

**PLANKTONIC BIOLUMINESCENCE
OFF THE SOUTH COAST AND
PUTTALAM LAGOON OF
SRI LANKA**

By

Hewa Bashithage Udeshika Gangani Manike Wimalasiri

MPhil

2020

**Planktonic bioluminescence off the South coast and Puttalam
Lagoon of Sri Lanka**

By

Hewa Bashithage Udeshika Gangani Manike Wimalasiri

Thesis submitted to the University of Sri Jayewardenepura
for the award of the Degree of Master of Philosophy

This thesis is dedicated

To

All kind people who

Willing or unwilling support me to achieve my goal

And

To all ocean lovers.

DECLARATION

The work described in this thesis was carried out by me under the supervision of Dr. D.C.T. Dissanayake, Senior Lecturer, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura Mr. S.U.P. Jinadasa, Senior lecturer, Ocean University of Sri Lanka, Dr A. D. Weidemann, Naval Research Laboratory, Ocean Sciences Branch, Stennis Space Center United States of America and Dr. H. W. Wijsekera, Naval Research Laboratory, Ocean Sciences Branch, Stennis Space Center United States of America. This has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma.

.....

Date

.....

Signature

H. B. U. G. M. Wimalasiri

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

.....
Dr. D.C.T. Dissanayake,
Senior Lecturer,
Department of Zoology,
Faculty of Applied Sciences,
University of Sri Jayewardenapura,
Gangodawila, Nugegoda, Sri Lanka.

.....
Mr. S.U.P. Jinadasa
Senior lecturer
The Ocean University of Sri Lanka,
Crow Island, Colombo 15, Sri Lanka.



Dr. Alan D. Weidemann
Ocean Sciences Branch
Naval Research Laboratory, Code 7330
Stennis Space Center, MS 39529



Dr. Hemantha W. Wijesekera
Ocean Sciences Branch
Naval Research Laboratory, Code-7332
Stennis Space Center, MS 39529

CERTIFICATION OF THE SUPERVISOR

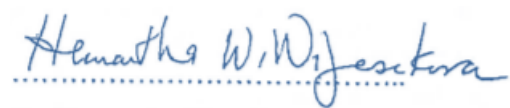
We certify that the candidate has incorporated all corrections, additions and amendments recommended by the examiners to the final version of the MPhil thesis.

.....
Dr. D.C.T. Dissanayake,
Senior Lecturer,
Department of Zoology,
Faculty of Applied Sciences,
University of Sri Jayewardenapura,
Gangodawila, Nugegoda, Sri Lanka.

.....
Mr. S.U.P. Jinadasa
Senior lecturer
The Ocean University of Sri Lanka,
Crow Island, Colombo 15, Sri Lanka.



Dr. Alan D. Weidemann
Ocean Sciences Branch
Naval Research Laboratory, Code 7330
Stennis Space Center, MS 39529



Dr. Hemantha W. Wijesekera
Ocean Sciences Branch
Naval Research Laboratory, Code-7332
Stennis Space Center, MS 39529

TABLE OF CONTENT

TABLE OF CONTENT	i
LIST OF TABLES	iv
LIST OF FIGURES	vi
LIST OF PLATES	x
ACKNOWLEDGEMENT	xi
LIST OF ABBREVIATIONS	xiii
ABSTRACT	xiv
CHAPTER ONE.....	1
INTRODUCTION	1
CHAPTER TWO	5
LITERATURE REVIEW.....	5
2.1 Introduction to bioluminescence	5
2.2 Evolution of bioluminescent organisms	7
2.3 Biochemistry of bioluminescence	9
2.4 Bioluminescent organisms	11
2.4.1 Bioluminescence in microorganisms	12
2.4.2 Bioluminescence macro-organisms	18
2.5 Important of Bioluminescence	19
2.6. Applications of Bioluminescence	21

2.7 Bioluminescence Measurements	22
2.8 Bioluminescence research in world oceans	25
2.9 Bioluminescence in the Indian Ocean	29
2. 10 Lagoon bioluminescence	30
CHAPTER THREE	33
MATERIALS AND METHODS	33
3.1 Study area.....	33
3.2 Data collection and analysis.....	33
3.2.1 Bioluminescence intensity.....	36
3.2.2 Oceanographic measurements	37
3.2.3 Zooplankton sampling and analysis	39
3.2.4 Water sample collection and nutrient analysis	43
3.2.5 Fixed station data	45
3.3 Statistical analysis	46
CHAPTER FOUR	47
RESULTS.....	47
4.1 Variation in Bioluminescence intensity off the south coast, the Puttalam lagoon and fixed sampling station	47
4.1.1. South coast.....	47
4.1. 2 The Puttalam lagoon.....	54
4.1.3 Fixed sampling station	56

