COMPARATIVE STUDY ON GROWTH AND *VIBRIO* VARIATION BETWEEN NORMAL AND STUNTED GROWTH IN POND-CULTURED WHITELEG SHRIMP, *Litopenaeus vannamei*

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DECLARATION

I hereby declare that the material in this thesis is my own except for quotations, equations, summaries and references, which have been duly acknowledged.

4 October 2021

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CERTIFICATION

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DEGREE	:	**MASTER OF SCIENCE**
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VIVA DATE	:	**4 OCTOBER 2021**

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ABSTRACT

The occurrence of stunted growth in *Litopenaues vannamei* culture causing large size variation in harvested shrimps which may lead to huge profit loss. Despite causing concerns to farmers, detailed information of stunted growth in *L. vannamei* in relationship to its growth and *Vibrio* composition was limited. Normal and stunted growth shrimp were collected from shrimp ponds in Tuaran, Malaysia. Further investigation of the length-weight relationship and *Vibrio* variation between normal and stunted growth shrimp were conducted. Statistical analysis showed that size variations were present in all age groups between normal shrimps and stunted shrimps. The relationship between the length and weight was calculated using the equation $W = aL^b$. The regression coefficient (b value) for normal shrimp was 2.92 which not significantly less than 3.00 indicating isometric growth pattern. Meanwhile, positive allometric growth pattern was observed in stunted growth shrimp with b value of 3.41. The coefficient of correlation (r value) of normal shrimp and stunted shrimp was 0.95 and 0.94, respectively. No significant difference was found regarding the total bacteria count and the total *Vibrio* count between normal and stunted growth shrimp. However, the tests result revealed that *Vibrio* isolates obtained from stunted shrimp showed higher variation of phenotypic characteristics compared to isolates from normal shrimp. The finding in this study also indicates that stunted growth shrimp might have more diverse and complex bacterial variation than the normal shrimp. This present study managed to provide information on the size difference and length-weight relationship of normal and stunted growth *L. vannamei*. Therefore, this opens up the opportunity to conduct more research to find out the possible cause of stunted shrimp problems.

ABSTRAK

KAJIAN PERBANDINGAN MENGENAI PERTUMBUHAN DAN VARIASI VIBRIO PADA UDANG PUTIH, LITOPENAEUS VANNAMEI, TERNAKAN KOLAM YANG NORMAL DAN TERBANTUT PERTUMBUHAN

Masalah udang dengan pertumbuhan terbantut dalam pengkulturan L. vannamei menyebabkan variasi saiz yang besar dan boleh mengakibatkan kerugian. Walaupun keadaan ini menyebabkan masalah kepada penternak, maklumat terperinci mengenai pertumbuhan terbantut pada L. vannamei dan kaitannya dengan kandungan dan variasi bakteria Vibrio adalah terhad. Sampel udang normal dan udang terbantut diperoleh dari kolam udang yang terletak di daerah Tuaran, Malaysia. Selain itu, hubungan antara panjang dan berat udang serta komposisi bakteria Vibrio antara udang normal dan udang terbantut telah dikaji. Analisis statistik menunjukkan bahawa terdapat variasi saiz di antara udang normal dan udang terbantut dalam kesemua kumpulan umur. Hubungan antara panjang dan berat udang dihitung menggunakan persamaan, $W = aL^b$*. Pekali regresi (nilai b) bagi udang normal ialah 2.92, menunjukkan pola pertumbuhan isometrik kerana tidak berbeza secara signifikan dengan nilai 3.00. Manakala udang terbantut menunjukkan pola pertumbuhan alometrik positif kerana nilai 3.41 adalah berbeza secara signifikan dengan nilai 3.00. Pekali korelasi (nilai r) bagi udang normal ialah 0.95 dan 0.94 bagi udang terbantut. Tidak ada perbezaan yang signifikan di antara jumlah bakteria dan jumlah Vibrio yang terdapat pada udang normal dan udang terbantut. Walau bagaimanapun, keputusan ujian menunjukkan bahawa Vibrio yang diperoleh daripada udang terbantut mempunyai lebih banyak variasi dari segi karakter fenotip berbanding udang normal. Kajian ini juga menunjukkan bahawa udang terbantut mungkin mempunyai variasi bakteria yang lebih komplex dan pelbagai berbanding udang normal. Kajian ini dapat menyediakan informasi mengenai perbezaan saiz dan perhubungan di antara panjang dan berat pada L. vannamei yang normal dan terbantut. Oleh itu, terdapat lebih banyak keperluan untuk menjalankan penyelidikan bagi mengkaji punca masalah pertumbuhan terbantut dalam udang.*

