

FECAL CONTAMINATION IN CULTURED CYSTERS
(*Crassostrea irradialis*) AND SURROUNDING
WATERS OF SETIU LAGOON, TERENGGANU

KESAVEN BHUBALAN

FACULTY OF SCIENCE AND TECHNOLOGY
UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

2005

FECAL CONTAMINATION IN CULTURED OYSTERS (*Crassostrea iredalei*)
AND SURROUNDING WATERS OF SETIU LAGOON, TERENGGANU.

By

KESAVEN BHUBALAN

Research Report in partial fulfillment of
the requirements of the degree of Bachelor of
Science (Marine Biology)

Department of Marine Science

Faculty of Science and Technology

KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA

2005

1100034625

This project report should be cited as:

Kesaven, B. Fecal contamination in cultured oysters (*Crassostrea iredalei*) and surrounding waters of Setiu lagoon, Terengganu. Undergraduate thesis, Bachelor of Marine Biology, Faculty of Science and Technology, Kolej Sains dan Teknologi Malaysia, Terengganu. 122p.

No part of this project report may be reproduced by any mechanical, photographic, or electronic process, or in the form of phonographic recording, nor may it be stored in a retrieval system, transmitted, or otherwise copied for public or private use, without written permission from the author and the supervisor of the project.



**JABATAN SAINS SAMUDERA
 FAKULTI SAINS DAN TEKNOLOGI
 KOLEJ UNIVERSITI SAINS DAN TEKNOLOGI MALAYSIA**

**PENGAKUAN DAN PENGESAHAN LAPORAN
 PROJEK PENYELIDIKAN I DAN II**

Adalah ini diakui dan disahkan bahawa laporan penyelidikan bertajuk:

FECAL CONTAMINATION IN CULTURED OYSTERS (*Crassostrea*
iredalei) AND SURROUNDING WATERS OF SETIU LAGOON, TERENGGANU

oleh KESAVEN BHUBALAN....., No. Matrik UK 6490.....

telah diperiksa dan semua pembetulan yang disarankan telah dilakukan. Laporan ini dikemukakan kepada Jabatan Sains Samudera sebagai memenuhi sebahagian daripada keperluan memperoleh Ijazah SARJANA MUDA SAINS BIOLOGI MARIN....., Fakulti Sains dan Teknologi, Kolej Universiti Sains dan Teknologi Malaysia.

Disahkan oleh:

.....

Penyelia Utama
 Nama: **PROF. DR. LAW AH THEEM**
 Pensyarah
 Cop Rasmi: Jabatan Perikanan dan Sains Samudera
 Fakulti Sains dan Teknologi
 Kolej Universiti Sains dan Teknologi Malaysia
 21030 Mengabang Telipot
 Kuala Terengganu.

Tarikh: 31/3/05.....

.....
 Penyelia Kedua (jika ada)

Nama:
 Cop Rasmi:

Tarikh:

.....

Ketua Jabatan Sains Samudera
 Nama: **DR. AHMAD SHAMSUDDIN B. AHMAD**
 Cop Rasmi: Ketua
 Jabatan Sains Samudera
 Fakulti Sains dan Teknologi
 Kolej Universiti Sains dan Teknologi Malaysia
 21030 Kuala Terengganu

Tarikh: 31/3/05.....

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude and deepest appreciation to my supervisor Professor Dr. Law Ah Theem for his continuous support and guidance in completing this thesis. His valuable information, comments, criticisms and patience have guided me in completing my study successfully. I am also thankful to Dr. Siti Aishah Abdullah and Dr. Antonina for their guidance.

I am also indebted to Professor Dr. Law Ah Theem's post graduate students, Mr. Chin Kam Yee, Mr. Yong Jaw Chuen and Mr. John Chuah for their unlimited guidance and support throughout this study which has benefited me in countless ways. Special thanks also go to the Oceanography lab assistants Mr. Kamari, Mr. Sulaiman, Mr. Raja and Mr. Kamarun for their assistance and guidance.

