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Chemical toxicity on coral reefs: Bioassay protocols utilizing benthic foraminifers

Benjamin J. Ross *, Pamela Hallock

College of Marine Science, University of South Florida, St. Petersburg, FL 33701, USA

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ABSTRACT

Coral-reef communities worldwide are in decline and one category of threats is chemical pollution. Although the Loop Current ultimately isolated the Florida reef tract from oil and oil dispersants in the aftermath of the Deepwater Horizon oil spill in 2010, the initial threat of exposure highlighted the need for reliable bioassay protocols for predicting the effects of chemical pollutants on coral-reef communities, as well as in monitoring possible exposure. *Amphistegina* spp. are relatively large, benthic, shelled protists (Class Foraminifera) that are common on coral reefs at euphotic depths worldwide. These foraminifers harbor algal symbionts in a relationship analogous to that of corals that host zooxanthellae, making such foraminifers useful proxies for responses of zooxanthellate corals to environmental insults. *Amphistegina* and other large foraminifers are easy to collect and work with in statistically rigorous sample sizes, features that have led to their use as general bioindicators of reef water quality. The goal of this study was to develop bioassay protocols for chemical pollutants utilizing *Amphistegina gibbosa* d'Orbigny, the species found ubiquitously on Caribbean and western Atlantic reefs. A protocol was developed to identify the 48-h Lethal Concentration (LC) 50, the concentration of a test chemical in seawater that killed 50% of the specimens during 48-h exposure. Two chemicals found in oil dispersants employed in the clean-up efforts in the Gulf of Mexico, propylene glycol and 2-butoxyethanol, were used as test chemicals. Some individuals, which had appeared to be dead at the end of the 48-h exposure period, recovered following rinsing and removal to clean seawater. This observation required further definition of an Acute Concentration (AC) 50, the concentration of chemical in seawater that killed or rendered inactive 50% of the specimens during a 48-hour exposure. We also evaluated several indicators of chronic effects of the short-term exposure. All concentrations of propylene glycol tested resulted in significantly higher incidences of bleaching (color loss in the foraminifers due to loss of, or damage to, algal symbionts). As bleaching is a common stress response in zooxanthellate corals, even short-term exposure to dispersant chemicals may increase susceptibility to bleaching.

1. Introduction

Coral-reef communities worldwide are in decline (e.g. De'ath et al., 2012; Perry et al., 2013; many others); those of the Florida reef tract are no exception (e.g. Callahan et al., 2007; Ruzicka et al., 2010). Although fortuitous behavior of the Loop Current during the summer of 2010 prevented direct exposure of the Florida reef tract to oil released during the Deepwater Horizon oil spill, or to the dispersant chemicals deployed during cleanup efforts, this tragedy further highlights the range of potential threats to coral-reef and other coastal-marine environments.

How do chemical pollutants affect corals? Possibilities include not only direct toxicity, but also additive and sublethal effects. Negri et al. (2011), for example, found that exposure to herbicides amplifies the effects of thermal stress on corals. Previous studies (i.e. Epstein et al., 2000; Negri and Heyward, 2000) have found that both oil and oil dispersants can be toxic to larvae of a number of coral species, as well as reduce larval settlement rates, causing deformations, tissue degradation, and other sub-lethal effects, with the effects increased by exposure to combined oil–dispersant mixtures. Elgershuizen and De Kruijf (1976) reported a similar effect on established colonies of the stony coral *Madracis mirabilis*, which was able to recover from the effects of surface oil, but showed higher toxicity and significantly reduced recovery when exposed to a combination of oil and dispersants. The effects of oil, dispersants and other chemicals have a broad range of negative effects on shallow-water stony corals. However, because experimenting with corals poses both logistical and permitting challenges, model organisms have important advantages as bioindicators of stress, both in situ and in laboratory experiments. This paper presents a bioassay protocol for testing chemical pollutants utilizing common reef-dwelling foraminifers.

