

A SANGUINICOLID BLOOD FLUKE IN SEA BASS
(LATES CALCARIFER BLOCH)
IN COASTAL PENINSULAR MALAYSIA

BY
BRETT W. HERBERT

MASTER OF SCIENCE
UNIVERSITI PERTANIAN MALAYSIA
1992

**A SANGUINICOLID BLOOD FLUKE IN SEA BASS (*LATES
CALCARIFER* BLOCH) IN COASTAL PENINSULAR MALAYSIA**

By

BRETT W. HERBERT

Thesis submitted in partial fulfilment of the
requirements for the Degree of Master of Science in the
Faculty of Fisheries and Marine Science,
Universiti Pertanian Malaysia.

June 1992

1000385377

ACKNOWLEDGEMENTS

I wish to thank my supervisor, Dr. Faizah Shaharom, who provided guidance, support and assistance at all times during the course of this study, and who, as course coordinator, eased the period of adjustment into a new country.

My sincere thanks to Dr. Hassan Daud and Dr. Rohana Subasinghe on the supervisory committee, who also provided their time, comments and suggestions where necessary. I also wish to thank the technical staff at the Faculty of Fisheries and Marine Science, UPM, for their assistance, particularly Mrs. Kartini Mohamad who did the electron microscopy, and Mr. Rosli Aslim, who taught me the finer points of histological procedures and photography. Also, thanks to Mr. Soh Keh Seng, Mr. Zubir Baharuddin, Miss Noraini Abu Hassan, and Miss Jariah Sulaiman who assisted in the collection of sea bass. I am most grateful to Mr. Yap Khiam Leong and Mr. Jeremy Yap, who provided fish and transport to their net cages at Pulau Ketam gratis, and who were most helpful in providing information and were very co-operative at all times.

Thanks also to Prof. J. C. Pearson and Dr. R. M. Overstreet who gave helpful comments and suggestions on the description part of the study, and to Dr. Hassan Daud and Dr. G. Nash, who assisted in interpretation of some of the histological material. I am very grateful to Dr. I. G. Anderson who arranged for supply of sea bass and working facilities at Oonoonba Veterinary Laboratory in Australia, and who gave me access to his histological material from both Malaysian and Australian sea bass infected with sanguinicolids. Thanks also to Dr. Leong Tak Seng who furnished facilities at Universiti Sains Malaysia for examination of sea bass from Penang, and who provided access to his collection of parasites of sea bass.

Finally, my sincere thanks to my family, friends and course mates, whose support and encouragement throughout the course of this study were invaluable.

TABLE OF CONTENTS

	Page
ACKNOWLEDGEMENTS	ii
LIST OF TABLES	vi
LIST OF FIGURES	vii
LIST OF PLATES	ix
LIST OF ABBREVIATIONS	xi
ABSTRACT	xii
ABSTRAK	xv
CHAPTER	
I	INTRODUCTION
	Foreword
	Sanguinicolids
	Classification and Taxonomy
	Pathological Effects on the Host
	Sea Bass Culture in Malaysia
	1 2 9 12 22
II	DESCRIPTION OF A NEW BLOOD FLUKE, <i>CRUORICOLA LATES</i> N.G., N.SP. (DIGENEA: SANGUINICOLIDAE), FROM CULTURED SEA BASS, <i>LATES CALCARIFER</i> BLOCH 1790 (CENTROPOMIDAE).
	Introduction
	Materials and Methods
	Genus <i>Cruoricola</i> gen. nov.
	<i>Cruoricola lates</i> sp. nov.
	Discussion
	Summary
	24 25 26 27 36 46
III	HISTOPATHOLOGY AND HAEMATOLOGY OF <i>LATES CALCARIFER</i> INFECTED WITH SANGUINICOLID BLOOD FLUKES
	Introduction
	Materials and Methods
	Results
	Discussion
	Conclusion
	Summary
	48 51 53 72 91 92

