Parasitol Res
DOI 10.1007/s00436-008-1143-9

ORIGINAL PAPER

# Biomonitoring of surface and coastal water for *Cryptosporidium*, *Giardia*, and human-virulent microsporidia using molluscan shellfish

**Frances E. Lucy · Thaddeus K. Graczyk · Leena Tamang · Allen Miraflor · Dan Minchin**

Received: 4 April 2008 / Accepted: 21 July 2008


**Abstract** Surface inland and coastal waters in Ireland were surveyed for the human waterborne enteropathogens; *Cryptosporidium parvum*, *Giardia lamblia, Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Enterocytozoon bieneusi* by utilizing bivalve mussel species, i.e., *Mytilus edulis* (blue mussel), *Anodonta anatina* (duck 'mussel', actually a unionid clam), and the invasive *Dreissena polymorpha* (zebra mussel) as biomonitors at twelve sites located in three Irish river-basin districts with various water-quality pressures. Biomolecular techniques were utilized to assess the presence and concentration of these pathogens. At least one pathogen species was detected in shellfish at each site. *Cryptosporidium*, implicated in several recent Irish gastrointestinal epidemics, was recorded at all sites subjected to agricultural runoff and at one sewage discharge site, linking source-track directly to human and animal fecal wastes. *G. lamblia* was present at eleven of the twelve sites in a range of concentrations. A coastal bay with raw urban sewage discharge was 100% positive for all analyzed enteropathogens. Overall, the results demonstrate long-term human enteropathogen contamination of Irish waters with consequent public-health risk factors for drinking-water abstraction and water-based activities.

## Introduction

*Cryptosporidium parvum*, *Giardia lamblia*, and microsporidia such as *Encephalitozoon intestinalis*, *Encephalitozoon hellem*, and *Enterocytozoon bieneusi* are human enteropathogens, which are commonly waterborne, deriving from various environmental sources of fecal contamination

F. E. Lucy (✉)
Department of Environmental Science, School of Science,
Institute of Technology,
Sligo, Ireland
e-mail: lucy.frances@itsligo.ie

F. E. Lucy
Centre for Biomolecular Environmental and Public Health
Research, School of Science, Institute of Technology,
Sligo, Ireland

F. E. Lucy
Environmental Services Ireland, Lough Allen,
Carrick on Shannon, Co.,
Leitrim, Ireland

T. K. Graczyk · L. Tamang
Department of Environmental Health Sciences,
Division of Environmental Health Engineering,
Johns Hopkins Bloomberg School of Public Health,
Baltimore, MD 21205, USA

T. K. Graczyk
Johns Hopkins Center for Water and Health,
Johns Hopkins Bloomberg School of Public Health,
Baltimore, MD 21205, USA

T. K. Graczyk
Department of Molecular Microbiology and Immunology,
Johns Hopkins Bloomberg School of Public Health,
Baltimore, MD 21205, USA

A. Miraflor
Johns Hopkins University,
Baltimore, MD 21218, USA

D. Minchin
Marine Organism Investigations, Ballina, Killaloe, Co,
Clare, Ireland

in water catchments (Fournier et al. 2000; Graczyk et al. 2004, 2007; Rimhanen-Finne et al. 2004). In terms of public health, *Cryptosporidium* and *Giardia* are anthropozoonotic parasites, which cause diarrheal disease worldwide (Graczyk et al. 2008; Thompson et al. 1993). *Cryptosporidium* is a life-threatening protozoan parasite in immunocompromised patients, while immunocompetent individuals can suffer mild, moderate, or severe acute illness (Tzipori and Ward 2002). The contamination of catchments used for drinking-water abstraction has resulted in outbreaks of cryptosporidiosis worldwide (EPA 2004; Pollock et al. 2008). Drinking water contaminated with *Cryptosporidium* oocysts is an internationally recognized risk factor for human illness (McAnulty et al. 2000) and *Giardia* is also frequently transmitted *via* water (Thompson et al. 1993; Graczyk et al. 1999). Microsporidia are considered to be emerging opportunistic pathogens among both immunocompromised and immunocompetent people. Considerable evidence gathered to date indicates the involvement of water as a vector in the epidemiology of human microsporidiosis (Cotte et al. 1999; Fournier et al. 2000).

