

## Effects of food limitation and pharmaceuticals compounds on the larval development of a marine invertebrate

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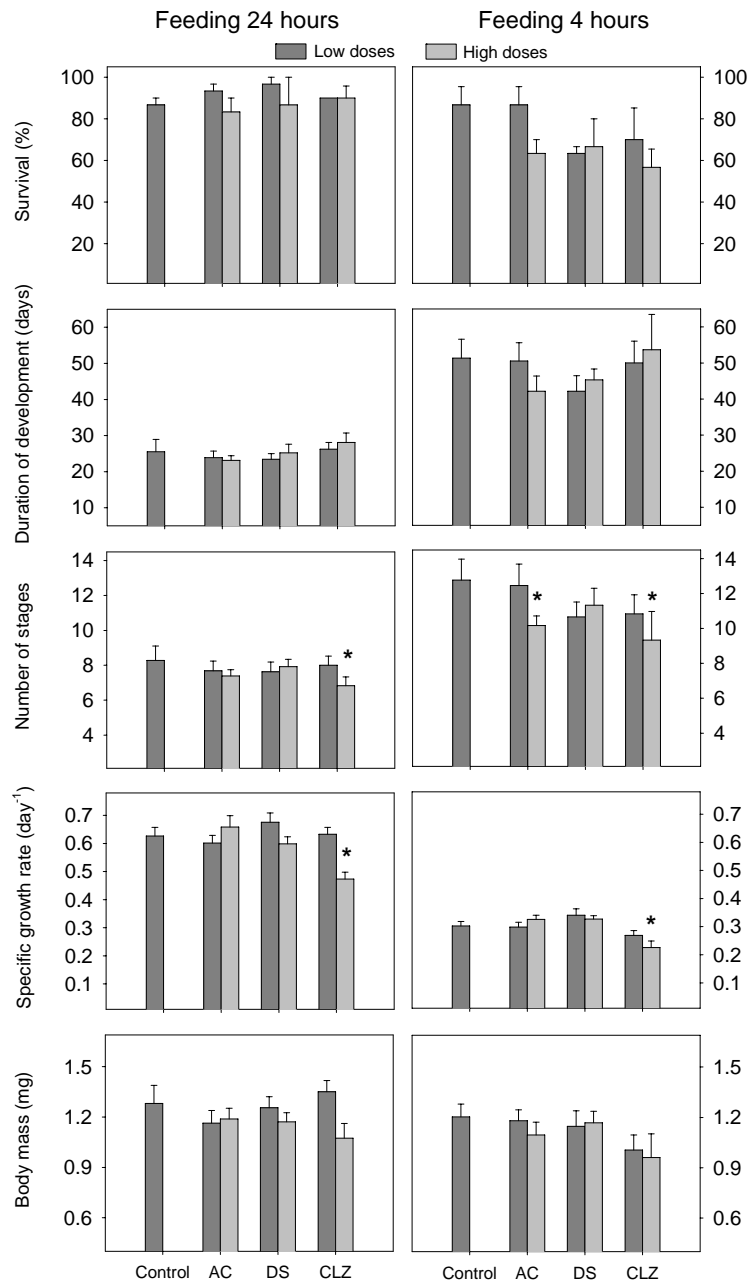
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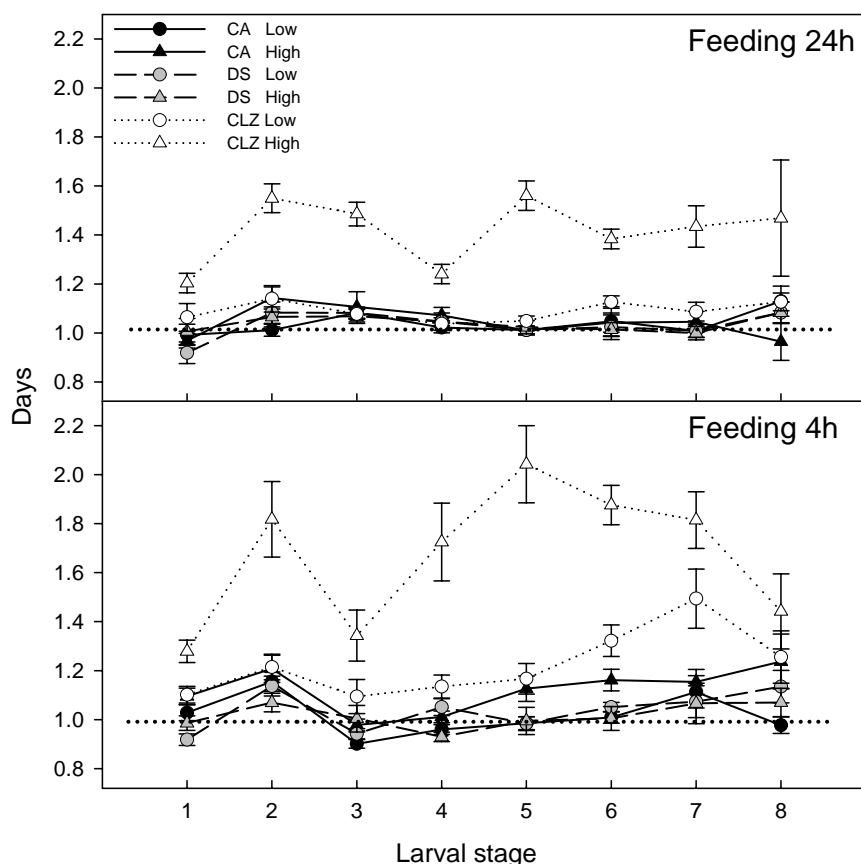
We studied the combined effects of pharmaceutical compounds and food limitation on growth, development and body mass of larval stages of the marine shrimp *Palaemon serratus*. Effects of emergent compounds on growth and development of early life stages may be stronger when organisms are under some additional stress (González-Ortegón et al 2013). Coastal marine organisms such as the marine shrimp *Palaemon serratus* develop in a heterogeneous environment characterised by variations in food conditions (Mann & Lazier 2006). Under food limited conditions larvae of this marine species developed through an increased number of stages, especially in warmer waters, resulting in increased duration of development (González-Ortegón and Giménez 2013).

