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Static tank depuration and chronic short-term experimental contamination of Eastern oysters (*Crassostrea virginica*) with *Giardia duodenalis* cysts

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ABSTRACT

Cysts of the protozoan parasite *Giardia* have been detected in many bivalve shellfish species worldwide. The detection of zoonotic *Giardia duodenalis* assemblages A and B is of public health concern, yet there is limited data available demonstrating the bioaccumulation and elimination of *Giardia* cysts in bivalve shellfish. This study quantified *G. duodenalis* cysts that were filtered and retained by oysters (*Crassostrea virginica*) over a one week chronic exposure period, or 24 hour exposure followed by a 6 day depuration period, using static tank systems containing 10 L of 29 ppt water inoculated with 1000 or 10,000 cysts. Under chronic exposure, each oyster retained a mean of 13.4 and 87.4 cysts during the first 24 h of exposure at low and high doses, respectively, and the cysts bioaccumulated at a rate of 1.2 and 6.8 cysts/oyster/day, respectively, for the remaining duration of the trials. In acute exposure trials, oysters retained 13.8 cysts or 78.9 cysts at low and high doses, respectively, during the initial 24 hour exposure and naturally depurated cysts at a rate of −0.92 cysts/oyster/day and −2.2 cysts/oyster/day, respectively, after transfer. Although most *G. duodenalis* cysts were eliminated within the first 24 h via pseudofeces and feces, detection of some cysts in the fecal material on day 7 of acute exposure trials was indicative of cysts which passed through the digestive tract and released in feces. Only 48–53% of the initial tank inocula were recovered and may indicate that some cysts were selectively filtered by oysters but degraded through digestion.

1. Introduction

Giardia duodenalis is a protozoan parasite which causes gastroenteritis in humans worldwide, with an estimated 280 million symptomatic infections (known as giardiasis) each year (Ankarklev et al., 2010). *G. duodenalis* has at least 7 molecularly distinct genotypes (A–G) including non-zoonotic, host specific assemblages (C–G) and zoonotic assemblages (A–B). The zoonotic assemblages possess wide host ranges including humans as well as many species of wild, domestic, and farmed animals (Budu-Amoako et al., 2012b; O'Handley and Olson, 2006). At peak shedding, infected animals and humans can emit 10^8–10^9 cysts/day (Danciger and Lopez, 1975; O'Handley et al., 1999; Ralston et al., 2003). The parasite is transmitted directly via fecal–oral route, or indirectly through ingestion of contaminated water or food containing the infective, chlorine-resistant ovoid cyst stage (8-12 μm × 7–10 μm) (Ivanov, 2010). The ease of environmental dispersal, worldwide distribution, resistance to common disinfectants, low infectious dose of only 10 cysts, disease burden of infection, and broad host range of known zoonotic assemblages are all factors that make *G. duodenalis* an important public health risk (Ankarklev et al., 2010; Budu-Amoako et al., 2012b; Erickson and Ortega, 2006; Feng and Xiao, 2011; Rendtorff, 1954).

G. duodenalis is commonly transmitted via contaminated drinking water supplies, but has also been implicated in waterborne outbreaks across the globe due to ingestion of untreated recreational water, sewage contaminated ground water, and contaminated water sources/reservoirs (Baldursson and Karanis, 2011; Huang and White, 2006). Water contamination may originate from human sewage, agricultural run-off, or wild animal feces (Heitman et al., 2002; Ivanov, 2010). Bivalve molluscs are considered sentinels of environmental contamination with protozoan parasites such as *Giardia* because they filter large amounts of water and bioaccumulate cysts from surrounding water (Ivanov, 2010; Thompson, 2013). There are few studies that have examined the presence of *Giardia* cysts in various commercially important bivalve species (Willis et al., 2013), some of which have been able to successfully genotype and confirm the presence of zoonotic assemblages

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(Gómez-Couso et al., 2004). In clams (*Macoma* sp.), genotyping identified that all isolates belonged to *G. duodenalis* assemblage A, which indicated that water was likely contaminated via a human source of origin (Graczyk et al., 1999). The detection of protozoan parasites in harvestable bivalves poses a potential public health risk, particularly for species such as oysters which are often consumed raw (Robertson, 2007; Smith and Nichols, 2010).

