

UNIVERSITI PUTRA MALAYSIA

HISTOPATHOLOGY AND HEMATOLOGY OF EPIZOOTIC ULCERATIVE SYNDROME (EUS)-POSITIVE SNAKEHEAD (OPHICEPHALUS *STRIATUS*)

ERLINDA C. LACIERDA

FPSS 1995 9

HISTOPATHOLOGY AND HEMATOLOGY OF EPIZOOTIC ULCERATIVE SYNDROME (EUS)-POSITIVE SNAKEHEAD (OPHICEPHALUS STRIATUS)

By

ERLINDA C. LACIERDA

Dissertation Submitted in Fulfillment of the Requirements for the Degree of Doctor of Philosophy in the Faculty of Fisheries and Marine Science, Universiti Pertanian Malaysia

July 1995



ACKNOWLEDGEMENTS

My sincerest gratitude are extended to the following:

- Prof. Mohamed Shariff, my committee chairman, for his unselfish support, continuing guidance, interest, and enthusiasm throughout the completion of this programme;

- International Development Research Centre (IDRC) of Canada for the financial support under the Fish Microbiology Project 3-P-88-1053-02;

- SEAFDEC/AQD for giving me the opportunity to pursue graduate studies and financial support;

- Dr. James Richard Arthur, then IDRC Project Coordinator, for awarding me this research project;

- Prof. Law Ah Theem , Dr. Noor Azhar Mohd. Shazili, and Dr. Hassan Mohd. Daud, members of my supervisory committee, for their valuable suggestions and going through the manuscript;

- The Staff of Fish Health Section and Binañgonan Freshwater Substation for their technical assistance during the conduct of this study;

- Mr. O.K. Ho of the Electron Microscopy Unit and Prof. Tengku Azmi B.T. Ibrahim, Dean of Faculty of Veterinary Medicine and Animal Science, UPM for the technical expertise on transmission electron microscopy and use of their facilities;

- Dr. Juan D. Albaladejo for teaching me the rudiments of TEM processing;



- Larni Angeli Espada for assistance in statistical analyses, Mila Castaños for editorial assistance, R. Elison and E. Ledesma for doing the figures, R. Buendia, Yusaini, and Dr. Gerald F. Quinitio for helping me with the black and white pictures, and Milagros Paner, Gregoria Erazo and the Staff of Microtechnique Laboratory, SEAFDEC/AQD for their technical assistance;

- All my friends and colleagues from SEAFDEC/AQD, UPM, the first batch of Tropical Fish Health Program, Mr. and Mrs. Paul L. Manalo and family, Dr. James L. Torres, Adrian and Zonie Vidjiarungam, Gopinath Nagaraj, Ron and Vicky Cheong, and the Filipino community in Serdang for their support, encouragement, concern, and joyous company;

- My family and most especially my husband, Rod, for their love, understanding, moral and spiritual support, faith, and concern.



TABLE OF CONTENTS

Page

ACKNOWLED	GEMENTS	ii
LIST OF T	ABLES	vii
LIST OF F	IGURES	viii
LIST OF P	LATES	xi
LIST OF A	BBREVIATIONS	xix
LIST OF F	ISH SPECIES	xxi
ABSTRACT		xxiv
ABSTRAK		xxvii
CHAPTER		
I	GENERAL INTRODUCTION	1
II	REVIEW OF LITERATURE	7
	Historical Review of EUS and Related Epizootics	8
	EUS in Southeast Asia and the Indo-Pacific	8
	Mycotic Granulomatosis in Japan	14
	Red Spot Disease in Australia .	14
	Ulcerative Mycosis in Southeast United States	15
	Socio-Economic Impact of EUS	16
	Pathogens Associated with EUS and	

Socio-Economic	Impa	ct of	EUS	•	•	16
Pathogens Asso Related Epizoo	ciate tics	d wit	h EUS	and •		18
Viruses	•	•		•	•	19
Bacteria						21



