Aspects of life history and ecology of *Dicathais orbita* Gmelin, 1781 related to potential aquaculture for bioactive compound recovery

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Abbreviations

FSW	Fresh seawater
GSI	Gonadosomatic index
DAD	Parallel UV/Vis diode-array
ESI-MS	electrospray ionisation-mass
RAS	Recirculating aquaculture system
P	Pigment
Bf	Bifurcation
H	Hypobranchial gland
DG	Digestive gland
G	Gonad
SBwt	Soft body weight
SL	Shell length
to	Tuberculate ornementation
V	Velum
Ft	Foot
Sc	Siphonal canal
Op	Operculum
Es	Eye spots
SGR	Specific growth rate
dph	days post hatching
KCl	Pottasium chloride
HPLC	High performance liquid
AUD	Australian dollars

Abstract

World aquaculture production, including the production of pharmaceuticals and nutraceuticals, is increasing to supplement fisheries harvest from wild stocks. Muricids (Neogastropoda) are widely fished around the globe and produce a range of interesting bioactive compounds (Chapter 1). Several species of muricids have been successfully cultured to supply seafood markets. The southern Australian muricid, *Dicathais orbita*, is recreationally harvested for food and more recently has been shown to produce potent bioactive compounds of interest for development as pharmaceutical leads. Successful aquaculture of *D. orbita* would provide a sustainable supply for ongoing development of pharmaceutical leads, as well as for seafood markets. Information on aspects of the life history of *D. orbita* will underpin successful aquaculture production.

Neogastropods are dioecious, but morphologically identical when in their shell. In order to non-destructively assess effective population size of wild stocks of *D. orbita* and manipulate lab held stock, it was necessary to develop a means to identify the sex of the species. A suite of anaesthetics were trialled for their efficacy in relaxing *D. orbita* out of the shell to identify sex organs and for stimulating bioactive compound production through a stress response (Chapter 2). Magnesium chloride proved most effective in relaxing *D. orbita* specimens enough to identify sex. Benzocaine and the carrier solvent ethanol were less effective for identifying sex, but stimulated expulsion of the bioactive precursors. The presence of bioactive brominated indoles in the expelled mucus of *D. orbita* was confirmed by liquid chromatography/mass spectrometry and provides a novel, sustainable means for obtaining these compounds without killing the snails.

The reproductive cycle of *D. orbita* was studied using a gonadosomatic index in wild and captive populations, along with spawning and post hatching larval development (Chapter 3). *D. orbita* follows an annual reproductive cycle, peaking in early summer (December) in South Australia. Female *D. orbita* spawn ~ 40 egg capsules in a session and each capsule contains an average of 5542 eggs with an average diameter of 105.2 µm. Post-hatching larval development proceeded through 5 stages over 41 days with average shell length increasing from 253 to 974.3 µm and shell width increasing from 203.8 to 980.5 µm. Information on the reproductive cycle and larval development patterns of *D. orbita* will allow for enhancement of reproductive condition and larval production.

Larval rearing experiments, to determine the effects of temperature and diet on the growth and survival of *D. orbita* larvae under laboratory conditions, used five different unicellular algal diets for larvae maintained at 16°C and 22°C (Chapter 4). Larvae reared at 22°C on a mixed diet, or diatoms alone, performed significantly better than those reared on green microalgal diets alone. Trials with settlement cues were undertaken on newly hatched to 38 day old *D. orbita* larvae to determine when larvae become competent. An array of natural cues (carrion, *Xenostrobus pulex*, adult mucus and *Ulvella lens*), as well as concentrations of KCl were tested. 20 mM KCl induced the greatest settlement, however no larvae metamorphosed under the conditions provided.

In conclusion, *D. orbita* can be grown under laboratory conditions and are highly fecund. Bioactive compounds can be extracted non-destructively from *D. orbita*, providing an extra resource that can be collected to value-add to seafood aquaculture. Larvae are planktotrophic with higher development rates at the higher water temperatures within their local range and grow to greater size on a diet comprised of

both green microalgae and diatoms. *D. orbita* is iteroparous with an annual reproductive cycle. High fecundity indicates that larval survival and recruitment is the limiting phase of the life cycle for *D. orbita*. Further studies will optimise culture conditions and cues for settlement and metamorphosis in order to close the life cycle of *D. orbita* for aquaculture production.

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Thesis structure

This thesis is presented in manuscript format. Thus some repetition of background and methods will be evident between chapters. To reduce repetition between chapters, a single reference list is contained at the end of the thesis. The introductory chapter (Chapter 1) provides context for the thesis through a broad overview of aquaculture, molluscan resources and neogastropod life history and concludes with overall aims of the thesis. Chapters 2-4 have independent hypotheses and aims which support the overall aims of the thesis. Chapter 2; Application of anaesthetics for sex identification and bioactive compound recovery from wild *Dicathais orbita.* and Chapter 4; Growth, settlement and survival of *Dicathais orbita* (Neogastropoda, Mollusca) larvae in response to temperature, diet and settlement cues, have been published, and Chapter 3; Reproductive cycle, spawning and post hatching larval development of *Dicathais orbita* (Neogastropoda: Muricidae), has been provisionally accepted for publication. The full details of publication are provided at the start of each chapter. Within each manuscript, the "study" refers to the particular set of experiments associated only with that chapter. Chapter 5 provides an overall, concluding discussion of all previous chapters. Appendix A and B consist of large tables referred to in the introductory and discussion chapters, and appendix C provides information on initial experiments on reproductive conditioning o *D. orbita*.

To maintain continuity in presentation, all chapters have been formatted in a consistent manner. Although I am first author on all chapters and personally responsible for the experimental design, conducting the research and preparing manuscripts, Kirsten Benkendorff and James Harris were supervisors who provided intellectual input for experiments and editorial input to facilitate publication. The contributions of an additional author on one published manuscript is outlined in the description of chapter objectives.