Amphistegina spp. are relatively large, benthic, shelled protists (Class Foraminifera). The genus is abundant in warm seas nearly circumtropically, living primarily on phytal and hard substrata in coral-reef and open-shelf environments. *Amphistegina* host diatom

* Corresponding author at: College of Marine Science, University of South Florida, 830 1st Street South, St. Petersburg, FL 33701, USA. Fax: +1 727 553 1189.
E-mail address: benjaminross@mail.usf.edu (B.J. Ross).

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symbionts in a relationship analogous to that of reef corals and their zooxanthellae (e.g. Lee, 2006). Although these protists are somewhat more tolerant of temperature changes than most reef-building corals (Talge and Hallock, 2003), they are highly sensitive to light and water-quality stressors (Hallock et al., 2003; Williams and Hallock, 2004). *Amphistegina* are particularly useful for experimental studies because their small size makes working with statistically robust sample sizes relatively easy, yet they are sufficiently long lived (several months) and are large enough (~0.5–1.5 mm diameter) to respond visibly to stressors by changes in color, motility, growth rates, shell structure, and reproductive success over time (e.g. Hallock, 2000a; Hallock et al., 1986). These characteristics make *Amphistegina* spp. useful bioindicators for water and sediment quality.

The calcite shells of *Amphistegina*, and of other benthic foraminifers that host algal endosymbionts, remain in the environment after death. These shells provide key data for the FoRAM (Foraminifera in Reef Assessment and Monitoring) Index, a single-metric index of water quality developed for use in the western Atlantic and Caribbean (Hallock et al., 2003). The index has been effectively adapted to other regions, including reef environments in Kiritimati Island (Carilli and Walsh, 2012), Brazil (Barbosa et al., 2009), and Australia (e.g. Schueth and Frank, 2008; Uthicke and Nobes, 2008; Uthicke et al., 2010, 2012a), where it is now used by the inshore monitoring program for the Great Barrier Reef. The FoRAM Index also has been used effectively as an indicator of water quality and pollution in estuarine environments in Florida (Carnahan et al., 2009), Australia (Narayan and Pandolfi, 2010), and even in the Mediterranean (Koukousioura et al., 2011). In addition, examination of live *Amphistegina* populations can provide a relatively quick, low-cost method to assess environmental conditions on a reef to determine if stressors are emerging and to evaluate the need for more costly chemical analyses of water and sediments or of biopsies of macrofauna (Hallock, 2012; Hallock et al., 2006b).

The features that make benthic foraminifers appropriate for bioindicator research also make them excellent candidates for bioassay applications in reef environments. Such uses have emerged, to date, primarily to assess chronic responses to nutrient pollution, rising temperatures and elevated pCO_2 (e.g. Reymond et al., 2013; Schmidt et al., 2011; Uthicke et al., 2012b, 2013). Van Dam et al. (2012a, 2012b) demonstrated that the herbicide diuron increases the sensitivity of symbiont-bearing foraminifers to elevated temperatures, similar to what Negri et al. (2011) found in zooxanthellate corals. Moreover, de Freitas Prazeres et al. (2011, 2012) demonstrated oxidative-stress responses in *Amphistegina* exposed to zinc and other potentially toxic metals.

Short-term, acute-lethality tests typically are carried out by exposing organisms to chemicals for a specific exposure period, between 24 and 96 h depending on the organism and test, to determine the concentration expected to kill 50% of a population (LC50), as well as determining the No Observable Adverse Effect Concentration (NOAEC), the highest concentration which does not cause toxic effects significantly different from control (EPA, 2002). Both of these measurements are useful in establishing endpoints in risk assessments and are integral in environmental planning strategies, especially for waterways (i.e. Jones et al., 2004; Sappington, 2013). According to EPA guidelines, for acute toxicity tests to be statistically rigorous, a minimum of 20 specimens must be exposed to each concentration of interest. Foraminifers are thus an attractive option, owing to their small size and amenability to laboratory culture. In addition to descriptive assessment parameters, other short-term response variables can be applied or developed, such as oxidative-stress biomarkers (de Freitas Prazeres et al., 2011, 2012), contributing further to their potential applications.

