Ecotoxicology and Environmental Safety 133 (2016) 188–194

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

# Bioaccumulation of *Toxoplasma* and *Cryptosporidium* by the freshwater crustacean *Gammarus fossarum*: Involvement in biomonitoring surveys and trophic transfer

Aurélie Bigot-Clivot [a,*], Mélissa Palos Ladeiro [a], Alexandra Lepoutre [a], Fanny Bastien [a], Isabelle Bonnard [a], Jitender P. Dubey [c], Isabelle Villena [b], Dominique Aubert [b], Olivier Geffard [d], Adeline François [d], Alain Geffard [a]

[a] Université de Reims Champagne Ardenne, Unité Stress Environnementaux et BIOSurveillance des Milieux Aquatiques, UMR-I 02 (SEBIO), Reims, France
[b] Laboratoire de Parasitologie-Mycologie, EA 3800, SFR CAP-Santé FED 4231, Hôpital Maison Blanche Reims, France
[c] United States Department of Agriculture, Agricultural Research Service, Beltsville Agricultural Research Center, Animal Parasitic Diseases Laboratory, Beltsville, MD 20705, USA
[d] Institut National de Recherche en Sciences et Technologies pour l'environnement et l'agriculture, Unité de Recherche Milieux Aquatiques, Ecologie et Pollutions, Ecotoxicologie, Villeurbanne Cedex, France

ARTICLE INFO

*Article history:*
Received 30 March 2016
Received in revised form
4 July 2016
Accepted 6 July 2016
Available online 22 July 2016

*Keywords:*
Protozoa
Oocysts
Gammarids
Molecular detection

ABSTRACT

The protozoa *Toxoplasma gondii* and *Cryptosporidium parvum* are public health priorities because their oocysts can persist in recreational, surface, drinking, river, and sea water sources for a long time. To evaluate the capacity of the freshwater crustacean *Gammarus fossarum* to accumulate *T. gondii* and *C. parvum* oocysts, gammarids were exposed to 200, 2000 or 20,000 oocysts per gammarid and per day for 21 days followed by 5 days of depuration. *C. parvum* DNA was detected by qPCR in *G. fossarum* in only one out of four pools for the highest concentration and after 14 days of exposure, and *T. gondii* DNA was detected after 7 days of exposure to the two highest concentrations. Our results document the capacity of *G. fossarum* to accumulate *T. gondii* in its tissues proportionally to the ambient concentration; the maximum number of oocysts was detected in gammarid tissues after exposure to 20,000 oocysts per day. Mean values of 3.26 ( ± 3), 21.71 ( ± 15.18), and 17.41 ( ± 10.89) oocysts were detected in gammarids after 7, 14, and 21 days, respectively, and after 5 days of depuration, *T. gondii* oocysts were still present in gammarid tissues. These results show for the first time that a freshwater crustacean can bioaccumulate *T. gondii* oocysts, suggesting that *G. fossarum* is a potential effective bioindicator of protozoan contamination in biomonitoring studies. Moreover, due to its key position in freshwater food webs, *G. fossarum* could also play a role in the trophic transfer of protozoa.



## 1. Introduction

*Toxoplasma gondii* and *Cryptosporidium parvum* represent a threat to human health because they are infectious at low doses and their oocysts can remain infectious in the environment for months or even years (Lindsay and Dubey, 2009; Tamburrini and Pozio, 1999). Protozoan oocysts are difficult to eliminate from the environment because they are surrounded by a robust wall that withstands chemical disinfection and mechanical damage (Belli et al., 2006; Dumètre et al., 2008). *Escherichia coli* and other enterococci are currently used as indicators of fecal contamination of drinking water. Compared with these bacteria, protozoa are difficult to detect in environmental samples. Helmi et al. (2011) highlighted that some sites could be protozoa positive while no bacterial fecal indicators were found; conversely, some samples were bacterial-indicator positive but protozoa-negative. The protozoa *Cryptosporidium* and *Giardia* are currently detected in samples from 100l of filtered water, using indirect immunofluorescence after immunomagnetic separation (ISO 15,553:2006). Limitations have been identified in this method: it requires large volumes of filtered water and high parasite concentrations, is costly and time-consuming, and does not allow for rapid routine detection. Moreover, filtration and purification techniques from water supplies can yield variable results depending on water quality, sampling period, place, and quantity (Gallas-Lindemann et al., 2013).