		Fungi	٠	٠	•	•	•	23
		Parasi	tes	•	•	•	•	25
	Envi EUS	ronment and Rel	al Fac ⁺ ated E ₁	tors A pizoot	ssoci ics	ated w	with	27
	Hist Rela	opathol ted Epi	ogical zootics	Studi s .	es on	EUS a	and •	30
	Hema Rela	tologic ted Epi	al Stu zootic:	udies s	on	EUS a	and	32
	Stud	ies on	Natura	l Indu	ction	of EU	JS	33
	Rese	arch Pr	oblems	•		•		33
111	LIGH POSI	T AND E TIVE SN	LECTRO	N MICR	ROSCOP	Y OF I	EUS-	34
	Mate	rials a	nd Met	hods	•	30.	•	35
		Study	Areas	٠	•	•	•	35
		Collec Specim	tion and the state of the state	nd Tra •	nspor •	t of •		37
		Gross	Examina	ation	•	•	•	39
		Light	Micros	сору	•	•	•	39
		Transm	ission	Elect	ron M	icros	сору	41
	Resu	lts .		•	•	•	•	41
		Occurr	ence o	f EUS	•	•	•	41
		Gross	Lesion	s.	•	•	•	42
		Histop Snakeh	atholog ead	gy of	EUS-p	ositiv	7e	53
	Disc	ussion	•	•	•	•		80
IV	hema Snak	TOLOGIC EHEAD	AL CHAI	NGES I	N EUS	-POSI	rive	87
	Mate	rials a	nd Metl	hods		•	•	88
		Study	Areas	•	•	•		88
		Hemato	logica	l Para	meter	s.	•	88
		Statis	tical A	Analys	es		•	90



	Resul	lts	•		•	•	•	٠	91
		Gross	s Le	sions	5.	•			91
		Hemat	tolo	gical	Para	ameter	s.	•	92
	Discu	issior	ı	•	•	•	•	•	101
V	EXPEI SNAKI	RIMEN' EHEAD	PAL '	TRANS	SMISS:	ION OF	EUS	EN	111
	Mate	rials	and	Meth	nods	•	•	•	112
		Ехреі	cime	ntal	Fish	•		•	112
		Trans	smis	sion	Expe	riment	s.	•	113
		Gross	s Ex	amina	ntion	•	•		117
	Resu	lts	•			•		•	118
		Clini	ical	Sign	ns.		•		118
		Gross	s Pa	tholo	рду	•		•	124
	Discu	issior	ı	٠	•			•	130
VI	LIGH		ROSC	OPY,	ULTR	ASTRUC	TURE,		
VI	LIGH HEMA TRAN	r Mici rology smissi	ROSC 7 O LON	OPY, F SN EXPER	ULTRA IAKEHI RIMEN'	ASTRUC EAD F TS	TURE, ROM I	AND SUS-	134
VI	LIGHT HEMAT TRANS	F MICI FOLOGY SMISSI rials	ROSC 7 O 1 ON and	OPY, F SN EXPEN Meth	ULTRA IAKEHI RIMEN nods	ASTRUC EAD F TS	TURE, ROM I	AND SUS-	134 135
VI	LIGHT HEMAT TRANS	r MICI FOLOGY SMISS rials Colle	ROSC 7 O 10N and ecti	OPY, F SN EXPEN Meth on of	ULTRA IAKEHI RIMEN hods f Samj	ASTRUC EAD F TS ples	TURE, ROM I	AND SUS-	134 135 135
VI	LIGHT HEMAT TRANS	r MICH FOLOGY SMISS rials Colle Light	ROSC CON ION and ecti	OPY, F SN EXPER Meth on of croso	ULTRA JAKEHI RIMEN nods Samj copy	ASTRUC EAD F TS ples	TURE, ROM I	AND SUS-	134 135 135 135
VI	LIGH HEMAT TRANS	r MICI FOLOGY SMISSI rials Colle Light Trans	ROSC 7 O ION and ecti t Mi smis	OPY, F SN EXPEN Meth on of croso sion	ULTRA IAKEHI RIMEN nods Samj copy Elec	ASTRUC EAD F TS ples tron M	TURE, ROM I	AND EUS- · ·	134 135 135 135 135
VI	LIGH HEMA TRANS	r MICI FOLOGY SMISS cials Colle Light Trans Hemat	ROSC ZON And ecti t Mi smis	OPY, F SN EXPEN Meth on of croso sion gical	ULTRA IAKEHI RIMEN Dods E Samj Copy Elec Exai	ASTRUC EAD F TS ples tron M minati	TURE, ROM I	AND EUS- copy	134 135 135 135 136 136
VI	LIGH HEMA TRANS Mater Resul	r MICI FOLOGY SMISSI rials Colle Light Trans Hemat	ROSC CON ION and ections tolo	OPY, F SN EXPEN Meth on of crosc sion gical	ULTRA IAKEHI RIMEN LODS COPY Elec Exai	ASTRUC EAD F TS ples tron M minati	TURE, ROM I	AND BUS- copy	134 135 135 135 136 136 136
VI	LIGH HEMAT TRANS Mater Resul	r MICI FOLOGY SMISSI rials Colle Light Trans Hemat Its Histo	ROSC CON ION and ecti t Mi smis tolo	OPY, F SN EXPEN Meth on of croso sion gical y .	ULTRA IAKEHI RIMEN nods Samj copy Elec Exai	ASTRUC EAD F TS ples tron M minati	TURE, ROM I	AND EUS- · · · · · · · · · · · · · · · · · · ·	134 135 135 135 136 136 137 137
VI	LIGH HEMAT TRANS Mater	r MICI FOLOGY SMISS Colle Light Trans Hemat Its Histo Hemat	ROSC CON ION and ecti t Mi smis tolo	OPY, F SN EXPEN Meth on of crosc sion gical y . gy	ULTRA IAKEHI RIMEN Dods E Samj Copy Elec Exai	ASTRUC EAD F TS ples tron M minati	TURE, ROM I	AND EUS- · · · · · · · · · · · · · · · · · · ·	134 135 135 135 136 136 137 137 169
VI	LIGH HEMA TRANS Mater Resul	r MICI FOLOGY SMISSI rials Colle Light Trans Hemat Its Histo Hemat	ROSC CON ION and ecti t Mi smis tolo	OPY, F SN EXPEN Meth on of croso sion gical y . gy	ULTRA IAKEHI RIMEN Dods E Samj COPY Elec Exai	ASTRUC EAD F TS ples tron M minati	TURE, ROM I	AND EUS-	134 135 135 135 136 136 137 137 169 180
VII	LIGH HEMAT TRANS Mater Mater Resul	r MICI FOLOGY SMISS cials Colle Light Trans Hemat lts Histo Hemat	ROSC CON ION and ecti tolo tolo tolo tolo	OPY, F SN EXPEN Meth on of croso sion gical y . gy SSION	ULTRA IAKEHI RIMEN Dods E Samj COPY Elec Exai	ASTRUC EAD F TS ples tron M minati	TURE, ROM I	AND EUS- · · · · · · · · · · · · · · · · · · ·	134 135 135 135 136 136 137 137 169 180 186
VI VII BIBLIOGRA	LIGH HEMAT TRANS Mater Mater Discu Discu GENEI PHY	r MICI FOLOGY SMISS Collect Light Trans Hemat Its Histo Hemat Issior RAL D	ROSC CON ION and ecti tolo tolo tolo tolo tolo	OPY, F SN EXPEN Meth on of crosc sion gical y . gy SSION	ULTRA JAKEHI RIMEN' nods E Samj copy Elec: Exai	ASTRUC EAD F TS ples tron M minati	TURE, ROM I	AND EUS- · · · · · · · · · · · · · · · · · · ·	134 135 135 135 136 136 137 137 169 180 186 198



