Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.
The physiological stress response to amputation in the eleven-armed sea star (Coscinasterias muricata)

A thesis presented in partial fulfilment of the requirements for the degree of

Masters of Science in Zoology
Massey University
Palmerston North, New Zealand

Anne Bailey Kim

2016
Chapter 4

– Initial steps made in the development of an in vitro cytotoxicity bioassay using coelomocytes harvested from sea stars
ABSTRACT

The eleven-armed sea star, *Coscinasterias muricata*, is subject to human-induced stressors, such as invasive fishing activities, that can cause limb loss (amputation), and heavy metal discharge into their habitat. The well-being and survival of a keystone predator such as *C. muricata* has important ecological implications as their presence influences community structure in the marine environment.

Understanding the stress physiology of an animal can provide insight into their overall health and survival. While the stress physiology of northern asteroid species (such as *Asterias rubens*) has been well documented, this has not been well studied with *C. muricata*. In this thesis, I was able to identify time-dependent changes in two physiological parameters (total coelomocyte count and dopamine levels in the coelomic fluid) in *C. muricata* subjected to amputation. There was a synchronous increase in both of these parameters 24 hours post amputation.

Dopamine in the coelomic fluid was measured by using high performance liquid chromatography (HPLC). I adapted a pre-existing method involving pre-column derivatisation and fluorescent detection, which was initially developed for the detection of dopamine in porcine muscle. However, this method requires further development as it could not detect dopamine to the same sensitivity as previously reported HPLC methods using electrochemical detection.

Lastly, the initial attempts at developing an *in vitro* cytotoxicity bioassay using *C. muricata* coelomocytes is described in detail. The initial aim of this experiment was to understand the effect of heavy metals on cellular parameters. However the experiment was hampered by unusually low cell counts in this species, which has not been previously reported. The knowledge gleaned from this study may provide the groundwork for future studies that use *C. muricata* coelomocytes for cytotoxicity testing or as a biomarker.
Initial steps made in the development of an in vitro cytotoxicity bioassay using coelomocytes harvested from sea stars.
ACKNOWLEDGEMENTS

First of all, I’d like to thank my two supervisors, Professor Murray Potter and Dr. Wei-Hang Chua, for the academic and emotional support I have received through the good, bad and out-right atrocious times. I’m extremely grateful for everything you have taught me, and all the opportunities you have provided me. I hope I can make both of you proud for the scientist you have raised me to be. I must also thank my third supervisor Dr. Preet Singh, for all the tremendous help I have received with the HPLC. I am also greatly indebted to Antony Jacob, for his time and help with the HPLC. I’m grateful to my emergency supervisor, Corrin Hulls for his colossal contribution to sampling and lab work. And last but not least, I’d like to thank Assoc. Prof. Kevin Pedley for all his kind words of encouragement and wisdom during times of devastation.

I owe a huge thanks to the technicians; Paul Barrett, Cleland Wallace, Tracy Harris and Shaun Nielsen for helping me with all my endeavours in the lab and in the field. I’d also like to thank Anne Broomfield who kindly lent me chilly bins for housing my starfish. This thesis would not have been written without the help of everyone, thank you all once again.

I’m grateful to my parents who made a lot of sacrifices for me to be here. Thank you for trusting me with all your hard-earned money and allowing me to stay sane with regular retail therapy. I’d like to thank my sister Katy for all the constructive criticism, laughter and support she has given me throughout my life. You have been my dad, mum, sister, best-friend and teacher when I had so little. A big thank you to Felix, the most kind-hearted and understanding person I will ever meet. I am extremely fortunate to have had you in my life. I am forever in your debt.

I would like to express my unconditional love towards my Labrador siblings, Chubb and Bailey. Thank you for being the ray of sunshine when I had lost everything, and showing me unconditional love like I have never seen before. I would also like to thank my beloved feline baby Ruha for all the trust and affection she has shown me. Thank you for choosing me, my life would have been incomplete without you.

