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Long-term exposure to caffeine and carbamazepine: Impacts on the regenerative capacity of the polychaete *Diopatra neapolitana*

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HIGHLIGHTS

- Carbamazepine and caffeine affected the regenerative capacity of *Diopatra neapolitana*.
- Under environmentally relevant concentrations, carbamazepine induced higher impacts.
- The regenerative capacity can be used as a biomarker of pharmaceuticals effects.

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ABSTRACT

The toxicity induced in non-target organisms by pharmaceutical drugs has been the focus of several studies. In the aquatic environment, most of the studies have been devoted to fish and bivalves, while little is known on the impacts induced in polychaetes. The present study evaluated the impacts of carbamazepine and caffeine on the regenerative capacity of *Diopatra neapolitana*, a polychaete species with high ecological and economic relevance. Under laboratory controlled conditions polychaetes were exposed, during 28 days, to carbamazepine (Ctl-0.0; 0.3; 3.0; 6.0; 9.0 μg/L) and caffeine (Ctl-0.0; 0.5; 3.0; 18.0 μg/L). During the experiment, at days 11, 18, 25, 32, 39 and 46 after amputation, for each specimen, the percentage of the body width regenerated was determined and the number of new segments was counted. The regenerative capacity was assessed considering the number of days needed to achieve full regeneration and the total number of new segments. The obtained results revealed that with the increase of drugs concentrations organisms regenerated less new segments and took longer to completely regenerate.

1. Introduction

Pharmaceuticals are among the most common contaminants worldwide, since they are widely consumed by humans and largely used in aquaculture and agriculture (Heberer et al., 2002). The ubiquity of pharmaceuticals in the aquatic environment, their persistent biological activity, and/or the lack of information regarding their toxicity explains the concern over this group of pollutants. Given their properties, when in the aquatic environment, several pharmaceuticals can be bioaccumulated and cause toxic effects in non-target organisms (e.g., Aguirre-Martínez et al., 2013; Crane et al., 2006; Gagné et al., 2006; Martin-Diaz et al., 2009; Halling-Sørensen et al., 1998; Huggett et al., 2002; Santos et al., 2010). However, although an increasing number of studies have been addressing the impacts of different drugs in various aquatic species (among others, Almeida et al., 2015, 2014; Antunes et al., 2013; Gonzalez-Rey and Bebianno, 2012; Li et al., 2011; Martin-Diaz et al., 2009), still scarce toxicity data is available for the majority of pharmaceuticals in use.

Two of the most widely distributed and abundant drugs in aquatic environment are carbamazepine and caffeine (e.g. Clara et al., 2004; Daly and Fredholm, 1998), being commonly used as anthropogenic markers for wastewater contamination of surface waters (Buerge et al., 2003; Clara et al., 2004; Kurissery et al., 2012;

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Peeler et al., 2006; Seiler et al., 1999; Standley et al., 2000). Carbamazepine, an antiepileptic, is commonly detected in the environment since in wastewater treatment plants (WWTPs) this drug is not significantly degraded by activated sludge (below 10%), thus passing almost unchanged to the receiving water bodies (Ternes, 1998; Zhang et al., 2008). For this reason, it has been reported in WWTP influents and effluents, surface waters, groundwater and even in treated drinking water, with concentrations ranging from 0.03 to 11.6 μg/L (Bahlmann et al., 2012, 2009; Calisto et al., 2011a,b; Li, 2014; Loos et al., 2009; Metcalfe et al., 2003; Sacher et al., 2001; Ternes, 1998, Zhang et al., 2008). Besides its biological activity and persistence capacity, CBZ presents a low octanol–water partition coefficient being expected to remain in the aqueous phase (Andreozzi et al., 2003; Lam and Mabury, 2005; Ternes et al., 2004; Tixier et al., 2003). Moreover, carbamazepine is also highly resistant to bio- and photodegradation, resulting in long-term persistence in aquatic environments (e.g. Calisto et al., 2011a,b; Cunningham et al., 2010; Martin-Diaz et al., 2009). Caffeine, a stimulant psychoactive substance, is the most consumed drug worldwide, particularly in beverages (e.g. Daly and Fredholm, 1998). Usually employed as a stimulant, caffeine is a component of hundreds of prescription pharmaceuticals, including some aimed to reduce physical fatigue and to restore alertness when drowsiness occurs (Nehlig et al., 1992; Potera, 2012). Caffeine is frequently found in surface water (Kolpin et al., 2002) since it is relatively stable under variable environmental conditions, has high water solubility, and negligible volatility (Buerge et al., 2003; Kurissery et al., 2012). Caffeine has also been detected in several water bodies, with concentrations in the range of ng/L to μg/L. Weigel et al. (2004) reported concentrations as high as 293 μg/L in a hospital sewer in Tromsø (Norway). Bahlmann et al. (2012) found caffeine concentrations of 230 μg/L and 18 μg/L for influent and effluent wastewater, respectively, in WWTPs in Berlin (Germany). In USA, concentrations of 0.028 μg/L were recorded in water from San Francisco Bay (Klosterhaus et al., 2013). Martínez Bueno et al. (2011) found concentrations of 0.475–0.515 μg/L in the Henares River (Madrid, Spain). Silva et al. (2014) quantified caffeine in waters from public fountains of Aveiro region (Portugal) with concentrations of 0.14–0.58 μg/L. Despite the concentrations of these two drugs are very low in worldwide aquatic systems (ng/L to μg/L), their constant release to the aquatic environment via wastewater effluent and other anthropogenic activities have shown to induce alterations in non-target organisms (e.g. Aguirre-Martínez et al., 2013; Almeida et al., 2014; Bahlmann et al., 2012; Fent et al., 2006); yet, the risk from exposure to low concentrations of carbamazepine and caffeine, including long-term exposure and sublethal effects, remains unclear. In fact, the majority of the studies looking at the biological effects of pharmaceuticals have thus far concentrated on acute exposures (Quinn et al., 2008) but subtle long-term effects could be occurring in apparently healthy ecosystems. Furthermore, although the toxicity of these compounds have been tested on organisms belonging to different trophic levels (including fish and bivalves; Almeida et al., 2014; Canesi et al., 2007; Li et al., 2011; McEneff et al., 2014; van den Brandhof and Montforts, 2010; Yang et al., 2008), little is known on the effects of pharmaceuticals on polychaetes (Freitas et al., 2015c,d; Maranho et al., 2014; Mendéz et al., 2013), often the most abundant group of benthic invertebrate communities in estuaries and lagoons (Rodrigues et al., 2011). Polychaete species (including *Diopatra neapolitana*), are suitable and highly pertinent to be used in toxicological studies, as they are usually available all the year in high densities and have a wide geographic range, making them easily available for environmental quality assessment (Lewis and Watson, 2012; Wehe and Fiege, 2002). Furthermore, in polychaetes, physiological and biochemical markers have been considered an adequate approach of organism-level effects (among others, Ayoola, et al., 2011; Freitas et al., 2012; Solé et al., 2009), but few works assessed the impacts of anthropogenic or natural stressors on the regenerative ability of these organisms (Carregosa et al., 2014; Freitas et al., 2015a, 2015b, 2015c; Nusetti et al., 2005).

