Environmental Pollution 213 (2016) 240–253

Contents lists available at ScienceDirect

Environmental Pollution

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

Improving the ecological relevance of toxicity tests on scleractinian corals: Influence of season, life stage, and seawater temperature☆,☆☆

Laetitia S. Hédouin [a, *, 1, 2], Ruth E. Wolf [b], Jeff Phillips [c], Ruth D. Gates [a]

[a] Hawai'i Institute of Marine Biology, 46-007 Lilipuna Road, HI 96744, United States
[b] Crustal Geophysics and Geochemistry Science Center, U.S. Geological Survey, Bldg 20, MS 964D, Denver Federal Center, Denver, CO 80225, United States
[c] U.S. Fish and Wildlife Service, Pacific Islands, 300 Ala Moana Blvd, Room 3-122, Honolulu, HI 96850, United States

ARTICLE INFO

Article history:
Received 15 October 2015
Received in revised form
24 January 2016
Accepted 27 January 2016
Available online xxx

Keywords:
Toxicology
Coral
Lethal concentration
Metal
Larva

ABSTRACT

Metal pollutants in marine systems are broadly acknowledged as deleterious: however, very little data exist for tropical scleractinian corals. We address this gap by investigating how life-history stage, season and thermal stress influence the toxicity of copper (Cu) and lead (Pb) in the coral *Pocillopora damicornis*. Our results show that under ambient temperature, adults and larvae appear to tolerate exposure to unusually high levels of copper (96 h-LC_{50} ranging from 167 to 251 μg Cu L^{-1}) and lead (from 477 to 742 μg Pb L^{-1}). Our work also highlights that warmer conditions (seasonal and experimentally manipulated) reduce the tolerance of adults and larvae to Cu toxicity. Despite a similar trend observed for the response of larvae to Pb toxicity to experimentally induced increase in temperature, surprisingly adults were more resistant in warmer condition to Pb toxicity. In the summer adults were less resistant to Cu toxicity (96 h-LC_{50} = 175 μg L^{-1}) than in the winter (251 μg L^{-1}). An opposite trend was observed for the Pb toxicity on adults between summer and winter (96 h-LC_{50} of 742 vs 471 μg L^{-1}, respectively). Larvae displayed a slightly higher sensitivity to Cu and Pb than adults. An experimentally induced 3 °C increase in temperature above ambient decreased larval resistance to Cu and Pb toxicity by 23–30% (96 h-LC_{50} of 167 vs 129 μg Cu L^{-1} and 681 vs 462 μg Pb L^{-1}).

Our data support the paradigm that upward excursions in temperature influence physiological processes in corals that play key roles in regulating metal toxicity. These influences are more pronounced in larva versus adult corals. These findings are important when contextualized climate change-driven warming in the oceans and highlight that predictions of ecological outcomes to metal pollutants will be improved by considering environmental context and the life stages of organism under study.

1. Introduction

Coral reefs are some of the most biologically complex and threatened marine ecosystems on Earth. Scleractinian corals create reefs through the deposition of calcium carbonate and their survival frames the maintenance of a habitat that protects coastline and host high levels of biodiversity (Tanner et al., 1994). The high rates of productivity and calcification in corals reflect the functional capacity made possible by their intimate mutualistic association with dinoflagellates in the genus *Symbiodinium* that live symbiotically inside coral tissues. Under normal circumstances, the *Symbiodinium* photosynthesize and supply up to 95% of the coral host's energy requirements for growth and reproduction (Muscatine, 1990). However, the functionality of this association is vulnerable to a variety of environmental stressors (e.g., temperature, metal pollution, ultraviolet light), and the *Symbiodinium* are lost from coral tissues, which makes corals appear pale or bleached (e.g. Bastidas and García, 2004; Harland and Brown, 1989; Jones, 1997). The fragility of this pivotal symbiosis in the face of environmental disturbance raises questions regarding the capacity of corals to sustain and adapt to detrimental effects of anthropogenic activities

