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# Combined thermal and herbicide stress in functionally diverse coral symbionts

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## ABSTRACT

Most reef building corals rely on symbiotic microalgae (genus *Symbiodinium*) to supply a substantial proportion of their energy requirements. Functional diversity of different *Symbiodinium* genotypes, endorsing the host with physiological advantages, has been widely reported. Yet, the influence of genotypic specificity on the symbiont's susceptibility to contaminants or cumulative stressors is unknown. Cultured *Symbiodinium* of presumed thermal-tolerant clade D tested especially vulnerable to the widespread herbicide diuron, suggesting important free-living populations may be at risk in areas subjected to terrestrial runoff. Co-exposure experiments where cultured *Symbiodinium* were exposed to diuron over a thermal stress gradient demonstrated how fast-growing clade C1 better maintained photosynthetic capability than clade D. The mixture toxicity model of Independent Action, considering combined thermal stress and herbicide contamination, revealed response additivity for inhibition of photosynthetic yield in both tested cultures, emphasizing the need to account for cumulative stressor impacts in ecological risk assessment and resource management.

## 1. Introduction

Coral reefs are primarily formed by calcification processes of scleractinian corals and coralline algae, providing a structural basis for a host of reef-dwelling organisms. Most coral species rely on an obligate symbiotic association with intracellular dinoflagellates of the genus *Symbiodinium* for the majority of their energy acquisition (LaJeunesse and Thornhill, 2011; Muscatine, 1990). These microalgae convert sunlight into chemically bound energy through photosynthesis and translocate photosynthates to the coral host, allowing the coral to maintain growth and reproduction in an oligotrophic environment (Muscatine and Porter, 1977). However, coral reefs worldwide are in decline due to a range of anthropogenic pressures including climate change and reduced water quality (Hoegh-Guldberg et al., 2007; Wilkinson, 2008). These pressures are likely to increase in future years under projected climate scenarios (Collins et al., 2013), expanding industrialization, urbanization and agricultural activities, presenting a substantial risk to the biodiversity of tropical coral reefs and the services they provide (Moberg and Folke, 1999).

The physical and biological properties of a coral's (micro)environment predominantly determines the specific genotypes of *Symbiodinium* the host partners with, whose qualities in turn are important regulators of the host's distribution (Baker, 2003; Cooper et al., 2011; Iglesias-Prieto and Trench, 1994; Rowan and Knowlton, 1995; van Oppen et al., 2001). Most species can host several taxa simultaneously, and may shift the dominant *Symbiodinium* type following an environmental cue to increase tolerance to prevailing conditions (Baker et al., 2004; Berkelmans and van Oppen, 2006). For example, some corals maintained under conditions of thermal stress displaced sensitive symbionts in favor of a more thermally tolerant type (Jones et al., 2008). While beneficial to overcome periods of adverse conditions, the increase in thermal tolerance has been described as a trade-off with major implications (Stat and Gates, 2011), as several empirical studies have shown how hosting a more thermal-tolerant strain of *Symbiodinium* is likely to come at the cost of reduced photophysiological output, calcification

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and growth (Cantin et al., 2009; Jones and Berkelmans, 2010; Little et al., 2004; Mieog et al., 2009).

Reef-building corals are vulnerable to environmental change as they live close to their upper thermal limits (Berkelmans and Willis, 1999) and are sensitive to a range of contaminants (Fabricius, 2005). When sea surface temperatures (SSTs) rise above certain thresholds, high solar irradiance can overexcite the algal photosystems, overwhelming protective mechanisms and potentially damaging sensitive tissues in both symbiont and host. In corals and other symbiotic reef species, this may lead to the dissociation of the symbiotic relationship (bleaching) (Lesser, 2011).

On Australia's Great Barrier Reef (GBR), highest SSTs occur in January and February (Lough, 2007), coinciding with monsoonal rainfall and river flooding events that deliver vast loads of fresh water, suspended sediments, dissolved nutrients and agrochemical residues into estuaries and coastal seas (Kroon et al., 2012). Thus nearshore reefs in the GBR lagoon are prone to suffer from combinations of cumulative stressors during episodes of thermal stress. Recent decades have seen increased agricultural applications of fertilizers and pesticides in coastal river catchments which has resulted in the ubiquitous presence of chemical residues in waterways and inshore areas of the GBR (Kennedy et al., 2012; Lewis et al., 2009; Shaw et al., 2010). Of particular ecological concern are the photosystem II (PSII) herbicides including diuron and atrazine as they are highly mobile and persistent (Brodie et al., 2012), while safety margins between chronic environmental concentrations and toxic concentrations are relatively small (van Dam et al., 2011). Although the highest concentrations of PSII herbicides on the GBR have been found in conjunction with major river floodings (Lewis et al., 2009), some highly potent ones such as diuron can be detected year round in the central region of the GBR (Kennedy et al., 2012). These compounds inhibit photochemical electron transport through PSII after binding to an electron-acceptor protein, outcompeting the normal ligand for binding sites. In corals, this limits photosynthesis in the symbionts and energy flow to the host (Cantin et al., 2009). Chronic exposure can lead to coral bleaching (Cantin et al., 2007; Jones, 2004), as excitation energy cannot be transferred through the electron transport chain but is instead passed over to form damaging reactive oxygen. Despite the relatively low direct risk associated with herbicide pollution to corals as chronic environmental concentrations are generally low (Kennedy et al., 2012; Shaw et al., 2010), evidence is emerging that prolonged, low level herbicide exposure may increase the sensitivity of corals and other symbiotic phototrophs to further environmental stressors such as high SSTs (Negri et al., 2011; van Dam et al., 2012).

