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# Bioactive molluscan resources and their conservation: biological and chemical studies on the egg masses of marine molluscs

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Benkendorff, Kirsten, Bioactive molluscan resources and their conservation: Biological and chemical studies on the egg masses of marine molluscs, PhD thesis, Department of Biological Sciences, Department of Chemistry, University of Wollongong, 1999.  
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**BIOACTIVE MOLLUSCAN RESOURCES**  
**AND THEIR CONSERVATION:**  
**Biological and Chemical Studies on the Egg Masses of**  
**Marine Molluscs**

**\* A thesis submitted in fulfilment of the  
requirement for the award of the degree**

**DOCTOR OF PHILOSOPHY**

**from**

**UNIVERSITY OF WOLLONGONG**

**by**

**KIRSTEN BENKENDORFF, B.Sc. Hons.**

**Department of Biological Sciences  
Department of Chemistry**

**March, 1999**

*The edge of the sea is a strange and beautiful place.*

Rachael Carson

*Certainly Divinity is here in these shells in their humble form of life.*

Frank Lloyd Wright

*By Tyre the old, with ocean-plunder, A netful, brought to land.*

Robert Browning



Dedicated to my Grandmother, Jutta Benkendorff

For inspiring me to take a philosophical approach to evolution,

And my parents, Robin and Peter Benkendorff

For endless support and encouragement.

## **Thesis Declaration**

This thesis contains no material that has been accepted for the award of any other degree or diploma at any University, and to the best of my knowledge contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

## Acknowledgments

I am indebted to Dr Andy Davis and Prof. John Bremner from the University of Wollongong for supervising this project. I would like to especially thank Dr Andy Davis (Department of Biological Sciences) for initially suggesting that I study the egg masses of marine molluscs. Having never before studied in the marine environment, Andy's knowledge of marine biology has greatly assisted me during this project. Andy has also significantly improved my understanding of experimental design, as well as providing enthusiasm, encouragement and valuable comments throughout the research project. I am also extremely grateful to Prof. John Bremner (Department of Chemistry) for agreeing to take on a biology student and making available his knowledge of organic and natural products chemistry. John's advice has been essential to some aspects of this research project and his comments on all aspects of the study have been valuable and are much appreciated.

Sincere thanks are extended to my parents for, in the words of my mother, "providing a good set of genes" but perhaps more importantly, for providing the perfect environment in which to grow and learn. I would also like to thank the rest of my family, particularly Carlo Pisanu, for being supportive and accepting my asocial behaviour over the last few years.

I am grateful to Dr. Bill Rudman, Mr Ian Loch and Dr. Winston Ponder from the Australian Museum, for assisting me with species identification and for generally improving my knowledge of marine molluscs. I have also appreciated the

opportunity to access the wonderful resources (specimens and literature) that are available in the Malacology Department of the Australian Museum.

I am grateful to Dr. John Korth from the University of Wollongong (UOW, Chemistry Department) for assisting me on the GC/MS. Thanks also to Dr. Renate Griffiths from the Department of Chemistry (UOW) for performing the conformational search on Tyriverdin in the SPARTAN modelling program. Darren Saunders (Department of Biological Sciences, UOW) kindly cultured the human lymphoma cells that were used in this project and explained the methods used for cytotoxicity testing. My appreciation is extended to Mr. George Gray and Dr. Alistair Lochhead from Southern Pathology, Wollongong, NSW, for the use of the cytopsin and light microscope/camera equipment. Dr. David Muir from the Royal North Shore Hospital kindly provided two strains of *Candida albicans* and Dr. Jeremy Carson from the Fish Health Unit, Department of Primary Industry and Fisheries, Tasmania, provided the strains of marine pathogens.

I would like to further thank Dr. Andy Davis, for providing the egg masses of a subtidal nudibranch, a terrestrial snail and several Mediterranean molluscs. The assistance of Dr. Manuel Ballesteros (University of Barcelona, Spain) in the collection of the egg masses from *Spurilla neopolitana* and *Ceratostoma erineceum* is also appreciated.

The Australian specimens collected for this study were authorised under a general scientific permit for the collection of marine invertebrates outside

reserve areas in the waters of N.S.W (reference no. F95/269). This license was authorised in accordance with the provisions of Section 37 of the Fisheries Management Act 1994.

