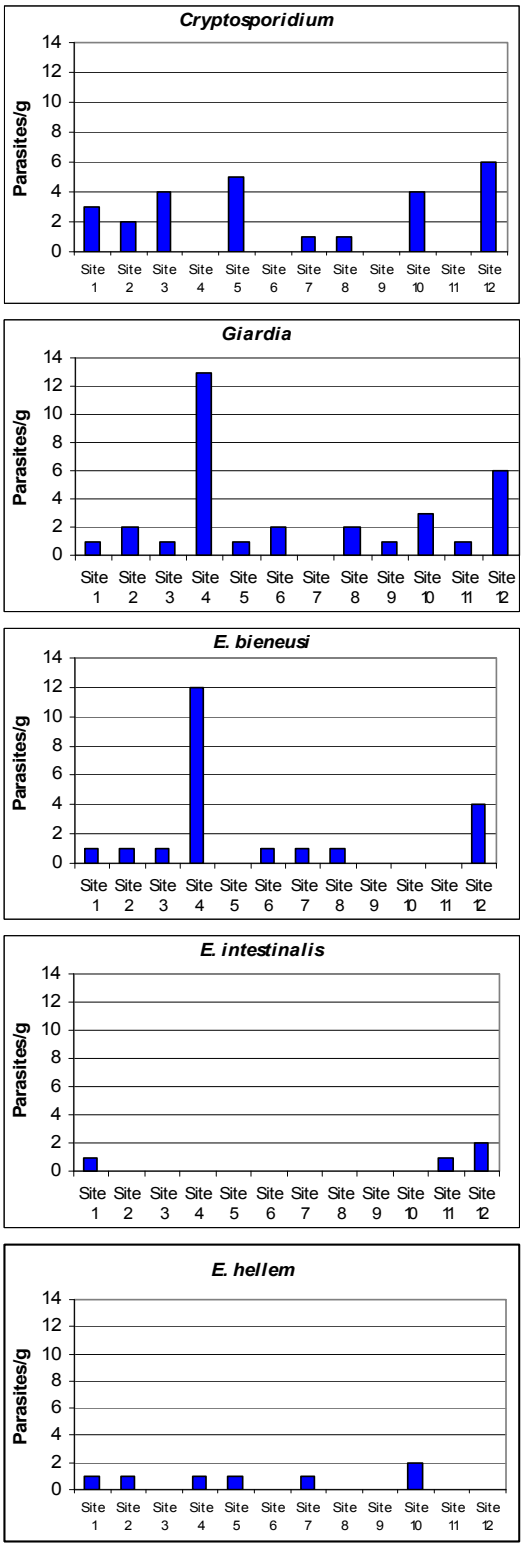

Figure 3. Number of *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and spores of *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Encephalitozoon hellem* per gram of molluscan shellfish wet weight.

In contrast, only *G. lamblia* was identified (1 cyst/g and 1 cyst/zebra mussel) at Site 9 (Figures 2 and 3), which is predominantly used for leisure craft and angling (Table 1). Shellfish at the other ten sites had an average of three species of enteropathogen present (Figure 2). In general, bivalves at the sites subjected to agricultural runoff (Site 7, 8, and 12) contained the highest cumulative numbers of human pathogens (Figure 2); the bivalves from sites used only for recreation (Site 9 and 11) had the lowest (Figure 2). Interestingly, cumulative numbers of pathogen species identified in molluscan shellfish at sites subjected to wastewater effluent discharges (Sites 1, 3, 4, and 5) varied considerably (Figure 2).

Regarding the pathogen prevalence, *G. lamblia* was most commonly found, and occurred at 11 of the 12 sites (Figure 3). Concentration of *G. lamblia* cysts at Site 4 was lower in duck mussels (3 cysts/g; 97 cysts/mussel) than in zebra mussels (13 cysts/g; 13 cysts/mussel) (Figure 3). Both *C. parvum* and *E. bieneusi* were found at eight sites (Figure 3). For *C. parvum*, 3 oocysts/g and 22 oocysts/blue mussel were recorded at Site 1 whereas a range of 1 to 6 oocysts/g and 1 to 4 oocysts/zebra mussel were detected at other sites (Figure 3). At the 8 sites where it was recorded, E. bieneusi varied in concentration: <1 spore/g and 1 spore/blue mussel; 2 spores/g and 67 spores/duck mussel; 1-12 spores/g and 1-12 spores/zebra mussel (Figures 2 and 3). *E. hellem* (1 spore/g and 18 spores/blue mussel; 1 spore/g and 3 spores/duck mussel; 1-2 spores/g and 1-2 spores/zebra mussel) and *E. intestinalis* (1 spore/g and 6 spores/blue mussel; 1-2 spores/g and 1-2 spores/zebra mussel) were found at only 5 and 3 of the 12 sites, respectively (Figure 3).

