Egyptian Journal of Aquatic Research (2016) 42, 341–347

HOSTED BY
ELSEVIER

National Institute of Oceanography and Fisheries

Egyptian Journal of Aquatic Research

http://ees.elsevier.com/ejar
www.sciencedirect.com

# Effects of selected pharmaceuticals (ibuprofen and amoxicillin) on the demography of *Brachionus calyciflorus* and *Brachionus havanaensis* (Rotifera)

**Brenda Karen González-Pérez** [a], **S.S.S. Sarma** [b,*], **S. Nandini** [b]

[a] *Posgrado en Ciencias del Mar y Limnología, Universidad Nacional Autónoma de Mexico, Mexico City 04510, Mexico*
[b] *Universidad Nacional Autónoma de México, Campus Iztacala. Av. De los Barrios #1, Col. Los Reyes Iztacala, Tlalnepantla, Estado de México C.P. 54090, Mexico*

Received 14 March 2016; revised 29 June 2016; accepted 7 September 2016
Available online 13 October 2016

**KEYWORDS**

Drugs;
Life table;
Sublethal effects;
Zooplankton

**Abstract** The levels of emerging chemicals have increased dramatically during the last two decades posing problems for human and environmental health. Pain-killers such as ibuprofen and antibiotics such as amoxicillin are generally consumed together and hence are discharged into waterbodies as effluents. The lack of a rigorous control of pharmaceutical discharges into natural waterbodies is a concern for limnologists and ecotoxicologists because of their possible effects on non-target organisms. Rotifers, due to their sensitivity, short generation time and high reproductive rates, are widely used as bioassay organisms in testing the effects of different substances including pharmaceuticals. Here we quantified the demographic responses of *Brachionus calyciflorus* and *Brachionus havanaensis* exposed to three sublethal concentrations of ibuprofen (25, 12.5 and 6.25 mg $L^{-1}$) and amoxicillin (200, 100 and 50 μg $L^{-1}$). Our data showed that both survivorship- and reproduction-related variables were negatively affected with increasing concentrations of both pharmaceuticals. The rate of population increase of *B. calyciflorus* (0.63–0.72 $d^{-1}$) was not affected by amoxicillin or by ibuprofen but for *B. havanaensis*, it was decreased significantly (from 0.89 to 0.38 $d^{-1}$). Compared to ibuprofen, amoxicillin had more adverse effects on both the rotifer species.


## Introduction

During the last two decades, levels of emerging chemicals including pharmaceuticals have increased dramatically and these pose a threat to environmental and human health (Burkhardt-Holm, 2010). Personal care products, endocrine disruptors and pharmaceuticals are abundant in domestic effluents (Halling-Sørensen et al., 1998a,b; Daughton and Ternes, 1999). Since most of these substances are sold without a medical prescription, their production, availability and use are a source of concern for effective management of freshwater ecosystems. There is little information on the ecotoxicological effects of most pharmaceuticals to non-target organisms, especially zooplankton. Pharmaceutical products including drugs for human and/or veterinary medical practices are produced and

* Corresponding author.
E-mail address: sarma@unam.mx (S.S.S. Sarma).
Peer review under responsibility of National Institute of Oceanography and Fisheries.

http://dx.doi.org/10.1016/j.ejar.2016.09.003
1687-4285 © 2016 National Institute of Oceanography and Fisheries. Hosting by Elsevier B.V.

consumed in large quantities. For example, Latin America accounts for more than 3% of the global production of these substances (Pérez Zazueta, 2013). In spite of their high production, consumption and inadvertent release into aquatic bodies, there is no rigorous control over these substances for safe disposal. Therefore, they are found in many water bodies at concentrations ranging from ng to μg per litre. At these concentrations, it is difficult to detect and quantify them in effluent waters (Daughton and Ternes, 1999; Bila and Dezotti, 2003).

Among the commonly used pharmaceuticals in Mexico for treating bacterial infections and for pain-killing the broad-based antibiotics and nonsteroidal anti-inflammatory drugs (NSAIDs), respectively, are important. Although for purchasing the antibiotics such as amoxicillin a medical prescription is needed, for common pain-killers such as ibuprofen no such requirement exists due to their possibly low risk from side effects to humans (Dresser et al., 2008). In Mexico ibuprofen is one of the pharmaceuticals mentioned in the *Essential Drug List* (CSG, 2014). Amoxicillin is one of the most widely administered antibiotics in Mexico (Vargas-Jiménez et al., 2011).

The occurrence of pharmaceuticals in aquatic environments and their possible ecotoxicological effects to the non-target species has received much less attention compared to other chemicals such as pesticides and heavy metals (Daughton and Ternes, 1999; Cleuvers, 2004; Ali and Al-Qahtani, 2012). Effluents from hospitals, industries, domestic and veterinary sources contain pharmaceuticals which eventually reach treatment plants or rivers and in most cases without pre-treatment (Araujo and McNair, 2007). The presence of ibuprofen and amoxicillin in freshwaters and in effluents of sewage treatment plants has been frequently detected in many countries (Andreozzi et al., 2004; Brun et al., 2006; Gómez et al., 2007; Kim et al., 2007; Kasprzyk-Hordern et al., 2008). In Mexico too, ibuprofen in wastewaters from the Valley of Tula (State of Hidalgo), at concentrations ranging from 0.7 to 1.4 $\mu g\ L^{-1}$, has been documented (Gibson et al., 2010).

