THE DEVELOPMENT OF LIGHT ORGAN AND BACTERIAL INFECTION IN SLIP-MOUTHS (LEIOGNATHIDAE)

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DECLARATION

I, hereby declare that this dissertation is my original writing, except the data, notes and facts that have been already stated with its sources and origins.

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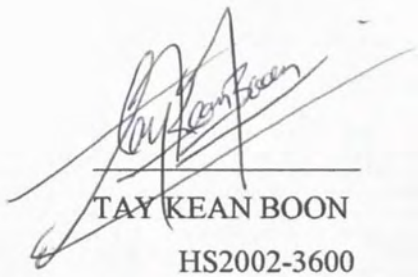

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ABSTRACT

To date, there are no previous studies to describe the development of the light organ in slip-mouths and at the stage at which bacterial infection occurs. Thirty four juveniles of *Leiognathus rapsoni*, a new record for Sabah, were captured by using modified triple layering gill net at Karambunai Lagoon (NE 06° 04' 39.5", E 116° 06' 59.7") for this investigation. Six juveniles of *Leiognathus splendens* were bought from roadside along Jalan Sulaman for culturing the bacterial presence in a leiognathid. Light organs from six of *L. rapsoni* (3.8 to 5.2 cm SL) were histologically examined. Results show that the light organ increases in height, width and thickness with increasing size of the leiognathids (3.8 to 5.2 cm SL). Furthermore, the pattern of development of the light organs might develop in two different ways. There are single sided growing pattern with a ring-liked surrounding, and dual sided growing pattern with a croissant-liked surrounding. All *L. splendens* juveniles (6.9 to 7.9 cm SL) have bacteria, *P. leiognathi*, that are gram negative. It is apparent from the results that the light organ in *L. rapsoni* develops and gets infected by *P. leiognathi* in individuals smaller than 3.8 cm. Since luminous bacteria can be bioindicators of the water quality, these findings indicate that the Karambunai Lagoon is not polluted by toxicants.

ABSTRAK

Sehingga kini, tiada kajian menghuraikan pembangunan organ bercahaya dalam slip-mouths dan daripada peringkat mana infeksi bakteria berlaku. Tiga puluh empat juvenil *Leiognathus rapsoni*, catatan sebuah rekod baru bagi Sabah, telah ditangkap dengan menggunakan sebuah pukat insang berlapisan tiga yang telah dimodifikasi di Teluk Karambunai (NE 06° 04' 39.5", E 116° 06' 59.7") untuk pemeriksaan. Enam juvenil *Leiognathus splendens* telah dibeli daripada tepi jalan sepanjang Jalan Sulaman untuk penentuan kehadiran kultur bakteria dalam leiognathid. Organ bercahaya daripada enam individu *L. rapsoni* (3.8 hingga 5.2 cm SL) telah dijalani pemeriksaan histologi. Keputusan menunjukkan bahawa organ bercahaya bertambah dalam ketinggian, lebar dan ketebalan dengan pertambahan saiz leiognathid(3.8 hingga 5.2 cm SL). Tambahan ini, corak perkembangan organ bercahaya mungkin berkembang dalam dua jenis yang berlainan, iaitu corak pertumbuhan sisian-bujang dengan lingkaran bentuk cincin, serta corak pertumbuhan sisian-dedua dengan lingkaran bentuk roti berbentuk anak bulan. Semua juvenil *L. splendens* (6.9 to 7.9 cm SL) mempunyai bakteria *P. leiognathi* yang bergram negatif. Daripada keputusan yang diperolehi, adalah jelas bahawa organ bercahaya dalam *L. rapsoni* berkembang dan dapat infeksi oleh *P. leiognathi* dalam individu yang lebih kecil daripada 3.8 cm. Oleh disebabkan bakteria yang bersinar boleh menjadi sebuah penunjuk biologi bagi kualiti air, maka kajian ini telah menunjukkan bahawa Teluk Karambunai adalah bebas daripada pencemaran toksik.