Most studies on bioaccumulation as well as most surveys of naturally contaminated shellfish have focused primarily on another protozoan parasite, *Cryptosporidium* (Levesque et al., 2010). It is important to understand the bioaccumulation dynamics of *Giardia* in bivalve species because both protozoans are not necessarily present together at the same site, with several studies reporting the presence of *Giardia* in bivalves, but no *Cryptosporidium* (Lucy et al., 2008; Robertson and Gjerde, 2008). In sites contaminated with both protozoa, bivalves sampled at the same time period may be positive for either parasite, both, or neither (Schets et al., 2007).

Although the cyst stage is resistant to many common disinfectants, the survival of *Giardia* cysts is impacted by environmental parameters of the water containing these cysts. For example, *Giardia* cysts in tap water retain 89% infectivity after 49 days when held between 0 and 4 °C, whereas cysts that are held in tap water at 20–28 °C were rendered non-infective within 14 days (Feng and Xiao, 2011). In seawater (28–35 ppt), only 10% of *Giardia* cysts survived (as measured by excystation) for 65 days of storage at 4 °C (Erickson and Ortega, 2006; Olson et al., 1999). Although some studies have examined the bioaccumulation of *G. duodenalis* in oysters (Graczyk et al., 2006; Nappier et al., 2010), there has been no examination of cyst bioaccumulation in Eastern oysters (*Crassostrea virginica*) in highly saline conditions. As an estuarine species with a wide geographic range extending from the Gulf of St. Lawrence in Canada to the Gulf of Mexico and parts of Brazil, Eastern oysters have adapted to survive in a wide range of salinities (~5–30 ppt) (Galtsoff, 1964; Gosling, 2003).

The first aim of this study was to determine whether Eastern oysters were capable of bioaccumulating *G. duodenalis* cysts when chronically exposed in a static tank system at a high estuarine salinity (29 ppt). The second aim of this study was to determine whether oysters would begin a natural elimination of cysts after an acute (24 h) exposure that was observable by a reduction of cysts in oyster tissue and an accumulation of cysts in fecal material (pseudofeces, feces, and settled debris).

2. Materials and methods

2.1. Source of G. duodenalis cysts

Cysts were isolated by fecal flotation methods following previously published methods (Budu-Amoako et al., 2012a) from clinical cattle fecal samples submitted to the Atlantic Veterinary College for diagnostic testing. Subsamples of cysts were stained by direct immunofluorescence antibody (IFA) as described by Budu-Amoako et al. (2012a), and identity was confirmed as assemblage A by nested polymerase chain reactions of the 16S rRNA (Appelbee et al., 2003) and β-giardin (Castro-Hermida et al., 2009) genes with subsequent sequencing of amplification products in both directions (Genome Quebec Innovation Centre at McGill University, Montreal, Quebec). Sequences were independently compared to sequences of *G. duodenalis* available in GenBank via BLAST analysis, and alignments were made using BioEdit (Altschul et al., 1990; Hall, 1999). The average number of cysts counted from four 20 μL IFA subsamples of a *G. duodenalis*-confirmed stock was used to determine the approximate number of cysts per experimental treatment. The volume required for 1000 or 10,000 cysts was calculated and used to directly inoculate aerated tank water.

2.2. Exposure trial sampling

A total of 330 market sized *C. virginica* (shell length of 73–88 mm) were secured from a local aquaculture company in Prince Edward Island, Canada, and held in a recirculating tank system at 20 °C in artificial seawater (Instant Ocean Sea Salt, United Pet Group, Blacksburg, VA) with salinity of 29 ppt. Oysters were fed daily with 1.5 Tbsp algae paste (IAP Innovative Aquaculture Products Ltd., Lasqueti Island, BC). The algae paste did not fluoresce when exposed to the *Giardia* specific direct immunofluorescence assay (IFA) and thus did not impede sample analysis,

Groups of 15 oysters were randomly selected and transferred to static aquaria containing 10 L of 20 °C artificial seawater (29 ppt) aerated with a single air stone. Oysters were fed with algae paste 1 h after transfer to the static tank system and allowed to acclimatize for 24 h prior to initial sampling and subsequent introduction of cysts. Oysters were subsequently fed with algae paste at 1 and 3 days post-exposure.

A set of 10 tanks were used for both chronic (7 days) and acute (1 day) exposures. For each exposure trial (chronic and acute) 5 tanks were spiked with a low dose (1000 cysts/tank) and 5 tanks were spiked with a high dose (10,000 cysts/tank) of parasites. Oysters in the chronic exposure trial were kept in the same water throughout the 7 day trial period; whereas oysters in the acute exposure trial were exposed to cyst-contaminated water for 1 day then transferred to a clean static tank system for the remainder of the week (6 days). Each trial (chronic and acute) possessed one negative control tank (15 oysters with no cysts) and one positive control tank (10,000 cysts in an aerated system with no oysters) which was used to assess whether cysts would settle out of the water or remain suspended as desired.