Zooplankton forms an important component of freshwater ecosystems including shallow waterbodies (Moss, 2007). Rotifers, which are a numerically dominant group of zooplankton, have been widely used in aquatic risk assessments of pharmaceuticals and their metabolites due to their great sensitivity and high reproductive rates (Snell and Joaquim-Justo, 2007). Species of *Brachionus*, mainly *B. plicatilis*, *B. havanaensis* and *B. calyciflorus* have already been used as bioassay organisms for testing various chemicals (Snell and Janssen, 1995).

Life table demography is useful to monitor the sublethal effects of different chemicals including pharmaceuticals. Using this approach it is possible to derive both survivorship- and reproduction-related variables, which are considered as sensitive indicators of stress (Calow, 1995; Forbes and Calow, 1999). This study focuses on the chronic effects of two common pharmaceuticals, ibuprofen and amoxicillin on the life table demography of two rotifers *B. calyciflorus* and *B. havanaensis*.

## Material and methods

*B. calyciflorus* and *B. havanaensis* were isolated from Lake Xochimilco (Mexico City). Monoclonal cultures were separately established using the single-celled green alga, *Chlorella vulgaris*, as exclusive food. The rotifers were cultured using a synthetic medium, moderately hardwater (here after EPA medium), which was daily prepared by dissolving 96 mg of NaHCO3, 60 mg of CaSO4, 60 mg of MgSO4 and 4 mg of KCl in 1 L of distilled water (Weber, 1993). *Chlorella vulgaris* was batch-cultured using standard medium (Bold's basal, Borowitzka and Borowitzka, 1988). Log phase algae were harvested and concentrated by centrifugation at 3000 rpm for 5 min. The concentrated algae were then resuspended in 5 ml of distilled water. The algal density was estimated using a haemocytometer.

For the rotifers, in the stock cultures and in the experiments, the temperature was set at 22 ± 1 °C, pH 7.0–7.4, and fluorescent illumination was continuous but diffuse. To obtain neonates of a known age, we separated a large number of egg-bearing individuals from the mass cultures in the exponential phase of growth. Neonates hatched within 3 h were used in the experiments. Ibuprofen and amoxicillin were first dissolved separately in distilled water following Hussein et al. (2008). Based on $LC_{50}$ data available in literature (Heckmann et al., 2007), we chose the respective sublethal concentrations for both the drugs, prepared daily. For ibuprofen we considered 25, 12.5 and 6.25 $mg\ L^{-1}$ and for amoxicillin we chose 200, 100 and 50 $\mu g\ L^{-1}$, in addition to controls (containing alga but no drug).

Standard cohort life table experiments were separately conducted for each pharmaceutical (ibuprofen and amoxicillin) (Krebs, 1985). The rotifers in the test jars were fed *Chlorella vulgaris* at a density of $1 \times 10^6$ cells $mL^{-1}$ per day. The experimental design consisted of 16 transparent jars of 50 mL capacity, each with 20 mL of test medium. For each treatment we used 4 replicates. Using a finely drawn Pasteur pipette under a stereomicroscope, we individually introduced 20 neonates (i.e., initial density 1 ind. $mL^{-1}$) of each rotifer species into the test jars containing *Chlorella* of the chosen density and one of the selected concentrations of each pharmaceutical.

Following the initiation of the life table experiment, in each replicate the number of neonates produced and dead adults, if any, were counted and discarded after every 12 h interval. Daily, the surviving individuals of the original cohort were transferred to fresh jars containing appropriate pharmaceutical and food concentration. The experiments for *B. calyciflorus* and *B. havanaensis* were discontinued when every individual from each cohort had died. We used standard formulae to derive the life table variables (survivorship, fecundity, average lifespan and gross, net reproductive rates, generation time and rate of population increase) (Krebs, 1985).

$l_x$ = Proportion of surviving at the start of age $x$

$m_x$ = Offspring produced per female at age $x$

Life expectancy at birth $x$: $e_x = \frac{T_x}{n_x}$

where $T_x$ = cumulative number of individuals from the age $x$

$n_x$ = number living at the age $x$ (days)

Net reproductive rate $R_o = \sum_0^\infty l_x \cdot m_x$

Generation time: $T = \frac{\sum l_x \cdot m_x \cdot x}{R_o}$

Rate of population increase per day, $r$ $1 = \Sigma e^{-rx} l_x m_x$ (Euler-Lotka equation, solved iteratively).

In order to test if the differences among the treatments were significant, we used a one-way analysis of variance (ANOVA) and for multiple comparisons, we used post hoc (Tukey tests) following standard statistical methods (Sokal and Rohlf, 2000).

**Results**

The age-specific survivorship ($l_x$) of *B. calyciflorus* and *B. havanaensis* exposed to ibuprofen and amoxicillin declined with increasing drug concentrations (Fig. 1). The age-specific fecundity ($m_x$) curves for *B. calyciflorus* and *B. havanaensis* in controls showed higher offspring production (3 neonates per female per day) than in treatments containing amoxicillin or ibuprofen (2 offspring per female per day). The fecundity was lowest for both rotifer species at the highest concentrations of ibuprofen or amoxicillin (Fig. 2). Although both pharmaceuticals adversely affected the fecundity of the brachionid species, amoxicillin had a far greater effect on *B. calyciflorus* and *B. havanaensis*.