CHAPTER		Page
IV	DISTRIBUTION OF BLOOD FLUKES OF CULTURED SEA BASS IN MALAYSIA	
	Introduction	94
	Materials and Methods	95
	Results	97
	Discussion	103
	Summary	108
V	CONCLUSION AND FUTURE DIRECTIONS	
	Conclusion	109
	Future Directions	113
	BIBLIOGRAPHY	121
	APPENDIX	133
	BIOGRAPHICAL SKETCH	143

LIST OF TABLES

Table		Page
1	Selected Characteristics of Marine Sanguinicolid Trematodes	4
2	Distribution of Adult and Juvenile <i>Cruoricola</i> in <i>Lates calcarifer</i> from Pulau Ketam as Determined from Histological Studies	54
3	Distribution of <i>Cruoricola</i> Eggs in <i>Lates calcarifer</i> Tissues as Determined from Histological Studies	55
4	Infection and Prevalence Rates of Cultured Sea Bass Examined for <i>Cruoricola lates</i> in Various Locations in Malaysia	98

LIST OF FIGURES

Figure	Page
1 <i>Cruoricola lates</i> n.g., n.sp. Holotype. Dorsal View	33
2 Terminal Genitalia of <i>C. lates</i> n. g., n. sp. Slightly Conventionalised to show Relationships of Ducts and Auxiliary Seminal Vesicle	34
3 Transverse Sections of <i>C. lates</i> n. g., n.sp. ...	36
4 Sea Bass Culture Sites in Peninsular Malaysia Sampled for <i>C. lates</i>	96
5 Numbers of <i>C. lates</i> in <i>Lates calcarifer</i> stocked in May 1991 at Pulau Ketam	100
6 Regression of Haematocrit on Weight- Pulau Ketam	135
7 Regression of Serum Protein on Weight- Pulau Ketam	135
8 Regression of Plasma Protein on Weight- Pulau Ketam	136
9 Regression of Haematocrit on Number of Worms- Pulau Ketam	136
10 Regression of Serum Protein on Number of Worms- Pulau Ketam	137
11 Regression of Plasma Protein on Number of Worms- Pulau Ketam	137
12 Regression of Haematocrit on Weight- Pulau Aceh	138
13 Regression of Serum Protein on Weight- Pulau Aceh	138
14 Regression of Plasma Protein on Weight- Pulau Aceh	139

Figure	Page
15 Regression of Haematocrit on Number of Worms- Pulau Acheh	139
16 Regression of Serum Protein on Number of Worms- Pulau Acheh	140
17 Regression of Plasma Protein on Number of Worms- Pulau Acheh	140
18 Regression of Haematocrit on Weight- Setiu	141
19 Regression of Serum Protein on Weight- Setiu	141
20 Regression of Plasma Protein on Weight- Setiu	142
21 Regression of Haematocrit on Number of Worms- Setiu	142

LIST OF PLATES

Plate		Page
1	Ventrolateral Submarginal Spines of <i>Cruoricola lates</i>	34
2	Subterminal Mouth of <i>C. lates</i>	34
3	Two Adult <i>C. lates</i> in Wall of Rectum of <i>L. calcarifer</i>	37
4	Dorso-ventral duct-like Structures in the Ovary and Vitellaria of <i>C. lates</i>	39
5	Separate Genital Pores of <i>Cruoricola lates</i> ...	41
6	Four <i>Cruoricola lates</i> in a Hepatic Vein of <i>Lates calcarifer</i>	56
7	Transverse Section of <i>C. lates</i> in Pericardial Vein and a Degenerated Egg in Fibrocytic Reaction in Ventricle	56
8	Necrotic Juvenile Worm Encapsulated in Fibrocytic Reaction in Hepatopancreas of Sea Bass	57
9	Necrotic Juvenile Presumed <i>C. lates</i> in Connective Tissue at Base of Gill Filament....	57
10	Adult <i>C. lates</i> in Mesenteric Venule of Sea Bass	58
11	Live <i>C. lates</i> Eggs Adhered to Afferent Filamental Artery Wall in Gill of <i>Lates</i> <i>calcarifer</i>	60
12	Two Miracidia Prior to Escape from Primary Lamellar Epithelium	60
13	Single Miracidium Immediately Prior to Escape from Gill	61