The transmissive stages of *C. parvum*, *G. lamblia*, and microsporidia, i.e. oocysts, cysts, and spores, respectively, are shed in millions in the feces of infected people or animals and because they are resistant to environmental stressors, they are ubiquitous in the environment (Karanis et al. 2007; Wolfe 1992). *Cryptosporidium* oocysts can remain viable for almost a year in the environment (Tamburrini and Pozio 1999) and in animal liquid waste (Hutchison et al. 2005). Diffuse or point-source discharges of human sewage or agricultural wastes within catchments often contaminate surface or coastal waters of river-basin districts, following precipitation events leading to contamination by these pathogens (Beach 2008; http://www.wfdireland.ie) leading to public-health risks for drinking-water abstraction or for recreational purposes (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), *G. lamblia* (Graczyk et al. 2004), and *E. intestinalis* and *E. bieneusi* (Graczyk et al. 2004) in Irish river basins.

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; 1999; 2001; 2004; 2007). These molluscs include the marine blue mussel (*Mytilus edulis*) and two freshwater species, the zebra mussel (*Dreissena polymorpha*) and the duck mussel (*Anodonta anatina*, a unionid clam). Of these, *Anodonta* is the largest and can attain a shell length of ~11 cm. *Mytilus edulis* attains ~6 cm, and *D. polymorpha*, 2 to 3 cm (Lucy et al. 2005; Zotin and Ozernyuk 2004). The zebra mussel is an abundant species which arrived in Ireland's Shannon river basin in the early 1990's (Lucy 2006; Minchin et al. 2006). It has since spread to other Irish waterbodies (Minchin et al. 2006) and provides a readily accessible biomonitoring tool to detect human pathogens in water catchments characterized under the water framework directive as being 'at risk' from diffuse or point-source organic pollution (http://www.wfdireland.ie).

Because *C. parvum*, *G. lamblia*, 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 labeled oligonucleotide probes that target species-specific sequences of 18S rRNA, leading to identification. (Hester et al. 2000; Graczyk et al. 2004). Also, rRNA has a short half-life and is only present in numerous copies in viable organisms and so, FISH allows for differentiation between viable and non-viable pathogens (Vesey et al. 1998; Hester et al. 2000; Dorsch and Veal 2001; Graczyk

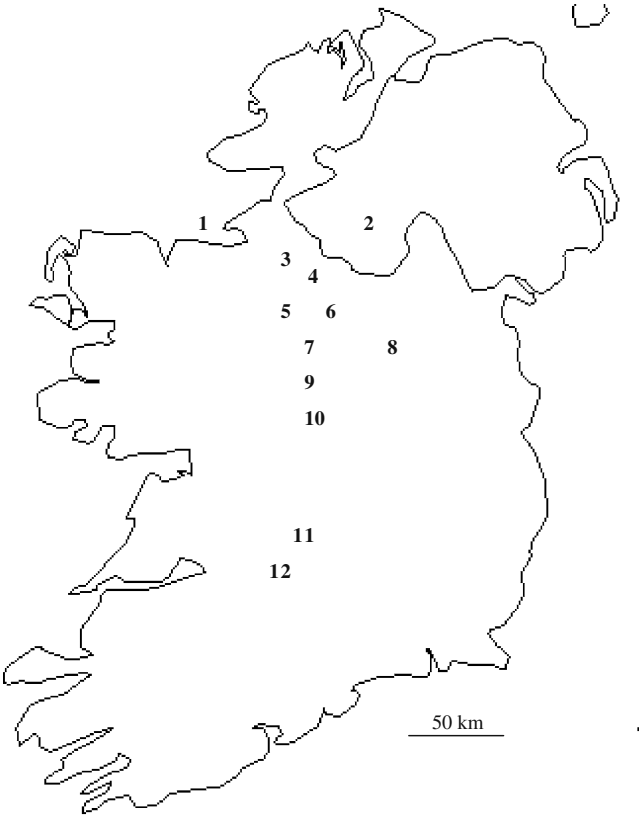


**Fig. 1** Map of Ireland showing sites where blue mussels (*Mytilus edulis*) (*1*), zebra mussels (*Dreissena polymorpha*) (*2–12*) and duck mussels (*Anodonta anatina*) (*4*) were collected: (*1*) Sligo Bay; (*2*) Lough Erne; (*3*) Lough Arrow; (*4*) Lough Meelagh; (*5*) Lough Key; (*6*) Shannon River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

et al. 2004; Jenkins et al. 2000). 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 *G. lamblia* in environmental samples (Graczyk et al. 2003, 2004). 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* and *E. bieneusi* (Graczyk et al. 2004).