The average lifespan in controls of both *B. calyciflorus* and *B. havanaensis* was about 12 days and at the highest amoxicillin level, it was reduced by about 20% for either rotifer species. When exposed to ibuprofen, the decrease in the lifespan, compared to the controls, of *B. havanaensis* was much greater than that of *B. calyciflorus* (Table 1). Statistically, the average lifespan of either rotifer species was significantly different from controls and at the two highest concentrations of amoxicillin or ibuprofen for *B. calyciflorus* and for *B. havanaensis* ($<0.001$, Tukey, Table 1). Gross (GRR) and net reproductive ($R_0$) rates of *B. calyciflorus* in controls were 33 and 21 offspring female$^{-1}$ and for *B. havanaensis* in controls, they were 40 and 23 offspring female$^{-1}$, respectively. The GRR and $R_0$, for both species, decreased with an increase in the level

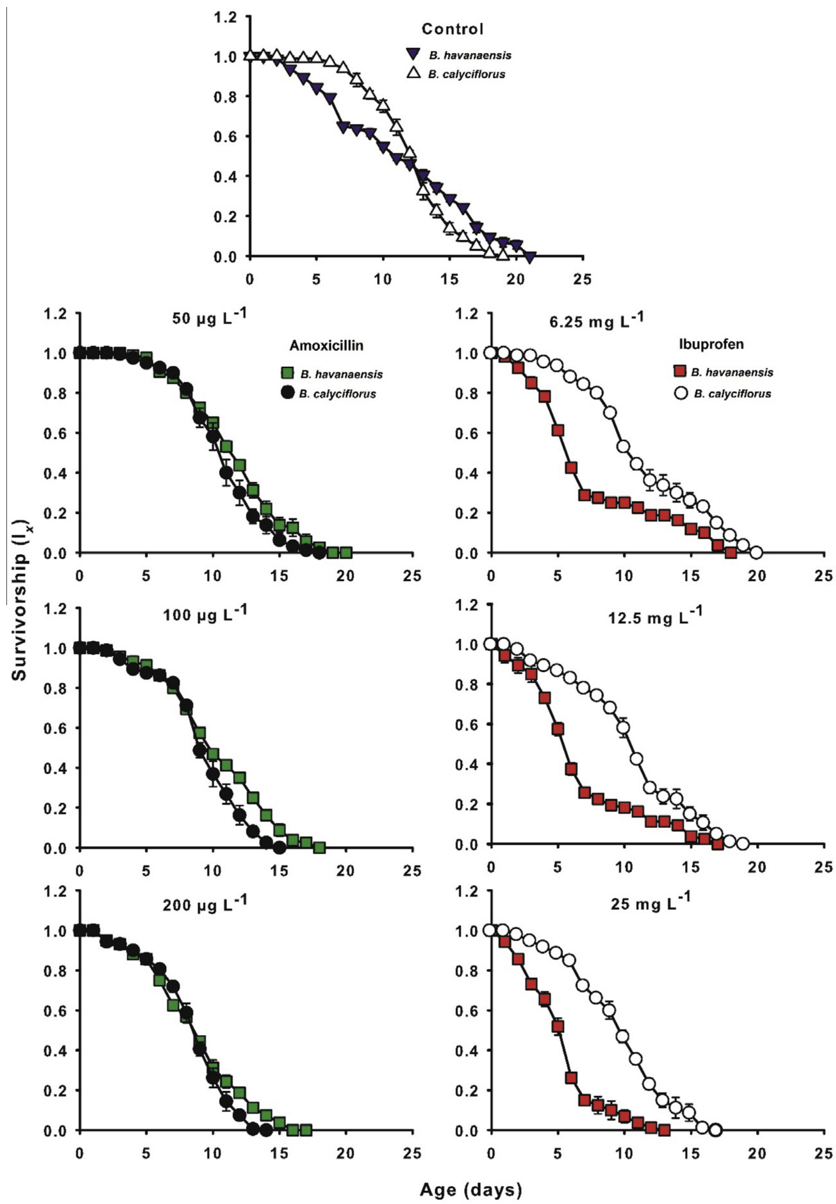


**Figure 1** Age-specific survivorship ($l_x$) curves of *Brachionus calyciflorus* and *B. havanaensis* in controls (triangle) and exposed to three sublethal concentrations of amoxicillin (closed (black) circle and closed (green) square) (50, 100 and 200 μg L$^{-1}$) and ibuprofen (open circle and closed (red) squares) (6.25, 12.5 and 25 mg L$^{-1}$). Shown are the mean ± SE of 4 replicates.

