Chronic effects of herbicide exposure on photosynthesis, symbiosis and reproduction of reef building corals

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This thesis includes collaborative work with my supervisors Dr. Andrew Negri (Australian Institute of Marine Science) and Prof. Bette Willis (James Cook University) and Dr. Madeleine van Oppen (Australian Institute of Marine Science). While undertaking these collaborations, I was responsible for the project concept and design, data collection, analysis and interpretation and the final synthesis of the results in a form suitable for publication. My collaborators provided intellectual support, financial support, technical instruction and editorial assistance.

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Abstract

The herbicide, diuron, is found at levels equivalent to 1 μg l^{-1} within sediments in the Great Barrier Reef lagoon, where it potentially reduces photosynthesis and carbon fixation within *Symbiodinium*, the dinoflagellate symbiont associated with reef corals. Little is known about the potential of diuron to reduce energy acquisition and change energy allocation strategies to reproduction in corals. The objective of this study was to examine the importance of carbon-based energy (carbohydrates) derived from photosynthesis for gametogenesis, gamete viability and larval quality of corals following the first long-term, experimental exposures to diuron and to investigate the influence of symbiont type on energy provisioning to host tissues under normal conditions and in the presence of diuron. This is the first study to investigate the chronic sub-lethal effects of herbicide-induced photoinhibition on coral symbionts and the subsequent flow on effects to the fitness of the coral host.

Two broadcast spawning corals, *Acropora tenuis* and *A. valida,* and a brooding coral, *Pocillopora damicornis,* were exposed to 0 (control), 1.0 (low) and 10 (moderate) μg l^{-1} diuron treatments for 2 to 3 months prior to spawning or planulation. Diuron caused rapid and consistent declines in effective quantum yields of approximately 20% at 1.0 μg l^{-1} and 75% at 10 μg l^{-1} in each species compared to controls (Chapter 2). Total lipid content (coral tissue, oocytes and planulae) was reduced by 2.5- to 5-fold for the three species in the presence of diuron, indicating significant use of storage lipid to meet nutritional demands under conditions of chronic photoinhibition. Polyp fecundity in *A. tenuis* was not impacted, however it was reduced by 6-fold in *A. valida,* and both *A. valida* and *P. damicornis* were unable to spawn or planulate following long-term exposures to 10 μg l^{-1} diuron.

Maternal provisioning of lipids, pigments and antioxidants to coral eggs that lack *Symbiodinium* provides energy and protection essential for the development, survival and dispersal of coral larvae. For corals that were able to spawn or planulate following 2-3 month experimental exposures to diuron (i.e. *A. valida* and *P. damicornis* in the 0 and 1 μg l^{-1} treatments; *A tenuis* in the 0, and 10 μg l^{-1} treatments), gamete fertilisation was not affected (Chapter 3). Larvae from each of these species also successfully metamorphosed into juvenile corals following parental exposures to the above diuron treatments. Although gametes were viable, gamete quality was reduced in *A. valida*

following even low exposures to diuron. Peridinin, the major carotenoid pigment identified in *A. valida* eggs, was 10-fold lower in eggs derived from corals exposed to 1.0 µg l^{-1} diuron compared with tank controls. The tank controls in turn contained 5-fold less peridinin than field controls. In contrast, no difference in vitamin E (α – tocopherol) was detected in any of the treatments. Peridinin in combination with xanthophylls may enhance the capacity of buoyant coral eggs to absorb potentially harmful high-energy photosynthetically active radiation (PAR, 400 - 530 nm) that is not absorbed by mycosporine-like amino acids (MAAs) and vitamin E. All of these compounds are likely to work synergistically to protect eggs from oxidative damage.

Algal endosymbionts of the genus *Symbiodinium* play a key role in fulfilling the nutritional requirements of reef building corals, however comparisons of photosynthetic capacity among different *Symbiodinium* types *in hospite* within the same coral species have only recently become possible. A sensitive quantitative PCR assay was developed for *Symbiodinium* spp. (Chapter 4) based upon chloroplast (cp) large subunit (23S) ribosomal DNA sequences, to detect low level background strains of *Symbiodinium* spp. It was then applied to verify symbiont assemblages within juvenile colonies of *Acropora millepora* that had been experimentally infected with two different symbiont types (Chapter 5). Using experimentally infected C1- and D-juveniles of *A. millepora*, relative electron transport ($rETR_{MAX}$) of PSII, was found to be 87% greater in *Symbiodinium* C1 than in *Symbiodinium* D *in hospite* in the control treatment, resulting in a doubling of ^{14}C photosynthate incorporation (energy) into juvenile tissues of *A. millepora* (Chapter 5). *Symbiodinium* C1 corals, however, lost this competitive advantage in the presence of diuron, due to inhibition of rapid electron transport. There was no observable difference in phytotoxicity of diuron between genetically distinct symbionts *in situ*. The finding that genetically distinct *Symbiondinium* spp. are not functionally equivalent, highlights the importance of symbiont identity in the nutritional physiology of the coral-algal holobiont.

These results provide evidence of a link between reduced energy acquisition due to diuron exposure causing significant PSII photoinhibition and reduced reproductive output in zooxanthellate corals. Energy allocated to reproduction was directed towards maintaining and releasing fewer eggs and larvae, while ensuring the full developmental viability of these progeny. Along with diuron, other herbicides such as atrazine and Irgarol 1051 that are designed to target the PSII in the same manner as diuron, are commonly found entering the marine environment, which could create an additive

effect on the chronic impacts induced by diuron exposure within the natural environment. The observed reductions in reproductive development (*A. valida*) and reproductive output (*A. valida* and *P. damicornis*) caused by the inhibition of energy acquisition from photosynthesis following long-term diuron exposure, highlights the importance of carbon-based energy from photosynthesis for coral reproduction and provides further evidence of physiological trade-offs that can result following events that limit the availability of energetic resources to individual coral colonies.

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Publications arising from this Thesis

1. From Chapter 2: Cantin, N.E., Negri, A.P. and Willis, B.L. (2007) Photoinhibition from chronic herbicide exposure reduces reproductive output of reef-building corals. Marine Ecology Progress Series **344**: 81-93.

2. From Chapter 4 (in part): Mieog, J.C., van Oppen, M.J.H., Cantin, N.E., Stam, W.T. and Olsen, J.L. (2007) Real-time PCR reveals a high incidence of *Symbiodinium* clade D at low levels in four scleractinian corals across the Great Barrier Reef: implications for symbiont shuffling. Coral Reefs **26**:449-457.

3. From Chapter 5: Cantin, N.E., van Oppen, M.J.H, Willis, B.L., Mieog, J.C. and Negri, A.P. (2008) Juvenile corals acquire more carbon from high-performance algal symbionts. Coral Reefs: *in review*.

4. From Chapter 3: Cantin, N.E., Willis, B.L. and Negri, A.P. (2008) Quality of coral gametes following chronic parental herbicide exposure. Marine Ecology Progress Series: to be submitted (*in prep.*).