

INCREASING LIVE FOOD VISIBILITY FOR JUVENILE
SEAHORSES, *Hippocampus kuda* WITH COLORANTS

FARAHANIS BINTI MAT YAMAN

FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
UNIVERSITI MALAYSIA TERENGGANU

2013

INCREASING LIVE FOOD VISIBILITY FOR JUVENILE SEAHORSES,

***Hippocampus kuda* WITH COLORANTS**

By

Farahanis binti Mat Yaman

Research Report submitted in partial fulfilment of
the requirement for the degree of
Bachelor of Science (Marine Biology)

Department of Marine Science
Faculty of Marine Science and Maritime Studies
UNIVERSITI MALAYSIA TERENGGANU
2013

This project report should be cited as:

Farahanis, M. Y. 2013. Increasing live food visibility for juvenile seahorses, *Hippocampus kuda* with colorants. Undergraduate thesis, Bachelor of Science (Marine Biology), Faculty of Marine Science and Maritime Studies, Universiti Malaysia Terengganu, Terengganu. 92p.

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 permission from the author and the supervisor(s) of the project.

1100091324

4
5
17/11
2
2013



DEPARTMENT OF MARINE SCIENCE
 FACULTY OF MARITIME STUDIES AND MARINE SCIENCE
 UNIVERSITI MALAYSIA TERENGGANU

DECLARATION AND VERIFICATION REPORT
 FINAL YEAR RESEARCH PROJECT

It is hereby declared and verified that this research report entitled:
 Increasing live food visibility for juvenile seahorses, *Hippocampus kuda* with Colorants

by Farahanis binti Mat Yaman, Matric No. UK23058 have been
 examined and all errors identified have been corrected. This report is submitted to the
 Department of Marine Science as partial fulfillment towards obtaining the Degree
 Bachelor of Science (Marine Biology), Faculty of Maritime Studies and Marine
 Science, Universiti Malaysia Terengganu.

Verified by:

Principal Supervisor

Name: Prof. Dr. Mohd Effendy Abd. Wahid

Official stamp: PROF. DR. MOHD EFFENDY ABD. WAHID
 Timbalan Naib Canselor
 (Penyelidikan dan Inovasi)
 Universiti Malaysia Terengganu
 21030 Kuala Terengganu, Terengganu

Date: June 19th., 2013

Second Supervisor

Name: Associate Prof. Liew Hock Chark

Official stamp: PROF. MADYA LIEW HOCK CHARK
 Pensyarah
 Jabatan Sains Marin
 Fakulti Pengajian Maritim Dan Sains Marin
 Universiti Malaysia Terengganu
 21030 Kuala Terengganu

Date: 28/6/13

ACKNOWLEDGEMENTS

Alhamdulillah. Greatest and deepest gratitude goes to the supervisors, Prof. Dr. Mohd Effendy Abd. Wahid and Associate Prof. Liew Hock Chark for their invaluable ideas and help, their kind words of encouragements and their patience to bear with the author throughout the process. Author thanks them as well for allowing the use of the hatchery, equipments and other resources.

The project was made possible by the Institute of Marine Biotechnology, UMT for financially supporting the project especially with seahorse acquirement and to Mr. Thiru for his great assistance taking time from his busy schedule. The author thanks all the staffs of the Department of Marine Science, Freshwater Hatchery of Faculty of Fisheries and Aqua-Industries and Institute of Marine Biotechnology, UMT for their assistance in preparing and supplying equipments and live samples especially Mr. Mohd Zan and Mr. Abdul Manaf from the Biodiversity Lab.

Special mention to Dr. Siti Aishah Abdullah, without whom, the author shall have not learnt this much during her three years and without whom, would never even consider of studying *Artemia* culture and of course, biomimicry. Thank you for always being there for all Bio-Mariners. Of course, to the author's family, friends and other individuals that had not been mentioned, the author owes them her innermost gratitude. To the author's father, who divulged her into a book about sea creatures at the age of five. Thank you.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS	ii
LIST OF TABLES.....	v
LIST OF FIGURES	vi
LIST OF PHOTOGRAPHS.....	viii
ABBREVIATIONS	x
ABSTRACT.....	xii
ABSTRAK.....	xiii
1.0 INTRODUCTION	1
1.1 Research Background.....	1
1.2 Objectives and Scope	4
2.0 LITERATURE REVIEW	5
2.1 <i>Hippocampus kuda</i>	5
2.2 Seahorse Ophthalmology and Relations to Feeding Efficiency.....	9
2.2 Prey Colour and Contrast Manipulation.....	11
3.0 METHODOLOGY	14