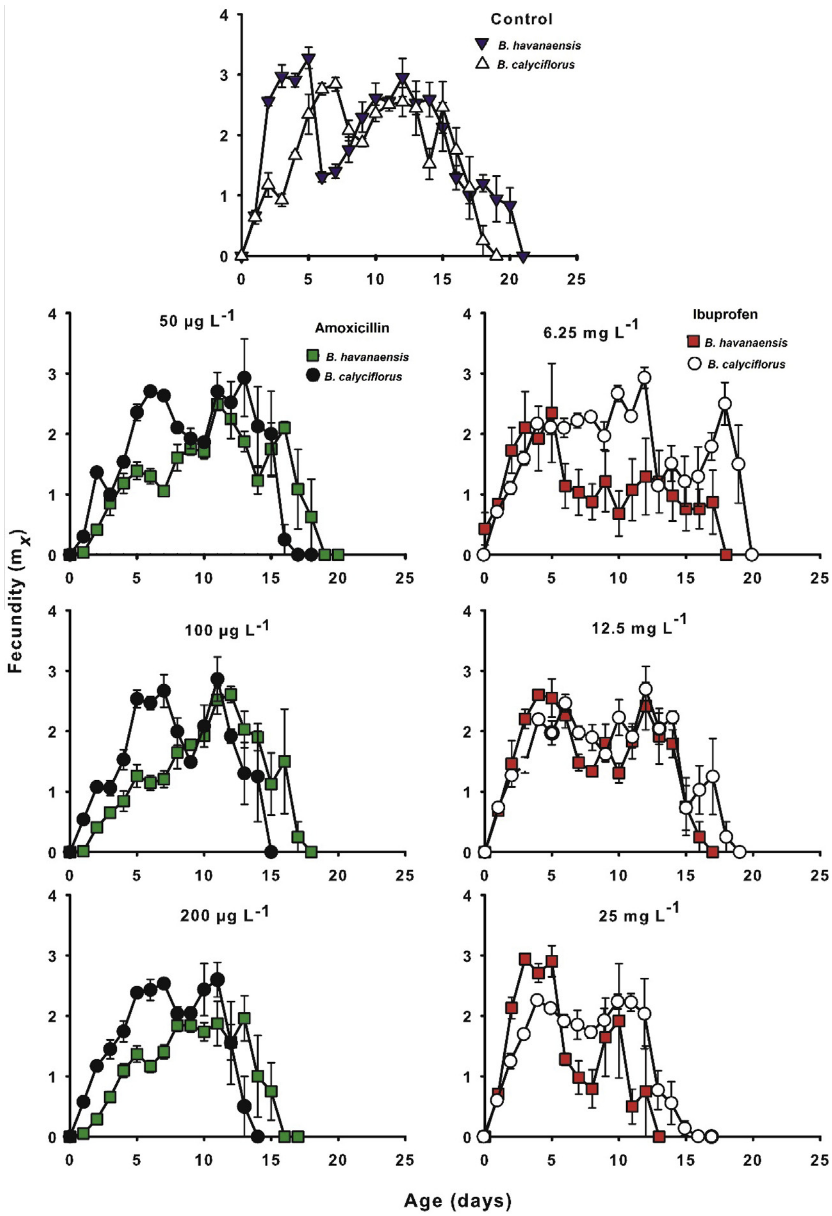


**Figure 2** Fecundity ($m_x$) (offspring female$^{-1}$ lifespan$^{-1}$) curves of *Brachionus calyciflorus* and *B. havanaensis* in controls (triangle) and exposed to three sublethal concentrations of amoxicillin (closed (black) circle and closed (green) square) (50, 100 and 200 µg L$^{-1}$) and ibuprofen (open circle and closed (red) squares) (6.25, 12.5 and 25 mg L$^{-1}$). Shown are the mean ± SE of 4 replicates.

of amoxicillin or ibuprofen. There were also significant differences ($p < 0.001$, Tukey) in the reproductive rates of *B. havanaensis* even at the lowest drug concentrations; reproduction in *B. calyciflorus*, on the other hand, was not significantly influenced by either drug at the lowest tested concentration ($p > 0.05$, Tukey).

In general, the generation time (T) of both the rotifer species was significantly lower at the highest pharmaceutical concentrations when compared to controls. The shortest T was observed for either rotifer species at the highest concentration of amoxicillin or ibuprofen. Depending on the concentration or drug type, the generation time of *B. calyciflorus* and *B. havanaensis* varied from 4–8 days. Under the higher ibuprofen levels, the generation time of *B. havanaensis* was more adversely affected than under lower drug concentrations.

In treatments containing no drugs (controls), the rate of population increase ($r$) of *B. havanaensis* (0.89 d$^{-1}$) was higher than that of *B. calyciflorus* (0.68 d$^{-1}$). When *B. havanaensis* was exposed to amoxicillin and ibuprofen, the $r$ values ranged from 0.38 to 0.43 and 0.71 to 0.85 d$^{-1}$, respectively. However, $r$ of *B. calyciflorus* when exposed to amoxicillin (0.63–0.67 d$^{-1}$) or ibuprofen (0.71–0.72 d$^{-1}$) showed no significant differences compared to control. With reference to the sensitivity of $r$ for both rotifer species to amoxicillin or ibuprofen, there were some differences; *B. havanaensis* was more sensitive to the antibiotic and ibuprofen ($p < 0.001$, Tukey) while *B. calyciflorus* was not.

**Table 1** Selected life history traits of *B. calyciflorus* and *B. havanaensis* in relation to three sublethal concentrations (Conc.) of amoxicillin (50, 100 and 200 μg $L^{-1}$) and ibuprofen (6.25, 12.5 and 25 mg $L^{-1}$ amoxicillin. ALS: Average lifespan, GRR: Gross reproductive rate, $R_0$: Net reproductive rate, T: Generational time, *r*: Rate of population increase. Gross reproductive rate (offspring $female^{-1}$), net reproductive rate (offspring $female^{-1}$), generation time (days) and rate of population increase $day^{-1}$. For a given variable of *B. calyciflorus* or *B. havanaensis*, data carrying similar alphabet are not significant ($p > 0.05$, Tukey test) in treatment with Amoxicillin or Ibuprofen. For each rotifer species, the superscripts (a–d) indicate the results of Tukey test.