Previous research in Ireland demonstrated the presence of these pathogens in waters in Sligo Bay, by monitoring blue mussels (Chalmers et al. 1997), and in the Shannon navigation by monitoring zebra mussels (Graczyk et al. 2004). 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 analysis of bivalves over a greater range of Irish sampling sites (Fig. 1) with various water-quality pressures (Table 1).

## Materials and methods

Molluscan shellfish were collected from 12 sites (Fig. 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 from horizontal 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, complete with shells, 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 were then pooled by species and homogenized. The homogenates were gravity-sedimented overnight at 4°C, and 50-ml samples of the top sediment were collected into a plastic tube and centrifuged (10,000×$g$, 10 min), supernatant discharged, and the pellet stored in 75% ethanol. Ethanol was washed from the pellets by centrifugation (10,000×$g$, 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. 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. 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 (22). 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. All combined FISH and direct IFA reactions were carried out in Eppendorf tubes in a total volume of 100 μl of hybridization buffer at 48°C for 1 hour (Graczyk et al. 2004). 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 h. The concentration of each oligonucleotide probe, i.e., HEL 878, INT-1, BIEN-1 was 1 mmol $l^{-1}$. Positive and negative controls were used as described previously (Graczyk et al. 2004). After hybridization, the tubes were centrifuged twice at 4°C (2,000×$g$, 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 (Carlson Scientific,

**Table 1** Location and characteristics of molluscan shellfish sampling sites with associated water-quality pressures

| Site | Lat/long | | Description and water-quality pressures |
|---|---|---|---|
| 1 | N54° 17′ W08° 31′ | Sligo Bay | Sheltered bay: discharge of untreated sewage |
| 2 | N54° 20′ W07° 39′ | Lower Lough Erne | Urban lakeside park: leisure craft, waterfowl, surface water runoff |
| 3 | N54° 01′ W08° 19′ | Lough Arrow | Rural lake: sewage-treatment outfall, agriculture |
| 4 | N54° 03′ W08° 09′ | Lough Meelagh | Rural lake: sewage-treatment outfall |
| 5 | N53° 59′ W08° 14′ | Lough Key | Rural lake: leisure craft, waterfowl, waterside park, adjacent sewage-treatment plant, agriculture |
| 6 | N53° 56′ W08° 06′ | River Shannon (A) at Carrick on Shannon | Urban river boat mooring: leisure craft |
| 7 | N53° 46′ W07° 52′ | Lough Forbes | Rural lake: agriculture |
| 8 | N53° 48′ W07°19′ | Lough Sheelin | Rural lake: agriculture |
| 9 | N53° 28′ W07° 56′ | Lough Ree | Rural lake: leisure craft |
| 10 | N53° 19′ W07° 59′ | River Shannon (B) at Clonmacnoise | River: agriculture (managed wetland area) |
| 11 | N52° 53′ W08° 23′ | Castlelough, Lough Derg | Rural lake: human bathing area |
| 12 | N52° 42′ W08° 35′ | Ardnacrusha Headrace | Canal, sheep grazing on sloping banks |

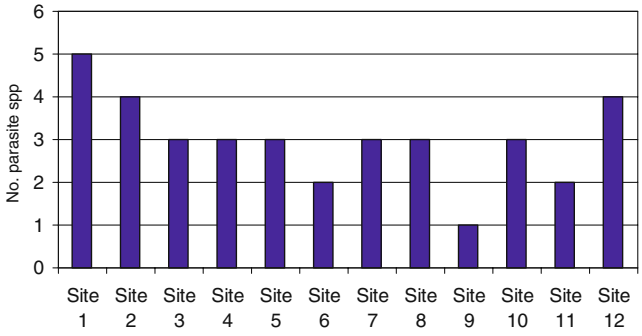


**Fig. 2** Cumulative number of species of human waterborne parasites identified in molluscan shellfish

Inc., Peotone, IL, USA) and air-dried. The entire area of a 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 then enumerated, and the samples uncoded.