3.1	Location of Experiment.....	14
3.2	Experiment Design and Implementation.....	14
3.2.1	Preliminary Assay.....	14
3.2.2	Laboratory Work.....	17
3.3	Data Analysis.....	21
4.0	RESULTS.....	22
4.1	Experiment A: Mixed Prey Colour against Three Backgrounds.....	22
4.2	Experiment B: One Prey Colour against Three Backgrounds.....	26
4.3	Behavioural Observations.....	31
5.0	DISCUSSION.....	36
6.0	CONCLUSION AND SUGGESTION.....	43
	REFERENCES.....	44
	APPENDICES.....	50
	APPENDIX I Raw Data.....	51
	APPENDIX II Preliminary Assay.....	55
	APPENDIX III Statistical Data and Calculations.....	58
	VITAE.....	79

LIST OF TABLES

Table 4.1	Mean and percentage of prey ingestion of seahorse juveniles (\pm SEM) fed with mixed prey colour against different background treatments	24
Table 4.2	Mean and percentage of prey ingestion of seahorse juveniles (\pm SEM) fed with one type of prey colour against different background treatments	27

LIST OF FIGURES

Figure 2.1. Illustration of adult <i>H. kuda</i> external morphology (Lourie <i>et al.</i> , 2004)....	6
Figure 2.2. Illustration of adult <i>H. kelloggi</i> external morphology (Lourie <i>et al.</i> , 2004), a similar species with <i>H. kuda</i> , to be used as comparison.	7
Figure 2.3. Illustration of adult <i>H. spinosissimus</i> external morphology (Lourie <i>et al.</i> , 2004) to be used as a general comparison with <i>H. kuda</i>	8
Figure 3.1. A simplified diagram of survival and toxicity test procedures. R= red, G= green, B= blue and C= clear while numbers indicate replicates.	15
Figure 3.2. Simplified diagram of colour fastness and length record procedures. RA= red, GA= green, BA= blue and CA= clear.	17
Figure 3.3. A simplified illustration to Experiment A procedures.	19
Figure 3.4. A simplified illustration to Experiment B procedures.	20

Figure 4.1. Prey ingested by juvenile seahorses (\pm SEM) fed with mixed prey colour against three tank backgrounds.....24

Figure 4.2. Prey ingested by juvenile seahorses (\pm SEM) fed with one prey colour against three tank backgrounds.....27

Figure 4.3. Samples of *H. kuda* juveniles of different colours and sizes encountered during study.35

LIST OF PHOTOGRAPHS

- Photograph 4.1.** Apparatus setup for one replicate of Experiment A.23
- Photograph 4.2.** Aerial view of three tanks (white, black and clear) during experiment A. Observations were made from above with minimal shadow blockage.25
- Photograph 4.3.** Apparatus in mid-setup for one replicate of experiment B.....28
- Photograph 4.4.** Sample result collection in five minutes from Experiment B of green prey colour against white background. Sample result shows 24 successful strikes and zero unsuccessful strikes.....28
- Photograph 4.5.** Juvenile *H. kuda* hunting for prey during Experiment B; green prey against clear background where prey were visible and colour-distinguishable with naked eye during experiment execution.29
- Photograph 4.6.** *Artemia* recollection from 80µm plankton net with a dropper after thoroughly rinsing the sides of net.....29

Photograph 4.7. *Artemia* recounting procedures using light microscopy at 100 x total magnification. Clockwise from top-left: *Artemia* in red and clear, blue *Artemia* and green *Artemia* and light microscope used. Prey may considerably lose colour after rinsing during recollection thus looks paler during recounting.30

Photograph 4.8. Left: 500 mL tank used to culture dyed *Artemia* with the respective non-toxic food colouring (Red Ponceau 4R, Brilliant Blue and Apple Green). Cultures were maintained to be between 3 – 4 days of age. Right: Method used to count *Artemia* to be used during experiment A. *Artemia* were counted carefully during the one hour acclimatisation period.30

Photograph 4.9. Weekly stock tank cleaning and daily water change procedure.31

Photograph 4.10. *H. kuda* juveniles swimming at water surface during the day.34

Photograph 4.11. At night, juvenile *H. kuda* aggregates at substratum bases to rest. 34

Photograph 4.12. This particular seahorse was temporarily isolated to observe for any signs of illness due to its pale colouration and weak movements compared to other seahorses after arrival. The seahorse was later introduced to the stock tank.35