Despite a wide body of research on the thermal stress physiology of corals and their diverse range of symbionts, very little is known regarding the sensitivity of different types of *Symbiodinium* to further aspects of water quality, e.g. their ability to tolerate contaminants or sedimentation, let alone how these parameters may influence their thermal sensitivity. Physical conditions such as temperature are likely to affect membrane properties, diffusion rates and biokinetic pathways of contaminants, thereby largely influencing a compound's toxicity (Holmstrup et al., 2010). In a similar fashion, contaminants may effectively lower threshold temperatures at which a stress response is initiated. Elevated SSTs in conjunction with PSII herbicides have previously been reported to elicit additive adverse effects in adult corals (Negri et al., 2011) and symbiotic foraminifera (van Dam et al., 2012), yet the physiological properties of the hosted symbionts were never considered in these mixed stressor studies.

Here, we aim to further elucidate what role the genotype may play in a symbiont's response to combined physical and chemical stress, and how environmentally relevant concentrations of a PSII herbicide may influence the thermal susceptibility of different strains of *Symbiodinium*. We apply a comparative approach to investigate the photochemical capacity of two distinct types of *Symbiodinium* dominant on the central section of the GBR: a generalist (fast-growing) strain of clade C1 against a specialist (thermal-tolerant) strain of clade D. First, PSII electron turnover in response to the commonly detected PSII herbicide diuron was assessed in long-term cultures (1–3 months; proxies for free-living populations) and freshly isolated *Symbiodinium* (symbiotic cells outside the host's influence) of both strains. Next, cultures of both strains were exposed to increasing concentrations of diuron over a thermal stress gradient to examine any cumulative effects. With globally increasing SSTs potentially driving corals towards associations with more thermal-tolerant symbiont types (Stat and Gates, 2011; van Oppen et al., 2009), their capacity to respond to and manage additional environmental pressure may be reduced.

## 2. Methods

### 2.1. Preparation of Symbiodinium cultures

Several healthy colonies (~30 cm diameter) of both *Acropora tenuis* and *Acropora millepora* were randomly sampled at ~6 m depth from an inshore, central GBR reef (19°10.200′S, 146°51.157′E). Colonies were maintained outdoors in partially shaded, 1000 L flow-through aquaria containing 5 μm filtered seawater (FSW) at 26–27 °C. At high noon, maximum irradiance observed was 350 μmol quanta $m^{-2}s^{-1}$ PAR. The dominant *Symbiodinium* clades C1 (*A. tenuis*) and D (*A. millepora*) as hosted by the corals were confirmed by single strand conformation polymorphism (SSCP) following van Oppen et al. (2001). Symbiont types were selected due to their dominance on the central section of the GBR and their history as a laboratory test species. Part of each coral was stripped of its tissue using compressed air; tissue and algae were collected, filtered through a 20 μm mesh and centrifuged at 1600 × g for 3 min. The supernatant was then gently decanted and the algal pellet resuspended in 0.2 μm FSW. This process was repeated 3 times before algal suspensions were pooled by type. Antibiotics (penicillin, neomycin, streptomycin and nystatin; at 100 μg $mL^{-1}$ each) and a diatom growth inhibitor ($GeO_2$; 5 mg $mL^{-1}$) were added to the suspensions. After 24 h, the suspensions were washed 3 times before resuspension in sterile IMK medium (Wako Chemicals, USA) containing antibiotics (as above). Each parent coral hosted several strains of the dominant clades C1 and D *Symbiodinium*, resulting in polyspecific cultures for each clade. These will subsequently be referred to as the clade C1 and clade D cultures. Cultures were maintained in log growth phase by weekly transfer to fresh growth media for 1–3 months before use; dominant genotypes were reconfirmed prior to the start of each experiment. Cultures and test plates were maintained at 26 °C, on a 14L:10D photoperiod under 50–60 μmol quanta $m^{-2}s^{-1}$ PAR irradiance, unless stated otherwise.

### 2.2. Chlorophyll fluorescence techniques

Photochemical performance of the different symbiont types was estimated using the saturation pulse method (Schreiber, 2004) in a Maxi Imaging-PAM (I-PAM) (Walz GmbH) fluorometer. The effective quantum yield of PSII, $\Delta F/Fm'$, is directly proportional to photosynthetic energy conversion in PSII and the recommended parameter for use in ecotoxicology (Ralph et al., 2007). This is particularly important when dealing with PSII-inhibitors as these chemicals generally require light to elicit an effect (Ralph et al., 2007; Schreiber et al., 2007). Test procedures followed a standard