## Results

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

Of the five enteric parasite species tested, there was at least one species detected in molluscan shellfish at each of the twelve sampling sites; and on average, three pathogen species were present (Table 2, Fig. 2). Sligo Bay, Site 1, (Fig. 1) had the highest cumulative number of all five pathogen species present in the *M. edulis* samples. Sligo Bay is a site to which raw and secondary-treated wastewater is routinely discharged (Table 1). In contrast, *Giardia lamblia* was the only pathogen identified at Site 9, an area which is predominantly used for leisure craft and angling. Shellfish at the other ten sites (Fig. 1) had an average of three species of enteropathogen present (Table 2, Fig. 2). In general, bivalves at the sites subjected to agricultural runoff (Site 7, 8, and 12) contained the highest cumulative numbers of human pathogens (Table 1, Fig. 2), and the bivalves from sites used only for recreation (Site 9 and 11), the lowest (Table 1, Fig. 2). Interestingly, cumulative numbers of pathogen species identified in molluscan shellfish at sites subjected to effluent discharges from wastewater-treatment plants (Sites 1, 3, 4, and 5) varied considerably (Table 1, Fig. 2).

In terms of the prevalence of individual pathogen species in shellfish, *G. lamblia* was most commonly found, occurring at eleven of the twelve (92%) sites (Fig. 3), in a range of concentrations (Table 2). Concentration of *G. lamblia* cysts at Site 4 was lower in *Anodonta* (3 cysts/g; 97 cysts/mussel) than in *Dreissena* (13 cysts/g and 13 cysts/mussel), and no *C. parvum* oocysts were found in either *Anodonta* or *Dreissena* at this site. Both *C. parvum* and *E. bieneusi* were found in different concentrations at eight of the sampling sites (Table 2, Fig. 3). *E. hellem* and *E. intestinalis* were found at only six and three of the twelve sites, respectively (Table 2, Fig. 3).

## Discussion

Increasing the geographical spread of sites previously 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-basin districts (RBDs; Shannon, Western, and North-Western RBDs) as defined by the European Union (EU) water framework directive (2000/60/EEC).

The environmental sources of the aforementioned enteropathogens and their route to surface waters have been studied internationally and are anthropozoonotic in nature (Graczyk et al. 2008; Zintl et al. 2006). In Ireland, the most common source of waterborne *Cryptosporidium* oocysts, *Giardia* cysts, and microsporidian spores are agricultural lands which contain (1) animal feces from grazing herds and spreading of stored waste from winter-housing and (2) human sewage sludge end products spread on agricultural land. Cryptosporidiosis is one of the chief causes of diarrhea in neonatal ruminants (Zintl et al. 2006), and cattle and sheep feces from grazing animals can contami-

**Table 2** Mean concentration of human waterborne parasites (parasites/mussel, parasites/g) at Sites 1–12

| Enteropathogen | Site 1 | Site 2 | Site 3 | *Zm* Site 4 | *Dm* Site 4 | Site 5 | Site 6 | Site 7 | Site 8 | Site 9 | Site 10 | Site 11 | Site 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Cryptosporidium* | 22.3 | 1.1 | 3.4 | 0.0 | 0.0 | 4.5 | 0.0 | 1.1 | 1.1 | 0.0 | 3.4 | 0.0 | 4.6 |
| *Giardia lamblia* | 6.1 | 1.1 | 1.1 | 13.13 | 97.3 | 1.1 | 2.2 | 0.0 | 1.2 | 1.1 | 2.3 | 1.1 | 4.6 |
| *E. bieneusi* | 1.1 | 1.1 | 1.1 | 12.12 | 67.2 | 0.0 | 1.1 | 1.1 | 1.1 | 0.0 | 0.0 | 0.0 | 3.4 |
| *E. intestinalis* | 6.1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 1.1 | 2.2 |
| *E. hellem* | 3.1 | 1.1 | 0.0 | 0.0 | 17.1 | 1.1 | 0.0 | 1.1 | 0.0 | 0.0 | 1.2 | 0.0 | 0.0 |

Molluscan shellfish sampled and analyzed as follows: marine mussels (*Mytilus edulis*) at Site 1; duck mussels (*Anodonta anatina*) at Dm Site 4; zebra mussels at all other sites.
*Zm* Zebra mussels, *Dm* duck mussels