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

☆☆ Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

* Corresponding author. USR 3278 EPHE CNRS CRIOBE, Laboratoire d'excellence "Corail", BP1013, Papetoai, 98729, Moorea, French Polynesia.
E-mail address: laetitia.hedouin@criobe.pf (L.S. Hédouin).
[1] Current address: USR 3278 CNRS EPHE UPVD CRIOBE, B1013, 98729 Papetoai, Moorea, French Polynesia.
[2] Current address: Laboratoire d'Excellence "CORAIL", B1013, 98729 Papetoai, Moorea, French Polynesia.

http://dx.doi.org/10.1016/j.envpol.2016.01.086
0269-7491/

at global and local scales (e.g., climate change, sedimentation, pollution, ocean acidification). To survive, corals will need to acclimatize and adapt to both global-scale climate changes as well as local land-based pollution.

Dense human populations along the coastline have increased the exposure of adjacent coral reefs to pollutants. Of these pollutants, metal compounds are one of the major constituents due to industrial discharges, roadway pollutants and other anthropogenic sources. Metals are non-biodegradable so they may persist through food chains. Against the backdrop of deteriorating reef integrity worldwide, recent efforts have focused on assessing metal contamination in reef locations and on metal bioaccumulation processes in corals. The metal bioaccumulation capacities of annual growth bands of coral skeletons have been used for several decades as an environmental proxy to track historical events of metal contamination in various coral reef ecosystems (e.g., David, 2003; Fallon et al., 2002; Rasmussen et al., 1993; St. John, 1974). However, recent research investigating how corals bioaccumulate metals indicates higher metal concentrations in coral tissues as opposed to coral skeleton (e.g., Bastidas and García, 1997; Esslemont, 1998; Metian et al., 2015) as only a fraction of metals bioaccumulated in coral tissues is transferred and stored in the coral skeleton. Consequently, estimates of the exposure to metals based on coral-skeleton concentrations alone may underestimate the total exposure. Furthermore, within coral tissues (an animal-algae component), contrasting trends have been observed on the role of *Symbiodinium* in the metal bioaccumulation process. Some studies indicated that metals were preferentially bioaccumulated in the *Symbiodinium* zooxanthellae harboring high efficient metal bioconcentration capacities (e.g., Metian et al., 2015; Reichelt-Brushett and McOrist, 2003): yet no preferential accumulation of Cu and Pb in zooxanthellae was apparent in the coral *Pocillopora damicornis* (Mitchelmore et al., 2007).

Although metals toxicity has been widely characterized in marine temperate organisms, the effects of metals on tropical corals have received comparatively little attention (Peters et al., 1997). Existing ecotoxicological research in tropical areas has identified metal contamination in various areas with high metal concentrations detected in corals, seawater and/or sediment (e.g., Hanna and Muir, 1990; Hédouin et al., 2008, 2009, 2011; Howard and Brown, 1987). In the context of risk assessment, a significant and fundamental knowledge gap is whether life-history stages of coral exhibit differences in vulnerability to metals. This is especially important in coastal regions experiencing high levels of land-based metal contamination such as the heavily populated Hawaiian Islands (De Carlo et al., 2004; Hallacher et al., 1985; Hédouin et al., 2009). Toxicological studies on temperate marine invertebrates have shown that early life stages are generally more sensitive to pollutants than adult organisms (His et al., 1999). Notably toxicity tests are generally performed on early life stages by using an endpoint of fertilization success. For example published data on corals indicate that metals, such as copper, have deleterious effects on adult corals at low concentrations for a short period of time (*Galaxea fascicularis*, LC_{50} of 0.032 mg L^{-1} after 96 h of exposure, Sabdono, 2009) and on the early life stages of corals by reducing fertilization or impairing their normal development (*Goniastrea aspera*, Reichelt-Brushett and Harrison, 1999; *Montipora capitata*, Hédouin and Gates, 2013; *Acropora pulchra* and *Acropora cytherea*, Puisay et al., 2015; *Acropora japonica*, Suwa et al., 2014). However, we know of no published data that directly compare the responses of different coral life-history stages (e.g., adults, gametes and larvae) to metal exposure. Furthermore published toxicological tests have generally been performed at a single time period and so do not consider potential effects associated with seasonal fluctuations of the marine environment and the changing seasonal physiological state of adult corals (e.g., *Symbiodinium* density, Fagoonee et al., 1999; reproduction states, Vargas-Ángel et al., 2006; lipid and protein content (Ben-David-Zaslow and Benayahu, 1999). Research has shown synergy between metal pollution and temperature for a wide range of temperate organisms, which are able to adapt to large temperature variations (e.g., crayfish, Khan et al., 2006; copepod, Kwok and Leung, 2005): however this pattern is not true in tropical marine ecosystems. Scleractinian corals have a narrow window of thermal tolerance, and an increase of just a few degrees above ambient seawater temperature may induce abnormal development of early life stages and cause adult mortality (e.g. Bassim et al., 2002; Loya et al., 2001; C. Randall and Szmant, 2009). Recent work has emphasized that warmer temperature reduce the tolerance of coral larvae *Acropora millepora* and *Acropora tenuis* to copper toxicity (Negri and Hoogenboom, 2011). That work questions whether metal toxicity will also be enhanced during the warmer season (summer compared to winter) or only when the temperature of seawater is a few degrees above ambient. Importantly current, water-quality guidelines for metals used to protect tropical corals are derived from temperate systems, which might be under-protective for corals in tropical areas (Chapman et al., 2006).