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LIST OF SYMBOLS

%	percentage
°C	degree of celcius
g	gram
ml	mililitre
m	metre
cm	centimetre
mm	milimetre
µm	micrometer
s	second(s)
min	minute(s)
h	hour(s)
kPa	kilo Pascal
kCal	kilo Calories
<	less than
>	more than

LIST OF ABBREVIATIONS

Approx.	approximately
SL	standard length
SD	standard deviation

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND

Natural bacteria in the marine environment are important to the ecology of fishes but this natural population may be disturbed by pollution. Slipmouths (Leiognathidae), which often known as ponyfish are bioluminescent fishes, having a culture of bioluminescent fishes in a light organ around the esophagus. The bacteria is from the natural, free-living *Photobacterium leiognathi* (Nealson and Hastings, 1979). No study has been done on the development of the light-organ and culture of *Photobacterium leiognathi.*

Leiognathidae is mainly habitat marine though some species enter freshwater. It occurs in estuarine and coastal waters of the Indo-West Pacific (Pauly, 1976). It feeds on benthic invertebrates, therefore, it is commonly found in shallow coastal waters and tidal creeks. It can be easily caught by gill net during adult, nevertheless, obviously not so easy juvenile. Thereafter, Karambunai Lagoon, Sabah is to be chosen to set as a spot (divided into several stations-close or mid of sea grass region) to collect specimens.

Karambunai Lagoon suits the best to all required factors for habitat and living environment for Leiognathidae.

1.1.1 The Development of Light Organ of Leiognathids

Of the more than 30 families of bioluminescent marine fishes, a dozen or more rely on luminous bacteria for light production (Nealson and Hastings, 1979). Among these bacterially bioluminescent fishes, leiognathids is found sufficiently abundant and accessible that extensive behavioral studies of their luminescence are feasible. Bioluminescent in leiognathids is produced by an apparently pure culture of *Photobacterium leiognathi* (Hastings and Mitchell, 1971) housed in a circumesophageal, gland-like light organ located just anterior to the stomach. This internal position of the light organ allows for the incorporation of other parts of the fishes' anatomy in the expression and control of luminescence. Proximal control of light expression is by the operation of muscular shutters on the light organ itself. Therefore, to study the slowly development of the light organ, histology method is in use.

1.1.2 The Development of Bacterial Infection in Leiognathids

Symbiotic luminescence is known in many species of fishes in shallow marine environments. These unique bacteria-animal interactions are unusual in that the hosts harbor monospecific cultures of one species of luminous bacteria at high density as their light source in specialized organs. To maintain this specific interaction between the hosts

and their symbiotic bacteria over ensuing generations, the symbiotic must be passed on to the offspring with fidelity (Haneda and Tsuji, 1976). In this project, the colonization and establishment of the bacterial population within the light organ are to be directly assessed by Gram staining from the larval to juvenile leiognathids.

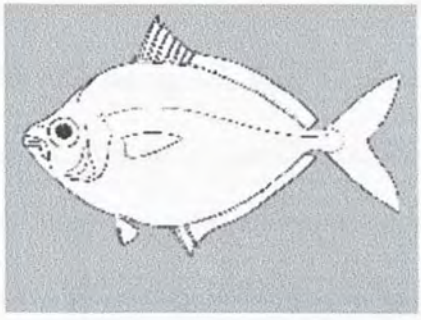

Figure 1.1 Draft of ponyfish (Shiino, 1976)

Table 1.1 Taxonomic Hierarchy

Family	Leiognathidae	-- ponyfishes
Genus	Gazza	
Genus	Leiognathus	
Genus	Secutor	

(Shiino, 1976)

1.2 OBJECTIVES

There are mainly two objectives to this project: firstly, to describe the development of light organ in slipmouths (Leiognathidae); and secondly, to determine when bacterial (*Photobacterium leiognathi*) infection occurs.

By having these two objectives succeeded, there is one method for field work, and two methods of laboratory work have to be carried out. In field work, specimens have to be captured by sampling. Sampling consist three basic steps, briefly, vary net capturing, data labeling and formalin preserving. Whereas, in laboratory work, methods of histology (Samoilys and Roelofs, 2000) and Gram staining (Standard procedure introduced by Brock, 2000) are to be chosen to describe and determine the objectives. These two methods are closely guided and followed by standard procedures.

1.3 HYPOTHESIS

There are two sets of hypotheses: hypothesis null and two hypothesis alternatives. Whereby, first hypothesis is that the light organ develops slowly in the out-pocketing of the esophagus, with the hypothesis alternative of the light organ does not develop slowly in the out-pocketing of the esophagus. Secondly, the hypothesis null is that the bacterial infection occurs at 5mm long in leiognathidae, with the hypothesis alternative of the bacterial infection does not occur at 5mm long in Leiognathidae.

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