I am also fortunate to have great friends like Mr. Karthigeyan, Mr. Kuhan Chandru, Mr. Satheesh Rao, Mr. Soma Sundram, Mr. Saravanan, Mr. Vickneswaran, Mr. Tanabalan, Mr. Arul Das and Ms. Sathiya Bharathi. I am thankful for their interest and enthusiasm in helping me all the way.

Finally, I am deeply grateful to my parents, Mr. Bhubalan and Mdm. Kalaiselvi for their boundless support and encouragement which kept me motivated.

KESAVEN BHUBALAN

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	i
TABLE OF CONTENT	ii
LIST OF TABLES	v
LIST OF FIGURES	vi
LIST OF ABBREVIATIONS	ix
LIST OF APPENDICES	xi
ABSTRACT	xiii
ABSTRAK	xiv
CHAPTER 1 INTRODUCTION	1
CHAPTER 2 LITERATURE REVIEW	4
2.1 Bacteria as Bio-indicators of Aquatic Pollution	4
2.2 Indicators of Fecal Contamination in Aquatic Environment	6
2.2.1 Total Coliforms	6
2.2.2 <i>Escherichia coli</i> : The Fecal Coliform	7
2.3 Water Quality Standards	9
2.3.1 Microbiological Water Quality	9
2.3.2 Microbiology Water Quality for Malaysia	10
2.3.3 Interim National Water Quality Standards for Malaysia (INWQS)	11
2.3.4 Total Coliform and Fecal Coliform Studies in Malaysian Waters	13
2.4 Oysters	17
2.4.1 Standards for Shellfish Growing Water	18
2.5 Treatment of Molluscs	20
2.6 Multiple Test Tube Fermentation Technique of Standard Method	22
2.6.1 Standard Test for Coliforms	23

2.7	Biochemical Oxygen Demand (BOD)	24
CHAPTER 3	MATERIAL AND METHODS	26
3.1	Sampling Techniques	26
3.2	Sampling Stations (Map)	27
3.3	Medium and Reagent Preparations	28
3.3.1	Lauryl Tryptose Broth Preparation	28
3.3.2	EC Broth Preparation	29
3.3.3	Glutamate Decarboxylase (GAD) Reagent Preparation	29
3.3.4	Phosphate Buffer Solution Preparation (1×10^{-3} M)	30
3.3.5	Hydrochloride Acid (0.1N) Preparation	30
3.4	Analytical Techniques	31
3.4.1	Total Coliform and Fecal Coliform Counts	31
3.4.2	<i>Escherichia coli</i> Determination	34
3.4.3	BOD ₃ and BOD ₅ Analysis	35
CHAPTER 4	RESULTS	
4.1	Estimation of Total coliform, Fecal coliform and <i>Escherichia coli</i> in Setiu Lagoon	37
4.1.1	Estimation of total coliform in the waters of Setiu lagoon	37
4.1.2	Estimation of fecal coliform in the waters of Setiu lagoon	39
4.1.3	Estimation of <i>Escherichia coli</i> in the waters of Setiu lagoon	41
4.1.4	Estimation of total coliform in the sediments of Setiu lagoon	42
4.1.5	Estimation of fecal coliform in the sediments of Setiu lagoon	43
4.1.6	Estimation of <i>Escherichia coli</i> in the sediments of Setiu lagoon	44
4.1.7	Estimation of total coliform in cultured oysters of Setiu lagoon	45
4.1.8	Estimation of fecal coliform in cultured oysters of Setiu lagoon	47
4.1.9	Estimation of <i>Escherichia coli</i> in cultured oysters of Setiu lagoon	48
4.2	BOD ₅ levels in the waters of Setiu lagoon	49