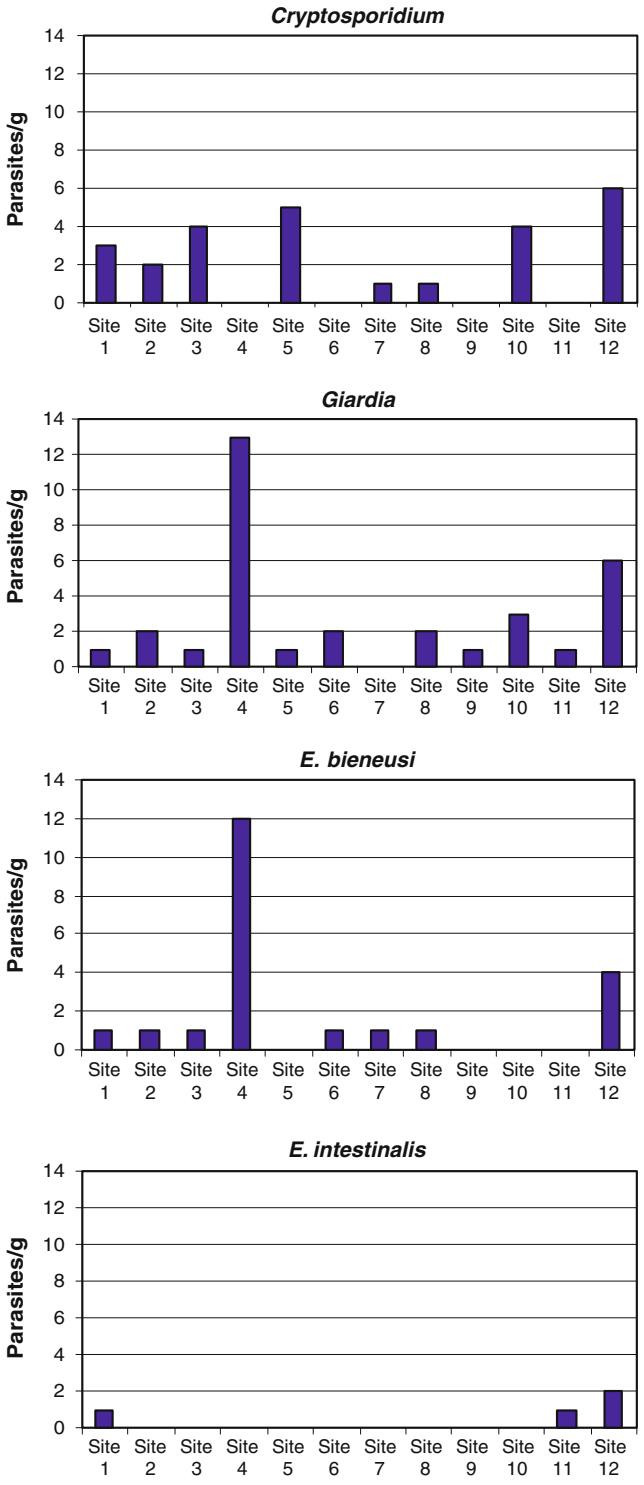


**Fig. 3** Number of *Cryptosporidium parvum* oocysts, *Giardia lamblia* cysts, and spores of *Enterocytozoon bieneusi*, *Encephalitozoon intestinalis*, and *Encephalitozoon hellem* per gram of molluscan shellfish wet weight

nate proximal waterbodies via surface runoff (Zintl et al. 2006), as was found in this study.

Since 2000, there has been an increase both in the overall percentage and volumes of sewage sludges spread on Irish farmland, with an estimated 45,543 tonnes spread in 2005 (EPA 2007). These large volumes of spread biosolids increase the risk of *Cryptosporidium* and other pathogens being introduced via surface runoff into waters used for drinking-water abstraction. Various international studies have identified high levels of *Cryptosporidium* in both treated and untreated sewage sludges (Rimhanen-Finne et al. 2004; Graczyk et al. 2007). It is likely that spreading of sewage sludge 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, and10). *C. parvum* was found in *Dreissena* at all farmed sites (Fig. 3) including Site 12, which was proximate to sheep grazing pastures (Table 1). This indicates the close association between *Cryptosporidium* and the agricultural anthropozoonotic environment. *G. lamblia* was also widespread with the exception of *Dreissena* from Site 7; *E. bieneusi* was found in *Dreissena* at Site 3, 7, and 8; *E. intestinalis* at Site 11 and *E. hellem* at Sites 5, 7, and 10 (Fig. 3).

