This thesis is dedicated to Kimberly
for years of love and support.
Acknowledgments

Thank you to my graduate advisor, Stephen A. Smith, for the opportunity to work in the Aquatic Medicine Laboratory. I also appreciate the work of my committee, Nammalwar Sriranganathan and Gerhardt G. Schurig, and the help from those who were not on my committee, Robert M. Gogal, Jr., Theresa C. Hrubec, Ramesh Vemulapalli, Daniel L. Ward, and Jeffrey Wolf.

Special thanks to the graduate students in the Aquatic Medicine Laboratory: Kathleen H. Hartman and Brent C. Bowden. I would also like to thank the staff of the VMRCVM, especially Mary E. Mainous, Joan Kalnitsky, and Sandy S. Brown.

This work was supported by Animal Health and Disease grant #39095.

And finally, I would like to extend special appreciation to Judy, Michael, and Eleonore for their unwavering support.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>v</td>
</tr>
<tr>
<td>TABLE OF CONTENTS</td>
<td>vi</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>ix</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>x</td>
</tr>
<tr>
<td>CHAPTER 1 INTRODUCTION</td>
<td></td>
</tr>
<tr>
<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td><em>Mycobacterium marinum</em></td>
<td>2</td>
</tr>
<tr>
<td>Vaccination and Immunity</td>
<td>7</td>
</tr>
<tr>
<td>Piscine Animal Model</td>
<td>12</td>
</tr>
<tr>
<td>Specific Research Objectives</td>
<td>15</td>
</tr>
<tr>
<td>References</td>
<td>16</td>
</tr>
</tbody>
</table>

| CHAPTER 2 EFFICACY OF A RECOMBINANT VACCINE EXPRESSING A MAMMALIAN MYCOBACTERIUM SP. ANTIGEN AGAINST ACUTE MYCOBACTERIOSIS IN FISH | 29 |
| Abstract                                                               | 30   |
| Introduction                                                           | 30   |
| Materials and methods                                                 | 32   |
| Results                                                                | 37   |
| Discussion                                                             | 38   |
| Objectives Met                                                         | 41   |
| Acknowledgments                                                        | 41   |
| References                                                             | 41   |
| Figures                                                                | 47   |
CHAPTER 3 IMMUNOGENIC AND PROTECTIVE EFFECTS OF A DNA VACCINE FOR *MYCOBACTERIUM MARINUM* ..................51

Abstract .................................................................52
Rationale .................................................................52
Introduction ............................................................52
Materials and methods ...............................................54
Results .................................................................62
Discussion ............................................................64
Objectives Met ........................................................68
Acknowledgments ......................................................68
References ............................................................68
Tables ....................................................................73
Figures ................................................................74

CHAPTER 4 PROTECTION OF HYBRID STRIPED BASS AGAINST *MYCOBACTERIUM MARINUM* AFTER DNA VACCINATION .............82

Abstract .................................................................83
Rationale .................................................................83
Introduction ............................................................83
Materials and methods ...............................................84
Results .................................................................90
Discussion ............................................................93
Objectives Met ........................................................96
Acknowledgments ......................................................96
References ............................................................96
Tables ....................................................................101
Figures ................................................................104
CHAPTER 5 APPENDICES

Raw data tables.................................................................109

CURRICULUM VITAE.............................................................179
LIST OF TABLES

CHAPTER 3
Table 1. Comparisons of mean survival time, splenic bacterial counts, and relative percent survival following live bacterial challenge of DNA vaccinated hybrid striped bass.................................73

CHAPTER 4
Table 1. Comparisons of mean survival, percent survival, and splenic bacterial counts on Day 14 post-challenge following live bacterial challenge of DNA vaccinated hybrid striped bass.........................101

Table 2. Comparisons of mean survival, percent survival, and splenic bacterial counts on Day 28 post-challenge following live bacterial challenge of DNA vaccinated hybrid striped bass.........................102

Table 3. Comparison of mean granuloma counts following live bacterial challenge of DNA vaccinated hybrid striped bass.................103
LIST OF FIGURES

CHAPTER 2

Fig. 1. Percent identity of M. marinum Ag85A amino acid sequence (248/295 = 84%) to that of M. bovis and M. tuberculosis…………………47

Fig. 2. ELISA detection of Ag85A-specific antibodies in serum of vaccinated striped bass…………………………………………………..48

Fig. 3. Proliferation of anterior kidney lymphocytes from vaccinated striped bass after in vitro stimulation with saline, Ag85A, or ConA……49

Fig. 4. Cumulative mortality curve for vaccinated striped bass following challenge on Day 70 post-vaccination with live M. marinum…50

CHAPTER 3

Fig. 1. The 1,100 bp M. marinum fbpA PCR product containing the entire open reading frame, ribosomal binding site, and stop codon was cloned into the pcDNA-3.1 eukaryotic expression vector to generate the pCMV-85A construct………………………………………..74

Fig. 2. Nucleotide sequence of Ag85A from M. marinum
(ATCC no. 927)………………………………………………………………….75

Fig. 3. ELISA detection of Ag85A-specific antibodies from serum of vaccinated hybrid striped bass……………………………………….76

Fig. 4. Proliferation of anterior kidney lymphocytes from vaccinated hybrid striped bass after in vitro stimulation with ConA………………….77
Fig. 5. Proliferation of anterior kidney lymphocytes from vaccinated hybrid striped bass after in vitro stimulation with Ag85A………………..78

Fig. 6. Phagocytic uptake by macrophages from vaccinated hybrid striped bass………………………………………………………………...79

Fig. 7. Macrophage respiratory burst from vaccinated hybrid striped bass………………………………………………………………..80

Fig. 8. Cumulative mortality for vaccinated hybrid striped bass following challenge on Day 90 post-vaccination with live M. marinum……………………………………………………………….81

CHAPTER 4

Fig. 1. Cumulative mortality for vaccinated hybrid striped bass following challenge with live M. marinum on Day 120 post-vaccination……………………………………………………………….……..104

Fig. 2. Histopathology of typical lesions found in hybrid striped bass challenged with live M. marinum……………………………………………………105

Fig. 3. Detection of Ag85A-specific antibodies by ELISA from serum of vaccinated and challenged hybrid striped bass…………………106

Fig. 4. Proliferation of anterior kidney lymphocytes from vaccinated and challenged hybrid striped bass after in vitro stimulation with ConA……………………………………………………107

Fig. 5. Proliferation of anterior kidney lymphocytes from vaccinated hybrid striped bass after in vitro stimulation with Ag85A……………………………………………………………………...108