4.2 Bioluminescent zooplankton.....	59
4.2.1 Variations in bioluminescent zooplankton abundance off the south coast of Sri Lanka	63
4.2.3 Bioluminescent zooplankton fixed station	73
4.3 Variation in surface nutrients off the south coast, the Puttalam lagoon and fixed sampling station	77
4.3.1 South coast.....	77
4.3.2 Puttalam lagoon	79
4.3.3 Fixed sampling station	80
4.4 Effects of surface nutrients on the abundance of bioluminescent zooplankton....	80
CHAPTER FIVE	89
DISCUSSION.....	89
CHAPTER SIX.....	100
CONCLUSION	100
REFERENCE	
APPENDICES	

LIST OF TABLES

Table 2.1. Evolution of bioluminescence measuring instruments from 1965 to present.....	24
Table 3.1. Geographical location and average depth of sampling locations off the South coast and the Puttalam lagoon of Sri Lanka	35
Table 4.1. Surface bioluminescence zooplankton species identified off the south coast and the Puttalam lagoon of Sri Lanka from January to December 2016.....	59
Table 4.2. Abundance of surface bioluminescent zooplankton (individuals /m ³) off the south coast of Sri Lanka during the study period from January to December 2016 in all five sampling locations.....	64
Table 4.3. Surface bioluminescence zooplankton diversity, richness and evenness at 5 sampling locations off the south coast of Sri Lanka from January to December 2016.....	69
Table 4.4. Abundance of surface bioluminescence zooplankton (individuals /m ³) in the the Puttalam lagoon of Sri Lanka during the study period from January to December 2016 in all six sampling locations.....	71
Table 4.5. Surface bioluminescence zooplankton diversity, richness and evenness at 6	

sampling locations at the Puttalam lagoon of Sri Lanka during 2016	73
Table 4.6. Variations in the abundance of surface bioluminescence zooplankton (individuals /m ³) at the fixed sampling location off the south coast of Sri Lanka during the northeast (January) and southwest (May) monsoon periods.....	75
Table 4.7. Variation in surface nutrient concentration (\pm SD) at six sampling locations, in the Puttalam lagoon of Sri Lanka during 2016	81
Table 4.8. Correlation between bioluminescent zooplankton abundance with surface nutrient parameters at 5 sampling locations, off south coast of Sri Lanka during January to December 2016.	83
Table 4.9. Correlation between bioluminescent zooplankton abundance with surface nutrient parameters at 6 sampling locations, the Puttalam lagoon of Sri Lanka during January to December 2016.	85

LIST OF FIGURES

Figure 1.1.A bioluminescence tree of life proposed by Dunn et al. (2008): Marine (blue) and terrestrial/ freshwater (green) bioluminescence is spread throughout the tree relative to the nonluminous phyla (grey)	08
Figure 1.2.Chemical structure of Luciferin extracted from <i>Cypridina</i> spp. (left) and luminous bacteria (right) (Source: Shimomura, 2008).....	11
Figure 1.3.Structure of <i>Cypridina</i> sp. luciferin as proposed by Kishi et al. (1966).....	16
Figure 3.1. (a.) Selected sampling sites for the measurement of planktonic bioluminescence, water and plankton samples collection off the south coast and the Puttalam lagoon of Sri Lanka (b.) Sampling locations in the the Puttalam lagoon of Sri Lanka (c.) Sampling location off south coast.....	35
Figure 3.2. Recoverable bathyphotometer (a) and schematic representation of internal structure (b) of the bathyphotometer (Fulicle, 2003) 1. Data cable 2. Pressure transducer 3. Chamber 4. Bioluminescence detector 5. Associated electronic 6. Transparent inner envelop 7. Viewing chamber 8. Zinc ring weight 9. Excitation grid 10. Flow of water (inlet).....	37
Figure 3.3. Diagrammatic representation of CTD SBE 19 plus SeaCat profiler (Sea-Bird Electronic Inc. 2009).....	38

Figure 3.4. Zooplankton net figure (a) and schematic representation of zooplankton net (b).....40

Figure 3.5. Sampling location of the fixed station off the South coast of Sri Lanka.....45