4.3	Determination of Total Coliform, Fecal Coliform and <i>Escherichia coli</i> in wild oysters and the depuration rate	50
4.3.1	Determination of total coliform in seawater and wild oysters during 14 days of depuration	50
4.3.2	Determination of fecal coliform in seawater and wild oysters during 14 days of depuration	51
4.3.3	Determination of <i>Escherichia coli</i> in seawater and wild oysters during 14 days of depuration	52
4.4	Regression analysis between fecal coliform (MPN.100 mL ⁻¹) and various parameters in Setiu lagoon	53
4.5	Regression analysis between BOD ₅ (mg.L ⁻¹) and DO (mg.L ⁻¹) in Setiu lagoon	55
4.6	Regression analysis between BOD ₅ (mg.L ⁻¹) and temperature (°C) in Setiu lagoon	56
4.7	Regression analysis between DO (mg.L ⁻¹) and temperature (°C) in Setiu lagoon	57
CHAPTER 5	DISCUSSION	58
CHAPTER 6	CONCLUSION	76
LITERATURE CITED		78
APPENDICES		81
CURRICULUM VITAE		122

LIST OF TABLES

Table		Page
2.1	Microbiology Water Quality Standards for Malaysia (DOE, 2002-2003)	11
2.2	DOE Interim National Water Quality Standards (INWQS) for Malaysia (DOE, 2002-2003)	12
2.3	Total coliform and fecal coliform counts in the water and sediment of Perhentian Island (Thong, 2002)	14
2.4	Total coliform, fecal coliform and <i>E. coli</i> counts in riverine, coastal waters and estuarine zone of Terengganu River Estuary (Chan, 2003)	15
2.5	Total coliform, fecal coliform and <i>E.coli</i> counts in water, sediment and oyster in Setiu lagoon (Neoh, 2004)	16
2.6	Criteria for the classification of shellfish harvesting areas in United Kingdom (Neoh, 2004)	19
2.7	Bacteriological quality of the French and European water according to the number of <i>Escherichia coli</i> for 100g of mussel flesh (Neoh, 2004)	20
3.1	Locations of the sampling stations	27
5.1	Average monthly precipitation and evaporation in Kuala Terengganu for the year 2004	62
5.2	Comparison of Total coliform, Fecal coliform and <i>Escherichia coli</i> counts with previous studies in Setiu lagoon	70
5.3	Comparison of Total coliform, Fecal coliform and <i>Escherichia coli</i> counts in cultured oysters with previous studies in Setiu lagoon	71
5.4	Comparison of BOD ₅ levels with previous studies in Setiu lagoon	71
5.5	R ² values of regression analysis between various parameters in the waters of Setiu lagoon	72

LIST OF FIGURES

Figure		Page
3.1	Sampling stations along Setiu lagoon	27
4.1	Total coliform counts in waters of Setiu lagoon	37
4.2	Fecal coliform counts in the waters of Setiu lagoon	39
4.3	<i>Escherichia coli</i> counts in the waters of Setiu lagoon	41
4.4	Total coliform counts in sediments of Setiu lagoon	42
4.5	Fecal coliform counts in sediments of Setiu lagoon	43
4.6	<i>Escherichia coli</i> counts in the sediments of Setiu lagoon	44
4.7	Total coliform counts in cultured oysters of Setiu lagoon	45
4.8	Fecal coliform counts in cultured oysters of Setiu lagoon	47
4.9	<i>Escherichia coli</i> counts in cultured oysters of Setiu lagoon	48
4.10	BOD ₅ levels in the waters of Setiu lagoon	49
4.11	Total coliform counts in seawater and wild oysters during 14 days of depuration	50
4.12	Fecal coliform counts in seawater and wild oysters during 14 days of depuration	51
4.13	<i>Escherichia coli</i> counts in seawater and wild oysters during 14 days of depuration	52
4.14(a)	Regression between fecal coliform (MPN.100 mL ⁻¹) and BOD ₅ (mg.L ⁻¹) in the waters of Setiu lagoon on first sampling (21 st August 2004)	53
4.14(b)	Regression between fecal coliform (MPN.100 mL ⁻¹) and BOD ₅ (mg.L ⁻¹) in the waters of Setiu lagoon on second sampling (23 rd October 2004)	53
4.14(c)	Regression between fecal coliform (MPN.100 mL ⁻¹) and BOD ₅ (mg.L ⁻¹) in the waters of Setiu lagoon on third sampling (27 th November 2004)	53
4.15(a)	Regression between fecal coliform (MPN.100 mL ⁻¹) and salinity (ppt) in the waters of Setiu lagoon on first sampling	54

