

**IDENTIFICATION OF ETIOLOGICAL AGENTS CAUSING MASS
MORTALITY OF STRIPED CATFISH (*Pangasianodon
hypophthalmus*) DURING THE YOLK-SAC STAGE**

by

JONARD FERNANDO CABADING

An Undergraduate Thesis presented to the faculty of the
College of Fisheries in partial fulfillment of the
requirements of the degree of

BACHELOR OF SCIENCE IN FISHERIES

**Department of Aquatic Resources, Ecology and Management
COLLEGE OF FISHERIES
CENTRAL LUZON STATE UNIVERSITY
Science City of Muñoz, Nueva Ecija**

2016



**IDENTIFICATION OF ETIOLOGICAL AGENTS CAUSING MASS MORTALITY
OF STRIPED CATFISH (*Pangasianodon hypophthalmus*)
DURING THE YOLK-SAC STAGE**

by

JONARD FERNANDO CABADING

is an Undergraduate Thesis presented to the Faculty of the
College of Fisheries in partial fulfillment of the
requirements for the degree of


BACHELOR OF SCIENCE IN FISHERIES

APPROVED:


JOSE S. ABUCAY
Adviser

1/24/17

Date


REMEDIOS B. BOLIVAR
Critic

1/24/17

Date

NOTED:


REMEDIOS B. BOLIVAR
Department Chair

1/24/17

Date

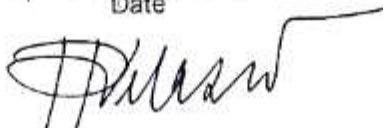
RECORDED:


REMEDIOS B. BOLIVAR
College Research Coordinator

1/24/17

Date

ACCEPTED:


RAVELINA R. VELASCO
Dean

1/24/17

Date

BIOGRAPHICAL DATA



Personal Background

Name: Jonard Fernando Cabading
Birthdate: March 25, 1992
Birthplace: Tallang, Baggao, Cagayan
Address: #187, ZONE 3, Sta. Margarita, Baggao, Cagayan
Father: Jessie Domingo Cabading
Mother: Benilda Padre Fernando Cabading

Educational Attainment

Elementary: Sta. Margarita East Elementary School
Sta. Margarita, Baggao, Cagayan
Secondary: Baggao National Agricultural School Sta. Margarita Annex
Sta. Margarita, Baggao, Cagayan
Tertiary: Central Luzon State University
Science City of Muñoz, Nueva Ecija

ACKNOWLEDGEMENT

The author would like to extend his sincerest gratitude to the following persons who contributed in the preparation of this thesis:

To the College of Fisheries faculty members who mentored the author in different fields, especially to his ever supportive advisers, Dr. Karl Marx Quiazon (former) and Dr. Jose S. Abucay (present) who shared so much of their time checking and helping the Author to complete this paper even beyond office times and even when when Dr. Quiazon is out of town or country just to share his expertise, and to his critic and thesis coordinator, Dr. Remedios B. Bolivar, for her undying support and diligence in checking this paper, and to Dr. Tereso A. Abella, Director of Freshwater Aquaculture Center, for allowing the author to use different Laboratory apparatus to complete this thesis. Thank you ma'ams and sirs.

Thanks to Sir Hamzer Saway for allowing the author to conduct his thesis using their water and infected samples and sharing his expertise in managing the farm, for extending his support especially for sending the water and fish sample early in the morning everyday and also to Sir James and Ma'am Mary Jane Dela Cruz, the owner of the farm whom the Century company rents, for coordinating the author to be permitted to conduct his thesis on the farm, and for touring the author around the farm.

To Tita Marie, Kuya Onat and Ma'am Wye for always motivating the author to finish his thesis offering the author what they can help, and for treating the author as part of their family and welcoming the author to their respective houses whenever the author has nowhere to go, and to his Coaches, Prof. Ramon L. Sabacan, Prof. Anna Ison, Dr. Jose S. Abucay and Ma'am Melaine Fernandez, Coach Rod and Coach Donnis for

sharing their lives to the author and giving game and life advises, and to CLSU Varsity Swimming Team members to Kuya Ven, Kuya Kim, Kuya Harold, Kuya Cristino, Kuya Meynard, Kuya Michael, Ate Eriel, Ate Rizza, Ate Cherry, Ate Bia, Jewel, Rina, Christwell, Genesse, Ina, Harold, Kathlene, Catherine, Timut, Jonas, Ellie, Tiryu, Jem, Ralph, Kenneth, Crosby, Cocoy, Von, Ness, Abe, Julie, Limuel, Jaynard, Dominic, Vanessa, Russell, Lando Yuri, Melvin, Carlo Geryldine, Joana, Dianne, Xab, Jed, Lamberto, Obama, Ciren, Emil, Gabaldon, Geanne, Candace, Windy, Lyka, Kim for the memories shared together with the daily dose of chlorine during training, and for showing to the author that they are not just a team, but a family. Thank you po.