Figure 4.1. Distribution of bioluminescence profile (black line), Chlorophyll profile (green line) density profile (blue line) and temperature profile (red line) at the 5 sampling locations off the south coast of Sri Lanka from January to March 2016. Five sampling locations represented as (a) Location 1 (b) Location 2 (c) Location 3 (d) Location 4 (e) Location 5.....48

Figure 4.2. Distribution of bioluminescence profile (black line), Chlorophyll profile (green line) and temperature profile (red line) at the 5 sampling locations off the south coast of Sri Lanka from April to June 2016. Five sampling locations represented as (a) Location 1 (b) Location 2 (c) Location 3 (d) Location 4 (e) Location 5.....50

Figure 4.3. Distribution of bioluminescence profile (black line), Chlorophyll profile (green line) and temperature profile (red line) at the 5 sampling locations off the south coast of Sri Lanka from July to September 2016. Five sampling locations represented as (a) Location 1 (b) Location 2 (c) Location 3 (d) Location 4 (e) Location 552

Figure 4.4. Distribution of bioluminescence profile (black line), Chlorophyll profile (green line) and temperature profile (red line) at the 5 sampling locations off the south coast of Sri Lanka from October to December 2016. Five sampling locations

represented as (a) Location 1 (b) Location 2 (c) Location 3 (d) Location 4 (e) Location 5.....53

Figure 4.5. Variations in bioluminescence intensity at 6 sampling locations of the the Puttalam lagoon of Sri Lanka during January – March (a), April – June(b), July – September (c) , and October – December (d) 2016.....56

Figure 4. 6. Variations in bioluminescence intensity at fixed sampling location off the south coast of Sri Lanka on 20th January (a) and 5th May (b) 2016 from 7.00 pm to 5.00 am.....57

Figure 4.7. Variations in temperature at fixed sampling location off the south coast of Sri Lanka on 20th January(a) and 5th May(b) 2016 from 7.00 pm to 5.00 am.....57

Figure 4.8. Variations in salinity at fixed sampling location off the south coast of Sri Lanka on 20th January(a) and 5th May(b) 2016 from 7.00 pm to 5.00 am.....58

Figure 4.9. Variations in Chlorophyll concentration at fixed sampling location off the south coast of Sri Lanka on 20th January (a) and 5th May (b) 2016 from 7.00 pm to 5.00 am.....58

Figure 4.10. Total zooplankton abundance (%) and bioluminescent zooplankton abundance (%) variation during 2016 off the south coast of Sri Lanka.....67

Figure 4.11. Percentage abundance of surface bioluminescence zooplankton at 5 sampling locations off the south coast of Sri Lanka during 2016.....	68
Figure 4.12. Surface bioluminescence zooplankton abundance (%) at fixed station, off south coast of Sri Lanka in 20 th January and 5 th May 2016.....	74
Figure 4.13. Variation in surface total nitrogen concentration (\pm SD) at 5 sampling locations off the south coast of Sri Lanka during 2016.....	77
Figure 4.14. Variation in surface orthophosphate concentration (\pm SD) at 5 sampling locations, off the south coast of Sri Lanka during 2016	78
Figure 4.15. Variation in surface silicate concentration (\pm SD) at 5 sampling locations, off the south coast of Sri Lanka during 2016.....	79

LIST OF PLATES

Plate 4.1. Identified bioluminescent zooplankton species off the south coast and the Puttalam lagoon of Sri Lanka during January to December 2016.....	62
---	----

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my supervisors, Dr. D.C.T. Dissanayake, Senior Lecturer, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Mr. S.U.P. Jinadasa, Senior lecturer, Department of Oceanography, the Ocean University of Sri Lanka, Dr. Alan D. Weidemann, Ocean Sciences Branch, Naval Research Laboratory, Stennis Space Center, Dr. Hemantha W. Wijesekera, Ocean Sciences Branch, Naval Research Laboratory, Stennis Space Center, for their guidance, encouragement and sparing their valuable time to a successful completion of this study. I could not have imagined having better supervisors for my M.Phil study.

My sincere thanks also go to Prof. Harindra Fernando, University of Notre Dame and the Office of Naval Research, United States of America for financial and technical assistance during the study. Next I would like to thank National Aquatic Resources Research and Development Agency (NARA) for the opportunity they have given me. I would like to thank the staff of the National Institute of Oceanography and Marine Sciences (NIOMS) of NARA and the crew of RV Samuddrika for their immense support. Especially I would like to mention Mr Upul Adhikari, Mr Suresh Gunawardhana and Mr Sunil Shantha who support me during data collection.