The provision of an Australian Postgraduate Award from the Australian Government, as well as a Supplementary Postgraduate Scholarship from the Australian Flora and Fauna Research Centre (AFFRC), the Bioactive Molecules Research Centre and the Environmental Research Institute at the University of Wollongong is greatly appreciated. I would also like to thank the members of the AFFRC and John Bremner's research group for interesting discussions.

My most sincere thanks to all

Kirsten Benkendorff, 1999

## Abstract

Chemical prospecting for pharmaceuticals in natural organisms (bioprospecting) can be used as a tool for the conservation of biological diversity. However, bioprospecting can only be considered compatible with conservation if it is conducted in an environmentally sustainable manner. In order to prevent the overcollection of vulnerable organisms it is essential to gain an understanding of the local distribution and abundance of the target organisms. In this study, the egg masses of intertidal molluscs were targeted as a novel source of biologically active compounds. Surveys of the molluscan fauna were conducted on 13 intertidal reefs along the Wollongong Coast, New South Wales, Australia. In total, 161 species of intertidal molluscs were found and the benthic egg masses from 47 species were identified. Only 31% of these molluscs have been previously recorded from intertidal surveys in the region and 66% of the species may be regarded as regionally rare. Repeated surveys of the 13 reefs revealed that the species diversity recorded in a single inventory was representative of the cumulative diversity detected. 'Hotspots' of molluscan diversity were found on the northern side of two large headlands (Bass Point and Bellambi Point), which are characterised by a high habitat complexity and shelter from strong wave action.

Three selective pressures could potentially lead to the evolution of chemical defence in molluscan egg masses: predation, disease and surface fouling. Marine molluscs may rely on a range of alternative strategies to protect their egg masses from predators, including physical protection in leathery egg capsules, camouflage and rapid embryonic development, as well as behavioural mechanisms, such as brooding and the deposition of large aggregated egg masses. Predator feeding trials provided evidence of chemical defence in five out of eight species that were tested. On the other hand, observational studies provided no evidence to suggest that molluscan egg

masses are chemically defended against surface fouling by macroorganisms. A range of macrophytes and epizooites were observed on the surface of both gelatinous egg masses and leathery egg capsules. Nevertheless, the overall incidence of fouling was low, probably because of their ephemeral nature and the fact that most molluscs deposit egg masses on the underside of boulders.

Disease appears to be a significant selective pressure leading to the evolution of chemical defence in molluscan egg masses. Two assays were used to screen the egg masses of marine molluscs for antimicrobial activity against human and marine pathogens; a modified version of the traditional Zone of Inhibition assay and the Fluorescein Diacetate assay. These two assays have small sample requirements and thus it was possible to screen the egg masses of 42 molluscs and four polychaetes. Antimicrobial activity against at least one human pathogen was found in the egg masses of 36 species, including two polychaetes and a wide range of molluscs. The egg masses from a number of species clearly lose activity during embryonic development. The antimicrobial activity also appears to be greater in the internal matrix, rather than on the outer surfaces of molluscan egg masses. Surface bacteria could be responsible for the observed activity in some species but are unlikely to be the source of antimicrobial agents in leathery egg capsules, or the gelatinous egg ribbons of *Aplysia* spp. The egg masses of *Dicathais orbita* and *Aplysia juliana* were found to inhibit ecologically significant marine bacteria, as well as Gram negative and Gram positive human pathogenic bacteria and the yeast *Candida albicans*.

The compounds responsible for the antimicrobial activity in the egg masses of the common muricid *Dicathais orbita* were isolated using bioassay-guided fractionation. These were then identified by mass spectrometry and proton nuclear magnetic

resonance spectroscopy. Three antimicrobial compounds were characterised (tyrindoleninone, tyriverdin and 6-bromoisatin) and these are all known precursors to the ancient dye Tyrian Purple. Tyrindoleninone is the most abundant volatile organic compound found in the fresh eggs and this compound was shown to be toxic to bacteria at a concentration of 1mg/ml. As the eggs develop, most of the tyrindoleninone is converted into tyriverdin, which was found to be effectively bacteriostatic at 0.5 µg/ml but was not cytotoxic at 1 mg/ml. This compound is considered to be a useful new drug lead. The 6-bromoisatin, which is likely to be an oxidative artefact derived from the other precursors, exhibited mild cytolytic activity against a range of bacteria. As the larvae began hatching, most of the tyriverdin was converted into Tyrian Purple in the egg masses. Tyrian Purple did not exhibit any significant antimicrobial activity, although it is highly insoluble in aqueous media. Nevertheless, these studies provide evidence for a chemical ripening process in the egg masses of *Dicathais orbita*, which may provide a means of avoiding autotoxicity to the larvae during hatching.

