

## TABLE OF CONTENTS

<b>Abstract.....</b>	<b>i</b>
<b>Acknowledgement.....</b>	<b>iv</b>
<b>Table of Contents.....</b>	<b>v</b>
<b>List of Tables.....</b>	<b>ix</b>
<b>List of Figures.....</b>	<b>xi</b>
<b>Chapter 1.....</b>	<b>1</b>
<b>1.0 Introduction.....</b>	<b>1</b>
<b>Chapter 2.....</b>	<b>5</b>
<b>2.0 Literature review.....</b>	<b>5</b>
2.1 Importance of shrimp farming.....	5
2.2 Pathogenic bacteria of farm shrimp.....	6
2.2.1 <i>Vibrio species</i> .....	6
2.2.1.1 <i>Vibrio cholerae</i> .....	8
2.2.1.2 <i>Vibrio parahaemolyticus</i> .....	10
2.2.2 <i>Salmonella</i> .....	10
2.2.3 Coliforms.....	12
2.2.4 <i>Staphylococcus aureus</i> .....	15
2.3 Microflora of frozen shrimp.....	16
2.4 Biochemical identification.....	16
2.4.1 <i>Escherichia coli</i> .....	16
2.4.2 <i>Staphylococcus aureus</i> .....	18

2.5.1 Serological identification .....	19
2.5.1.1 <i>Salmonella</i> .....	19
<b>Chapter 3 .....</b>	<b>20</b>
<b>3.0 Materials and methods .....</b>	<b>20</b>
3.1 Materials .....	20
3.1.1 Sampling procedure .....	20
3.1.1.1 Water source .....	20
3.1.1.2 Shrimp, pond water, pond sediment and shrimp feed.....	21
3.1.1.3 Raw frozen shrimp.....	21
3.2 Methods.....	22
3.2.1 Sterilization of glassware .....	22
3.2.2 Plating technique.....	22
3.2.2.1 Shrimps .....	22
3.2.3 Enumeration of bacteria .....	22
3.2.3.1 Aerobic plate count .....	22
3.2.3.2 Total coliforms .....	23
3.2.3.3 Faecal coliforms.....	23
3.2.3.4 <i>Escherichia coli</i> .....	23
3.2.3.5 Isolation, identification and confirmation of <i>Salmonella</i> .....	24
3.2.3.5.1 Isolation.....	24
3.2.3.5.2 Identification of <i>Salmonella</i> .....	25
3.2.3.5.3 Biochemical confirmation of <i>Salmonella</i> .....	25

3.2.3.5.4 Serological identification .....	25
3.2.3.6 Isolation of <i>Vibrio parahaemolyticus</i> .....	25
3.2.3.7 <i>Vibrio cholerae</i> .....	27
3.2.3.8 <i>Staphylococcus aureus</i> .....	29
3.2.4 API Tests.....	29
3.2.5 Preparation of inoculum.....	30
3.2.6 Influence of salinity and pH for growth of isolated pathogens.....	30
3.2.6.1 <i>Vibrio parahaemolyticus</i> .....	30
3.2.6.2 <i>Salmonella</i> .....	31
3.2.6.3 <i>Escherichia coli</i> .....	31
3.2.7 Combined effect of salinity and pH on the growth of pathogens .....	31
3.2.7.1 <i>Vibrio parahaemolyticus</i> .....	32
3.2.7.2 <i>Salmonella</i> .....	32
3.2.7.3 <i>Escherichia coli</i> .....	32
3.2.8 Physical parameters .....	32
3.2.8.1 Suspended solids.....	32
3.2.8.2 Salinity .....	33
3.2.8.3 pH.....	33
<b>Chapter 4</b> .....	<b>34</b>
<b>4.0 Results and discussion</b> .....	<b>34</b>
4.1 Analysis of samples from water sources .....	34
4.1.1 API profiling .....	42

4.2 Analysis of samples from shrimp farms .....	43
4.3 Bacteriological analysis of frozen shrimp.....	47
4.4 Growth of isolated pathogen under varying condition of pH & salinity .....	53
<b>Chapter 5.....</b>	<b>57</b>
<b>5.0 Conclusion.....</b>	<b>57</b>
<b>References .....</b>	<b>58</b>

## LIST OF TABLES

Table 1: Human pathogenic bacteria in aquaculture .....	7
Table 2: Microbial characteristics of <i>Salmonella</i> contaminated samples .....	13
Table 3: Total bacterial count and incidence of <i>E.coli</i> in <i>Salmonella</i> positive samples of fishery products .....	14
Table 4: Faecal coliform most probable numbers (MPN) for samples obtain from a major shrimp production area in Southern Thailand .....	15
Table 5: The occurrence of <i>Vibrio parahaemolyticus</i> in prawn during processing....	17
Table 6: Summary of bacteriological results of the individually quick frozen raw and cooked ready to eat shrimp analyzed during the period Jan. 1994 -Dec. 1995 ... ..	18
Table 7: Biochemical reaction of <i>Salmonella</i> .....	26
Table 8: Biochemical identification of <i>Vibrio parahaemolyticus</i> .....	28
Table 9: Biochemical reactions of <i>Vibrio cholerae</i> .....	30
Table 10: Indicator organism and physico chemical factors associated with <i>S. arizonae</i> .....	42
Table 11: Results of API profiling of isolates of <i>Salmonella</i> and <i>Vibrio</i> <i>parahaemolyticus</i> .....	43
Table 12: Isolation of coliforms, Fecal coliforms and <i>E. coli</i> from farm shrimps, pond Water, pond sediment and shrimp feed .....	48

Table 13: Summary of aerobic plate counts, Coliforms, Fecal coliforms and *E.coli* of frozen shrimp ..... 50

Table 14: Microbiological limits for raw quick frozen shrimp ( Sri Lanka Standards) ..... 51

Table 15: Salinity levels and pH ranges permitting growth of pathogens .....53

## LIST OF FIGURES

Figure 1: Study area including sampling points in Dutch canal and Mundal lake ..	36
Figure 2: Total coliforms vs salinity of water sources .....	37
Figure 3: Total coliforms vs pH of water sources .....	37
Figure 4: Total coliforms vs suspended solid water sources .....	38
Figure 5: Fecal coliforms vs salinity of water sources .....	39
Figure 6: Fecal coliforms vs pH of water sources .....	39
Figure 7: Fecal coliforms vs suspended solids of water sources .....	40
Figure 8: <i>E.coli</i> vs salinity of water sources .....	40
Figure 9: <i>E.coli</i> vs pH water sources .....	41
Figure 10: <i>E.coli</i> vs suspended for water sources .....	41
Figure 11: Rainfall of sampling points during study periods .....	42
Figure 12: The combined effect of salinity and pH on the survival of <i>Salmonella</i> <i>arizona</i> .....	55
Figure 13: The combined effect of salinity and pH on the survival of <i>Vibrio parahaemolyticus</i> .....	55
Figure 14: The combined effect of salinity and pH on the survival of <i>Escherichia coli</i> .....	56