LIST OF TABLES

Table		Page
1	Number of EUS Lesions on the Body Surface of Snakehead Used for Histo- pathological Examination	50
2	Distribution of EUS Lesions on the Body Surface of Snakehead Used for Histopathological Examination	51
3	Number of EUS Lesions on the Body Surface of Snakehead Used for Hematological Examination	91
4	Distribution of EUS Lesions on the Body Surface of Snakehead Used for Hematological Examination	92
5	Differential Leucocyte Counts of Normal, Apparently Normal, and EUS- positive Snakehead from Natural Population	100
6	Differential Leucocyte Counts of Control, Apparently Normal, and EUS- positive Snakehead from Transmission Experiments without Additional Food.	178
7	Differential Leucocyte Counts of Control, Apparently Normal, and EUS- positive Snakehead from Transmission Experiments with Additional Food	179



LIST OF FIGURES

Figure		Page
1	Countries in Asia and the Indo-Pacific Affected by EUS	3
2	Provinces in Luzon Island, Philippines Affected by EUS	4
3	Countries in Asia and the Indo-Pacific Affected by Ulcerative Diseases (Dates Indicate First Confirmed Reports) .	9
4	Map of the Philippines Indicating Site of Sampling and Experimental Work: (1) Laguna de Bay; (2) Pangasinan; and (3) Oton, Iloilo where Snakehead with and without EUS Lesions were Collected. The Samples were Brought to (4) the Binañgonan Freshwater Station of SEAFDEC/AQD; (5) BFAR, Quezon City; and (6) the Tigbauan Main Station of SEAFDEC/AQD for Examination	36
5	Body Subdivision of the Snakehead Used for Recording EUS Lesions	40
6	Prevalence of Different Stages of EUS Lesions in the Philippines from 1990 to 1993	52
7	Histograms Showing Hematocrit (%) of Normal, Apparently Normal, and Different Stages of EUS in Snakehead.	93
8	Histograms Showing Hemoglobin (g/100g) of Normal, Apparently Normal, and Different Stages of EUS in Snakehead.	95
9	Histograms Showing Mean Corpuscular Hemoglobin Concentration (MCHC, %) of Normal, Apparently Normal, and Different Stages of EUS in Snakehead.	97