Sewage discharges or fecal seepage to surface waters, operational deficiencies at sewage-treatment plants or septic tank malfunction and leisure craft discharging untreated wastes overboard may also be implicated (Graczyk et al. 2004). A previous Irish study demonstrated the persistence of these pathogens throughout the wastewater-treatment process (Graczyk et al. 2007). In this study, Sites 3 and 4 had sewage outfalls to lakes; the former site had *C. parvum*, *G. lamblia*, and *E. bieneusi* present in bivalves (Fig. 3) and the latter site had the highest *G. lamblia* and *E. bieneusi* concentration in shellfish (Table 2, Fig. 3). These two latter pathogen species can be source-tracked to the sewage-treatment plant discharging to the lake (Graczyk et al. 2007). Overall, the results suggest long-term contamination of the lacustrine environment and consequent risk factors for recreational lake activities. Direct discharges of sewage from leisure craft utilizing the Shannon–Erne waterway may have resulted in bivalve bioaccumulation at Sites 2, 5, 6, and 9 (Table 1).

Waterfowl have also been identified as 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. 2008). Sites 2 and 5 (Table 1), located in recreational park areas, have flocks of resident and overwintering waterfowl which may have contributed to the presence of enteropathogens in *D. polymorpha*.

In terms of human health, the presence of enteropathogens, particularly *Cryptosporidium*, is of growing concern due to the increasing number of cryptosporidiosis outbreaks in Ireland and worldwide. Since 2004, several epidemics have been recorded in Ireland including a massive outbreak in Galway in 2007, with 182 clinically confirmed cases linked to drinking water; preliminary research indicated that both human sewage and animal wastes factored in the water contamination (Pelly et al. 2007).

Drinking-water outbreaks of cryptosporidiosis have been associated with heavy rainfall and flooding (Beach 2008). Ireland is a densely watered country with about 14,000 km of rivers and streams, a similar length of smaller tributaries, and approximately 4,000 Irish lakes greater than 5 ha (Reynolds 1998). In addition, many farms have developed their own drainage systems, which flow to local catchments. In Ireland, heavy periods of rainfall lead to: (1) runoff of slurries and sewage sludges from agricultural land into drainage channels and surface waters; (2) storm water overflows in sewage-treatment plants with subsequent release of untreated wastewater; and (3) increased turbidity in reservoirs used for drinking-water abstraction. Many Irish drinking-water treatment plants rely on chlorination for disinfection of water and this is ineffective in inactivation of *Cryptosporidium* oocysts.

In terms of recreational risk factors, water-sports are very popular in Ireland in inland and coastal waters. Collection of wild shellfish for human consumption is common on Irish shores, and, as demonstrated in the present study, this is a significant public-health risk factor in Sligo Bay, Site 1 (Table 1). Sligo is currently constructing a state-of-the-art sewage-treatment plant that conforms to EU legislation. In this survey, differences in cumulative number of species between sewage outfall sites may reflect the prevalence of these pathogens in the human population discharging to specific sewage-treatment plants.

This study strengthens the applicability of bivalves from marine and freshwater habitats as biomonitors for human pathogens, arising from both point and diffuse sources, in the aquatic environment. The three shellfish species used were all shown to be effective for accumulating several human enteropathogens. Although not present in the marine environment, the zebra mussel is the most highly applicable biomonitor in surface waters because it is easily sampled, occurs at a high abundance, is widely distributed, and continues to spread to different catchments.

As the transmissive stages of these enteropathogens remain viable for long periods in the environment and since the bivalves live for at least two (*D. polymorpha*) and as much as 10 years (*M. edulis* and *A. anatina*), these species are effective for long-term monitoring for animal and human fecal-water-pollutant inputs to surface and coastal waters of river-basin districts.

The widespread contamination of Irish river basins with human protozoan enteropathogens, reported in this and an earlier study (Graczyk et al. 2004), relates to water-quality pressure factors generated both by point-source and diffuse pollution sources. This study indicates that current utilization of Irish waters for drinking water and recreational uses may pose definite and far-reaching public-health risks.

**Acknowledgments** We acknowledge Owen Kieran, Moore Marine Services and Michael Duggan, West Coast Diving for providing molluscan shellfish samples. The OECD provided funding for this research under the co-operative research program (Grant AGR/PR (2007)1 to F.E. Lucy). 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. D. Minchin received support from the European Union 6th Framework project ALARM (GOCE-CT-2003-506675).