Extracts from the egg masses of 23 molluscs were then examined for the precursors of Tyrian Purple, as well as other potential antimicrobial agents, using gas chromatography/ mass spectrometry. The egg masses from six species of Muricidae were found to contain the precursors of Tyrian Purple. However, these compounds were not found in the egg masses of species from any other family. A range of other related indoles, as well as di- and tribromoimidazoles/pyrazoles were also found in the egg masses of the Muricidae. Most of these compounds have not been previously described from a natural source and they could all contribute to the observed antimicrobial activity in the muricid egg masses. The egg masses of the Aplysiidae were found to contain some bioactive polychlorinated hydrocarbons and a range of

long chain unsaturated fatty acids. Halogenated compounds were not found in the egg masses of any other species, although fatty acids could be partly responsible for the observed antimicrobial activity in most of the gelatinous egg masses. A high diversity of volatile organic compounds was found in the molluscan egg masses, but further work is required to identify the active components.

Clearly, bioprospecting can contribute to conservation through the development of comprehensive species inventories. Bioprospecting can be conducted with minimal impact on the environment and the discovery of novel bioactive compounds provides an incentive for conservation. All marine molluscs that deposit benthic egg masses have potential pharmaceutical value and therefore an effort should be made to conserve both them and their natural habitats. Bass Point would be an appropriate site for an intertidal protected area in the Wollongong region.

# Table of Contents

<b>Acknowledgments</b>	<b>V</b>
<b>Abstract</b>	<b>VIII</b>
<b>CHAPTER 1</b>	<b>1</b>
<b>MOLLUSCAN RESOURCES AND CONSERVATION</b>	
<b>1.1 General introduction</b>	<b>1</b>
<b>1.2 Molluscan resources</b>	<b>2</b>
1.2.1 The diversity of resources.	2
1.2.2 Bioactive molluscan resources.	3
<b>1.3 The biorational approach to drug discovery</b>	<b>7</b>
1.3.1 The biorational approach.	7
1.3.2 Egg masses as targets for drug discovery.	9
1.3.3 Molluscan reproductive strategies.	10
<b>1.4 Bioprospecting</b>	<b>14</b>
1.4.1 Conservation and bioprospecting.	14
1.4.2 The ethics of bioprospecting.	15
1.4.3 Is bioprospecting environmentally sustainable?	16
1.4.4 Requirements for sustainable bioprospecting.	17
<b>1.5 Molluscan conservation</b>	<b>20</b>
1.5.1 The conservation status of marine molluscs.	20
1.5.2 Habitat protection.	21
<b>1.6 Structure of this study</b>	<b>22</b>
<b>CHAPTER 2</b>	<b>25</b>
<b>MOLLUSCAN DIVERSITY: SPECIES INVENTORIES AND HABITAT ASSESSMENT</b>	
<b>2.1 Introduction</b>	<b>25</b>
2.1.1 Species diversity, species rarity and conservation implications.	25