10	Histograms Showing Total Plasma Protein (g/100g) of Normal, Apparently Normal, and Different Stages of EUS in	
	Snakehead	98
11	Diagram of Transmission Experiment to Unfed Snakehead	114
12	Diagram of Transmission Experiment to Fed Snakehead	116
13	Water Temperature in Transmission of EUS to Unfed Snakehead by (a) Co- habitation with EUS-positive Fish in EUS-endemic Environment and (b) Exposure to EUS-endemic Environment Alone.	e 119
14	Transmission Rates (%) of EUS to Unfed Snakehead by (a) Cohabitation with EUS-positive Fish in EUS-endemic Environment and (b) Exposure to EUS- endemic Environment Alone	120
15	Water Temperature in Transmission of EUS to Fed Snakehead by (a) Cohabi- tation with EUS-positive Fish in EUS- endemic Environment and (b) Exposure to EUS-endemic Environment Alone .	122
16	Transmission Rates (%) of EUS to Fed Snakehead by (a) Cohabitation with EUS-positive Fish in EUS-endemic Environment and (b) Expsoure to EUS- endemic Environment Alone	123
17	Number of Lesions in Transmission of EUS to (a) Unfed and (b) Fed Snakehead	128
18	Distribution of Lesions on the Body Surface of (a) Unfed and (b) Fed Snakehead from EUS Transmission Experiments	129
19	Mean Hematocrit (%) of Control, Apparently Normal, and Different Stages of EUS from Transmission of EUS to (a) Unfed and (b) Fed Snakehead by Cohabitation with EUS-positive Fish and Exposure to EUS-endemic Environment	171
		±,1





- 20 Mean Hemoglobin (g/100g) of Control, Apparently Normal, and Different Stages of EUS from Transmission of EUS to (a) Unfed and (b) Fed Snakehead by Cohabitation with EUS-positive Fish and Exposure to EUS-endemic Environment 172
- 22 Mean Plasma Protein (g/100g) of Control, Apparently Normal, and Different Stages of EUS from Transmission of EUS to (a) Unfed and (b) Fed Snakehead by Cohabitation with EUS-positive Fish and Exposure to EUS-endemic Environment 176



LIST OF PLATES

Plate		Page
1	Snakehead with Typical Advanced EUS Lesions	1
2	Snakehead with Stage 1 EUS Lesion: Flat, Hemorrhagic Areas with Scale Loss and Skin Erosion	43
3	Snakehead with Stage 1 EUS Lesion: Raised Hemorrhagic Areas with Scale Loss and Skin Erosion	43
4	Snakehead with Stage 2 EUS Lesion: Eroded Skin and Exposed Underlying Musculature	44
5	Snakehead with Stage 3 EUS Lesion: Edematous Necrotic Musculature .	45
6	Snakehead with Stage 3 EUS Lesion: Exposed Visceral Organs	46
7	Snakehead with Stage 3 EUS Lesion: Exposed Vertebral Column	46
8	Snakehead with Stage 3 EUS Lesion: Severe Erosion of the Head	47
9	Snakehead with Stage 3 EUS Lesion: Erosion of the Caudal Peduncle .	47
10	Snakehead with Healing Lesion: Ulcers with Blackened Margins	48
11	Stage 1 EUS Lesion: Hyperplastic, Spongiotic and Necrotic Dermatitis. Hypodermis and Muscle Fibers are Normal	54
12	Stage 1 EUS Lesion: Electron Micro- graph Illustrating the Break-up of Cell in the Dermal Laver	54
	AND A THE LEASE PARTY AND A THE A THE A	