Subsamples of 3 oysters were randomly selected for removal at each time point for analysis. Three oysters were removed just prior to the addition of cysts to tank water to ensure no natural contamination was present, and additional subsamples of 3 oysters were removed at 1, 2, 3, and 7 days post-exposure for analysis. Experimental design for both the chronic and acute exposure trials has been summarized in Fig. 1.

2.3. Processing of cysts from oyster tissue

Individual oysters (tissue and hemolymph) were excised from their shells by severing the adductor muscle with an oyster shucking knife, and contents were carefully poured into 50 mL tubes, and homogenized for 1 min using an Omni tissue homogenizer (Omni International, Kennesaw, GA). Homogenized samples were then incubated at 35 °C with 15 mL of pepsin–HCl solution for 75 min but were removed from the incubator every 25 min and vortexed for 20 s. Samples were centrifuged at 900 ×*g* for 5 min, and the resulting pellet was subsequently washed and re-centrifuged with deionized water, phosphate buffered saline (PBS) eluting fluid (pH 7.4), and again with water. The final pellet was suspended in 50 μL of PBS and stored at −20 °C. Pepsin–HCl digestion solution and PBS eluting fluid were prepared according to Robertson and Gjerde (2008).

2.4. Processing of cysts from oyster fecal material

For the chronic exposure trial, fecal material (feces combined with pseudofeces and tank debris) was recovered from the bottom of the holding tanks after removal of the last oyster (day 7). During the acute exposure trial, fecal material was collected from empty tanks immediately after oysters were removed (day 1) as well as at the end of the trial from the tanks into which oysters were transferred (day 7). Fecal material from each tank was analyzed individually. Samples were strained through 2 layers of surgical gauze to remove large debris and oyster shell, and captured material was washed twice with 2 mL of PBS. The resulting strained fecal solution was centrifuged at 8500 ×*g*

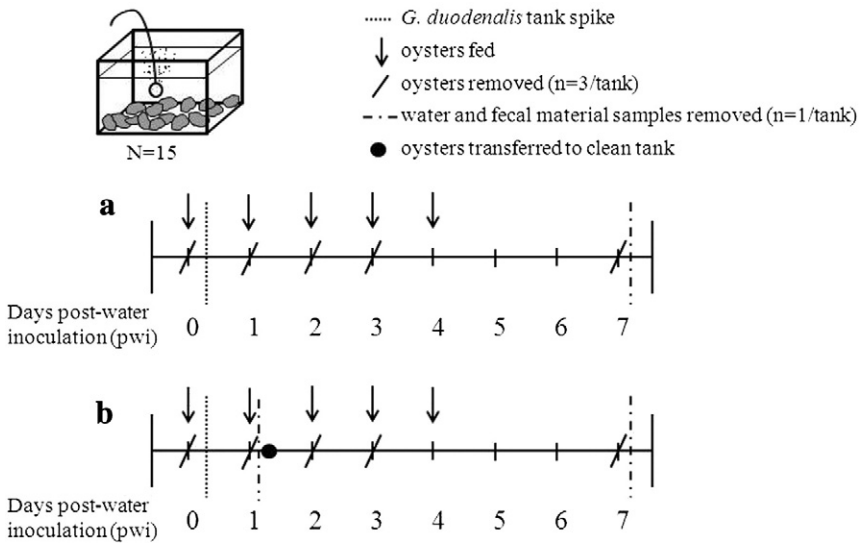

Fig. 1. Exposure trial outline for (a) chronic exposure and (b) acute exposure trials with *Giardia duodenalis* cysts. Each dose (10,000 cysts and 1000 cysts) had replicates of 5 tanks for each trial.

for 5 min, and the supernatant was discarded. The pellet was washed once in 15 mL of PBS eluting fluid and again in deionized water. The final product was re-suspended in 20–100 μL PBS to ensure samples could be homogenously mixed.