Plate	Page
14 Miracidium Escaping from Gill Epithelium of Sea Bass	61
15 Lesion Left by Escaped Miracidium in Gill Epithelium	62
16 Inflammatory Reaction Induced by Presence of Multiple Miracidia in One Location	62
17 Haemorrhage and Inflammation around Multiple Miracidia in Gill Filament	63
18 Eggs of <i>C. lates</i> Inside Afferent Filamentary Artery of Sea Bass	64
19 Miracidium in Ventricle of Heart Surrounded by Macrophages	65
20 Dead Miracidium in Ventricle of Heart	65
21 Necrotic Eggs and Dead Juvenile Worm in Pancreatic Tissue of Sea Bass	67
22 Pigmented Macrophage Aggregate Surrounded on Two Sides by Necrotic Eggs of <i>C. lates</i>	67
23 Replacement of Pancreatic Tissue in Mesentery by <i>C. lates</i> Eggs	68
24 MMCs, Melanomacrophages and Necrotic <i>C. lates</i> Eggs in Head Kidney of Sea Bass	70
25 Formation of Apparent MMCs in Caudal Kidney of <i>L. calcarifer</i>	70

LIST OF ABBREVIATIONS

ant. - anterior
c - caeca
ci - cirrus
co - anterior commissure
cp - cirrus pouch
EGC - eosinophilic granular cell
F. - female genital pore
FCR - food conversion ratio
fp - female pore
g - gland cells
M. - male genital pore
m - Mehlis' gland
MMC - melanomacrophage centre
n - nerve canal
o - ovary
oe - oesophagus
ov - oviduct
oo - ootype
PER - protein efficiency ratio
post - posterior
s - sperm
sd - sperm duct
sp - spine
sv - seminal vesicle
t - testis
u - uterus
ue - uterine egg
 μ - micrometre
v - vitellaria
vd - vitelline duct
vr - vitelline reservoir

Abstract of thesis submitted to the Senate of
Universiti Pertanian Malaysia in partial fulfilment of
the requirements for the degree of Master of Science.

**A SANGUINICOLID BLOOD FLUKE IN SEA BASS (*LATES³ CALCARIFER*
BLOCH) IN COASTAL PENINSULAR MALAYSIA**

By

BRETT W. HERBERT

June 1992

Chairman: Dr. Faizah Shaharom

Faculty: Fisheries and Marine Science

Cruoricola lates N. Gen., N. Sp. from the blood vessels of cultured sea bass (*Lates calcarifer* Bloch 1790) is described. It is a lanceolate sanguinicolid with a single column of submarginal, ventral spines; extensive vitellarium; and X-shaped intestine. The single, lobed testis extends laterally to the caeca. The cirrus lies dorsal to the spherical seminal vesicle. Auxiliary seminal vesicle present. The uterus is post-ovarian, partly situated between the lobes of the ovary; mid-portion is thick. *Cruoricola lates* N. Gen., N. Sp. is found in all sea bass over 15g weight in the type locality, Pulau Ketam.

The adults of *C. lates* primarily inhabit the venous circulation of *Lates calcarifer*. Eggs were found in the kidney, liver, ventricle of the heart and gills of all fish examined three months after stocking.

Cruoricola lates eggs in tissues evoke a cellular immune response consisting of encapsulation by either activated macrophages and/or endothelial cells. In the heart this is accompanied by macrophage infiltration. In the kidneys, encapsulation of eggs is followed by pigment deposition in and around the capsule. The main foci of pathological effect are the pancreatic acinar tissue, head kidney, and intertubular caudal kidney tissue. *Cruoricola lates* egg deposition in these tissues may have a negative effect on growth through reduction in food conversion ratio and depression of immunological capability.