13	Stage 1 EUS Lesion: Electron Micro- graph of Macrophage with Various Sizes and Shapes of Vacuoles	55
14	Stage 1 EUS Lesion: Electron Micro- graph of Mycotic Granulomatous Necrotic Skin	55
15	Stage 1 EUS Lesion: Electron Micro- graph of Fungal Hypha within a Granulomatous Skin. Membrane-bound Globules are Lysozomes	56
16	Stage 1 EUS Lesion: Electron Micro- graph of Necrotic Skin Showing Several Rod-Shaped Bacteria and Fungal Hyphae	56
17	Stage 1 EUS Lesion: Electron Micro- graph of a Blood Vessel with a Fungal Hypha in the Lumen	57
18	Stage 1 EUS Lesion: Higher Magnifica- tion of Plate 17 Showing the Aseptate Fungal Hypha in the Lumen of a Blood Vessel	57
19	Stage 2 EUS Lesion: Hyperplastic, Necrotic, Granulomatous Dermatitis. Muscle Fibers are Fragmented	58
20	Stage 2 EUS Lesion: Electron Micro- graph of a Macrophage with an Engulfed Fungal Material	59
21	Stage 2 EUS Lesion: Electron Micro- graph of Cells with Prominent Endo- plasmic Reticulum and Mitochondria .	59
22	Stage 2 EUS Lesion: Higher Magnifica- tion of Plate 21 Illustrating Mito- chondria with Tubular Cristae .	60
23	Stage 2 EUS Lesion: Electron Micro- graph of Necrotic Muscle Fibers Illustrating Numerous Mitochondria and Fungal Hyphae	60
24	Stage 2 EUS Lesion: Higher Magnifica- tion of Plate 23 Illustrating Mito- chondria with Cross-Sectioned and Longitudinally Sectioned Tubular Cristae	61



25	Stage 2 EUS Lesion: Electron Micro- graph of Necrotic Muscle Layer Showing Fragmentation and Loss of Striations of Muscle Fibers	61
26	Stage 3 EUS Lesion: Severe, Multifocal, Necrotic, Granulomatous Dermatitis and Myositis	62
27	Stage 3 EUS Lesion: Details of Plate 26. Caseo-Necrotic Granulomatous Muscle Fibers Surrounded by Fibrotic Tissue	63
28	Stage 3 EUS Lesion: Electron Micro- graph of an Eosinophilic Granular Cell	63
29	Stage 3 EUS Lesion: Spongiotic and Granulomatous Dermatitis	64
30	Stage 3 EUS Lesion: Numerous Aseptate Fungal Hyphae Surrounded by Granulomas in Dermis and Muscle Fibers	64
31	Stage 3 EUS Lesion: Electron Micro- graph of Necrotic Skeletal Muscle	65
32	Stage 3 EUS Lesion: Higher Magnifica- tion of Plate 31 Showing Numerous Aseptate Fungal Hyphae Between Necro- tic Muscle Fibers	65
33	Stage 3 EUS Lesion: Electron Micro- graph of Necrotic Muscle Showing Numerous Bacterial Cells	.66
34	Healing Lesion: Hyperplastic, Granu- lomatous Dermatitis with Numerous Melanin Pigments	67
35	Healing Lesion: Hyperplastic Dermis with Numerous Melanin Pigments .	68
36	Healing Lesion: Electron Micrograph of Macrophages with Extensive Pseudopods	68
37	Healing Lesion: Electron Micrograph of Fibroblast Tissue Surrounded by Collagen Fibers	69
38	Healing Lesion: Higher Magnification of Plate 37 Showing Fibroblast Cell xiii	69



39	Stage 1 EUS Lesion: Epithelial Lifting of Gill Lamellae	70
40	Stage 1 EUS Lesion: Multifocal Inters- titial Granulomas in Posterior Kidney	71
4 1	Gills of Stage 3 EUS Lesion: Severe Epithelial Lifting	72
42	Gills of Stage 3 EUS Lesion: Hyper- trophy of Epithelial Cells and Hyper- plasia of Basement Membrane with Fusion of Adjoining Lamellae	73
43	Stage 3 EUS Lesion: Multifocal Granulo- mas in the Spleen of Snakehead .	75
44	Stage 3 EUS Lesion: Necrotic Core of Granuloma in the Spleen with Gram- Negative Bacterial Colonies	75
45	Kidney of Snakehead with Stage 3 EUS Lesion: Severe, Multifocal Granulomas and Vacuolation of Renal Tubules .	76
46	Kidney of Snakehead with Stage 3 EUS Lesion: Gram-Negative Bacterial Colonies in the Granulomatous Tissue	76
47	Posterior Kidney of Snakehead with Stage 3 EUS Lesion: Severe Vacuolation of Renal Tubules	77
48	Kidney of Snakehead with Stage 3 EUS Lesion: Thickening of the Basement Membrane of Cells in the Glomerulus	77
49	Liver of Snakehead with Stage 2 EUS Lesion: Multifocal, Necrotic Hepato- cytes	78
50	Liver of Snakehead with Stage 3 EUS Lesion: Focal Granuloma with Necrotic Eosinophilic Center	78
51	Stomach of Snakehead with Stage 3 EUS Lesion: Focal Granuloma in the Sub- mucosal Layer	79
52	Higher Magnification of Plate 51 Showing the Granuloma Encased by Fibrous Tissue	79