2.1.2	Intertidal reefs and the determinants of species diversity.	28
2.1.3	Species inventories and rapid biodiversity assessment.	31
2.1.4	Molluscs of the Illawarra Coast.	33
<b>2.2</b>	<b>Objectives</b>	38
<b>2.3</b>	<b>Methods</b>	38
2.3.1	Study sites.	38
2.3.2	Surveys.	43
2.3.3	Habitat quality.	47
2.3.4	Statistical analysis.	50
<b>2.4</b>	<b>Results</b>	52
2.4.1	Species list of intertidal molluscs in the Wollongong region.	52
2.4.2	Comparisons to previous surveys.	59
2.4.3	Natural reefs vs. artificial reefs.	61
2.4.4	Egg laying habitats.	61
2.4.5	Spatial and temporal variation in molluscan diversity.	64
2.4.6	Molluscan diversity and the physical environment.	68
<b>2.5</b>	<b>Discussion</b>	72
2.5.1	Molluscan diversity and distribution in the Wollongong region.	72
2.5.2	Species rarity and implications for resource management .	73
2.5.3	Molluscan breeding habitats.	78
2.5.4	Molluscan diversity and the physical environment.	80
2.5.5	Methods for assessing intertidal molluscan diversity.	82
2.5.6	Intertidal management recommendations.	89
<b>2.6</b>	<b>Conclusion</b>	90
<b>CHAPTER 3</b>		<b>93</b>
<b>DEFENCE AGAINST PREDATION AND MACRO FOULING ON MOLLUSCAN EGG MASSES</b>		
<b>3.1</b>	<b>Introduction</b>	<b>93</b>

3.1.1	Marine predators and predation on molluscan egg masses.	93
3.1.2	Adaptations to predation: Behavioural and physiological defence.	95
3.1.3	Physical defence against predation.	96
3.1.4	Chemical defence mechanisms.	97
3.1.5	Predator feeding trials for assessing the role of chemical defence.	98
3.1.6	Macro fouling.	101
<b>3.2</b>	<b>Objectives</b>	104
<b>3.3</b>	<b>Methods</b>	104
3.3.1	Field and laboratory observations.	104
3.3.2	Collection and maintenance of predators.	105
3.3.3	Collection and preparation of egg material.	107
3.3.4	Preparation of artificial feeding disks.	107
3.3.5	Crab feeding experiments.	109
3.3.6	Isopod feeding experiments.	110
3.3.7	Starfish feeding trials	111
3.3.8	Field feeding trials	112
3.3.9	Statistical analysis	113
<b>3.4</b>	<b>Results</b>	115
3.4.1	Defensive strategies against predation.	115
3.4.2	Chemical defence: Crab feeding trials.	119
3.4.3	Chemical defence: Isopod feeding trials.	120
3.4.4	Chemical defence: Field feeding trials.	124
3.4.5	Starfish feeding trial.	125
3.4.6	Physical defence.	127
3.4.7	Macrofouling.	128
<b>3.5</b>	<b>Discussion</b>	131
3.5.1	Defensive strategies against predation.	131
3.5.2	Chemical defence and artificial feeding trials.	134
3.5.3	Physical defence against predation.	139
3.5.4	Macrofouling on molluscan egg masses.	140
<b>3.6</b>	<b>Conclusion</b>	

<b>CHAPTER 4</b>	143
<b>ANTIMICROBIAL ACTIVITY IN MOLLUSCAN EGG MASSES</b>	
<b>4.1 Introduction</b>	143
4.1.1 The need for novel antibiotics.	143
4.1.2 Antimicrobial activity in molluscs and molluscan egg masses.	144
4.1.3 Assays for detecting antimicrobial activity.	147
4.1.4 Microfouling and symbiosis.	155
<b>4.2 Objectives</b>	157
<b>4.3 Methods</b>	158
4.3.1 Collection and preparation of egg material.	158
4.3.2 Maintenance and preparation of microbial cultures.	162
4.3.3 Zone of Inhibition assay.	164
4.3.4 The Fluorescein Diacetate (FDA) assay.	165
4.3.5 Antimicrobial (cell lysis/ cell stasis) assay.	167
4.3.6 Microfouling and symbiosis.	168
<b>4.4 Results</b>	168
4.4.1 Antimicrobial activity in benthic invertebrate egg masses.	168
4.4.2 Antimicrobial properties at different stages of development.	169
4.4.3 Zone of Inhibition assay.	172
4.4.4 Fluorescein Diacetate assay.	178
4.4.6 Antimicrobial (cell stasis/lysis) activity.	186
4.4.7 Epibiosis and microfouling.	191
<b>4.5 Discussion</b>	192
4.5.1 Antibiotics in invertebrate egg masses.	192
4.5.2 The resource potential of molluscan antibiotics.	195
4.5.3 Developmental changes in antimicrobial properties.	197
4.5.4 Localisation of the antimicrobial components and autotoxicity.	199
4.5.5 Properties of the antimicrobial components.	202
4.5.6 Screening methods for detecting antimicrobial activity.	204
4.5.7 Epibiosis and symbiosis.	208