*Cryptosporidium parvum* is an intestinal protozoan found in mammals, fish and birds, and is one of four pathogens responsible

* Corresponding author.
*E-mail address:* aurelie.bigot@univ-reims.fr (A. Bigot-Clivot).

http://dx.doi.org/10.1016/j.ecoenv.2016.07.006
0147-6513/

for most of the moderate to severe diarrhea outbreaks in infants and toddlers (Kotloff et al., 2013). Its oocysts can be transmitted via drinking and recreational waters contaminated by agricultural and urban runoff (Gallas-Lindemann et al., 2013). *Cryptosporidium* oocysts have been reported in a range of bivalve mollusc species, including mussels (*Mytilus galloprovincialis*), clams (*Ruditapes decussatus*, *Ruditapes philippinarum*, *Venerupis pulastra*, *Dosinia exoleta*), oysters (*Ostrea edulis*) and cockles (*Cerastoderma edule*) (Gómez-Couso et al., 2003; Mladineo et al., 2009).

Unlike cryptosporidial species that can be shed by a wide range of animal species, including humans, *T. gondii* oocysts are only shed by felids, but a cat can excrete millions of oocysts in one week (Dabritz and Conrad, 2010). Terrestrial transmission of *T. gondii* occurs mainly through oocyst ingestion and through the carnivorism of infected tissues. Both marine and freshwaters can be contaminated with *T. gondii* oocysts (Robertson, 2007; Palos Ladeiro et al., 2013). *T. gondii* DNA was detected in oysters (*Crassostrea rhizophorae*) and mussels (*Mytella guyanensis*) from a commercial source in Santos City (São Paulo state) (Esmerini et al., 2010), and in crayfish (*Procambarus clarkii*), fish (*Hypophthalmichthys molitrix*) and freshwater shrimp (*Macrobranchium nipponense*) collected in parts of China (Zhang et al., 2014). Bioaccumulation in oysters or consumable mussels, which can be eaten raw, could transmit waterborne diseases and directly suggests a health risk to humans (Graczyk et al., 2006). It also demonstrates the interest of studying aquatic organisms to assess and monitor the quality of water bodies (Palos Ladeiro et al., 2013).

No data are currently available as to the possible accumulation of protozoan oocysts by crustacea. Gammarids have an interesting geographical distribution because they are located upstream of water bodies. Several studies have been carried out using these crustacea to evaluate the quality of freshwater ecosystems (Besse et al., 2013; Kunz et al., 2010). The amphipod species *Gammarus fossarum* selected in this study is widespread and common in rivers and streams of Western Europe. This crustacean is relatively robust, easily collected, and experiments can be performed using caging (Dedourge-Geffard et al., 2013; Schmidlin et al., 2015). It is considered as a key species that mainly acts as an efficient shredder, can feed on fine particulate organic matter (Dangles et al., 2004), and plays a fundamental role in organic matter breakdown in springs and spring brooks (Macneil et al., 1997). Gammarids play a determining ecological role in the food chain because they represent a food reserve for macroinvertebrates, fish, birds, and amphibians. Aquatic invertebrates can act as paratenic hosts by accumulating and concentrating protozoa in their tissues, so food transfer could be a significant transmission route of *T. gondii* and *C. parvum* oocysts.

The present study aims to determine whether *G. fossarum* can accumulate *T. gondii* and *C. parvum* oocysts in its tissues after experimental exposure, and whether it can be used as a matrix for biomonitoring protozoa concentrations in freshwater systems.

