Natural products and their supply from the tropical sponge *Luffariella variabilis*

Thesis submitted by

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STATEMENT OF THE CONTRIBUTION OF OTHERS

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ABSTRACT

This thesis examines the critical link between the fundamental biology and chemical ecology of the Great Barrier Reef sponge *Luffariella variabilis* (Poléjaeff 1884) for the aquaculture based supply of bioactive metabolites. *Luffariella variabilis* produces manoalide, a high value bioactive sesterterpene used as a molecular probe. The sponge is cryptic and distributed widely through the Indo-Pacific -- this study was done on on the central Great Barrier Reef at Orpheus Island in the Palm Islands group, Queensland, Australia where *L. variabilis* is common.

The first objective of the study was to examine the natural products chemistry of *L. variabilis*. Three new acetylated compounds, 25-acetoxyluffariellin A, 25-acetoxyluffariellin B and 25-acetoxyseco-manoalide were obtained from *L. variabilis* and the structures of the three new compounds elucidated on the basis of their spectroscopic data. The known major metabolites, manoalide monoacetate, manoalide, luffariellin A and seco-manoalide were also identified.

The known major metabolites were then monitored temporally and spatially to determine the potential yield from wild harvest or aquaculture. Production of the major metabolites was hardwired with little variation in space and time at the population level in the Palm Islands. Manoalide monoacetate (35 - 70 mg g^{-1} dry weight of sponge) was always the most abundant compound followed by manoalide (15 - 20 mg g^{-1} dry weight of sponge). Luffariellin A and seco-manoalide were always 10 –70 times less abundant and varied between 0 – 3 mg g^{-1} dry weight of sponge. Collections of *L. variabilis* made at Davies Reef and Magnetic Island yielded the same rank order and yields of compounds indicating a generality of pattern over at least 100 km. The ‘hardwiring’ of metabolite production at the population level by *L. variabilis* was also reflected in the lack of any inductive effect on metabolite production. In

addition, individually monitored sponges produced fixed ratios of the major metabolites over time. However, these ratios varied between individuals with some individuals consistently producing high levels of manoalide and manoalide monoacetate. The potential for selection of high yielding stocks is discussed.

In order to explore the sustainable production of natural products via wild harvest or aquaculture, the reproductive output of *L.variabilis* was quantified and correlated with sea temperature over two reproductive seasons (2004 and 2005). *Luffariella variabilis* is gonochoristic and viviparous. Gametogenesis commenced for females at a water temperature of 21 ºC, the lowest water temperature of the year, and spermatogenesis occurred above 22.5 ºC (with sperm asynchronously developed and released from August or September to October). Females asynchronously developed oocytes from July to September, embryos from September to December, and larvae from November to December. Female reproduction terminated in December (after larval release) prior to the highest mean annual water temperature of 30 ºC in January. There was a significant (30 %) decrease in female reproductive output in 2005 compared to 2004 as measured by the reproductive index (0.91 ± 0.14 female reproductive propagules mm^{-2} of choanoderm in 2005 compared with 1.27 ± 0.11 mm^{-2} in 2004). This corresponded with delayed oogensis and spermatogenesis, and a shortened larval development cycle because of a delayed minimum temperature (21 ºC) in August of 2005 compared with July 2004. Correspondingly, the maximum percentage of the choanoderm occupied by female reproductive propagules (eggs, embryos and larvae) was also reduced by 33 % in 2005 (5.09 % in October 2004 compared with 3.44 % in October 2005). However, the mean sizes of individual female propagules remained the same from year to year. Males in contrast, showed no overall difference in either reproductive index or percentage occupation of the choanoderm between 2004 and 2005. The significantly lower reproductive output (~30 %) for *L. variabilis* associated with delayed minimum water temperatures has significant implications for population reproductive success

where oogenesis, spermatogenesis and larval release are cued by minimum and maximum water temperatures, given the predicted increases in water temperatures associated with climate change.

Determining the settlement responses of *L.variabilis* larvae is crucial in determining on-growth potential for aquaculture. The response of *L. variabilis* larvae to a hierarchy of settlement cues was examined from mid-November to late December 2005. Light cued the daytime release (0700 – 1600 hrs) of up to 830 larvae day^{-1} $sponge^{-1}$ over 5 – 6 weeks. Newly released larvae initially swam upwards. However, at 20 - 40 min post release, larvae exhibited a clear negative phototaxis and light strongly influenced their settlement. Irradiance levels of 55 µmol m^{-2} s^{-1} and 14 µmol m^{-2} s^{-1} slowed the settlement rate of larvae and inhibited overall settlement after 18 hours by ~ 60 % and 35 % respectively compared with controls. The rate of settlement and overall settlement were still significantly reduced at irradiances of >3 µmol m^{-2} s^{-1}. This corroborated with the adult distribution of *L. variabilis* in dark areas. *Luffariella variabilis* larvae are gregarious settlers with increasing rates of settlement and overall settlement with increasing densities of larvae. Gregarious settlement of *L. variabilis* larvae is associated with a conspecific larval settlement cue(s). Individual and groups of ten larvae placed in 'conditioned' water (water in which 200 larvae had previously settled) initially settled faster than controls. Furthermore, this effect was highest on single larvae with a four fold increase in overall settlement. While the rate of settlement was faster for groups of ten larvae, overall settlement totals were similar to those of controls. In contrast, cues often associated with invertebrate larval settlement such as biofilms, crustose corraline algae and adult conspecifics had no effect on settlement at any time.

In summary, the production of the major *L. variabilis* metabolites was fixed in time and space. Manoalide monoaceteate and manoalide were produced in high amounts making the sponge an ideal target for either wild harvest or aquaculture. *Luffariella variabilis* is gonochoristic, released sperm in August,

September and October and asynchronously brooded embryos over six months culminating with larval release in November and December. Larvae settled rapidly in the dark and at faster overall rates, and higher overall totals with increasing density. This was because settling larvae release a settlement cue (athough there was no effect of other common invertebrate settlement cues). The rapid settlement of larvae in dark areas corroborates with the adult distribution of the sponge and strongly suggests that biomass of *L. variabilis* for the production of manoalide could be augmented by ongrowth and culture of larvae.

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