| Treatment | Conc. | Life history variable | | | | |
|---|---|---|---|---|---|---|
| Species | | ALS | GRR | $R_0$ | T | *r* |
| *B. calyciflorus* | | | | | | |
| Control | 0 | $12.3 \pm 0.3^{a}$ | $33.2 \pm 2.2^{a}$ | $21.8 \pm 1.6^{a}$ | $7.4 \pm 0.1^{a}$ | $0.68 \pm 0.0^{a}$ |
| Amoxicillin | 50 | $10.6 \pm 0.3^{b}$ | $30.3 \pm 1.0^{a}$ | $17.4 \pm 0.6^{b}$ | $6.6 \pm 0.1^{b}$ | $0.63 \pm 0.0^{a}$ |
| | 100 | $9.4 \pm 0.2^{c}$ | $25.2 \pm 2.0^{b}$ | $14.6 \pm 0.8^{b}$ | $6.1 \pm 0.1^{b}$ | $0.64 \pm 0.0^{a}$ |
| | 200 | $8.6 \pm 2.5^{c}$ | $23.4 \pm 0.9^{b}$ | $13.5 \pm 0.9^{b}$ | $5.6 \pm 0.3^{c}$ | $0.67 \pm 0.01^{a}$ |
| Ibuprofen | 6.25 | $11.8 \pm 0.3^{a}$ | $35.1 \pm 0.9^{a}$ | $19.9 \pm 0.7^{a}$ | $7.3 \pm 0.2^{a}$ | $0.72 \pm 0.0^{a}$ |
| | 12.5 | $10.7 \pm 0.1^{b}$ | $29.9 \pm 2.6^{a}$ | $17.2 \pm 0.7^{b}$ | $6.7 \pm 0.2^{a}$ | $0.72 \pm 0.0^{a}$ |
| | 25 | $9.9 \pm 0.2^{b}$ | $24.8 \pm 1.6^{b}$ | $15.4 \pm 0.6^{b}$ | $6.1 \pm 0.1^{b}$ | $0.71 \pm 0.02^{a}$ |
| *B. havanaensis* | | | | | | |
| Control | 0 | $11.5 \pm 0.1^{a}$ | $39.8 \pm 0.3^{a}$ | $23.1 \pm 0.3^{a}$ | $7.1 \pm 0.1^{a}$ | $0.89 \pm 0.0^{a}$ |
| Amoxicillin | 50 | $11.8 \pm 0.2^{a}$ | $24.7 \pm 1.7^{b}$ | $13.5 \pm 0.8^{b}$ | $8.2 \pm 0.3^{a}$ | $0.43 \pm 0.0^{b}$ |
| | 100 | $10.5 \pm 0.1^{b}$ | $23.3 \pm 2.3^{b}$ | $11.0 \pm 0.6^{c}$ | $8.1 \pm 0.1^{a}$ | $0.38 \pm 0.0^{b}$ |
| | 200 | $9 \pm 0.1^{c}$ | $18.6 \pm 0.9^{b}$ | $8.3 \pm 0.1^{d}$ | $6.9 \pm 0.05^{b}$ | $0.38 \pm 0.0^{b}$ |
| Ibuprofen | 6.25 | $8.8 \pm 1.1^{a}$ | $31.5 \pm 2.4^{b}$ | $13.9 \pm 0.8^{b}$ | $5.2 \pm 0.8^{b}$ | $0.85 \pm 0.0^{a}$ |
| | 12.5 | $6.8 \pm 0.1^{a}$ | $26.7 \pm 0.7^{b}$ | $10.3 \pm 0.7^{c}$ | $5.0 \pm 0.2^{b}$ | $0.71 \pm 0.0^{b}$ |
| | 25 | $5.5 \pm 0.1^{b}$ | $19.4 \pm 2.2^{c}$ | $9.0 \pm 0.2^{d}$ | $3.8 \pm 0.1^{c}$ | $0.78 \pm 0.0^{b}$ |

## Discussion

There are few studies, which quantify the effects of different drugs including painkillers and antibiotics such as ibuprofen and amoxicillin to rotifers (Snell and Janssen, 1995; Araujo and McNair, 2007; Iannacone and Alvariño, 2009; Sarma et al., 2014). Here we observed that both ibuprofen and amoxicillin affected the survivorship and reproductive variables of *B. calyciflorus* and *B. havanaensis*, especially when exposed to or above 100 μg $L^{-1}$ of amoxicillin or 12.5 mg $L^{-1}$ ibuprofen. Amoxicillin was much more toxic than ibuprofen for both the species. This is supported by previous works where Han et al. (2010) and Sanderson and Thomsen (2004) used higher doses for ibuprofen than for the antibiotics during the toxicity evaluations. The concentrations tested here are not considered high since humans are administered with these drugs at doses of 1000–7000 times (3,200,000 μg $d^{-1}$ per person for ibuprofen and 1,500,000 μg $d^{-1}$ of amoxicillin). In addition, a high percentage of these drugs (60–95%) is excreted out within a few hours into urine and then into aquatic systems. Though the half-life of these drugs in nature may just be a few minutes to a few hours, their continuous consumption and excretion ensure their permanent presence in untreated domestic and hospital effluents, later generally discharged into freshwater bodies.