To Carlo, Jaynard, and Buknoy, for accompanying the author during the conduct of this thesis even during beyond office hours and weekends, to Ate Juliet, for allowing the author to use the facilities of wet lab to incubate brine shrimp serves as food for the fish samples of the author, to Sir Ed and Kuya Balong for allowing the author to use the incubation facility of PhilFishGen in order hatch his samples, to Kuya Rey for always opening the laboratories even beyond office hours just for the author to analyse his samples, to Asher, Jemma, Mars, Flory and for assisting the author during his conduct. Without you this thesis won't be done. Thank you po.

To Von, Gabaldon and Lando his roommates, for their unending support whenever the Author is in need of any kind of help through thick and thin.

To his parents, for their undying support to the author for providing everything he needs especially in financial aspect, and for raising the author to become a better person. To his brothers and sisters: Julius for lending the author his motorcycle whenever he needs it and for spending Christmas to help the author just to finish this paper, Joel,

Jackie and Jenny for taking photo for the author during the conduct of this thesis, and for their moral support, and to his special someone who inspires the author to finish this paper especially when he felt like giving up.

Above all the author would like to thank the Almighty God, for granting him the enough diligence and knowledge to finish this paper.

JONARD FERNANDO CABADING

TABLE OF CONTENTS

	Page
BIOGRAPHICAL SKETCH	i
ACKNOWLEDGEMENT	ii
LIST OF TABLES	viii
LIST OF FIGURES	ix
LIST OF APPENDIX FIGURES	x
LIST OF APPENDIX TABLE	xii
ABSTRACT	xiii
INTRODUCTION	1
Background of the Study	2
Statement of the problem	3
Significance of the study	4
Objectives of the study	4
Scope and limitations	4
Time and Place of the Study	5
REVIEW OF RELATED LITERATURE	6
Analysis of mortality in <i>Pangasius fry</i> and appropriate treatments	6

Bacillary necrosis in farmed <i>Pangasius hypophthalmus</i>	7
Cannibalism during yolk-sac stage	8
Histopathology of microspora (<i>Kabatana arthuri</i>) infection in <i>Pangasius sutchi</i> catfish	9
MATERIALS AND METHOD	10
Experiment and Experimental Design	10
Egg incubation and larval rearing	12
Collection of water sample	13
Analysis of Water Samples	13
Total Bacterial Count	13
Preparation of Histological Sections	14
Statistical Analysis	14
RESULTS AND DISCUSSIONS	15
Incubation and hatching	15
Yolk-sac and Fry Stage	15
Water Quality	15
Temperature	17
Dissolved oxygen (DO)	17

pH	17
Total ammonia nitrogen (TAN)	18
Nitrite-nitrogen	19
Nitrite-nitrogen	19
Total bacterial count (TBC)	19
Histological examination	20
SUMMARY, CONCLUSION AND RECOMMENDATIONS	21
LITERATURE CITED	23
APPENDICES	26

LIST OF TABLES

Table No.		Page
1	Water Quality Parameters	16

LIST OF FIGURES

Figure No.		Page
1	Schematic presentation of the methodology of the study	11
2	Procedure for the analysis of Total Hardness	27
2	Procedure for the analysis of TAN	28
3	Procedure for the analysis of Nitrite-Nitrogen	28
4	Procedure for histological preparations	28

LIST OF APPENDIX FIGURES

Figure No.		Page
1	Upwelling artificial incubation system being used in Gapan City	37
2	Mechanical filter	37
3	Ultra Violet (UV) light sterilization treatment of water supply	38
4	Downwelling artificial incubation system being used in CLSU	38
5	Blue plastic barrel used as nursery for newly hatched fry in CLSU	39
6	White basin used as nursery for newly hatched fry in CLSU	39
7	Artemia in Gapan	40
8	Artemia and Tubifex in CLSU	40
9	Analysis of water quality using a multiparameter	41
10	Eppendorf tubes used to homogenize the fry sample	41
11	TBC results	42
12	Dissected view of an infected fry sample from Gapan City	42
13	Dissected view of a healthy fry sample hatched in CLSU	43
14	Microtome used in dissecting the sample	43
15	Staining of dissected samples	44
16	Ultra Violet light and degassing facility	44

17	Improvised cassette	45
18	Paraffin wax used to embed samples	45
19	Oven @ 58 °C	46
20	Embedded samples on the wax	46
21	Warm bath to flatten the sample	47
22	Dissecting poker	47
23	Microscope used to magnify the samples	48