<b>4.6</b>	<b>Conclusion</b>	210
<b>CHAPTER 5</b>		213
<b>ISOLATION AND CHARACTERISATION OF THE ANTIMICROBIAL COMPOUNDS FROM THE EGG MASS OF <i>DICATHAIS ORBITA</i></b>		
<b>5.1</b>	<b>Introduction</b>	213
<b>5.2</b>	<b>Objectives</b>	219
<b>5.3</b>	<b>Methods</b>	219
5.3.1	Collection and extraction of egg masses.	219
5.3.2	Analysis of the crude extract.	220
5.3.3	Isolation and identification of antimicrobial components.	221
5.3.4	Antimicrobial testing.	222
5.3.5	Cytotoxicity testing.	223
<b>5.4</b>	<b>Results</b>	224
5.4.1	Analysis of the crude egg extracts.	224
5.4.2	Antimicrobial activity.	229
5.4.3	Cytotoxicity of tyriverdin.	231
<b>5.5</b>	<b>Discussion</b>	238
5.5.1	Antimicrobial compounds from the egg mass of <i>Dicathais orbita</i> .	238
5.5.2	Tyriverdin – a novel drug lead.	241
<b>5.6</b>	<b>Conclusion</b>	248
<b>CHAPTER 6</b>		251
<b>VOLATILE ORGANIC COMPOUNDS IN MOLLUSCAN EGG MASSES</b>		
<b>6.1</b>	<b>Introduction</b>	251
6.1.1	Indole derivatives from muricids.	253
6.1.2	Other bioactive halogenated compounds from molluscs.	255
6.1.3	Nonhalogenated bioactive compounds from molluscs.	259
6.1.4	Metabolites from molluscan egg masses.	260
6.1.5	Chemical diversity and dereplication.	264
<b>6.2</b>	<b>Objectives</b>	

<b>6.3</b>	<b>Methods</b>	266
6.3.1	Specimen collection and sample preparation.	266
6.3.2	Derivatisation procedure.	267
6.3.3	Gas chromatography/mass spectrometry analyses.	267
6.3.4	Antimicrobial activity of identified egg constituents.	268
<b>6.4</b>	<b>Results</b>	270
6.4.1	Brominated compounds in muricid egg masses.	270
6.4.2	Other related compounds from muricid egg masses.	277
6.4.3	Halogenated compounds from Aplysiidae egg masses.	279
6.4.4	Fatty acids and methyl esters.	284
6.4.5	Other volatile components of molluscan egg masses.	286
6.4.6	Antimicrobial activity of some common egg metabolites.	294
<b>6.5</b>	<b>Discussion</b>	296
6.5.1	Bioactive compounds from muricid egg masses.	296
6.5.2	Bioactive metabolites in Aplysiidae egg masses.	301
6.5.3	Bioactive metabolites from other molluscan egg masses.	304
<b>6.6</b>	<b>Conclusion</b>	307
<b>CHAPTER 7</b>	<b>GENERAL DISCUSSION</b>	<b>309</b>
	<b>BIOACTIVE MOLLUSCAN RESOURCES AND CONSERVATION</b>	
7.1	Biodiversity and bioresources	309
7.2	The biorational approach to drug discovery	312
7.3	An interdisciplinary approach	314
7.4	Sustainable bioprospecting	317
7.5	Bioprospecting and conservation	319
7.6	Management recommendations	321
7.7	Conclusion	323
<b>REFERENCES</b>		<b>325</b>
<b>CHAPTER 1: APPENDIX 1</b>		<b>383</b>

Appendix 1.1	383
Appendix 1.2	390
<b>CHAPTER 2: APPENDIX 2</b>	<b>397</b>
Appendix 2.1	398
Appendix 2.2	431
Appendix 2.3	441
<b>CHAPTER 4: Appendix 4</b>	<b>445</b>
Appendix 4.1	445
Appendix 4.2	446
Appendix 4.3	448
<b>CHAPTER 6: Appendix 6</b>	<b>451</b>
Appendix 6.1	452
Appendix 6.2	515
Appendix 6.3	526
Appendix 6.4	527
Appendix 6.5	531
Appendix 6.6	534
Appendix 6.7	551
Appendix 6.8	553