2.5. Processing of cysts from tank water samples

One liter water samples were collected from each tank on day 7 of the chronic exposure trial after all oysters had been removed. For the acute exposure trial, one liter water samples were collected on day 1 (from tanks that received the initial exposure dose) as well as on day 7 after all remaining oysters were sampled from the transfer tanks. All water samples were stored in 1.5 L sterile Nalgene bottles at 4 °C prior to processing. Samples were centrifuged at 1050 ×*g* for 15 min, after which the supernatant was discarded and the resulting pellet re-suspended in deionized water and re-centrifuged. The volume of the packed pellet was recorded prior to slide preparation, and calculation of the number of cysts/L was as previously published (Budu-Amoako et al., 2012a; Farias et al., 2002).

2.6. Detection of cysts by direct immunofluorescence assay (DFA)

Subsamples of 50 μL of oyster homogenate, fecal material, or water suspension, were air dried to fluorescence microscopy slides (Waterborne, New Orleans, LA). Samples were stained with 50 μL of *Giardia*-specific fluorescein isothiocyanate (FITC)-labeled monoclonal antibody solution (Giardi-a-glo™, Waterborne Inc., New Orleans, LA). Slides were incubated in a humid chamber for 40 min, briefly rinsed with PBS, and allowed to air dry overnight in a dark slide box. Fluorescent antibody mounting fluid (AquaPoly-mount, Polysciences, Warrington, PA) was used to adhere coverslips, and cysts were detected and enumerated at 600× magnification using an epifluorescence microscope (Leica DM 2500, Leica Microsystems, Wetzlar, Germany). The number of cysts per sample was defined as the number of cysts per slide well divided by the μL of sample on slide and multiplied by the total μL of sample.

2.7. Recovery efficiency testing for spiked cyst doses in oysters and fecal material

To evaluate the recovery efficiency of the oyster processing method described above (Sections 2.3 and 2.4), individual commercial sized *C. virginica* (shell length of 74–90 mm) was shucked into 50 mL tubes and spiked with either 10, 10^2, 10^3, or 10^4 *G. duodenalis* cysts. For each spiked dose, 10 replicate oysters were tested. Samples were then processed and final cyst concentrations were determined by examination by DFA.

To evaluate the recovery efficiency of cysts from feces, samples of uncontaminated oyster fecal material were collected and allowed to settle over 4 h. Sample volumes were adjusted to 10 mL, and replicates of three were spiked with 10, 50, 100, ,000 or 5000 cysts. Tubes were vortexed for 20 s to ensure mixing of cysts with the samples, and further processing was as described above.

2.8. Statistical analyses

Linear regressions were used initially to determine whether there was a difference in cyst retention over time in oysters between tanks that received the same initial exposure dose. The effect of the tank was not a statistically significant source of variation between samples (P-value > 0.05; data not shown), and therefore data points from individual tanks were pooled based only on sampling time. Linear regressions of total cyst counts per individual oysters sampled were performed for data between days 1 and 7 post-exposure. Multiple comparisons were made to test the difference in mean cysts between days,

Table 1
Recovery efficiency for *G. duodenalis* cysts spiked into individual *C. virginica* processed with pepsin–HCl, and for *G. duodenalis* cysts spiked into *C. virginica* fecal material and processed via centrifugation.

	Mean recovery (%) efficiency ± SD
# Cysts/oyster (N = 10/group)	
5,000	79.1 ± 11.5
1,000	78.4 ± 18.1
100	75.5 ± 12.4
50	71.6 ± 12.6
10	67.0 ± 17.7
# Cysts/condensed fecal pellet (N = 3/group)	
5,000	77.2 ± 8.2
1,000	74.8 ± 2.7
100	72.8 ± 3.5
50	67.0 ± 10.3
10	64.6 ± 8.8

and p-values were corrected using Holm's method. The line of best fit was superimposed upon scatter plots to show the overall trend in cyst accumulation in 7 days of continuous exposure and depletion in 1 day acute exposure trials. All analyses were performed using the statistical software Stata 12 (StataCorp LP, College Station, Tx).

3. Results

3.1. Recovery efficiencies for cysts in oysters and fecal material

After processing, the mean recovery efficiency of cysts from individual spiked oysters ranged between 67.0% and 79.1% with 10 replicates for each tested dose of 10–5000 cysts (Table 1). The recovery efficiency of cysts from spiked oyster fecal pellets ranged from 64.6% to 77.2% with 3 replicates tested (Table 1).