Haematological parameters (haematocrit, serum protein, plasma protein) were so variable that no relationship between them and infection with blood flukes could be described.

Cruoricola lates was present in sea bass culture sites sampled in Penang, Johore, Pahang and Terengganu. Kelantan sites appeared not to have high incidence of infection, probably due to the freshwater influence. Intensity and prevalence of infection appear to increase with intensity of culture.

Histological and dissection techniques are complementary in giving a comprehensive picture of the location of worms and eggs in the host. As *Cruoricola lates* is readily available, and as sea bass are easily maintained under laboratory conditions, there is wide scope for further studies on this worm and its relationship with the host.

Abstrak tesis yang dikemukakan kepada Senat Universiti Pertanian Malaysia, sebagai memenuhi sebahagian daripada keperluan untuk mendapat Ijazah Master Sains.

FLUK DARAH SANGUINICOLID DALAM IKAN SIAKAP (*LATES CALCARIFER*) DI PANTAI SEMENANJUNG MALAYSIA

oleh

BRETT W. HERBERT

Jun 1992

Pengerusi: Dr. Faizah Shaharom

Fakulti: Perikanan dan Sains Samudera

Morfologi *Cruoricola lates* N. Gen., N. Sp. daripada saluran darah ikan siakap ternakan (*Lates calcarifer* Bloch 1790) dihuraikan. Ia adalah fluk darah sanguinicolid yang berbentuk daun dengan satu deretan spina bawah pinggiran di bahagian ventral, vitelarium yang luas dan usus berbentuk silang. Testis tunggal berlobus memanjang kebahagian sisi sekum. Sirus memanjang di bahagian dorsal vesikel sperma yang berbentuk sfera. Terdapat vesikel sperma auksiliari. Uterus terdapat di bahagian belakang ovari dan sebahagiannya terletak antara lobus ovari; bahagian tengah uterus adalah tebal. *Cruoricola lates* dijumpai dalam semua ikan siakap yang beratnya melebihi 15 gram di lokasi tertentu iaitu Pulau Ketam, Selangor. *Cruoricola*

lates yang matang tinggal di dalam peredaran vena *Lates calcarifer*. Telur dijumpai di dalam ginjal, hati, ventrikel jantung dan insang pada semua ikan yang diperiksa tiga bulan selepas pelepasan. Telur *C. lates* dalam tisu merangsangkan tindakan imun selular iaitu termasuk pengkapsulan sama ada oleh makrofaj teraktif dan/atau sel endotelium. Di dalam jantung tindakan ini disertai dengan penyusupan makrofaj. Di dalam ginjal pengkapsulan telur diikuti oleh endapan pigmen di dalam dan di sekeliling kapsul. Kesan patologi terutamanya terdapat di tisu asinar pankreas, kepala antara tubul ginjal dan tisu ginjal kauda. Ada kemungkinan telur *C. lates* di dalam tisu ini mempunyai kesan negatif ke atas pertumbuhan *L. calcarifer* melalui pengurangan dalam nisbah pertukaran makanan dan penurunan keupayaan keimunan. Parameter hematologi (hematokrit, protein serum, protein plasma) sangat berbeza hingga perhubungan antara parameter ini dan jangkitan fluk darah tidak dapat dihuraikan.

Cruoricola lates terdapat di dalam ternakan ikan siakap yang disampel dari Pulau Pinang, Johor, Pahang dan Terengganu. Kawasan di Kelantan tidak mempunyai insiden jangkitan yang tinggi mungkin disebabkan oleh pengaruh

air tawar. Keamatan jangkitan meningkat dengan peningkatan keamatan kultur ikan. Teknik histologi dan pembedahan adalah saling membantu dalam membuat gambaran menyeluruh tentang lokasi cacing dan telur dalam perumah. Oleh kerana *C. latens* mudah diperolehi dan ikan siakap mudah disimpan di dalam makmal maka terdapat skop yang besar bagi kajian lanjut tentang cacing ini dan perhubungannya dengan perumah.