## References

Beach MJ (2008) Waterborne: recreational water. In: Fayer R Xiao L (ed) *Cryptosporidium* and cryptosporidiosis. IWA, CRC, Florida, pp 335–370

Chalmers RM, Sturdee AP, Mellors P, Nicholson V, Lawlor F, Kenny F, Timpson P (1997) *Cryptosporidium parvum* in environmental samples in the Sligo area, Republic of Ireland: a preliminary report. Lett Appl Microbiol 25:380–384

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. J Infect Dis 180:2003–2008

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. 71 pp

Dorsch MR, Veal DA (2001) Oligonucleotide probes for specific detection of *Giardia lamblia* cysts by fluorescent in situ hybridization. J Appl Microbiol 90:836–842

EPA (2004) European communities (drinking water) regulations, 2000 (S. I. 439 of 2000) a handbook on implementation for sanitary authorities. Wexford, Ireland

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

Fournier S, Liguory O, Santillana-Hayat M, Guillot E, Sarfari C, Dumoutier N, Molina J, Derouin F (2000) Detection of microsporidia in surface water; a one-year follow-up study. FEMS Immunol Med Microbiol 29:95–100

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

Graczyk TK, Fayer R, Cranfield MR, Conn DB (1998) Recovery of waterborne *Cryptosporidium parvum* oocysts by freshwater benthic clams (*Corbicula fluminea*). Appl Environ Microbiol 64:427–430

Graczyk TK, Thompson RCA, Fayer R, Adams P, Morgan UM, Lewis EJ (1999) *Giardia duodenalis* of genotype a recovered from clams in the Chesapeake Bay subestuary, Rhode River. Am J Trop Med Hyg 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. Parasitol Res 87:231–234

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*). Parasitol Res 89:107–111

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. Parasitol Res 93:385–391

Graczyk TK, Lucy FE, Tamang L, Miraflor A (2007) Human enteropathogen load in activated sewage sludge and corresponding sewage sludge-end products. Appl Environ Microbiol 73:2013–2015

Graczyk TK, Majewska AC, Schwab KJ (2008) The role of aquatic birds in dissemination of human waterborne enteropathogens. Trends Parasitol 24:55–59

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. J Eukaryot Microbiol 47:299–308

Hutchison ML, Walters LD, Moore A, Avery SM (2005) Declines of zoonotic agent in liquid livestock wastes stored in batches on-farm. J Appl Microbiol 99:58–65

Jenkins MC, Trout J, Abrahamsen MS, Higgins J, Fayer R (2000) Estimating viability of *Cryptosporidium parvum* oocysts using reverse transcriptase-polymerase chain reaction (RT-PCR) directed at mRNA encoding amyloglucosidase. J Microbiol Methods 34:97–106

Karanis P, Kourenti C, Smith H (2007) Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J Water Health 5:1–38

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. J Appl Microbiol 91:774–779

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

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

MacAnulty JM, Keene WE, Leland DE, Hoesly F, Hinds B, Stevens G (2000) Contaminated drinking water in one town manifesting as an outbreak of cryptosporidiosis in another. Epidemiol Infect 125:79–86

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. Published Report. 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). Oceanol Hydrobiol Stud 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. Euro Surveill 12(5):E070503.3 http://www.eurosurveillance.org/ew/2007/070503.asp#3

Pollock KGJ, Young D, Smith HV, Ramsay CN (2008) Cryptosporidiosis and filtration of water from Loch Lomond, Scotland. Emerg Infect Dis 14:115–119

Reynolds JD (1998) *Ireland's Freshwaters*. Published 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. Lett Appl Microbiol 38:301–305

Skerrett HE, Holland CV (2000) The occurrence of *Cryptosporidium* in environmental waters in the greater Dublin area. Water Res 34:3755–3760

Tamburrini A, Pozio E (1999) Long term survival of *Cryptosporidium parvum* oocysts in seawater and in experimentally infected mussels (*Mytilus galloprovincialis*). Int J Parasitol 29:711–715

Thompson RC, Reynoldson JA, Mendis AH (1993) *Giardia* and giardiasis. Adv Parasitol 32:71–160

Tzipori S, Ward H (2002) Cryptosporidiosis: biology, pathogenesis and disease. Microb Dis 4:1047–1058

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. J Appl Microbiol 85:429–440

Wolfe MS (1992) Giardiasis. Clin Microbiol Rev 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 Vet Journal 59:442–447

Zotin AA, Ozernyuk ND (2004) Growth characteristics of the common mussel (*Mytilus edulis*) from the White Sea. Biol Bull 31:377–381