Discussion

Environmental studies undertaken by Chalmers et al. (1997) and Lowery et al. (2001) on *C. parvum* in molluscan shellfish in Ireland postulated the values of filter-feeders as alternative monitoring systems for aquatic habitats for waterborne contamination. Zebra mussels very efficiently concentrate *C. parvum* and *G. lamblia* in relation to low ambient concentration of oocysts and cysts, respectively (Graczyk et al. 2003); and actually serve already as excellent indicators for *C. parvum* in the St Lawrence River, Quebec, Canada (Graczyk et al.

2001). Together with the previous reports (Graczyk et al. 2004; Lucy et al. 2008), the present study showed that zebra mussels are able to recover spores of human-virulent species of microsporidia. All these studies together, leave no doubt that zebra mussels are highly applicable as a biomonitoring tool for assessing Ireland's river basins for contamination with human waterborne parasites. This study also strengthened the applicability of bivalves from marine habitats as sentinels for human pathogens in the aquatic environment. Although not present in the marine waters, the zebra mussel is the most highly applicable biomonitor in surface freshwaters, because it is easily sampled, occurs at a high abundance, is widely distributed and continues to spread to different catchments. Zebra mussels are convenient for such purposes because they (1) form dense populations and clumps (druses), which facilitate collection of a large sample, (2) do not have economic value, (3) have a relatively small size, and (4) are easily collected through-out the year. The transmissive stages of *C. parvum*, *G. lamblia* and microsporidia are resistant to environmental stressors (Wolfe 1992; Graczyk et al. 2007b), remaining viable for long periods in the environment (Tamburini and Pozio 1999). In general zebra mussels live and reproduce for two to three years in Irish waters (Lucy 2006), blue mussels and duck mussels may live in excess of 10 years and hence these species are effective for long-term biomonitoring for animal and human fecal water pollutant inputs to surface and coastal waters of river basin districts.

For identification of viable pathogens such as *C. parvum*, *G. lamblia* or human-virulent microsporidia, FISH is more advantageous than PCR because it allows species-specific identification, visualization, and viability assessment of up to a single pathogen cell. Such resolution is not available, or extremely impractical with any other technique. For example, using highly sensitive RT-PCR, the lowest number of *C. parvum* oocysts that can be assessed for viability was 103 (Jenkins et al. 2000). Considering the advantages of FISH for identification of viable pathogens of medical and veterinary importance it is surprising that this technique has not been widely implemented into screening of a variety of environmental samples. As only weak autofluorescence of nonstructural debris was observed in the present study, FISH is a suitable technique for identification of human pathogens in freshwater and marine molluscan shellfish.

Zebra mussels have an important ecological role in aquatic habitats because by filtering suspended particles they clarify the water and may generally improve water quality (McMahon 1991). In North America, zebra mussels serve as an excellent biological indicator of chemical, viral, and bacterial pollutants in the Great Lakes and the St. Lawrence River predominantly because they can bioaccumulate these pollutants in their tissue (see Graczyk et al. 2001, for review). Zebra mussels collected from the St. Lawrence River near a wastewater discharge site contained on average approximately 440 *C. parvum* oocysts per specimen (Graczyk et al. 2001). Knowing *C. parvum* retention rate as 4.9 x 102 oocysts/mussel/24 hr (Frischer et al. 1999) and *D. polymorpha* densities of approximately 30,000 specimens/m^2 for adult (>1-yr-old) mussels in St. Lawrence River (McMahon 1991), it has been calculated that during 24 hr approximately 1.3 x 10^7 waterborne *C. parvum* oocysts can be removed by a square meter of a zebra mussel bed in the St Lawrence river (Graczyk et al. 2001).