LIST OF APPENDIX TABLE

Table No.		Page
1	Temperature (°C) readings	33
2	Dissolved oxygen (ppm) readings	33
3	pH readings	34
4	Total ammonia nitrogen (ppm) readings	34
5	Nitrite nitrogen (ppm) readings	35
6	Total bacterial count	35

**IDENTIFICATION OF ETIOLOGICAL AGENTS CAUSING MASS
MORTALITY OF STRIPED CATFISH (*Pangasianodon
hypophthalmus*) DURING THE YOLK SAC STAGE^{1/}**

ABSTRACT

The study aimed to assess the etiological agents causing mass mortality of striped catfish in Gapan while the fry incubated in CLSU is not infected. Through comparing water quality and bacteriological analysis for four consecutive days of both samples from CLSU and from Gapan. In the TBC found out that the fry from Gapan has a higher number of bacterial colonies wherein there are 106.66667, while fry from CLSU only got 29.66667 bacterial colonies in average.

A total of 3 sample replicates in each site is collected everyday for four consecutive days for the TBC. A total 10 samples were collected everyday for four consecutive days in both sites were fixed in 70% alcohol for the histological analysis. The tissue of an infected fry is easily distorted while the healthy one is intact. The noticeable difference is that the development of the infected one is stagnant and they do not eat and eventually they die, while the healthy one consumed already its yolk-sac on the third day.

In general, it is assessed that the cause of mass mortality is bacterial infection in the belly portion of the fry.

Some management measures were recommended based on the current water management being practiced.

^{1/} Undergraduate thesis presented to the faculty of the College of Fisheries, Central Luzon State University as a partial fulfillment of the requirements for the degree of Bachelor of Science in Fisheries. Prepared at the Department of Aquatic Resources, Ecology and Management under the supervision of Dr. Jose S. Abucay.

LITERATURE CITED

- Arifin, Z. 1990. Rearing of patin fry (*Pangasius pangasius*) in different salinities. Bull. Penelitian Perikanan Barat (Indonesia), 9(1): 43-51.
- Baras, E., J. Slembrouck, C. Cochet, D. Caruso and M. Legendre. 2010. Interactions between temperature and size on the growth, size heterogeneity, mortality and cannibalism in cultured larvae and juveniles of the Asian catfish, *Pangasianodon hypophthalmus* (Sauvage). Wiley Inter Science. New York, U.S.A. p. 379-390.
- Bardach, J.E., J.H. Ryther and W.O. McLaren. 1972. Aquaculture - The Farming and Husbandry of Fresh Water and Marine Organisms. Wiley Inter Science. New York, U.S.A. 868 p.
- Bernet, D., H. Schmidt, W. Meier, P. Burkhardt-Holm and T. Wahli. 1999. Histopathology in fish: proposal for a protocol to assess aquatic pollution. Journal of Fish Diseases, 22: 25-34.
- Boyd, C.E. 1995. Bottom soils, sediment, and pond aquaculture. Chapman and Hall. New York, New York. 348 p.
- Canning, E.U., E. Elkan and P.I. Trigg. 1964. *Plistophora myotrophica* causing high mortality in the common toad (*Bufo bufo* L.), with notes on the maintenance of *Bufo* and *Xenopus* in the laboratory. J. Protozool., 11: 157-166.
- Canning E.U. and J. Lom. 1986. The Microsporidia of Fish. p. 1-16. In: E.U. Canning and J. Lom (eds.). The Microsporidia of Vertebrate. Academic Press. New York, N. Y., USA. 304 p.
- Crumlish, M. and T.T. Dung. 2006. Strategies to reduce risk and livelihood impact associated with outbreaks of bacillary necrosis of *Pangasius spp.* (BNP) farmed in the Mekong Delta, Viet Nam. Final Technical Report of the Department for International Development (Aquaculture and Fish Genetics Research Programme). London, England. 186 p.
- Crumlish, M., T.T. Dung, J.F. Turnbull, N.T.N. Ngoc and H.W. Ferguson. 2002. Identification of *Edwardsiella ictaluri* from diseased freshwater catfish, *Pangasius hypophthalmus* (Sauvage), cultured in the Mekong Delta, Vietnam. Journal of Fish Diseases, 25: 733-736.
- De Silva, S.S. and P.T. Nguyen. 2011. Striped catfish farming in the Mekong Delta, Vietnam: A tumultuous path to a global success. Reviews in Aquaculture, 3: 45-73.