Acute toxicity tests are helpful to select the range of concentrations of a given chemical needed for testing chronic effects. For example, the $EC_{50}$ value of ibuprofen for *Daphnia magna* is 51.4 mg $L^{-1}$ (Han et al., 2006) and for amoxicillin is 0.25 mg $L^{-1}$ (Sanderson and Thomsen, 2004). In this work, for sublethal concentrations, we chose the range that varied from half to one-tenth of $EC_{50}$ as the highest level. Demographic variables are highly sensitive to stress (Kammenga and Laskowski, 2000) and in this work, both survival and reproduction of both the rotifer species were affected by ibuprofen and amoxicillin.

Depending on the chemical nature and mode of action, certain substances affect mainly survival while others reproduction and yet a few others both these variables (Forbes and Calow, 1999; Roex et al., 2000). In addition, the same chemical may affect differentially depending on the sensitivity and the nature of the species. For example, amoxicillin inactivates the enzymes responsible for the synthesis of cell walls in bacteria and this leads to bacterial cell lysis from autolytic enzymes (Kaur et al., 2011). However, in zooplankton, this mode of action can be completely different since they do not have cell walls. In addition, the exposure route to zooplankton is different from that in bacterial systems. For example, rotifers in the test jars are exposed to the amoxicillin in the medium directly and/or indirectly through algal cells via bioaccumulation of the antibiotic. This may also be the case for ibuprofen, which is mainly investigated using the mammalian system, where this non-steroidal anti-inflammatory drug lowers the levels of prostaglandins thus affecting the body sensations (Vane and Botting, 1995). Therefore, the effects of drugs as well as their mode of action on non-target aquatic species need to be studied more extensively than to extend the mechanisms known from mammalian systems. In this work, for both the rotifer species amoxicillin had a far greater effect on reproduction than on survival. In addition, the lifespan of *B. havanaensis* was significantly reduced due to ibuprofen. Similarly, the reproductive rates of *B. calyciflorus* and *B. havanaensis* were decreased with an increase in the level of amoxicillin or ibuprofen. The *r* is considered to be one of the most sensitive variables in demographic studies as it integrates survival with reproduction (Stearns, 1992; Forbes and Calow, 1999). However, species-related differences influence this parameter (Araujo and McNair, 2007). Thus, the *r* of *B. havanaensis* was sensitive to the antibiotic and ibuprofen while that of *B. calyciflorus* was not. These results suggest the need to consider as many life history variables as possible for quantifying the impact of pharmaceuticals to zooplankton.

The selection of the test rotifer species in this work is based on their ecological relevance and sensitivity. Both *B. calyciflorus* and *B. havanaensis* are common in most Mexican freshwaters (Nandini et al., 2005). While a large set of information is available for *B. calyciflorus* as a bioassay organism by the American Society for Testing and Materials (ASTM, 1991), *B. havanaensis* is also becoming popular for toxicity tests of various toxic substances (Gama-Flores et al., 2007; Juárez-Franco et al., 2007). The current Mexican official norms recommend the use of *Daphnia magna* as a bioassay organism. Nevertheless, it may be equally important, if not more, to study locally available species because *D. magna* is not present naturally in Mexican waterbodies (Pica-Granada et al., 2000). Our results also showed that *B. havanaensis* was more sensitive compared to *B. calyciflorus* when exposed to both drugs. Considering the wide distribution of this rotifer species in Mexican freshwaters, and its sensitivity to these pharmaceuticals, it deserves to be included as a bioassay organism in the future Mexican official norms.

Organizations such as the US EPA (EDSTAC, 1996), the European Union and the Water Quality Guidelines of Canada are dedicated to determine the concentrations in which these pharmaceuticals can alter the normal metabolic processes of various organisms but mainly the fish and mammalian species. Thus, they have established maximum permissible limits for emerging chemicals and pharmaceuticals to protect aquatic ecosystems. For example, substances such as 4-nonylphenol and bisphenol A, are not to exceed $1\ \mu g\ L^{-1}$ (ISTS, 2002). There are also some attempts by the independent researchers from different countries urging the EU to improve the laws related to the release of certain chemicals into the environment (Gore et al., 2014). In the US, the FDA requires an assessment only if the concentration of the pharmaceutical being discharged is $>1\ \mu g\ L^{-1}$ (FDA, 1998). In Mexico there are no such rigorous laws that control or determine the maximum permissible limits of pharmaceuticals in aquatic systems. However, the Mexican Official Standard NOM- 001-SEMARNAT- 1996 establishes the maximum permissible limits of some pollutants for wastewater discharge.

## Conclusions

Ibuprofen and amoxicillin are the drugs administered together for curing bacterial infections. However, their effects to non-target species, especially the aquatic organisms are not well-known. Our results showed that though both these drugs had an adverse effect on *B. calyciflorus* and *B. havanaensis*, amoxicillin had far greater negative impact on the demographic variables of the latter. Due to the adverse effects observed on the population parameters of both rotifer species exposed to amoxicillin and ibuprofen, these drugs can be considered as ecologically harmful to zooplankton. Future studies need to consider the combined effect of both these pharmaceutical since they are often consumed together by the patients suffering from bacterial infections. Since drugs are generally discharged into water bodies, the Mexican governmental agencies responsible for the environmental law need to establish the minimal permissible limits for each of these drugs before they are discharged from industries and hospitals.

## Conflict of interest

The authors have no conflict of interest.