Bioassays of sublethal (chronic) effects of short-term exposure take place over longer time periods and have different goals. Foraminifers also are well suited for identifying sublethal effects. Growth is easily tracked using simple measurements of diameter (e.g. Hallock et al., 1986), area (e.g. Reymond et al., 2013), or fluorescent markers such as calcein (e.g. Denoyelle et al., 2012). *Amphistegina* host algal endosymbionts and are known to respond to stress by visible changes in color that have been verified ultrastructurally (Talge and Hallock, 2003) and fluorometrically (Schmidt et al., 2011). Thus, symbiont loss (bleaching) and other color changes can be used as sublethal indicators of stress. Moreover, the ease of culture of *Amphistegina* provides the potential for effects such as altered reproductive response or anomalies in calcification to be used as response variables; such effects have been documented in field populations (e.g. Hallock et al., 1995). Moreover, their shells can be assessed for cumulative toxicity (e.g. De'ath et al., 2012; Elberling et al., 2003; Frontalini and Coccioni, 2008; Le Cadre and Debenay, 2006).

An important consideration in designing bioassay experiments is the relevance of the test organism to the environments of concern. Organisms commonly used to assess acute toxicity in estuarine and marine systems (e.g. EPA, 2002) are estuarine fish and mysid shrimp. The latter are benthic and have a cosmopolitan distribution, and therefore are better models than estuarine fish. However, none of the common bioassay organisms harbor algal symbionts. The mixotrophic mode of life, which includes feeding by the host and photosynthesis by the symbiotic algae and therefore recycling of nutrients within the holobiont, has unique implications in terms of toxic exposure, uptake, and reactions within the organism. For example, Peters et al. (1981) found that long term exposure of the Caribbean coral *Manicina areolata* to oil did not lead to death, but did lead to a number of other effects, including the degeneration and loss of symbiotic zooxanthellae, effects that cannot be observed in assays employing fish or shrimp.

Amphistegina spp., along with other foraminifers that host algal symbionts, have already demonstrated applicability to monitor and assess water quality in coral-reef and other tropical coastal environments (Hallock, 2012; references therein). Because *Amphistegina* are known to react to photo-oxidative stress by visible symbiont loss (partial bleaching), their role as mixotrophic calcifiers is more analogous to that of zooxanthellate coral than other organisms commonly utilized in bioassay experiments. The bleaching response also imparts another benefit of *Amphistegina* spp. as bioassay organisms; as noted in Hallock et al. (2006a), the visible presentation of bleaching allows for application of laboratory results to the field, as well as field results to the laboratory (see also Talge and Hallock, 2003). In addition, *Amphistegina* live abundantly among corals in the reef ecosystem, and due to their small size, relatively limited mobility, and benthic habitat, are likely to be directly exposed to any chemical stressors that would affect corals, their algal symbionts, and associated benthos. Thus, there is limitless potential for use of *Amphistegina* spp. as both bioindicator and bioassay organisms for chemical and other threats to reef systems.

The purpose of this research was to develop protocols for use of *Amphistegina gibbosa* d'Orbigny as a bioassay organism for chemical pollutants. The chemicals used in the development of the protocols were propylene glycol and 2-butoxyethanol. These chemicals were chosen for their relatively low toxicity, thereby minimizing safety considerations, as well as for their presence in high proportions in COREXIT® oil dispersants (Nalco, 2005, 2008), which were used extensively in the Deepwater Horizon cleanup efforts (e.g. Rico-Martínez et al., 2013). The foraminifers were exposed separately to each chemical, with the primary goal to determine Lethal Concentration (LC50) of each chemical and to determine if sublethal responses were evident that can be useful as response variables in future applications.

2. Methods

2.1. Standard collection and culture methods

Samples were collected on Tennessee Reef in the Florida Keys at depths between 6 and 18 m. Foraminifers have been studied in this immediate area for nearly 30 years (e.g. Baker et al., 2009; Hallock et al., 1986; Williams et al., 1997). Collection methods for live foraminifers