This research was funded by the Massey University Masterate Scholarship, the Graduate Women Manawatu Postgraduate Scholarship, and the J P Skipworth Ecology Scholarship, for which I am extremely grateful for. I would like to express my sincere gratitude to all the animals that were sacrificed for this research.
Chapter 4 – Initial steps made in the development of an in vitro cytotoxicity bioassay using coelomocytes harvested from sea stars
# TABLE OF CONTENTS

Abstract .................................................................................................................................................. 3

Chapter 1 – General introduction ........................................................................................................ 11

1.1 Stressors ........................................................................................................................................... 12

1.1.1 Amputation ................................................................................................................................. 13

1.1.2 Heavy metals ............................................................................................................................. 14

1.1.2.1 Cadmium ................................................................................................................................. 15

1.1.2.2 Zinc ........................................................................................................................................ 16

1.2 Biology of C. muricata .................................................................................................................. 16

1.2.1 Coelomic fluid ............................................................................................................................ 18

1.2.2 Coelomocytes ............................................................................................................................ 19

1.3 Objectives and outlines .................................................................................................................. 20

1.3.1 Chapter outline ......................................................................................................................... 21

1.4 References ..................................................................................................................................... 23

Chapter 2 – Changes in total coelomocyte count in response to amputation .................................... 29

2.1 Abstract .......................................................................................................................................... 30

2.2 Introduction .................................................................................................................................... 30

2.3 Methods and materials .................................................................................................................. 32

2.3.1 Collection and field sampling ................................................................................................... 34

2.3.2 Transport and housing ............................................................................................................. 33

2.3.3 Experimental design ................................................................................................................ 33

2.3.4 Sample treatment ..................................................................................................................... 34

2.3.5 Total coelomocyte count .......................................................................................................... 34

2.3.6 Statistical analysis ................................................................................................................... 34

2.4 Results ........................................................................................................................................... 34

2.5 Discussion ..................................................................................................................................... 36

2.5.1 Differential coelomocyte count ............................................................................................... 39
Chapter 3 – Quantification of dopamine in coelomic fluid of sea stars using high-performance liquid chromatography (HPLC)

3.1 Abstract ..................................................................................................................48
3.2 Introduction ..............................................................................................................48
3.3 Methods and materials ............................................................................................51
  3.3.1 HPLC system configuration ..............................................................................51
  3.3.2 Reagents ............................................................................................................52
  3.3.3 Sample treatment ..............................................................................................52
  3.3.4 Method validation ..............................................................................................52
  3.3.5 Statistical analysis ..............................................................................................53
3.4 Results .......................................................................................................................54
  3.4.1 HPLC method development ..............................................................................54
    3.4.1.1 Intra- and inter- day variation ......................................................................55
    3.4.1.2 Recovery rate ..............................................................................................56
  3.4.2 Dopamine levels following amputation ..............................................................56
3.5 Discussion ..................................................................................................................57
3.6 References ................................................................................................................61

Chapter 4 – Initial steps made in the development of an in vitro cytotoxicity bioassay using coelomocytes harvested from sea stars

4.1 Abstract .....................................................................................................................64
4.2 Introduction ...............................................................................................................64
  4.2.1 Cellular heavy metal tolerance ..........................................................................66
  4.2.2 In vitro cytotoxicity testing ................................................................................67
    4.2.2.1 Cellular metabolic activity ............................................................................69
    4.2.2.2 Cellular membrane integrity .......................................................................70
    4.2.2.3 Phagocytosis activity ..................................................................................70
4.2.3 Chapter aims ........................................................................................................71
4.3 Method development.............................................................................................71
  4.3.1 First trial .............................................................................................................71
    4.3.1.1 Protein determination ....................................................................................73
  4.3.2 Second trial .........................................................................................................74
    4.3.2.1 Heavy metal exposure ......................................................................................75
    4.3.2.2 Cellular metabolic activity ..............................................................................75
    4.3.2.3 Cellular membrane integrity ...........................................................................76
    4.3.2.4 Phagocytosis activity ......................................................................................76
      4.3.2.4.1 Staining of zymosan particles ...................................................................76
      4.3.2.4.2 Quantification of zymosan uptake ..............................................................77
    4.3.2.5 Protein determination .....................................................................................77
4.4 Discussion ................................................................................................................77
  4.4.1 Heavy metal exposure on coelomocyte physiology ...........................................79
  4.4.2 Amputation and heavy metal challenge ...............................................................80
4.5 Conclusion .................................................................................................................81
4.6 Reference ..................................................................................................................82

Chapter 5 – General discussion ..................................................................................87
  5.1 Synthesis and future directions for assessing the stress response of amputated sea stars .................................................................................................................88
  5.2 Synthesis and future directions for in vitro experiments with sea star coelomocytes .........................................................................................................................90
5.3 References ...............................................................................................................93