I wish to express my sincere thanks to the head, academic and non-academic staff of the Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura for their support and provisions during the study. Furthermore my sincere thanks go to all the staff members of Faculty of Graduate studies, University of Sri Jayewardenepura for their support.

My heartiest gratitude goes to my husband, Chamika Siriwardhana, my mother, U.K Bisomanike, My father H.B. Wimalasiri and my brother H. B. Udaya Sadith for their love and encouragements during the whole time.

Finally I would like to thank all the people who support and encourage me to complete this study successfully.

LIST OF ABBREVIATIONS

NARA	– National Aquatic Resources Research and Development Agency
NIOMS	– National Institute of Marine Sciences
FRP	– Fiberglass Reinforced Plastic
HP	– Horse power
RBPM	– Recoverable Bathyphotometer
CTD profiler	– Conductivity, Temperature and depth profiler
HIDEX	– High intake defined excitation bathyphotometer

Planktonic bioluminescence off the South coast and Puttalam lagoon of Sri Lanka

Hewa Bashithage Udeshika Gangani Manike Wimalasiri

ABSTRACT

Marine bioluminescence is a unique phenomenon and widely studied area in the world. However, limited information on bioluminescence is available in the Indian ocean. This study was carried out to understand the spatial and temporal variations in planktonic bioluminescence in selected marine and lagoon waters to evaluate the relationship between planktonic bioluminescence organisms and oceanographic parameters, identify the bioluminescent zooplankton species and estimate their diversity and abundance in order to fill the existing knowledge gap in the region. Planktonic bioluminescence was assessed off the south coast ($5^{\circ}45'46.80''$ N - $6^{\circ}11'06.00''$ N, $80^{\circ} 6'57.60''$ E - $79^{\circ}47'34.80''$ E) and the Puttalam lagoon ($8^{\circ}30'14.40''$ N - $8^{\circ}19'44.40''$ N, $79^{\circ}47'24.00''$ E - $79^{\circ}49'22.80''$ E) of Sri Lanka from January to December 2016 in three months intervals. Sampling was carried out at selected five sampling locations off the south coast and six locations in the Puttalam lagoon. A fixed station ($6^{\circ}06'43.20''$ N, $79^{\circ}45'28.80''$ E) sampling was done off the south coast of Sri Lanka during monsoon and non-monsoon period. Variation in planktonic bioluminescence intensity and oceanographic parameters were measured using a Recoverable Bathypotometer (RBPM) and Conductivity Temperature Depth (CTD) profiler. Vertical hauls of zooplankton samples were collected from 10 m depth to the surface using WP-2 net with 180 μ m mesh size and lagoon zooplankton samples were collected by horizontal towing of the net, samples were preserved in 5% buffered formalin, and used to identify bioluminescent zooplankton species and their abundance (individuals/ m). Three water samples were collected at each sampling location and analyzed the nutrient level (nitrate,

orthophosphate and silicate) using UV-spectroscopic method. Bioluminescence intensity found to be varied with sampling time and sampling location and high intensity bioluminescence peaks were mostly concentrated to the mixed layer in most of the sampling sites. Analysis of bioluminescence and Chlorophyll profiles indicates the presence of both heterotrophic and autotrophic planktonic bioluminescence in the study area. Fixed station analysis indicates the variations in bioluminescence peaks showing the highest intensities at mid night and lowest at the dusk and dawn. Twenty two surface bioluminescent zooplankton species belonging to 15 families and 4 phyla were identified during this study. Of these, twenty one species were reported in offshore waters and eight were identified in the Puttalam lagoon. Seven species were common to off the south coast and the Puttalam lagoon of Sri Lanka. The most abundant bioluminescent zooplankton in the ocean is *Triconia conifera* and it is *Vargula hilgendorfi* in the lagoon. Surface bioluminescent zooplankton abundance exhibits significant difference ($p > 0.05$; Kruskal-Wallis test) with respect to the sampling season off the south coast of Sri Lanka. It was not revealed a significant relationship between bioluminescent zooplankton abundance and surface nutrient concentration ($p > 0.05$; Kruskal-Wallis test) though some positive and negative co-relations were observed. This study provides the first scientific evidence of the presence of bioluminescence in the marine and lagoon waters of Sri Lanka and preliminary results of intensity variations with respect to space and time and identified some zooplankton species responsible for the process of bioluminescence. This information will be useful to update the existing information regionally as well as globally.

Keywords: Bioluminescence, Recoverable bathyphotometer (RBPM), Chlorophyll profiles, Conductivity, Temperature, Depth, Zooplankton,