An interesting finding of the present study is the identification of human-virulent microsporidia in molluscan shellfish. Microsporidia infect a variety of vertebrate and invertebrate hosts, and approximately 14 species has been reported to infect people (Kotler and Orenstein 1999). Of these *E. intestinalis* and *E. bieneusi* have been reported to be zoonotic and to infect wildlife, domestic animals, and livestock (Graczyk et al. 2002). Although the actual route of transmission of spores of these species is no known, it is quite possible that spores of human or animal origin were secreted to the environment with animal feces or delivered by wastewater discharges. Spores of microsporidia have been detected from a variety of surface waters, and water has been implicated as a source of human infections, from epidemiological data (Cotte et al. 1999; Fournier et al. 2000).

In this study, increasing the geographical spread of sites monitored in Ireland (Chalmers et al. 1997; Skerrett and Holland 2000; Lowery et al. 2001; Graczyk et al. 2004), provides new data on the presence and abundance of *C. parvum*, *G. lamblia*, *E. intestinalis*, *E. bieneusi*, and *E. hellem* in a wide range of waters included in three Irish river basins districts (i.e., Shannon, Western and North-Western) as defined by the European Union water framework directive (Council of the European Communities 2000). In

Ireland, the most common source of *Cryptosporidium* oocysts, *Giardia* cysts and microsporidian spores most likely relates to the spreading of animal slurries and sewage sludge-end products (i.e., biosolids) on agricultural land. Due to the development of slatted houses for overwintering of farm animals, an estimated 29.3 million tones of animal slurry are spread on Irish farm lands annually (Hyde and Carton 2005). Since 2000, there has been an increase both in the overall percentage and volumes of sewage sludge spread on Irish farmland (EPA 2004; 2007). Various international studies have identified high levels of *Cryptosporidium* in both treated and untreated sewage sludges (Rimahen-Finne et al. 2004; Graczyk et al. 2007b; 2008a). It is likely that spreading of biosolids has occurred in watersheds surveyed for this study, as 50% of the waterbodies were in catchments used for semi-intensive livestock agriculture (Sites 3, 5, 7, 8 and 10). *C. parvum* was found in zebra mussels at all farmed sites (Figure 3), including Site 12 which was proximate to sheep grazing pastures (Table 1). This indicates the close association between *Cryptosporidium* and the agricultural environment (Zintl et al. 2006). *G. lamblia* was also widespread, with the exception of Site 7.

Wastewater discharges or sewage seepage to surface waters, operational deficiencies at wastewater treatment plants, septic tank malfunctioning, and leisure craft discharging wastes overboard may also be implicated in waterborne pollution (Graczyk et al. 2004; Lucy et al. 2008). A previous Irish study demonstrated the persistence of these pathogens throughout the wastewater treatment processes (Graczyk et al. 2007b). Overall, the results suggest long-term contamination of the Irish lacustrine environment and consequent risk-factors for recreational lake activities.

Waterfowl have also been identified as mechanical carriers of protozoan pathogens in a number of studies and *Cryptosporidium* spp. have been reported in more than 30 avian species worldwide (Graczyk et al. 2008b). Sites 2 and 5 (Table 1), located in recreational park areas, have flocks of resident and overwintering waterfowl, which may contributed to the presence of enteropathogens in water. Many of the diffuse sources of human and animal fecal pollution have been identified as risk factors in the water framework directive characterization report because they are definitive pressures for organic pollution (http://www.wfd.ireland.ie). In terms of human health, the presence of enteropathogens, particularly *Cryptosporidium*, is of growing concern due to the increasing number of waterborne cryptosporidiosis outbreaks in Ireland and worldwide (Pelly et al. 2007). Since 2004, when cryptosporidiosis became a notifiable disease in Ireland, several outbreaks have been recorded in Ireland including a massive outbreak in Galway in 2007, with 242 clinically confirmed cases (Pelly et al. 2007). Up to 90,000 households were without drinking water and a boil water notice was issued (Pelly et al. 2007).

Drinking water outbreaks of cryptosporidiosis have been associated with heavy rainfall, stream flow, and flooding (Beach, 2008). Ireland is a densely watered country that has high rainfall associated with a temperate oceanic climate; there are about 14,000 km of rivers and streams with a similar length of smaller tributaries. There are approximately 4,000 Irish lakes greater than 5 ha (Reynolds 1998). In addition, many farms have developed their own drainage systems, which flow directly to local catchments. Many Irish drinking water treatment plants rely on disinfection by chlorination of finished drinking water and this is ineffective in inactivation of *Cryptosporidium* oocysts.