53	The Concrete Tanks with Net Cages Used in EUS Transmission Experiments	114
54	Snakehead with Stage 1 EUS Lesion: Flat, Hemorrhagic Area with Scale Loss and Superficial Skin Erosion .	125
55	Snakehead with Stage 2 EUS Lesion: Eroded Skin and Surface Muscular Layer	126
56	Snakehead with Stage 3 EUS Lesion: Large, Open, Edematous, Necrotic Musculature	126
57	Control Snakehead: Longitudinal Section of Skin Showing Epidermis and Dermis	137
58	Control Snakehead: Dermal and Muscular Layers	138
59	Stage 1 Lesion: Hyperplastic, Necrotic Dermatitis. Hypodermis and Muscle are Normal	140
60	Stage 1 Lesion: Electron Micrograph of a Blood Vessel Congested with Erythrocytes and Leucocytes	141
61	Stage 1 Lesion: Electron Micrograph of Macrophages with Engulfed Fungal Material	141
62	Stage 1 Lesion: Electron Micrograph of a Lymphocyte with an Engulfed Aseptate Fungus	142
63	Stage 1 Lesion: Granulomatous Derma- titis. Muscle Fibers are Normal .	142
64	Stage 1 Lesion: Fungal Hyphae Sur- rounded by Granulomas in Epidermal Layer	143
65	Stage 1 Lesion: Electron Micrograph Illustrating Aseptate Fungal Hyphae within a Granulomatous Skin Tissue .	143
66	Stage 1 Lesion: Electron Micrograph Showing Aseptate Fungal Hypha within a Necrotic, Granulomatous Skin Tissue	144

67	Stage 1 Lesion: Electron Micrograph of an Old Fungal Hypha within a Necrotic Skin Tissue. Moderate Amount of Bacteria are also Present	144
68	Stage 1 Lesion: Cross-section of a New Fungal Hypha Showing a Nucleus and Peripheral Cytoplasm with Several Wall Vesicles	145
69	Stage 1 Lesion: Electron Micrograph Illustrating Fungal Hyphae in Between Normal Muscle Bands	145
70	Stage 2 Lesion: Hyperplastic, Necrotic Granulomatous Dermatitis and Myositis	146
71	Stage 2 Lesion: Several Fungal Hyphae Surrounded by Granulomas in Dermal Layer. Necrotic Muscle Fibers with Few Fungal Hyphae	147
72	Stage 2 Lesion: Electron Micrograph Illustrating Long, Aseptate, Thick- walled Fungal Hypha within Granulo- matous Tissue	147
73	Stage 2 Lesion: Electron Micrograph Showing a Fungal Hypha Containing Mitochondria with Tubular Cristae within a Necrotic Tissue .	148
74	Stage 2 Lesion: Necrotic, Granulomatous Dermatitis and Fragmentation of Muscle Fibers	148
75	Stage 3 Lesion: Severe, Diffuse, Necro- tizing Mycotic Granulomatous Dermatitis and Myositis	149
76	Stage 3 Lesion: Numerous Fungal Hyphae Surrounded by Granulomas in Dermal and Muscular Layers	150
77	Stage 3 Lesion: Electron Micrograph of Necrotic Muscle Fibers Completely Replaced by Fungus	150
78	Stage 3 Lesion: Electron Micrograph of Fungal Hypha in Necrotic Tissue Illustrating the Characteristic Tubular Mitochondrial Cristae .	151