(21st August 2004)

4.15(b)	Regression between fecal coliform (MPN.100 mL ⁻¹) and salinity (ppt) in the waters of Setiu lagoon on second sampling (23 rd October 2004)	54
4.15(c)	Regression between fecal coliform (MPN.100 mL ⁻¹) and salinity (ppt) in the waters of Setiu lagoon on third sampling (27 th November 2004)	54
4.16(a)	Regression between BOD ₅ (mg.L ⁻¹) and DO (mg.L ⁻¹) in the waters of Setiu lagoon on first sampling (21 st August 2004)	55
4.16(b)	Regression between BOD ₅ (mg.L ⁻¹) and DO (mg.L ⁻¹) in the waters of Setiu lagoon on second sampling (23 rd October 2004)	55
4.16(c)	Regression between BOD ₅ (mg.L ⁻¹) and DO (mg.L ⁻¹) in the waters of Setiu lagoon on third sampling (27 th November 2004)	55
4.17(a)	Regression between BOD ₅ (mg.L ⁻¹) and Temperature (°C) in the waters of Setiu lagoon on first sampling (21 st August 2004)	56
4.17(b)	Regression between BOD ₅ (mg.L ⁻¹) and Temperature (°C) in the waters of Setiu lagoon on second sampling (23 rd October 2004)	56
4.17(c)	Regression between BOD ₅ (mg.L ⁻¹) and Temperature (°C) in the waters of Setiu lagoon on third sampling (27 th November 2004)	56
4.18(a)	Regression between DO (mg.L ⁻¹) and Temperature (°C) in the waters of Setiu lagoon on first sampling (21 st August 2004)	57
4.18(b)	Regression between DO (mg.L ⁻¹) and Temperature (°C) in the waters of Setiu lagoon on second sampling (23 rd October 2004)	57
4.18(c)	Regression between DO (mg.L ⁻¹) and Temperature (°C) in the waters of Setiu lagoon on third sampling (27 th November 2004)	57
5.1	Comparison of mean total coliform counts in Setiu lagoon with the total coliform standards according to the microbiological water quality standards for Malaysia	60
5.2	Monthly rainfall and evaporation in Kuala Terengganu at different monsoon season for the year 2004	62
5.3	Comparison of mean fecal coliform counts in the waters of Setiu lagoon with the fecal coliform standards according to the microbiological water quality standards for Malaysia	65
5.4	Comparison of mean fecal coliform counts in the cultured oysters in Setiu lagoon with microbiological criteria for shellfish in United Kingdom, 2002	66

5.5	Comparison of mean <i>E. coli</i> counts in the cultured oysters in Setiu lagoon with microbiological criteria for shellfish in United Kingdom, 2002	66
5.6	Regression between total coliform in the seawater and wild oysters	74
5.7	Regression between fecal coliform in the seawater and wild oysters	74
5.8	Regression between <i>Escherichia coli</i> in the seawater and wild oysters	74

LIST OF ABBREVIATIONS

APHA	-	American Public Health Association
Apr	-	April
Aug	-	August
ASEAN	-	Association of South East Asia Nations
BOD	-	biological oxygen demand
BOD ₃	-	biological oxygen demand after 3 days incubation in 30 ⁰ C
BOD ₅	-	biological oxygen demand after 5 days incubation in 20 ⁰ C
COD	-	chemical oxygen demand
⁰ C	-	degree centigrade
Dec	-	December
DO	-	dissolved oxygen
DOE	-	Department of Environment
<i>E. coli</i>	-	<i>Escherichia coli</i>
FAD	-	Food and Drug Administration
FAO	-	Food and Agricultural Organization
FC	-	fecal coliform
g	-	gram
GAD	-	glutamic acid decarboxylase
HCL	-	hydrochloric acid
INWQS	-	Interim National Water Quality Standards
Kg	-	kilogram
M	-	molarity
Mg	-	milligram

mL	-	milliliter
mm	-	millimeter
MPN	-	Most Probable Number
NaCl	-	sodium chloride
NaOH	-	sodium hydroxide
Nov	-	November
NSSP	-	National Shellfish Sanitation Program
Oct	-	October
pH	-	potential of hydrogen
ppt	-	parts per thousand
Sept	-	September
TC	-	total coliform
WHO	-	World Health Organization