For practical reasons, investigations of pollution effects on larvae are conducted without taking into account environmental variability. However, multiple-stressor approaches are necessary to study and model the effect of emergent compounds on larvae of marine coastal species. Previous results on larvae of *Palaemon serratus* under different combinations of temperature and salinity conditions demonstrate that the effects on survival and larval growth were compound-specific and depended on salinity (González-Ortegón et al 2013). Following the multiple-stressor approach, we now studied the effect of three common pharmaceuticals (Diclofenac, Clofibric Acid and Clotrimazole) on larval development under food limitation. We hypothesised that the previous effects of these pharmaceuticals on larval survival, growth and development should be magnified under food limited conditions.

The pharmaceuticals compounds tested were the anti-inflammatory and analgesic Diclofenac Sodium (DS), the lipid regulator Clofibric Acid (CA) and the fungicide Clotrimazole (CLZ). The larvae were exposed to concentrations 2 times higher than found in natural habitats (validated concentrations: DS: 70 µg/l, CA: 40 µg/l and CLZ: 0.07 µg/l) and also concentrations 20 times higher than those found in nature (700, 400 and 4 µg/l, respectively). Larvae were reared at 24°C and full salinity seawater (32 PSU). Freshly hatched *Artemia* sp. nauplii was added and removed with the culture water after a period of 4 h (food limited condition) or 24 hours (permanent access to prey).

Clotrimazole had toxic effect at lower concentrations than the others two compounds. Survival varied between 56 and 96% and was reduced by 30% under the combined effect of food limitation and all pharmaceuticals treatments (exception: Clofibric Acid at low concentration). The duration of larval development and number of instars required to reach the juvenile stage was affected by feeding condition. While food limitation doubled the larval duration of development, the pharmaceuticals did not have any significant effect. By contrast, the number of stages tended to be fewer in larvae exposed to all tested pharmaceuticals especially in those growing under food limitation. Significant reductions (31%) in the number of stages were found when larvae were exposed to Clotrimazole at high concentration and to Clofibric Acid at high concentration under food limitation. The reduction in the number of stages without any significant variation in the developmental time in larvae exposed to Clotrimazole can be explained by an increment in the intermoult duration. Growth rate was reduced by Clotrimazole at high concentrations. However, the significant increase in the intermoult duration under the exposure of Clotrimazole with respect to the rest of treatments explained why juvenile body mass was not affected.





In conclusion, the study identifies the toxic effects of Clotrimazole in the marine environment and shows that the effects of emergent compounds on larval survival appear to be stronger under food limitation.

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