79	Stage 3 Lesion: Higher Magnification of Plate 78 Illustrating the Mito- chondrial Tubular Cristae	151
80	Stage 3 Lesion: Hyperplastic, Necrotic Mycotic Granulomas in the Dermal Layer	152
81	Stage 3 Lesion: Congestion of Blood Vessels	152
82	Stage 3 Lesion: Electron Micrograph Showing a Blood Vessel Densely Packed with Erythrocytes	153
83	Stage 1 Lesion: Slight Epithelial Lifting of Gill Lamellae	155
84	Stage 1 Lesion: Multifocal Mycotic Granulomas in Submucosal Layer of Stomach	155
85	Stage 1 Lesion: Flagellated Protozoans in the Lumen of Stomach	156
86	Stage 1 Lesion: Mycotic Granuloma in the Spleen	156
87	Stage 1 Lesion: Granulomas in the Spleen Contain Bacterial Colonies and Inflammatory Cells	157
88	Stage 1 Lesion: Multifocal Mycotic Granulomas in the Anterior Kidney .	157
89	Stage 2 Lesion: Severe Hyperplasia of Adjoining Lamellae	159
90	Stage 2 Lesion: <i>Scyphidia</i> -like Attached to a Hyperplastic Gill Lamella .	15 9
91	Stage 2 Lesion: Granuloma in the Liver Surrounded by Thick Fibrotic Tissue. The Necrotic Core of Granuloma Contain Bacterial Colonies	160
92	Stage 2 Lesion: Multifocal Mycotic Granulomas in the Submucosal Layer of Stomach	160
93	Stage 2 Lesion: Severe, Multifocal Necrotic Granulomatous Splenitis. The Necrotic Core of Granulomas Contain Bacterial Colonies and Inflammatory Cells xvii	161



94	Stage 2 Lesion: Severe, Multifocal Necrotic Interstitial Granulomas in Anterior Kidney. The Necrotic Core of Granulomas Contain Bacterial Colonies	161
95	Stage 2 Lesion: Interstitial Granulo- mas Surrounded by Thick Fibrous Tissue in Posterior Kidney	162
96	Stage 2 Lesion: Severe Vacuolation of Renal Tubules	162
97	Stage 3 Lesion: Extensive Hyperplasia of Gill Lamellae Resulting to Fusion of Entire Gill Filaments	164
98	Stage 3 Lesion: Focal Area of Necrosis in the Liver. Bottom Part Appears Normal	164
99	Stage 3 Lesion: Dilation of Sinusoids in the Liver	165
100	Stage 3 Lesion: Multifocal Mycotic Granulomas in Muscularis and Submuco- sal Layers of the Stomach	165
101	Stage 3 Lesion: Mycotic Granulomas in the Spleen	166
102	Stage 3 Lesion: Severe, Multifocal Necrotic Granulomatous Splenitis. The Necrotic Core of Granulomas Contain Bacterial Colonies and Inflammatory Cells	166
103	Stage 3 Lesion: Severe, Multifocal Necrotic Granulomas in Posterior Kidney. The Necrotic Core of Granulomas Contain Bacterial Colonies	167
104	Stage 3 Lesion: Severe Atrophy of Renal Tubules	167
105	Stage 3 Lesion: Numerous Fungal Hyphae in the Connective Tissue of the Ovary	168

LIST OF ABBREVIATIONS

ADB	-	Asian Development Bank						
ANOVA	-	Analysis of Variance						
B & B	-	Brown and Brenn's Gram Stain						
BF-2	-	Bluegill Fry						
BFAR	-	Bureau of Fisheries and Aquatic Resources						
BFS	-	Binañgonan Freshwater Substation						
CFU	-	Colony Forming Unit						
EPC	-	Epithelioma Papulosum Cyprini						
EUS		Epizootic Ulcerative Syndrome						
G	-	Giemsa Stain						
GMS	-	Gomori Methenamine Silver Stain						
нсі	-	Hydrochloric Acid						
H & E	-	Hematoxylin and Eosin Stain						
IDRC	-	International Development Research Centre of Canada						
LSD	-	Least Significant Difference						
МСНС	-	Mean Corpuscular Hemoglobin Concentration						
MG	-	Mycotic Granulomatosis						
MS 222	-	Tricaine Methanesulfonate						
NACA	-	Network of Aquaculture Centres in Asia and the Pacific						

xix

ODA	-	Overseas Development Administration
PAS	-	Periodic acid-Schiff Stain
RSD	-	Red Spot Disease
SEAFDEC/AQD		Aquaculture Department of Southeast Asian Fisheries Development Center
SGV	-	Sand Goby Virus
SHRV	-	Snakehead Rhabdovirus
SHV	-	Snakehead Fish Virus
TMS	-	Tigbauan Main Station
UDRV	-	Ulcerative Disease Rhabdovirus
UM	-	Ulcerative Mycosis
ZN	-	Ziehl Neelsen Acid Fast Stain