## Acknowledgments

We thank two anonymous reviewers for helpful comments. Marisa Mazari Hiriart, Marcelo Silva Briano, Ma. del Rosario Sánchez Rodríguez and Martín Frías Espericueta offered suggestions which improved the manuscript greatly. This study was supported by CONACYT (Ref-492489) through the Postgraduate programme in Limnology and Marine Sciences of our university (PCMyL, UNAM). Partial support was also obtained from PAPIIT- IA203315.

## References

Ali, M.H.H., Al-Qahtani, K.M., 2012. Assessment of some heavy metals in vegetables, cereals and fruits in Saudi Arabian markets. Egypt. J. Aquat. Res. 38, 31–37.

Andreozzi, R., Campanella, L., Fraysse, B., Garric, J., Gonella, A., Giudice, R.L., Marotta, R., Pinto, G., Pollio, A., 2004. Effects of advanced oxidation processes (AOPs) on the toxicity of a mixture of pharmaceuticals. Water Sci. Technol. 50, 23–28.

Araujo, A., McNair, J.N., 2007. Individual and population level effects of antibiotics on the rotifers, *Brachionus calyciflorus* and *B. plicatilis*. Hydrobiologia 593, 185–199.

ASTM, 1991. Standard guide for acute toxicity test with the rotifer Brachionus. Annual Book of ASTM Standards, vol. 11.04, E 1440, American Society for Testing and Materials, Philadelphia, PA, USA.

Bila, D.M., Dezotti, M., 2003. Fármacos no medio ambiente. Química Nova 26, 523–530.

Borowitzka, M.A., Borowitzka, L.J., 1988. Micro-Algal Biotechnology. Cambridge University, London, p. 480.

Burkhardt-Holm, P., 2010. Endocrine disruptors and water quality: a state-of-the-art review. Int. J. Water Resour. Dev. 26, 477–493.

Brun, L., Bernier, M., Losier, R., Doe, K., Jackman, P., Lee, H., 2006. Pharmaceutically active compounds in Atlantic Canadian sewage treatment plant effluents and receiving waters, and potential for environmental effects as measured by acute and chronic aquatic toxicity. Environ. Toxicol. Chem. 25, 2163–2176.

Calow, P., 1995. Risk assessment: principles and practice in Europe. Austr. J. Ecotoxicol. 1, 11–13.

Cleuvers, M., 2004. Mixture toxicity of the anti-inflammatory drugs diclofenac, ibuprofen, naproxen, and acetylsalicylic acid. Ecotoxicol. Environ. Saf. 59, 309–315.

CSG, 2014. Consejo de Salubridad General. Cuadro Básico y Catálogo de Medicamentos, Mexico City, Mexico, p. 685.

Daughton, C.G., Ternes, T.A., 1999. Pharmaceuticals and personal care products in the environment: agents of subtle change? Environ. Health Perspect. 107, 907–942.

Dresser, A., Wirtz, V.J., Corbett, K.K., Echániz, G., 2008. Uso de antibióticos en México: revisión de problemas y políticas. (Use of antibiotics in Mexico: review on problems and policies). Salud Publica Mexicana 50, 480–487.

Endocrine Disruptor Screening and Testing Advisory Committee (EDSTAC), 1996. Final report, EPA/743/R-96/00.

FDA, 1998. Guidance for Industry: Environmental Assessment of Human Drug and Biologics Applications, Food and Drug Administration (Center for Drug Evaluation and Research), CMC 6, Revision 1.