3.2. Cysts recovered from oyster homogenates

Oysters chronically exposed to *G. duodenalis* displayed two-phase retention kinetics for cysts, with faster uptake within the initial 24 hour post-water inoculation (pwi) than between day 1 and day 7 of static holding in the contaminated system (Fig. 2). Oysters at 24 h of exposure (combined measurement from both chronic and acute exposure trials) possessed a mean of 13.5 cysts (low dose group) or 83.1 cysts (high dose group). Linear regression analysis of chronic exposure trial data determined that there was a statistically significant (P-value < 0.05) average increase of 1.2 cysts/oyster/day (95% CI; 0.64, 1.7) and 6.8 cysts/oyster/day (95% CI; 4.2, 9.3) between day 1 and day 7 pwi in the low and high dose groups, respectively (Fig. 3a; b). In the high dose acute exposure trial, there was a statistically significant average decrease of 2.2 cysts/oyster/day (95% CI: −3.2, −1.2) between day 1 and day 7 pwi (Fig. 3c). Similarly, a statistically significant reduction of 0.92 cysts/oyster/day (95% CI: −1.3, −0.53) between day 1 and day 7 pwi was observed in the low dose exposure group (Fig. 3d).

3.3. Cysts recovered from oyster fecal material

At the end of the 7 day chronic exposure trials, a mean of 34.1% and 39.6% of the total spiked inoculum was recovered from fecal suspensions for the low dose (1000 cysts/tank) and high dose (10,000 cysts/tank) exposure groups, respectively (Fig. 4). Analysis of debris at the bottom of the positive control tank (no oysters) on day 7 identified 0.16% and 0.38% of the total tank inoculum of 1000 cysts or 10,000 cysts, respectively. Fecal material collected after 24 hour pwi during the acute exposure trials possessed an average of 30.0% and 37.0% of the total tank inoculum (low and high dose groups, respectively). A subsequent analysis of fecal material collected on day 7 from the clean transfer tank (which had been housing contaminated oysters for 6 days) found that an average of 5.1% and 2.9% of the total initial tank inoculum (low and high doses, respectively) had been excreted by oysters into the feces (Fig. 4).

3.4. Processing of cysts from tank water samples

Giardia cysts were not detected in any of the 1 L water samples, including the positive control tank.

4. Discussion

Protozoan parasites such as *Giardia* or *Cryptosporidium* are within the size range of particulate matter that most bivalves filter with high efficiency, including the Eastern oyster (particles of >5 μm) (Riisgaard, 1988; Zu Ermgassen et al., 2013). Some studies indicate that *Cryptosporidium* oocysts (spheres of ~4–6 μm diameter) may be preferentially ingested by shellfish over *Giardia* cysts (Graczyk et al., 2003). Size, shape, as well as other chemical factors (such as cell surface characteristics like carbohydrates) may result in some particles being "passively selected" during the differential selection process with pallial organs sorting captured particles (Rosa et al., 2013). Eastern oysters have been shown to be capable of distinguishing between particles of the same size by their surface characteristics (Rosa et al., 2013). In blue mussels (*Mytilus edulis*) it has been demonstrated that particle size (3–15 μm range) alone may not be responsible for differential clearance/elimination rates (Ward and Shumway, 2004). The capture, retention, and elimination of varying particulate matter constitute a multifactorial process that is highly dependent on species-specific bivalve characteristics as well as particle characteristics.

After an initial exponential uptake of cysts during the first 24 h, oysters held under chronic exposure conditions showed a smaller but

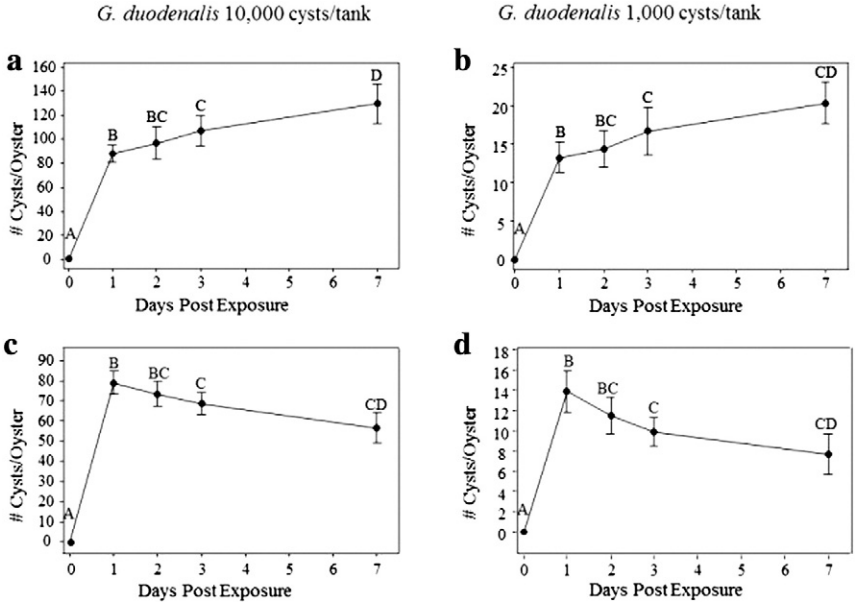

Fig. 2. Average bioaccumulation and elimination of *G. duodenalis* cysts in Eastern oysters during 7 day chronic (a–b) or 24 hour acute (c–d) exposure periods.

