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Research paper

Synergy effects of fluoxetine and variability in temperature lead to proportionally greater fitness costs in Daphnia: A multigenerational test

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ABSTRACT

Increased variability in water temperature is predicted to impose disproportionally greater fitness costs than mean increase in temperature. Additionally, water contaminants are currently a major source of human-induced stress likely to produce fitness costs. Global change models forecast an increase in these two human-induced stressors. Yet, in spite the growing interest in understanding how organisms respond to global change, the joint fitness effects of water pollution and increased variability in temperature remain unclear. Here, using a multi-generational design, we test the hypothesis that exposure to high concentrations of fluoxetine, a human medicine commonly found in freshwater systems, causes increased lifetime fitness costs, when associated with increased variability in temperature. Although fluoxetine and variability in temperature elicited some fitness cost when tested alone, when both stressors acted together the costs were disproportionally greater. The combined effect of fluoxetine and variability in temperature led to a reduction of 37% in lifetime reproductive success and a 17.9% decrease in population growth rate. Interestingly, fluoxetine and variability in temperature had no effect on the probability of survival. Freshwater systems are among the most imperilled ecosystems, often exposed to multiple human-induced stressors. Our results indicate that organisms face greater fitness risk when exposed to multiple stressors at the same time than when each stress acts alone. Our study highlights the importance of using a multi-generational approach to fully understand individual environmental tolerance and its responses to a global change scenario in aquatic systems.

1. Introduction

Although it has been long recognized that change is a constant in natural system, because of human actions (Kerr, 2007) environmental conditions are forecast to become more unpredictable (Morice et al., 2012; Mora et al., 2013). Despite the importance of understanding if and how species adapt to global change, few studies have explored how individuals respond to unpredictable changes in environmental conditions (Visser, 2008; Barbosa et al., 2015). This gap is surprising given that increased variability in temperature poses a disproportionally greater threat to individuals than an increase in mean temperature (Vasseur et al., 2014). Integrating variability in temperature in ecological studies provides a better and stronger understanding of how individuals respond adaptively to a noticeable scenario of global change.

Thermoregulation and the associated metabolic costs make temperature a determinant factor for growth, reproduction and development of all ectotherm species (Angilletta, 2009). Daily fluctuations in temperature also influence metabolic rate, and consequently the energetic balance between growth, maintenance and reproduction (Kooijman, 2001). Unpredictable changes in temperature (i.e. heterogeneity) have been shown to lead to greater physiological stress than mean changes in temperature (Barbosa et al., 2014). Increased energy expenditure due to stress caused by variability in temperature is therefore likely to hamper the ability of individuals to respond adaptively to additional stressors (West-Eberhard, 2003; Vasseur et al., 2014).

Freshwater systems, are the main recipients of environmental contaminants, including human pharmaceuticals (Kolpin et al., 2002; Blair et al., 2013; Kümmerer, 2013). Among the most commonly detected pharmaceuticals released by humans into the aquatic system are antidepressants like fluoxetine (Brooks et al., 2003). The main route by which fluoxetine enters the aquatic system is through sewage water plants (Kolpin et al., 2002; Vaswani et al., 2003). Fluoxetine is highly resistant to the processes of hydrolysis, photolysis and to microbial degradation and therefore is not always eliminated by treatment processes (Heberer, 2002; Fent et al., 2006; Peake et al., 2015). As a result, fluoxetine finds its way into the aquatic environment where it affects vital functions such as reproduction and feeding of many aquatic

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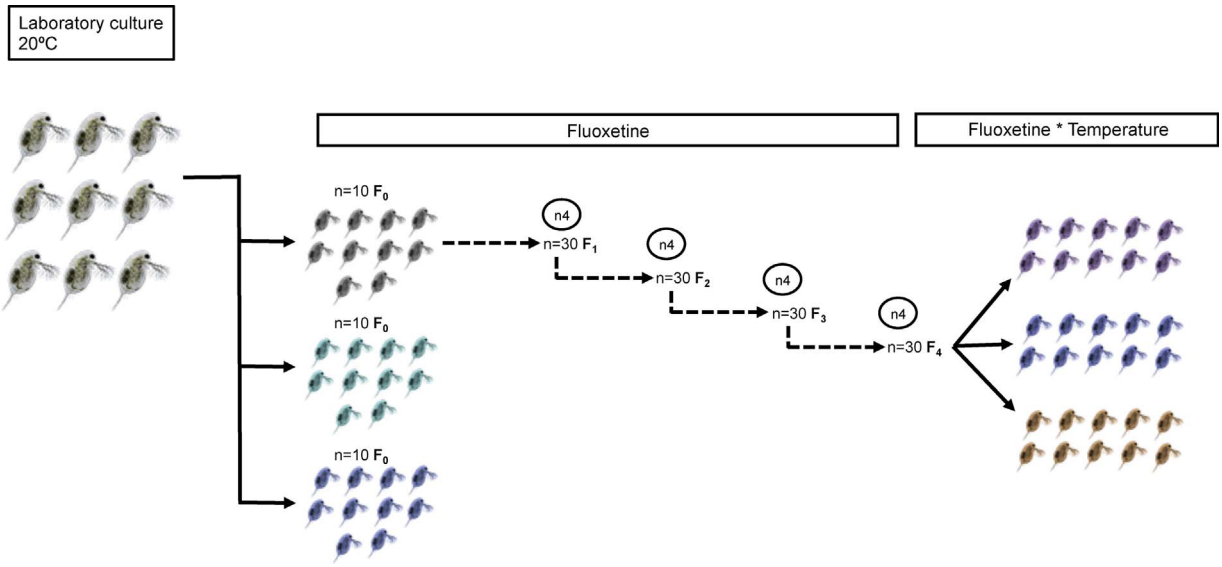

