Diseases of Asian seabass (or barramundi), *Lates calcarifer* Bloch

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Declaration

I declare that this thesis is an account of my research and contains work which has not been previously submitted for a degree at any tertiary education institution. Contributions by co-authors have been duly acknowledged.

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Preface

Chapter 1 serves as a brief introduction to husbandry practices and diseases previously reported in cultured *Lates calcarifer*. It also includes a section on the interactions between the host, environment and pathogens which need to be considered in the investigation and managing of disease outbreaks. Chapters 2 to 4 are based on published papers while Chapter 5 is a manuscript intended for publication in a scientific journal. There has been a need to adapt the chapters based on published papers to integrate them into a thesis. Chapter 6 discusses management strategies in relation to specific diseases in *L. calcarifer* at the htachery, nursery and growout levels. Citations style is in keeping with that used in Journal of Fish Diseases. Citations with more than 2 authors are quoted in full when it appears in text within each chapter for the first time, and thereafter only as first author & *et al.*

Abstract

Other than the study by Griffiths (2009) on gill diseases, there has been no comprehensive study and report on the major diseases of Asian seabass (or barramundi) *Lates calcarifer* Bloch. It is a food fish species of growing importance in Asia and Australia. This study investigates some of the major diseases encountered in the various stages of the culture of *L. calcarifer,* at the histopathological, ultrastructural and molecular levels. Culture practices can have significant impacts on fish health. Disease outbreaks are influenced by factors involving the host, environment and pathogen. Current knowledge on diseases of *L. calcarifer*, and these factors which may influence disease outbreaks are discussed in Chapter 1.

This is the first report of an intestinal *Eimeria* infection in *L. calcarifer*. The *Eimeria* infection was associated with severe pathology and significant mortality in the absence of other pathogens. It was detected in diseased L. calcarifer in all five nurseries in Ca Mau, Vietnam. Although these were small scale nurseries which stocked an average of 3000 to 5000 fish at any one time, a mortality rate of up to 30% was reported and is the cause of significant economic losses for these nurseries. Moderate to heavy *Eimeria* infestation were observed in greater than 80% of diseased fish examined. This high rate of *Eimeria* infestation is suspected to be linked to the low daily water exchange rates practised in these nurseries. However, the examination of only diseased fish does not allow the determination of prevalence. A systemic iridovirus infection was concurrently observed in some of the fishes but was not consistently present when compared to the *Eimeria* infection. Molecular analysis showed that the *Eimeria* of *L. calcarifer* from Vietnam formed clades with the *Eimeria* detected in

L. calcarifer cultured in Australia, but clustered separately from other known *Eimeria species*. Although *Cryptosporidium* was detected in these *L. calcarifer* tissues, it could not be demonstrated histologically or ultrastructurally, suggesting a low grade infestation or perhaps an environmental contaminant in fish tissues tested. *In situ* hybridization using labeled PCR products showed that labeled DNA probes generated from 18S PCR products could not be used to distinguish between closely related genera such as *Cryptosporidium* and *Eimeria*. Future investigation to determine the origin, transmission and risk factors associated with this *Eimeria* infestation in *L. calcarifer* are needed.

'Scale drop syndrome' is a novel disease first reported in *L. calcarifer* in Penang, Malaysia in 1992. Cases with similar gross and clinical presentations were observed in Singapore in 2002, 2006 and 2009. Affected fish have loose scales, which dropped off easily when handled. The disease was initially observed in 100-300g fish, and later in larger fish up to 5kg bodyweight. Cumulative mortalities of 40 to 50% were reported by farms, posing significant economic losses of larger more valuable fish. This investigation forms the first pathological description of 'scale drop syndrome' (SDS) in *L. calcarifer*. To aid recognition of new cases for study, a case definition was developed for 'scale drop syndrome' in *L. calcarifer* as a systemic vasculitis associated with tissue necrosis in all major organs including the skin, with apparent targeting of cells of epithelial origin. Attempts to isolate or detect the causative agent(s) by cell culture, PCR and immunohistochemistry have proven unsuccessful. Further studies to elucidate the definitive aetiology, isolate the causal agent(s) and reproduce the disease will help better understanding and control of SDS.

Although systemic iridoviral disease has been previously reported in many freshwater and marine fish species, this study forms the first report of this disease in *L. calcarifer*. Systemic iridoviral disease was observed in 5 to 20g *L. calcarifer* usually 2 to 3 weeks post-transfer into sea cages at two farms. Inclusion bodies suggestive of a systemic iridovirus infection were observed in clinically healthy *L. calcarifer* from the land-based nursery of one of these two farm; the presence of an iridovirus infection was supported by positive PCR results using Red Sea bream iridovirus (RSIV) primer 1. The presence of inclusions was not accompanied by any tissue necrosis in these clinically healthy fish. This finding suggested that the systemic iridovirus infection occurred before stocking at sea, and did not originate from wild fish or older fish in adjacent sea cages as initially suspected by this farm. Immunohistochemistry on tissues of clinical cases of systemic iridovirus gave positive results using the Red Sea bream iridovirus monoclonal antibody (RSIV M10), although intensity varied between tissues, possibly related to varying exposure of different tissues to fixation chemicals. Inclusion bodies in clinically healthy fish from the same farm did not show positive reaction with RSIV M10. This may be due to a lack of antigenic expression by the viral infected cells at this early stage of infection.