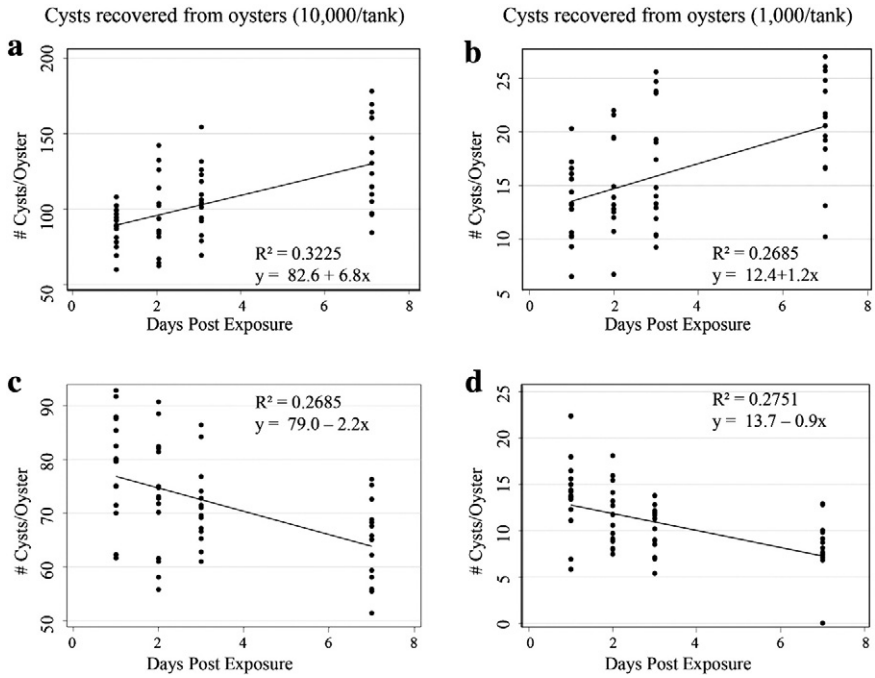

Fig. 3. Linear regressions of the number of cysts in individual oysters during a 7 day chronic (a–b) or 24 hour acute (c–d) exposure period.

still statistically significant increase in the number of cysts accumulated per day in both low and high dose exposure groups. In a study conducted by Graczyk et al. (2003), freshwater mussels (*Corbicula fluminea* and *Dreissena polymorpha*) were exposed daily to 80 cysts/L, an inoculum representative of the concentration of *Giardia* cysts that has been found in contaminated surface water samples. In that study, mussels were exposed to the inoculum for 31 consecutive days, and the number of cysts found in mussel tissue was correlated to the cumulative number of parasites inoculated (Graczyk et al., 2003). The current study demonstrates that oysters exposed to a single inoculum will continue to re-filter cysts, resulting in a gradual increase in parasite load when chronically exposed. Acute exposure trials demonstrate that oysters held under high salinities in static tank aquaria are still capable of gradually eliminating cysts over time, although at a slower rate. Both acute exposure trials (low and high dose) show a reduced cyst depuration between days 3 and 7 pwi compared to days 1 and 3 pwi, which could