LIST OF APPENDICES

Appendix		Page
I	Hydrological parameters of the waters of Setiu lagoon during the first sampling (21-08-2004)	81
II	Hydrological parameters of the waters of Setiu lagoon during the second sampling (23-10-2004)	82
III	Hydrological parameters of the waters of Setiu lagoon during the third sampling (27-11-2004)	83
IV	Estimation of total coliform in Setiu lagoon during first sampling (21-08-2004), second sampling (23-08-2004) and third sampling (27-11-2004)	84
V	Estimation of fecal coliform in Setiu lagoon during first sampling (21-08-2004), second sampling (23-08-2004) and third sampling (27-11-2004)	86
VI	Estimation of <i>E.coli</i> in Setiu lagoon during first sampling (21-08-2004), second sampling (23-08-2004) and third sampling (27-11-2004)	88
VII	Estimation of total coliform in cultured oysters during first sampling (21-08-2004), second sampling (23-08-2004) and third sampling (27-11-2004)	90
VIII	Estimation of fecal coliform in cultured oysters during first sampling (21-08-2004), second sampling (23-08-2004) and third sampling (27-11-2004)	91
IX	Estimation of <i>E.coli</i> in cultured oysters during first sampling (21-08-2004), second sampling (23-08-2004) and third sampling (27-11-2004)	92
X	BOD ₅ in Setiu lagoon during the first sampling (21-08-2004)	93
XI	BOD ₅ in Setiu lagoon during the second sampling (23-10-2004)	94
XII	BOD ₅ in Setiu lagoon during the third sampling (27-11-2004)	95
XIII	Two Way ANOVA analysis of total coliform in the waters of Setiu lagoon	96
XVI	Two Way ANOVA analysis of fecal coliform in the waters of Setiu lagoon	98

XV	Two Way ANOVA analysis of <i>E. coli</i> in the waters of Setiu lagoon	100
XVI	Two Way ANOVA analysis of total coliform in the sediments of Setiu lagoon	102
XVII	Two Way ANOVA analysis of fecal coliform in the sediments of Setiu lagoon	104
XVIII	Two Way ANOVA analysis of <i>E. coli</i> in the sediments of Setiu lagoon	106
XIX	Two Way ANOVA analysis of total coliform in the cultured oysters of Setiu lagoon	108
XX	Two Way ANOVA analysis of fecal coliform in the cultured oysters of Setiu lagoon	110
XXI	Two Way ANOVA analysis of <i>E. coli</i> in the cultured oysters of Setiu lagoon	112
XXII	Two Way ANOVA analysis of BOD ₅ in the waters of Setiu lagoon	114
XXIII	Estimation of total coliform in seawater on 0 day, 5 th day and 14 th day of depuration	116
XXIV	Estimation of fecal coliform in seawater on 0 day, 5 th day and 14 th day of depuration	117
XXV	Estimation of <i>E. coli</i> in seawater on 0 day, 5 th day and 14 th day of depuration	118
XXVI	Estimation of total coliform in wild oysters on 0 day, 5 th day and 14 th day of depuration	119
XXVII	Estimation of fecal coliform in wild oysters on 0 day, 5 th day and 14 th day of depuration	120
XXVIII	Estimation of <i>E. coli</i> in wild oysters on 0 day, 5 th day and 14 th day of depuration	121

