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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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BIOACTIVE MOLLUSCAN RESOURCES

AND THEIR CONSERVATION:

Biological and Chemical Studies on the Egg Masses of Marine Molluscs

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by

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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

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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.

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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.

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