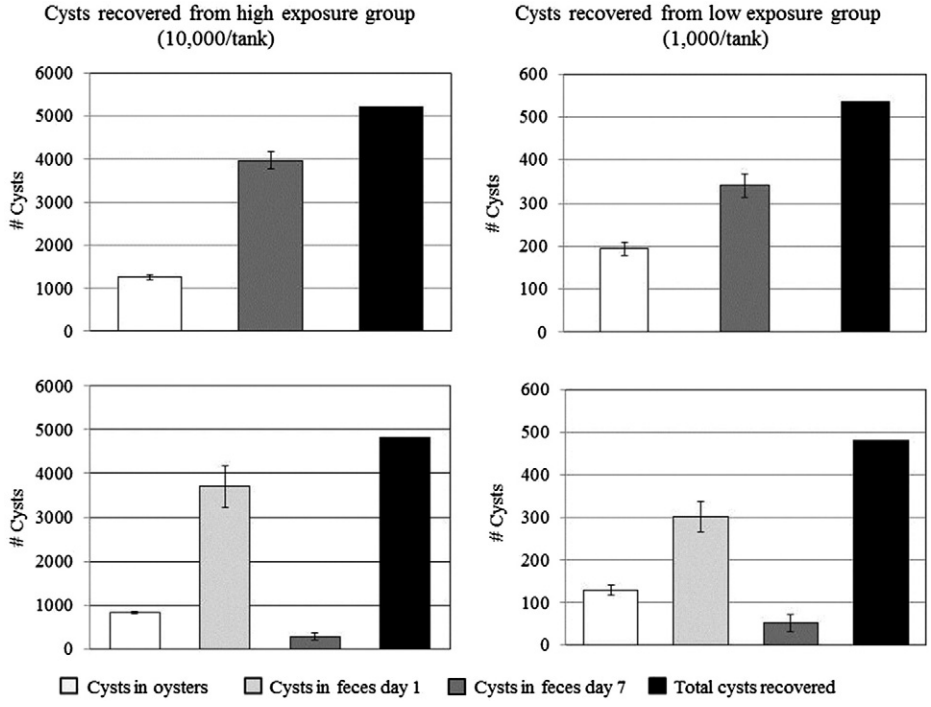

Fig. 4. Average total *G. duodenalis* cysts among 5 replicate tanks recovered from oyster tissue or oyster fecal material, from high and low exposure groups during chronic (a–b) or acute (c–d) exposure trials.

be due to a reduced filtration rate in remaining oysters due to lack of feeding over this time period. Nonetheless, 2/12 oysters examined on day 7 in the low dose acute exposure trial no longer harbored any *Giardia* cysts, demonstrating that oysters are still capable of gradually reducing parasite burden in the static tank system. *Giardia* cysts were not detected in any water samples, however water samples were stored for up to 6 weeks at 4 °C prior to processing which may have led to the deterioration of the cysts prior to analysis. Therefore, the centrifugation method was considered as an unsuccessful recovery method during these trials. Analysis of the debris (settled algae) at the bottom of the positive control tanks revealed only minor quantities of cysts, with only 0.16% and 0.38% of the total tank inoculum detected at the end of day 7 of the acute and chronic exposure trials, respectively, indicating that the majority of cysts remain suspended in the water during the exposure trial. Thus it is probable that the majority of the cysts detected from the fecal material in the experimental tanks were indeed excreted from the oysters and did not result from free-floating cysts settling to the bottom of the tank. Theoretical calculations by Ives (1990) determined sedimentation velocities of only 5.5 μm/s for *Giardia* cysts in stagnant water, and other studies have suggested that gravitational sedimentation is unlikely in aquatic habitats (Medema et al., 1998) and in a static tank system with good aeration it is unlikely that many cysts would settle to the bottom unless entwined in mucus/debris after being expelled by the bivalves.

Because oysters were transferred to clean, static tank systems during the acute exposure trials, the number of cysts recovered from the fecal material is indicative of the portion of cysts that were still present within the oyster at 24 h but subsequently expelled in the feces. The majority of cysts initially presented to oysters in tank water were rapidly filtered and removed, largely due to the production of pseudofeces. Our analysis of fecal material from day 7 of the acute exposure trials clearly indicates that some cysts were also likely retained, passed through the digestive system, and eliminated as feces after that initial 24 hour exposure. Significant reductions in the number of cysts in oyster tissue during days 1–7 pwi in the acute exposure trials support the findings within the fecal material as a portion of intact *Giardia* cysts was excreted in the feces during that time (Fig. 4). As only 48–53% of the total spiked tank inoculum was recovered in total, the possibility exists that cysts were lost during processing, present but unrecovered with our centrifugation method in the water, or some cysts are degraded by the bivalves' digestive system and thus unidentifiable by immunofluorescence methods, a possibility proposed by Graczyk et al. (2006).