ABBREVIATIONS

λ_{peak}	:	Approximate peak response to wavelength (Hawryshyn, 1992)
μm	:	Micrometre
ANOVA	:	Analysis of variance
<i>Artemia</i>	:	<i>Artemia</i> sp.
$^{\circ}\text{C}$:	Degrees Celsius
CITES	:	The Convention on International Trade in Endangered Species
cm	:	Centimetre
DIY	:	Do-It-Yourself
Exp	:	Experiment
<i>H.</i>	:	<i>Hippocampus</i>
<i>H. kuda</i>	:	<i>Hippocampus kuda</i>
Ind.	:	Individuals
IUCN	:	International Union for Conservation of Nature
L	:	Litre
mg	:	Milligram
mm	:	Millimetre
mL	:	Millilitre
MYT	:	Malaysia time
N/A	:	Not Available

NH ₃	:	Ammonia
NH ₄ ⁺	:	Ammonium cation
No.	:	Number
pH	:	Potential of Hydrogen
ppt	:	Parts per thousand
ppm	:	Parts per million
PVC	:	Polyvinyl chloride
SD	:	Standard deviation
SEM	:	Standard error of the mean
TM	:	Total magnification
UMT	:	Universiti Malaysia Terengganu
UV	:	Ultraviolet
x	:	Times

ABSTRACT

The study attempts to increase live food visibility for juvenile seahorses, *Hippocampus kuda* with edible non-toxic colorants by manipulating the prey and tank background colour. Effects of four different colours of dyed *Artemia* prey – red Ponceau 4R, brilliant blue, apple green and clear, normal *Artemia* – against three background colours: white, black and clear tanks, towards prey ingestion were determined. There were two experiments involved in this study: Experiment A, testing on the effects of mixed prey colours against three background colours towards *H. kuda* juvenile seahorses from 1 to almost 3 months old. Red prey colour was significantly more consumed ($p < 0.05$) than clear *Artemia* in all tank backgrounds. There were no significant effects ($p > 0.05$) of background colour toward prey ingestion. Experiment B was to test the effects of one prey colour against three backgrounds colours. No significant differences ($p > 0.05$) were found between coloured prey ingestion in all background colours and in between background colours with respect to one prey colour ($p > 0.05$). Two-way ANOVA tests also recorded no significant interaction ($p > 0.05$) between prey colour and background colour. Prey colour preference sequence by *H. kuda* according to percentage of prey ingestion in experiment A was red > blue > green > clear while experiment B sequence was red > green > blue > clear.

Meningkatkan Kadar Penglihatan Makanan Hidup untuk Kuda Laut Juvana,
Hippocampus kuda Dengan Menggunakan Pewarna

ABSTRAK

Kajian ini telah dijalankan untuk cuba meningkatkan penglihatan makanan hidup untuk kuda laut juvana, *Hippocampus kuda* menggunakan pewarna makanan tidak toksik dengan memanipulasi warna makanan dan latar belakang tangki. Kesan dari *Artemia* yang diwarnakan dengan empat warna berbeza – merah Ponceau 4R, biru “brilliant blue”, hijau epal dan *Artemia* normal yang jernih – terhadap tiga warna latar belakang: putih, hitam dan jernih, ke atas pengambilan mangsa telah dinilai. Terdapat dua eksperimen di dalam kajian ini: Eksperimen A, menguji kesan warna *Artemia* yang bercampur terhadap tiga warna latar belakang ke atas kuda laut *H. kuda* juvana dari umur 1 hingga hampir 3 bulan. Mangsa berwarna merah didapati dimakan secara lebih signifikan ($p < 0.05$) berbanding *Artemia* yang jernih dalam kesemua warna latar tangki. Juga, tiada kesan warna latar belakang yang signifikan ($p > 0.05$) ke atas pemakanan mangsa dapat dilihat. Eksperimen B menguji kesan hanya satu warna mangsa terhadap tiga warna latar belakang. Tiada perbezaan yang signifikan ($p > 0.05$) didapati antara pengambilan mangsa yang diwarnakan antara kesemua latar tangki dan juga di antara warna latar dengan mana-mana satu warna mangsa yang berkenaan ($p > 0.05$). Ujian ANOVA dua hala juga mencatatkan bahawa terdapat tiada interaksi yang signifikan ($p > 0.05$) antara warna mangsa dan warna latar tangki. Warna mangsa mengikut urutan keutamaan oleh *H. kuda* mengikut peratusan pengambilan mangsa dalam eksperimen A adalah merah > biru > hijau > jernih manakala eksperimen B urutan adalah merah > hijau > biru > jernih.