LIST OF FISH SPECIES

Common Name

Scientific Name

African Catfish	•	•	Clarias gariepinus
American Eel .	•	•	Anguilla rostrata
Atlantic Croaker	•	•	Micropogonias undulatus
Atlantic Menhaden		•	Brevoortia tyrannus
Atlantic Salmon	•		Salmo salar
Ayu		•	Plecoglossus altivelis
Barramundi .	•	•	Lates calcarifer
Bighead Carp .	•	•	Aristichthys nobilis
Black Fish .	•	-	Platycephalus fuscus
Bluegill		•	Lepomis macrochirus
Brook Trout .			Salvinelus fontinalis
Brown Trout .	•		Salmo trutta
Burnett Salmon .	•	•	Polydactylus sheridani
Catfish		•	Clarias macrocephalus
Channel Catfish			Ictalurus punctatus
Chinook Salmon			Oncorhynchus tshawytscha
Climbing Perch			Anabas testudineus
Coho Salmon .	•		Oncorhynchus kisutch
Common Carp .			Cyprinus carpio
Croaker			Johnius sp.
Crucian Carp .		•	Carassius carassius



Common Name

Scientific Name

Eel	•	•	•	Anguilla vulgaris
European Ee	1.	٠	•	Anguilla anguilla
Flat Fish	•	•	•	Psettodes sp.
Flat-tailed	Mullet	•	•	Liza dussumieri
Formosan Sna	akehead	•	•	Channa maculata
Freshwater	Eel	•	•	Fluta alba
Freshwater	Prawn	•	•	Macrobrachium sp.
Goat Fish	•	•		Upeneus bensasi
Goby .	ě		•	Glossogobius giurus
Goldfish	•		•	Carassius auratus
Gouramy .	•	•	-	Trichogaster pectoralis T. trichopterus
Gudgeons .	•			<i>Oxyeleotris</i> sp.
Indian Catf	ish	•	•	Heteropneustes fossilis
Japanese Ee	1.	•	•	Anguilla japonica
Japanese Tr	ident G	oby	•	Tridentiger obscurus
Java Carp	•		•	<i>Puntius</i> sp.
Largemouth	Bass	•	•	Micropterus salmoides
Manila Sea	Catfish	•		Arius manilensis
Mekong Catf	ish		•	Pangassius sutchi
Milkfish .	•	•	•	Chanos chanos
Mullet .	•	•	•	Mugil cephalus
Nile Tilapia	a .	•		Oreochromis niloticus
Perch .	•	•	•	Perca fluviatilis
Pike .	•	•		Esox lucius
Pinfish .	•	•		Lagodon rhomboides





Common Name

Scientifc Name

Plaice .	•	•	•	Pleuronectes platessa
Rainbow Trout	•		•	Salmo gairdneri
Rosy Barb .	•	•	•	Puntius conchonius
Thai Catfish	•	•	•	Clarias batrachus
Sand Goby	•	•	•	Oxyeleotris marmoratus
Siganid .	•	•	•	Scatophagus argus
Silver Barb	•	•	•	Puntius gonionotus
Silver Perch	•	•	•	Bairdiella chrysura
Silvery Therapo	nid	•	•	Therapon plumbeus
Snakehead	•	•	•	Ophicephalus striatus Channa striatus
Sockeye Salmon			•	Oncorhynchus nerka
Southern Flound	ler	•	•	Paralichthys lethostigma
Spot	•	•	•	Leiostomus xanthurus
Weakfish .	•	•		Cynoscion regalis
Whiting .	•	•	•	Sillago ciliata
Yellow Fin Brea	m	•	•	Acanthopagrus australis



Abstract of dissertation submitted to the Senate of Universiti Pertanian Malaysia in fulfillment of the requirements for the degree of Doctor of Philosophy

HISTOPATHOLOGY AND HEMATOLOGY OF EPIZOOTIC ULCERATIVE SYNDROME (EUS) - POSITIVE SNAKEHEAD (OPHICEPHALUS STRIATUS)

By

ERLINDA C. LACIERDA

July 1995

Chairman: Prof. Mohamed Shariff, Ph.D.

Faculty: Fisheries and Marine Science

Epizootic ulcerative syndrome is an ulcerative disease of wild and cultured freshwater fishes in Asia and the Pacific. Despite the bulk of research done on the syndrome for the past 10 years, the primary pathogen remains an enigma.

This study was undertaken to understand the progressive development of EUS lesions through histopathology and hematology of EUS-positive snakehead (*Ophicephalus striatus*).

Regular monitoring of snakeheads in Laguna de Bay, Philippines from October 1990 to February 1993

xxiv