The results of the present study highlights the effective use of naturally available and long-lived bivalves for biomonitoring of *C. parvum* oocysts, *G. lamblia* cysts, and human-virulent microsporidian spores in Irish aquatic environments. Evidence gathered to date indicate that zebra mussels should be utilized in freshwater biomonitoring, surveillance, and sanitary assessment of surface water quality. The widespread contamination of Irish river basins with human protozoan enteropathogens relates to water quality pressure factors that were generated by point and diffuse contamination sources. The presence of *C. parvum* in shellfish from at all sites subjected to agricultural runoff indicates contamination generated by current animal slurry and biosolid agricultural landspreading practices. As demonstrated by the present study, consumption of raw shellfish from contaminated coastal waters could cause gastroenteritis related to the investigated enteropathogens. The widespread utilization of Irish waters for drinking water and recreational purposes poses definite and far reaching public health risks. This has been indicated both by this and previous studies (Graczyk et al. 2004; Lucy et al. 2008) and by the recent cryptosporidiosis epidemic in County Galway (Pelly et al. 2007).

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References

Beach M (2008) Waterborne outbreaks: recreational water. In: Fayer R, Xiao L (eds), *Cryptosporidium* and Cryptosporidiosis. IWA Publishing, CRC Press, Florida, USA

Chalmers R, Sturdee M, Mellors AP, Nicholson V, Lawlor F, Kenny F, Timpson P (1997) *Cryptosporidium parvum* in environmental samples in the Sligo area, Republic of Ireland: a preliminary report. *Letters in Applied Microbiology* 25: 380-384, http://dx.doi.org/10.1046/j.1472-765X.1997.00248.x

Cotte L, Rabodonirina M, Chapuis F, Bailly F, Bissuel F, Raynal C, Gelas P, Persat F, Piens M, Trepo C (1999) Waterborne outbreak of intestinal microsporidiosis in persons with and without human immunodeficiency virus infection. *Journal of Infectious Diseases* 180: 2003-2008, http://dx.doi.org/10.1086/315112

Council of the European Communities (2000) Directive 2000/60/EC of the European parliament and of the council of 23 October 2000 establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* No L 327: 1-71

Dorsch MR, Veal DA (2001) Oligonucleotide probes for specific detection of *Giardia lamblia* cysts by fluorescent in situ hybridization. *Journal of Applied Microbiology* 90: 836-842, http://dx.doi.org/10.1046/j.1365-2672.2001.013 25.x

EPA (2004) European Communities (Drinking Water) Regulations, 2000 (S. I. 439 of 2000) A Handbook on Implementation for Sanitary Authorities. EPA, Wexford, Ireland

EPA (2007) Urban wastewater discharges in Ireland for population equivalents greater than 500 persons. A report for the years 2004 and 2005. EPA, Wexford, Ireland

Fournier S, Liguory O, Santillana-Hayat M (2000) Detection of microsporidia in surface water; a one-year follow-up study. *FEMS Immunology and Medical Microbiology* 29: 95-100, http://dx.doi.org/10.1111/j.1574-695X.2000.tb01510.x

Frischer ME, Nierzwicki-Bauer SA, Resto M, Toro A, Toranzos GA (1999) Zebra mussels as possible bio-monitors/filters of the protozoan pathogen *Cryptosporidium*. *Dreissena National Aquatic Nuisance Species Clearinghouse* 10: 1-4

Graczyk TK (2003) Human waterborne parasites in molluscan shellfish. *Journal of Parasitology* 89: 557-561

Graczyk TK, Cranfield MR (1996) Assessment of the conventional detection of fecal *Cryptosporidium serpentis* oocysts of subclinically infected captive snakes. *Veterinary Research* 27: 185-192

Graczyk TK, Fayer R, Cranfield MR, Conn DB (1998) Recovery of waterborne *Cryptosporidium parvum* ooocysyts by freshwater benthic clams (*Corbicula fluminea*). *Applied Environmental Microbiology* 64: 427-430

Graczyk TK, Fayer R, Lewis EJ (1999a) *Cryptosporidium* oocysts in Bent mussels (*Ischadium recurvum*) in the Chesapeake Bay. *Parasitology Research* 85: 518-521, http://dx.doi.org/10.1007/s004360050590