## 2. Materials and methods

### 2.1. Sampling and maintenance of gammarids

Adult *G. fossarum* were collected using a hand-held net at "La Tour du Pin", an unpolluted upstream part of the Bourbre River (France). This station displays good water quality according to RNB (Réseau National de Bassin) data records (French Watershed Biomonitoring Network). The crustacea were stored in plastic bottles containing ambient freshwater. In the laboratory, they were acclimatized for 15 days in 30-L tanks containing Cristaline Aurèle (Jandun, France) spring water ($Ca^{2+}$: 106 mg/L, $Mg^{2+}$: 4.2 mg/L, $Na^{+}$: 3.5 mg/L, $K^{+}$: 1.5 mg/L, $HCO_3^{-}$: 272 mg/L, $SO_4^{2-}$ 50 mg/L, $Cl^{-}$: 3.8 mg/L, $NO_3^{-}$: $<1$ mg/L, $F^{-}$: 0.9 mg/L) under constant aeration with a 10/14 h light/dark photoperiod and a temperature of $12 \pm 1$ °C. Gammarids were fed *ad libitum* with alder leaves (*Alnus glutinosa*) collected in the forests of the Beaujolais hills in autumn, at an altitude where there is neither farming nor urban activity. The leaves were previously conditioned in groundwater for at least 6 days (Charron et al., 2015).

*T. gondii* oocysts were collected from the feces of cats fed with mice infected with tissue cysts of the *T. gondii* genotype II strain ME49, as described by Dubey (2009). The sporulated oocysts were stored at 4 °C in an aqueous solution containing 2% $H_2SO_4$ until use. *C. parvum* oocysts were purchased from Waterborne™, Inc. (New Orleans, LA, USA) and were obtained from experimentally infected calves. They were stored in a phosphate-buffered saline (PBS) solution and kept at 4 °C until use.

### 2.2. Assessment of oocyst recovery rates

Oocysts were counted using a Kova slide using a Leitz Laborlux S fluorescence microscope. To determine oocyst recovery rates under different matrix conditions (gammarids or PBS buffer w/o Ca and Mg), four quantities of *C. parvum* or *T. gondii* oocysts were prepared: 20; 200; 2000 or 20,000 oocysts in 10 µL of PBS. Five pools of three gammarids per condition were spiked with the oocyst aliquots. Positive controls were oocysts in PBS. Extraction and DNA detection were performed immediately after spiking, as described below.

### 2.3. In vivo experimental design

Before the beginning of the exposure period, 4 pools of 3 gammarids were kept to ensure that they were free of *T. gondii* and *C. parvum* oocysts (day zero, D0). Then, gammarids were randomly divided into 7 groups of 60 gammarids: 1 control group and 6 exposed groups. Exposure was conducted at $13 \pm 1$ °C, under constant aeration in 9-L tanks containing 5 L of Cristaline Aurèle drinking water for the first week of exposure, and then 4 L for the second week and 3 L for the third week, according to the sampling dates, to maintain the same quantity of oocysts per organism and per liter. Gammarids were exposed to 200; 2000 and 20,000 *T. gondii* or *C. parvum* oocysts per gammarid and per day for 21 days (*i.e.* 40, 400 and 4000 oocysts/L for the first week of exposure, 50, 500 and 5000 oocysts/L for the second week, and 66.7, 666.7 and 6666.7 oocysts/L for the third week). Then they were transferred into new tanks filled with clean water and left there 5 days for depuration. Four pools of 3 gammarids were sampled per condition after 7, 14, and 21 days of exposure and after 5 days of depuration (Fig. 1).

Gammarids were fed with 1 alder leaf (*A. glutinosa*, same as maintenance conditions) disc (20 mm in diameter) per individual and per week. On the 7th, 14th and 21th days the water parameters were measured spectrophotometrically (colorimetric method, spectrophotometer DR/2000 Hach) (nitrites: $0.22 \pm 0.02$ mg/L, nitrates: $8.14 \pm 0.31$ mg/L, ammonium: $0.08 \pm 0.06$ mg/L), the tank water was filtered and replaced by fresh water. The remaining leaf discs were recovered, and oocyst DNA was searched for. Oocyst amounts were spiked again after water changes to maintain the previous oocyst concentrations in the tanks.

### 2.4. Oocyst extraction from different matrices

*T. gondii* and *C. parvum* oocyst DNA was searched for in gammarids, tank water and remaining leaf discs (biofilms on alder leaves can adsorb oocysts from the water (Shapiro et al., 2014). This molecular detection in the different compartments allowed us to draw a balance sheet of oocyst numbers.

Please cite this article as: Bigot-Clivot, A., et al., Bioaccumulation of *Toxoplasma* and *Cryptosporidium* by the freshwater crustacean *Gammarus fossarum*: Involvement in biomonitoring surveys and trophic transfer. Ecotoxicol. Environ. Saf. (2016), http://dx.doi.org/10.1016/j.ecoenv.2016.07.006