Here we examine the sensitivity of the scleractinian coral *P. damicornis* to copper (Cu) and lead (Pb) toxicity and assess how life stages (adults vs larvae) and thermal setting influence toxicity. Our goal is to provide new toxicological data for a common species of scleractinian corals with a testing framework of increased ecological relevance that may refine the current criteria for protecting tropical marine life and improve the capacity to manage coral reef ecosystems in the face of human-use patterns.

2. Materials and methods

2.1. Experimental design of the toxicity tests for adults and larvae

Toxicity tests with Cu and Pb were performed on adult and larvae of the coral *P. damicornis*. For each experiment, 10 adult colonies of *P. damicornis* were collected adjacent to Coconut Island (21°26′1.97″N, 157°47′20.10″W), Oahu, Hawaii, in January–February (Winter season) and July–August (Summer season) 2009. Each colony was split into multiple fragments (18 nubbins per colony, 2–4 cm length), and maintained in a common garden tank under natural light and flowing seawater aquaria (24.1 ± 0.7 °C and 26.7 ± 0.8 °C for winter and summer seasons, respectively) to allow them to recover for a month. In order to simulate light condition similar to those experienced by corals at collection site (Padilla-Gamiño et al., 2014), shade cloths were placed above tanks.

In each tank, 10 genetically distinct coral nubbins (one from each coral colony collected) were placed in each of 15-L plastic aquaria (n = 18 aquaria total, 3 replicates per treatment) containing 10 L of 5-μm natural filtrated seawater (closed circuit aquarium, natural light/dark cycle). Seawater salinity, temperature, and pH were checked twice daily throughout the 96 h duration of the experiment. Corals were exposed to six different seawater treatments: one control (C_0: no addition of Cu and Pb solution) and five containing increasing metal concentrations (added Cu concentration: $C_1 = 10$, $C_2 = 50$, $C_3 = 100$, $C_4 = 250$, and $C_5 = 500$ μg L^{-1}; added Pb concentration: $C_1 = 80$, $C_2 = 160$, $C_3 = 320$, $C_4 = 640$, $C_5 = 1280$ μg L^{-1}; Table 1). Metals were added (spiked) to seawater as either $Cu(Cl)_2$ or $Pb(NO_3)_2$ (purchased from Merck, synthesis quality). Half the seawater (plus the appropriate metal spikes) were replaced every 24 h over the 96 h. Seawater samples (n = 3 samples per concentration) were collected before and after each water change. Seawater samples collected after the first spike at 0 h and at the end of exposure (96 h) were analyzed separately, whereas