Graczyk TK, Thompson RCA, Fayer R (1999b) *Giardia duodenalis* of genotype A recovered from clams in the Chesapeake Bay subestuary, Rhode River. *American Journal of Tropical Medicine and Hygiene* 61: 526-529

Graczyk TK, Marcogliese DJ, deLafontaine Y, DaSilva AJ, Mhangami-Ruwende B, Pieniazek NJ (2001) *Cryptosporidium parvum* oocysts in zebra mussels (*Dreissena polymorpha*): evidence from the St. Lawrence River. *Parasitology Research* 87: 231-234, http://dx.doi.org/10.1007/s004360000293

Graczyk TK, Bosco-Nizeyi J, DaSilva A, Moura INS, Pieniazek NJ, Cranfield MR, Lindguist HDA (2002) A single genotype of *Encephalitozoon intestinalis* infects free-ranging gorillas and people sharing their habitats, Uganda. *Parasitology Research* 88: 926-931, http://dx.doi.org/10.1007/s00436-002-0693-5

Graczyk TK, Conn DB, Marcogliese DJ, Graczyk H, deLafontaine Y (2003) Accumulation of human waterborne parasites by zebra mussels (*Dreissena polymorpha*) and Asian freshwater clams (*Corbicula fluminea*). *Parasitology Research* 89: 107-111, http://dx.doi.org/10.1007/s00436-002-0729-x

Graczyk TK, Conn DB, Lucy F, Minchin D, Tamang L, Moura LNS, DaSilva AJ (2004) Human waterborne parasites in zebra mussels (*Dreissena polymorpha*) from the Shannon River drainage area, Ireland. *Parasitology Research* 93: 385-391, http://dx.doi.org/10.1007/s00436-004-1142-4

Graczyk TK, Lewis EJ, Glass G, DaSilva AJ, Tamang L, Girouard AS, Curriero FC (2007a) Quantitative assessment of viable *Cryptosporidium parvum* load in commercial oysters (*Crassostrea virginica*) in the Chesapeake Bay. *Parasitology Research* 100: 247-253, http://dx.doi.org/10.1007/s00436-006-0261-5

Graczyk TK, Lucy FE, Tamang L, Miraflor A (2007b) Human enteropathogen load in activated sewage sludge and corresponding sewage sludge-end products. *Applied Environmental Microbiology* 73: 2013-2015, http://dx.doi.org/10.1128/AEM.02412-06

Graczyk TK, Kacprzak M, Neczaj E, Tamang L, Graczyk H, Lucy FE, Girouard AS (2008a) Occurrence of *Cryptosporidium* and *Giardia* in sewage sludge and solid waste landfill leachate and quantitative comparative analysis of sanitization treatments on pathogen inactivation. *Parasitology Research* 101: 569-575, http://dx.doi.org/10.1007/s00436-007-0515-x

Graczyk TK, Majewska AC, Schwab KJ (2008b) The role of aquatic birds in dissemination of human waterborne enteropathogens. *Trends in Parasitology* 24: 55-59, http://dx.doi.org/10.1016/j.pt.2007.10.007

Hester FD, Linquist HDA, Bobst AM, Schaffer FW (2000) Fluorescent in situ detection of *Encephalitozoon hellem* spores with a 6-carboxyfluorescein-labeled ribosomal RNA-targeted oligonucleotide probe. *Journal of Eukaryotic Microbiology* 47: 299-308, http://dx.doi.org/10.1111/j.1550-7408.2000.tb00051.x

Hyde B, Carton OT (2005) Manure management facilities on farms and their relevance to efficient nutrient use. Presentation for the Fertilizer Association of Ireland, winter scientific meeting, UCD, Dublin

Jenkins MC, Trout J, Abrahamsen MS, Dorsch M, Veal D, Fayer R (2000) Estimating viability of *Cryptosporidium parvum* oocysts using reverse transcriptase-polymerase chain reaction (RT-PCR) directed at mRNA encoding amyloglucosidase. *Journal of Microbiological Methods* 34: 97-106, http://dx.doi.org/10.1016/S0167-7012(00)00198-6

Kotler DP, Orenstein JM (1999) Clinical syndromes associated with microsporidiosis. In: Wittner M, Weiss LM (eds), The microsporidia and microsporidiosis. ASM Press, Washington, DC, USA