Thus, the present study aimed to evaluate the impact of carbamazepine and caffeine on the regenerative capacity of the polychaete *Diopatra neapolitana* (Delle Chiaje, 1841), after a long-term exposure to each of these substances. *D. neapolitana* specimens present the capacity to regenerate their body when amputated (Pires et al., 2012a,b). As an important ecological and economic natural resource (Conti and Massa, 1998; Cunha et al., 2005), since it is a feeding source for different bird and fish species and extensively collected for fish bait, the study of *D. neapolitana* regenerative capacity is of extreme relevance to assess the sustainable manage of this resource.

2. Methodology

2.1. Sampling

To assess the toxicity of carbamazepine and caffeine, laboratory experiments were conducted with *Diopatra neapolitana* collected from the Mira channel, a low contaminated area at the Ria de Aveiro lagoon (Portugal).

Sampling was done at the end of October to avoid the reproductive period of the species (Pires et al., 2012a). In the field, specimens were collected inside their tubes and transported to the laboratory in plastic containers. Organisms that were already regenerating in the field were discarded and not used in this study.

2.2. Experimental setup and regeneration assessment

Organisms for exposure assays were maintained in the laboratory, in artificial seawater (28 g/L) and a mixture of sediment from the sampling site (3:1), for acclimatization, during 2 weeks, under continuous aeration. During the acclimatization period organisms were fed *ad libitum* with frozen cockles every 2–3 days.

After acclimatization and immediately prior to exposure, organisms were removed from their tubes and washed with artificial seawater. Specimens were anaesthetized with a solution of 4% $MgCl_2 \cdot 6H_2O$, during 15 min. Under a stereomicroscope, all individuals anaesthetized were amputated at 60th chaetiger. Amputation level at 60th chaetiger was selected since previous works, conducted by Pires et al. (2012b) revealed higher regenerative capacity of *D. neapolitana* when amputated at this level. No mortality was recorded during or immediately after the amputation procedure.

Chronic exposures to carbamazepine and caffeine were conducted, independently, following an adapted version of the ASTM E1562 – 00(2013) – Standard Guide for Conducting Acute, Chronic, and Life-Cycle Aquatic Toxicity Tests with Polychaetes, Annelids. Thus, organisms were exposed during 28 days to a concentration range of carbamazepine (Ctl-0.0; 0.3; 3.0; 6.0; 9.0 μg/L) and caffeine (Ctl-0.0; 0.5; 3.0; 18.0 μg/L), which reflects low to moderate contaminated areas (Bahlmann et al., 2012, 2009; Buerge et al., 2003; Calisto et al., 2011a; Clara et al., 2004; Kolpin et al., 2002; Kurissery et al., 2012; Loos et al., 2009; Martínez Bueno et al., 2011; Metcalfe et al., 2003; Sacher et al., 2001; Seiler et al., 1999; Silva et al., 2014; Ternes, 1998). To assess the effects of each drugs on the regenerative capacity of *D. neapolitana* individuals exposed to different conditions were placed in different containers, filled with a mixture of sediment (1 kg) from the sampling area and artificial seawater (3L). For each pharmaceutical drug and for each condition two aquaria with three containers/aquarium were used.