- Dung, T.T., F. Haesebrouck, N.A. Tuan, P. Sorgeloos, M. Baele, A. Decostere. 2008. Antimicrobial susceptibility pattern of *Edwardsiella ictaluri* isolates from natural outbreaks of bacillary necrosis of *Pangasianodon hypophthalmus* in Vietnam. *Microbial Drug Resistance*, 14(4): 311–316.
- Dyková, I. and J. Lom. 1980. Tissue reaction to microsporidian infection in fish. *J. Fish Dis.*, 3: 265-283.
- Fakayode, S.O. 2005. Impact of industrial effluents on water quality of the receiving, Alaro River in Ibadan, Nigeria. Retrieved on July 20, 2016 from <http://www.scopus.com/record/display.url?eid=2-s2.0-27644499667&origin=inward&txGid=14CC99AD1F4A9AF9FF9FA54A51BF756D.aqHV0EoE4xllF3hgVWgA%3a2>
- Folkvord, A. 1991. Growth, survival and cannibalism of cod juveniles (*Gadus morhua*): effect of feed type, starvation and fish size. *Aquaculture*, 97:41–59.
- Hassan, A., M.A. Ambak and A.A. Samad. 2011. Crossbreeding of *Pangasianodon hypophthalmus* (Sauvage, 1878) and *Pangasius nasutus* (Bleeker, 1863) and their larval development. *Journal of Sustainable Science and Management*, 6(1): 28-35.
- Islam, A. 2002. Some aspects of the embryonic and early development of Thai pangas (*Pangasius sutchi* Fowler, 1937). Conference abstracts proceeding Book. 26th Annual LFC. Bergen, Norway. 67 p.
- Ly T.T.L., D.N. Nguyen, H.P. Vo and C. Doan. 2009. Hemorrhage disease of cultured tra catfish (*Pangasianodon hypophthalmus*) in Mekong Delta (Viet Nam). *The Israeli Journal of Aquaculture – Bamidgeh* 61 (3): 215–224.
- Manat, C., W. Suraphong and L. Sanga,. 1990. Breeding of Chao Phya Giant catfish, *Pangasius sanitwongsei*. *Thai-Fish Gazette*, 43(2): 95-97.
- Nguyen, Q.T., T.D. Tu and H.W. Ferguson. 2004. Histopathology of white spot in internal organs of *Pangasius hypophthalmus*. *Scientific Journal of Can Tho University*, 51: 137–142.
- Nguyen, T.P., T.T.H. Tran, N.U. Vu, T.G. Huynh, T.A. Cao and T.T.H. Nguyen. 2007. Study on environment and disease pathogens of catfish farming Tra (*Pangasianodon hypophthalmus*) and basa (*Pangasius bocourti*) and giant freshwater prawn (*Macrobrachium rosenbergii*) in An Giang province. Report submitted to An Giang Science and Technology Department. 125 p.

- Nguyen, T.T.H., T.M.T. Dang, T.H.O. Dang and T.P. Nguyen. 2008. Investigation on parasitic infection in tra catfish (*Pangasianodon hypophthalmus*) farming in An Giang Province. Scientific Journal of Can Tho University, 1: 205– 213.
- Palma, A.L. 2009. Culture of *Pangasius hypophthalmus* in ponds. Paper presented at EAS (East Asian Seas) Congress on November 23-27, 2009 in Manila, Philippines.
- Phan, L.T., T.M. Bui, T.T.T. Nguyen, G.J. Gooley, B.A. Ingram and H.V. Nguyen. 2009. Current status of farming practices of striped catfish, *Pangasianodon hypophthalmus* in the Mekong Delta, Vietnam. Aquaculture, 296: 227–236.
- Subagja, J., J. Slembrouck, L.T. Hung, and M. Legendre. 1999. Larval rearing of an Asian Catfish *Pangasius hypophthalmus* (Siluroidei, Pangasiidae): analysis of precocious mortality and proposition of appropriate treatments. Aquat. Living Resour., 12(1): 37-44.
- Tu, T.D., M. Crumlish, T.N.N. Nguyen, Q.T. Nguyen and T.M.T. Dang. 2004. Investigation of the disease caused by the genus *Edwardsiella* from tra catfish (*Pangasianodon hypophthalmus*). Journal of Science, 51: 120–125.
- Wanpen, M. 1984. Taxonomical and behavioral difference of pla buk, *Pangasiandon gigas* (Chevy) and pla swai, *Pangasius sutchi* (Fowler) fingerling. Department of Fisheries, Bangkok, Thailand. 12 p.
- Wellborn, T.L. 1988. Channel catfish life history and biology. Southern Regional Aquaculture Center Publication No. 180. Texas Agricultural Extension Service, Texas A & M University. College Station, Texas. 4 p.
- Zaki, M.I. and A. Abdulla. 1984. The reproduction and development of *Clarias gariepinus* from Lake Manzula (Egypt). J. Ichthyol. 23 (6),48–58.
- Zulkafli, A.R. and A. Zahari. 1989. The supply of food and culture techniques of *Pangasius sutchi* Fowler in floating net cages. Fisheries Research Seminar, IPP, Penang, Malaysia, 78-85 p.