Fig. 1. Schematic diagram of the experimental design. A total of 30 F_0 individuals were randomly selected from a laboratory reared culture of *Daphnia magna* and allocated to one of three fluoxetine concentrations: control (n = 10, grey), low (n = 10, green), and high (n = 10, blue). Each individual was allocated to a single 50 mL glass beaker. Upon the production of the 4th brood, 30 neonates were randomly selected and individually exposed to the same fluoxetine treatment they were originated from. These neonates were considered as the first generation (F_1: control = 30; low = 30; high = 30). The same procedure was followed for F_2 and F_3. At brood number four of F_3 (i.e. F_4) the temperature treatment was introduced to each fluoxetine concentration. 30 F_4 individuals per fluoxetine treatment were randomly allocated and distributed into one of the three temperature treatments (total F4 = 90). For illustration purposes Figure 1 only shows $F_4{}^1$s via Control Fluoxetine line. A total of 30 F_4 were allocated to a Constant Mean (n = 10, purple), Constant Maximum (n = 10, blue) or Variable (n = 10, orange) temperature treatment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

organisms by acting on hormonal and neuronal pathways, by influencing food intake and assimilation, and by acting on reproductive allocation (Fent et al., 2006; Foster et al., 2010; Campos et al., 2012; Weinberger and Klaper, 2014; Rivetti et al., 2016). For example, exposure to fluoxetine reduces growth rate and lifetime reproductive success of Japanese medaka (*Oryzias latipes*) (Foran et al., 2004). The direct effect of fluoxetine on the reproductive biology of key organisms and its subsequent effect on the aquatic ecosystem led to fluoxetine being named as one of the most dangerous pharmaceuticals to the environment (Silva et al., 2016). In the water flea *Daphnia magna* the fitness effects of fluoxetine are not consistent. While some studies reported an increase in fecundity (Flaherty and Dodson, 2005; Campos et al., 2012; Rivetti et al., 2016), others have shown the opposite (Hansen et al., 2008). These contrasting results warrant to examine the long-term fitness costs of fluoxetine.

Due to growing anthropogenic pressure, freshwater systems are seriously threatened with declines in biodiversity far greater than for the most terrestrial ecosystems (Sala et al., 2000). This is particularly alarming since freshwater systems will be one of the hardest hit ecosystems from the effects of global change (Abell et al., 2008). The combined interaction of an already imperilled system together with the forecasted negative impacts from increased variability in temperature poses a great challenge for the sustainability of freshwater systems. Yet, despite this recognition our understanding of how freshwater organisms respond to multiple stressors and their fitness consequences remains limited (Schmitt-Jansen et al., 2016).

Individuals chronically exposed to stressful environments are expected to be less tolerant to additional changes in environmental conditions later in life (Harley et al., 2006). As result, we can predict that individuals exposed to fluoxetine and increased variability in temperature will have less adaptive responses (Nougué et al., 2016; Zhang et al., 2016). In this study we used *Daphnia magna* to investigate the fitness effects of chronic exposure to fluoxetine and to heterogeneity in temperature. Daphnia are unable to control their body temperature and thus, like all ectotherm species, they are susceptible to temperature changes, which have detrimental impact on fitness (Martin and Huey, 2008). For example, exposure to increased unpredictability in temperature disrupted time between broods, growth rate and the development of inducible defense traits in *Daphnia magna* (Barbosa et al., 2014). In addition to temperature, concentration of fluoxetine has a negative effect on fitness of daphnia (Groh et al., 2015). Studies revealed a non-monotonic effect of fluoxetine of *Daphnia magna*, which occurs within one or two orders of magnitude (Rivetti et al., 2016). It is important to note, however, that the scale of the fitness effects of fluoxetine on daphnia depends on the timing and duration of the exposure (Flaherty and Dodson, 2005).

In this study, we tested the prediction that the combination of both fluoxetine and variability in temperature reduces lifetime fitness. This prediction was tested using a multigenerational design in which three consecutive generations of *D. magna* were reared under different concentrations of fluoxetine and the fourth generation was additionally exposed to different temperatures.

2. Experimental

2.1. Test organisms

All individuals used in this study were 4th brood neonates, generated from *D. magna* clone F (Baird et al., 1991). This clone was chosen for this study due to its responsiveness to environmental stress (Barbosa et al., 2014; Barbosa et al., 2015). Cultured individuals were maintained at constant temperature of 20 °C, under a 16:8 h light:dark photoperiod, in ASTM (American Society for Testing Materials) medium (OECD). Individuals were fed every second day with green algae *Raphidocelis subcapitata* at a concentration of 3.0×10^5 cells mL^{-1}. Medium was changed every second day. The temperature, photoperiod and feeding rate used in our cultures follow the guideline 211 from the Organization for Economic and Co-operation and Development (OECD) recommended in reproduction and chemical tests with daphnia (OECD).

2.2. Setup of multigenerational D. magna experiments

Immediately after birth, a total of 30 F_0 individuals were randomly selected and individually allocated to a 50 mL glass beaker corresponding to one of three fluoxetine treatments: control (n = 10;