ABSTRACT

The aim of this study is to assess the level of fecal contamination caused by sewage pollution in Setiu lagoon and to determine whether the oysters cultured in the lagoon are safe for human consumption. Twelve sampling stations were established and three visits were conducted on 21st August 2004, 23rd October 2004 and 27th November 2004 respectively. Total coliform and fecal coliform counts in water, sediment and cultured oysters were estimated using the Multiple Test Tube Fermentation Technique of Standard Method. GAD test was conducted to confirm the presence of *E.coli* in the samples. BOD₅ levels in the waters of Setiu lagoon were also determined. The mean total coliform, fecal coliform and *E.coli* counts in the waters of Setiu lagoon were 82.78 MPN.100 mL⁻¹, 25.03 MPN.100 mL⁻¹ and 4.82 MPN.100 mL⁻¹ respectively. The mean total coliform, fecal coliform and *E.coli* counts in the sediments of Setiu lagoon were 1.97 MPN.g⁻¹, 0.55 MPN.g⁻¹ and 0.21 MPN.g⁻¹ respectively. The mean total coliform, fecal coliform and *E.coli* counts in the cultured oysters of Setiu lagoon were 35.06 MPN.g⁻¹, 6.19 MPN.g⁻¹ and 1.32 MPN.g⁻¹ respectively. The mean BOD₅ value of the waters in Setiu lagoon in August, October and November were 1.36±0.27 mg.L⁻¹, 1.66±0.30 mg.L⁻¹ and 0.94±0.39 mg.L⁻¹ respectively. The presence of total coliform, fecal coliform and *E. coli* in the water and sediment of Setiu lagoon indicates that the lagoon has been contaminated by domestic sewage. Although the *E.coli* counts in the oysters cultured in Setiu lagoon are still within the safety level for human consumption, sanitary quality of water in the lagoon has to be monitored in order to maintain a clean and healthy environment for aquaculture activities.

ABSTRAK

Tujuan kajian ini dijalankan ialah untuk menentukan tahap pencemaran najis akibat pembuangan bahan kumbahan ke dalam lagun Setiu dan menentukan sama ada tiram yang dikultur di lagun itu selamat untuk dimakan. Sebanyak dua belas stesen penyampelan dipilih di sepanjang lagun Setiu. Penyampelan dijalankan sebanyak tiga kali iaitu pada 21^{hb} Ogos 2004, 23^{hb} Oktober 2004 and 27^{hb} November 2004. Bilangan total coliform dan fecal coliform di dalam air, sedimen dan tiram yang dikultur ditentukan dengan menggunakan kaedah “Multiple Test Tube Fermentation Technique of Standard Method”. Ujian GAD telah dijalankan untuk mengesan kehadiran *E.coli* di dalam sampel. Tahap BOD₅ di dalam air di lagun Setiu juga ditentukan. Nilai purata bilangan total coliform, fecal coliform dan *E.coli* di dalam air di lagun Setiu masing-masing ialah 82.78 MPN.100 mL⁻¹, 25.03 MPN.100 mL⁻¹ dan 4.82 MPN.100 mL⁻¹. Bagi sedimen di lagun Setiu pula masing-masing ialah 1.97 MPN.g⁻¹, 0.55 MPN.g⁻¹ dan 0.21 MPN.g⁻¹. Nilai purata bilangan total coliform, fecal coliform dan *E.coli* di dalam tiram masing-masing ialah 35.06 MPN.g⁻¹, 6.19 MPN.g⁻¹ dan 1.32 MPN.g⁻¹. Nilai purata BOD₅ di dalam air di lagun Setiu pada Ogos, Oktober dan November masing-masing ialah 1.36±0.27 mg.L⁻¹, 1.66±0.30 mg.L⁻¹ dan 0.94±0.39 mg.L⁻¹. Kehadiran total coliform, fecal coliform and *E.coli* di dalam air dan sedimen di lagun Setiu menunjukkan bahawa lagun tersebut telah dicemari oleh bahan kumbahan. Walaupun bilangan *E.coli* di dalam tiram yang dikultur di lagun Setiu masih berada pada tahap yang selamat untuk dimakan, namun kualiti air di lagun tersebut harus dikawal untuk mewujudkan persekitaran yang bersih dan selamat untuk menjalankan aktiviti akuakultur.