Lowery CJ, Moore JE, Millar BC, McCorry KAJ, Xu J, Rooney PJ, Dooley JSG (2001) Occurrence and genetic genotyping of *Cryptosporidium* spp. in surface waters in Northern Ireland. *Journal of Applied Microbiology* 91: 774-779, http://dx.doi.org/10.1046/j.1365-2672.2001.01440.x

Lucy F (2006) Early life stages of *Dreissena polymorpha* (zebra mussel): the importance of long-term datasets in invasion ecology. *Aquatic Invasions* 1: 171-182, http://dx.doi.org/10.3391/ai.2006.1.3.12

Lucy F, Sullivan M, Minchin D (2005) Nutrient levels and the zebra mussel population in Lough Key. ERTDI Report Series No.34. EPA, Wexford, Ireland. http://www.epa.ie/EnvironmentResearch/ReportsOutput/FileUpload,8806,en.pdf

Lucy FE, Graczyk TK, Tamang L, Miraflor A, Minchin D (2008) Biomonitoring of surface and coastal water for *Cryptosporidium*, *Giardia* and human-virulent microsporidia using molluscan shellfish. *Parasitology Research* 103: 1369-1375, http://dx.doi.org/10.1007/s00436-008-1143-9

McMahon RB (1991) Mollusca: bivalvia. In: Thorp JH, Covich AP (eds) Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, USA

Minchin D, Lucy F, Sullivan M (2002) Monitoring of zebra mussels in the Shannon-Boyle navigation, other navigable regions and principal Irish lakes, 2000 and 2001. Marine Environment and Health Series, No. 5. Marine Institute, Dublin

Minchin D, Lucy F, Sullivan M (2006) Ireland: a new frontier for the zebra mussel *Dreissena polymorpha* (Pallas). *Oceanological and Hydrobiological Studies* 34: 19-30

Pelly H, Cormican M, O'Donovan D, Chalmers RH, Hanahoe B, Cloughley R, McKeowan P, Corbett-Feeney G (2007) A large outbreak of cryptosporidiosis in western Ireland linked to public water supply: A preliminary report. *European Surveillance* 12(5): E070503.3, http://www.eurosurveillance.org/ew/2007/070503.asp#3

Reynolds JD (1998) Ireland's Freshwaters. Report for Societas Internationalis Limnologiae (SIL) International Association of Theoretical and Applied Limnology XXVII Congress, Dublin, Ireland

Rimhanen-Finne R, Vuorinen A, Marmo S, Malmberg S, Hanninen ML (2004) Comparative analysis of *Cryptosporidium*, *Giardia* and indicator bacteria during sewage sludge hygienization in various composting processes. *Letters in Applied Microbiology* 38: 301-305, http://dx.doi.org/10.1111/j.1472-765X.2004.01487.x

Skerrett HE, Holland CV (2000) The occurrence of *Cryptosporidium* in environmental waters in the greater Dublin area. *Water Research* 34: 3755-3760, http://dx.doi.org/10.1016/S0043-1354(00)00135-4

Tamburini A, Pozio E (1999) Long term survival of *Cryptosporidium parvum* oocysts in seawater and in experimentally infected mussels (*Mytilus gallo-provincialis*). *International Journal of Parasitology* 29: 711-715, http://dx.doi.org/10.1016/S0020-7519(99)00033-8

Vesey G, Ashbolt N, Fricker EJ, Deere D, William KL, Veal DA, Dorsch M (1998) The use of ribosomal RNA targeted oligonucleotide probe for fluorescent labelling of viable *Cryptosporidium parvum* oocysts. *Journal of Applied Microbiology* 85: 429-440, http://dx.doi.org/10.1046/j.1365-2672.1998.853496.x

Wolfe MS (1992) Giardiasis. *Clinical Microbiology Review* 5: 93-100

Zintl A, Mulcahy G, de Waal, T, de Waele V, Byrne C, Clyne M, Holden N, Fanning S (2006) An Irish perspective on *Cryptosporidium*. *Irish Veterinary Journal* 59: 442-447, http://dx.doi.org/10.1186/2046-0481-59-8-442

Zotin AA, Ozernyuk ND (2004) Growth characteristics of the common mussel (*Mytilus edulis*) from the White Sea. *Biological Bulletin* 31: 377-381, http://dx.doi.org/10.1023/B:BIBU.0000036942.56020.d2