Viral nervous necrosis (VNN) is a serious disease of hatchery reared *L. calcarifer* fry in this study. Mortalities of 50 to 100% were reported in 3wo fry. VNN can be difficult to diagnose in older fry, where it can be associated with few vacuolations or an absence of viral inclusions

'Pot belly disease' (PBD) was previously reported in *L. calcarifer* fry less than 1g, in association with an intracellular coccobacillus infection and mortalities of 80 to 100%. In this study, PBD was observed in 120g *L. calcarifer*

at two sea cage farms, in association with significant granulomatous enteritis. The extent of the granulomatous enteritis is likely to have an effect on affected fish. It was observed concurrently with systemic iridoviral disease at one farm and nocardiosis at another farm. Diagnosis by histopathology and the lack of other confirmatory tests for PBD may result in underdiagnosis of this disease. The epidemiology of PBD needs further study to establish origin and modes of transmission, to facilitate better disease control.

Diseases associated with infections by ubiquitous bacteria such as *Vibrio*, *Tenacibaculum* were commonly observed in *L. calcarifer* post-handling. Tenacibaculosis and vibriosis often occurred concurrently with other diseases such as streptococcosis, systemic iridviral disease or PBD. Streptococcosis can affect fish up to 3kg bodyweight, resulting in significant mortalities greater than 40 to 50%. Like SDS, because streptococcosis can affect up to market size fish, they can cause considerable economic losses. Although vaccines against Streptococcosis are available, conflicting views are held on the efficacy of *Streptococcus* vaccines by various research groups. Overall, the South-east Asian *L. calcarifer* farms which practiced vaccination against *Streptococcus iniae* reported a reduction of mortality, especially in fish greater than 1 to 1.5kg bodyweight.

Nocardiosis has been reported as an emerging disease in marine food fish species caused by acid fast filamentous branching bacterium. Although nocardiosis was observed histopathologically in *L. calcarifer* at two sea cage farms, the numbers of samples examined were small and no other tests were attempted due to lack of suitable samples. More intensive and extensive study is needed to determine the significance of nocardiosis in *L. calcarifer*. Chronic

granulomatous enteritis was not uncommon in the cases submitted to the Fish Health Laboratory in Perth. Although the peritonitis was associated with heavy bacteria infection, it is unclear if these are secondary invaders. Schipps, Bosmans & Humphreys (2009) reported that *Vibrio harveyi* and *Photobacterium damsela damsela* vaccinations appeared to be not efficacious, suggesting that these bacteria were not the primary cause of the disease.

It is well recognized that disease outbreaks in farmed fish are influenced by the interaction between host, the environment and pathogens. While serious diseases are often reported in association with specific aquatic pathogens, not much is known about the risk factors which trigger fish disease outbreaks. Disease outbreaks often occur after stressful events such as net transfers, recent handling or poor water quality. In fact, diseases are often caused by ubiquitous pathogens that are commonly present in the culture environment. Although further research is necessary to gather more information to improve diagnosis and management of specific diseases, general health management strategies can be applied at the various stages in the culture of *L. calcarifer* to minimize disease outbreaks. This is discussed for *L. calcarifer* in Chapter 6.

Observations of types of disease agents may be influenced by site conditions or the types of tests or materials examined. For example, some parasites may be more prevalent in certain sites where intermediate hosts abound, or loosely attached ectoparasites may be lost unless wet mount microscopic examinations of fresh tissues were carried out. The study of emerging diseases such as scale drop syndrome (SDS) or pot belly disease (PBD) in *L. calcarifer* has been hampered by lack of confirmatory diagnostic tools and inadequate knowledge on critical epidemiological factors such as mode of

transmission or potential reservoirs. While ideally identification and isolation of the causal agent will help fulfil Koch's postulates, it may be possible to improve the understanding of disease via cohabitation or infectivity trials using tissue homogenates from diseased fish when pure isolates are not available. There is a need to conduct research to not only establish a definitive aetiology, but also to identify risk factors to facilitate successful disease control. The successful management of disease in aquaculture does not lie in any one strategy but an integrated management of all risks encountered during the culture cycle against disease occurrence or incursions.

Table of Contents

Legends of Figures

Lists of Tables

List of abbreviations

AP	alkaline phosphatase
DIG	dioxygenin
DO	dissolved oxygen
FFPE	formalin fixed paraffin embedded
IHC	immunohistochemistry
ISH	*in-situ* hybridization
PBD	pot belly disease (or big belly)
ppm	Parts per million
ppt	Parts per thousand
RSIV	Red Sea bream iridovirus
SDS	scale drop syndrome
SSC	saline sodium citrate
TBS	tris-buffered saline
VNN	viral nervous necrosis