Conducting exposure trials for bivalve species of interest is very important as it is apparent that the bioaccumulation of protozoan parasites is influenced by not only temperature, but also salinity and by the bivalve shellfish species being examined. Graczyk et al. (2003) noted that more *Giardia* cysts were retained by the freshwater mussels *D. polymorpha* compared to *C. fluminea* under identical experimental conditions. Similarly, Nappier et al. (2010) determined that the Suminoe oyster (*Crassostrea ariakensis*) was capable of retaining more *Giardia* cysts than Eastern oysters. In Suminoe oysters, low salinities (8 ppt) appear to hinder depuration of *Giardia* cysts compared to a higher salinity of 20 ppt (Graczyk et al., 2006). In that study, the end point day of detection of *Giardia* cysts determined by Graczyk et al. was 6 days at 20 ppt compared to 14 days at 8 ppt. The study by Nappier et al. (2010) detected *G. duodenalis* for 22 days pwi in Suminoe oysters compared to only 8 days in Eastern oysters, at a salinity of 12 ppt.

Extrapolating an elimination curve based on the data from the acute exposure trial, an estimated endpoint day of detection was 15 days post exposure for low dose groups and 36 days post exposure for high dose groups. These values are only estimates as the data points we had were limited and our extrapolations were made from relatively early elimination time points. Additionally, there were large variations in the number of cysts retained by each individual oyster at each time point ($R = 0.27–0.32$; Fig. 3). The increased time required by oysters to depurate in this study may be due to the use of a static tank system with low water volume, in which the water was only replaced after the initial oyster transfer on day 1 and may have enabled oysters to continually re-filter some re-suspended cysts even after transfer to a clean tank.

The current experimental tank exposures enable researchers to visualize the individual capacity of Eastern oysters to bioaccumulate as well as eliminate *G. duodenalis* cysts under high saline concentration conditions (29 ppt) that closely resemble natural conditions for many Eastern oyster populations. Elimination of *G. duodenalis* cysts by Eastern oysters is likely to vary across the broad salinity range of the species where 10–28 ppt is considered optimum for growth/reproduction, with some populations surviving up to 35 ppt (Buroker, 1983; Wilson et al., 2005). The use of detailed exposure tank trials involving only *Giardia* is helpful in understanding how bivalve shellfish responds to this specific protozoan parasite and enables direct comparisons to be made when a standardized experimental set up is used. When comparing the results of this study to a previously published study on *Cryptosporidium parvum* employing the same experimental methodology (Willis et al., 2014), several things are apparent: Firstly, *G. duodenalis* cysts are retained in smaller quantities compared to *C. parvum* oocysts during 7 day chronic exposure trials. For instance, when 10 L water was contaminated with either 10,000 (oo)cysts, individual oyster was found to retain a mean of 252 *C. parvum* oocysts compared to 87 *G. duodenalis* cysts (Willis et al., 2014). This finding supports the claim by Graczyk et al. (2003) that *Giardia* cysts may not be as readily taken up into or retained by the digestive system as *Cryptosporidium* oocysts. Secondly, *G. duodenalis* cysts depurate at a slower rate than *C. parvum* oocysts after an acute (24 h) exposure to 10,000 (oo)cysts, with a decrease of only 2.2 cysts/oyster/day compared to 9.5 oocysts/oyster/day (Willis et al., 2014). Although it is unclear why Eastern oysters process these two protozoan parasites differently, this research indicates that differential selection is likely taking place. Most studies that have detected *Giardia* cysts in shellfish have examined pooled samples including mixes of gill tissue, hemolymph, digestive tracts, etc. (Willis et al., 2013), and thus it is unclear whether or not cysts remain intact throughout the digestive tract or whether some are indeed digested and degraded, accounting for the lack of inoculum recoveries seen in this and other studies.

The data presented here illustrate that Eastern oysters held at high salinities are capable of filtering large numbers of *G. duodenalis* cysts, and that the small percentage of cysts that are initially not rejected as pseudofeces can persist in shellfish tissue and are eliminated slowly under static depuration conditions. This study also demonstrates that the elimination of cysts by oysters is observable not only by reductions in cyst concentrations in oyster homogenates, but also by the detection of cysts in the fecal material. Although the tank water in the present study did not reveal the presence of any cysts in the water, a complementary experiment of *C. parvum* oocysts conducted using the same experimental design and parameters showed that only a fraction of the initial tank inocula remained in water samples by day 7 pwi (Willis et al., 2014).

The potential health risks associated with consuming raw oysters or other undercooked shellfish potentially contaminated with *G. duodenalis* are likely dependent on several factors including the ability of a particular target bivalve species to filter and retain cysts. The salinity and temperature in regions considered at risk of contamination should also be considered as it may either improve or reduce the survival and/or retention of bioaccumulated cysts in oysters.

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