Forbes, V.E., Calow, P., 1999. Is the per capita rate of increase a good measure of population-level effects in ecotoxicology? Environ. Toxicol. Chem. 18, 1544–1556.
Gama-Flores, J.L., Castellanos-Paez, M.E., Sarma, S.S.S., Nandini, S., 2007. Life table demography of *Ceriodaphnia dubia* (Cladocera) exposed to copper at different levels and periods. J. Environ. Biol. 3, 691–696.
Gibson, R., Durán-Álvarez, J.C., León Estrada, K., Chávez, A., Jiménez Cisneros, B., 2010. Accumulation and leaching potential of some pharmaceuticals and potential endocrine disruptors in soils irrigated with wastewater in the Tula Valley, Mexico. Chemosphere 81, 1437–1445.
Gore, A.C., Crews, D., Doan, L.L., La Merril, M., Patisaul, H., Zota, A., 2014. Introduction to Endocrine Disrupting Chemicals (EDCs) – A Guide for Public Interest Organizations and Policy-makers. Endocrine Society, Washington, DC.
Gómez, M.J., Agüera, A., Mezcua, M., Hurtado, J., Mocholí, F., Fernández-Alba, A.R., 2007. Simultaneous analysis of neutral and acidic pharmaceuticals as well as related compounds by gas chromatography–tandem mass spectrometry in wastewater. Talanta 73, 314–320.
Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E., 1998a. Occurrence, fate and effects of pharmaceutical substances in the environment- a review. Chemosphere 36, 357–393.
Han, G.H., Hur, H.G., Kim, S.D., 2006. Ecotoxicological risk of pharmaceuticals from wastewater treatment plants in Korea: occurrence and toxicity to *Daphnia magna*. Environ. Toxicol. Chem. 25, 265–271.
Han, S., Choi, K., Kim, J., Ji, K., Kim, S., Ahn, B., Yun, J., Choi, K., Khim, J.S., Zhang, X., Giesy, J.P., 2010. Endocrine disruption and consequences of chronic exposure to ibuprofen in Japanese medaka (*Oryzias latipes*) freshwater cladocerans *Daphnia magna* and *Moina macrocopa*. Aquat. Toxicol. 3, 256–264.
Heckmann, L.H., Callaghan, A., Hooper, H.L., Connon, R., Hutchinson, T.H., Maund, S.J., Sibly, R.M., 2007. Chronic toxicity of ibuprofen to *Daphnia magna*: effects on life history traits and population dynamics. Toxicol. Lett. 172, 137–145.
Hussein, T.E., El-Bassat, R.A., El-Shimy, A., Harisa, G.I., 2008. Oxidative stress of plankton community and some isolated species during paracetamol toxicity test. J. Biol. Sci. 8, 13–23.
Iannacone, J., Alvariño, L., 2009. Aquatic risk assessment of seven pharmaceutical products on *Daphnia magna*. Ecología Aplicada 2, 71–80.
Halling-Sørensen, B., Nors Nielsen, S., Lanzky, P.F., Ingerslev, F., Holten Lützhøft, H.C., Jørgensen, S.E., 1998b. Occurrence, fate and effects of pharmaceutical substances in the environment—a review. Chemosphere 36, 357–393.
Juárez-Franco, M.F., Sarma, S.S.S., Nandini, S., 2007. Effect of cadmium and zinc on the population growth of *Brachionus havanaensis* (Rotifera: Brachionidae). J. Environ. Sci. Health Part A 42, 1489–1493.
Instituto Sindical de Trabajo, Ambiente y Salud (ISTAS), 2002. Curso de Introducción a los Disruptores Endocrinos: 1–34. Barcelona.
Kammenga, J., Laskowski, R., 2000. Demography in Ecotoxicology. John Wiley and Sons, New York, p. 318.
Kasprzyk-Hordern, B., Dinsdale, R.M., Guwy, A.J., 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface waters in South Wales, UK. Water Res. 42, 3498–3518.
Kaur, S.P., Rao, R., Nanda, S., 2011. Amoxicillin: a broad spectrum antibiotic. Int. J. Pharm. Pharm. Sci. 3, 30–37.
Kim, S., Cho, J., Kim, I., Vanderford, B., Snyder, S., 2007. Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking and wastewaters. Water Res. 41, 1013–1021.
Krebs, C.J., 1985. Ecology. Population biology; Biogeography; Methodology, Harper and Row, New York, p. 800.
Moss, B., 2007. Shallow lakes, the water framework directive and life. What should it all be about? Hydrobiologia 584, 381–394.
Nandini, S., Sarma, S.S.S., Ramírez-García, P., 2005. Seasonal variations in the species diversity of planktonic rotifers in Lake Xochimilco, Mexico. J. Freshwater Ecol. 20, 287–294.
Pérez Zazueta, G., 2013. Secretaria de Economía. Industria Farmacéutica. Unidad de Inteligencia de Negocios. ProMéxico, México, D.F..
Pica-Granada, Y., Trujillo, G.D., Hernández, H.S., 2000. Bioassay standardization for water quality monitoring in Mexico. Environ. Toxicol. 15, 322–330.
Roex, E.W.M., Van Gestel, C.A.M., Van Wezel, A.P., Van Straalen, N.M., 2000. Ratios between acute aquatic toxicity and effects on population growth rates in relation to toxicant mode of action. Environ. Toxicol. Chem. 19, 685–693.
Sanderson, H., Thomsen, M., 2004. Comparative analysis of pharmaceuticals versus industrial chemicals acute aquatic toxicity classification according to the United Nations classification system for chemicals. Assessment of the (Q) SAR predictability of pharmaceuticals acute aquatic toxicity and their predominant acute toxic mode-of-action. Toxicol. Lett. 187, 84–93.
Sarma, S.S.S., González-Pérez, B.K., Moreno-Gutiérrez, R.M., Nandini, S., 2014. Effect of paracetamol and diclofenac on population growth of *Plationus patulus* and *Moina macrocopa*. J. Environ. Biol. 35, 119–126.
Semarnat. Secretaría de Medio Ambiente y Recursos Naturales. 1996. Norma Oficial Mexicana NOM-001- SEMARNAT-1996. Diario Oficial de la Federación (DOF), 23 Abril del 2006.
Snell, T.W., Janssen, C., 1995. Rotifers in ecotoxicology: a review. Hydrobiologia 313 (314), 231–247.
Snell, T.W., Joaquim-Justo, C., 2007. Workshop on rotifers in ecotoxicology. Hydrobiologia 593, 227–232.
Stearns, S., 1992. The Evolution of Life Histories. Oxford University Press, New York, p. 262.
Sokal, R.R., Rohlf, F.J., 2000. Biometry. WH Freeman and Company, San Francisco.
Vane, J.R., Botting, R.M., 1995. New insights into the mode of action of anti-inflammatory drugs. Inflamm. Res. 44, 1–10.
Vargas-Jiménez, J.L., Silva-Hernández, R., de Lago-Acosta, A., Parada-Tapia, M.G., 2011. Estudio sobre eficacia y seguridad de azitromicina vs amoxicilina + ácido clavulánico en el tratamiento de rinosinusitis bacteriana aguda. Acta Pediatr. Mex. 32, 169–174.
Weber, C.I., 1993. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms.4th ed. United States Environmental Protection Agency, Cincinnati, Ohio, EPA/600/4-90/027F. 293 p.