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AHPND	- Acute hepatopancreatic necrosis disease
BLAST	- Basic Local Alignment Search Tool
CFU	- Colony forming unit
cm	- Centimetre
CV	- Coefficient of variation
DAR	- Days after restocking
DO	- Dissolved oxygen
DOF	- Department of Fisheries
EHP	- Enterocytozoon hepatopenaei
EMS	- Early mortality syndrome
FAO	- Food and Agriculture Organization
g	- Gram
ha	- Hectre
HP	- Hepatopancreas
HPM	- Hepatopancreatic microsporidiosis
ICMSF	- International Commission on Microbiological Specifications for Foods
IHHNV	- Infectious hypodermal and hematopoietic necrosis virus
IMNV	- Infectious myonecrosis virus
kg	- Kilogram
l	- Litre
m	- Metre
mg	- Miligram
N	- Number
PL	- Postlarvae
ppt	- Parts per thousand
RDS	- Runt Deformity Syndrome
rpm	- Revolutions per minute
SD	- Standard deviation
sp.	- Species
SPF	- Specific pathogen free
SPSS	- Statistical Program for the Social Sciences
subsp.	- Subspecies
TCBS	- Thiosulfate citrate bile salts sucrose
TSA	- Tryptic soy agar
TSB	- Tryptic soy broth
TSV	- Taura syndrome virus
WFS	- White feaces syndrome
WSD	- White spot disease
WSSV	- White spot syndrome virus

LIST OF APPENDICES

CHAPTER 1

INTRODUCTION

1.1 Background and rational

Traditionally, human has been getting their source of marine based protein from the sea or other natural waters by fishing (Clydesdale and Francis, 1985) .However, the catch of most fisheries products from the ocean and other natural waters has met or surpassed the sustainable level but the human population, on the other hand, continues to grow, resulting in increased demand for fisheries products (Coll *et al*., 2008). Thus, aquaculture, or farming of aquatic animals, is important to meet current and potential demand for fisheries products (Junda, 2018).

According to the Fisheries and Aquaculture Organization (FAO) (2018), shrimp aquaculture is one of the most profitable sectors of the global aquaculture industry. Shrimp are often deemed as a luxury choice of protein source by consumers due to its unique texture and delicious taste (Datta *et al*., 2010). China, India, and Southeast Asian countries such as Thailand, Vietnam, and Indonesia contributed the majority of shrimp aquaculture production (Fathi *et al*., 2018; Wati, 2018). Meanwhile, Ecuador has demonstrated spectacular growth in shrimp farming in Latin America (Lightner, 2011).

The FAO reported there are 11 species of penaeid shrimps produced through aquaculture in 2015, but the majority of the production came from two species: *Litopenaeus vannamei* (whiteleg shrimp) and *Penaeus monodon* (black tiger shrimp). The black tiger shrimp, *Penaeus monodon* was the largest cultivated species for shrimp farming before 2001 (Thitamadee *et al*, 2016). However, white spot disease (WSD) outbreak in black tiger shrimp culture has impacted negatively on shrimp farming industry (Flegel, 2012). This viral infection was caused by white spot syndrome virus (WSSV) (Lotz, 1997). Ravichandran (2012) stated that due the outbreak, shrimp farmers began to culture whiteleg shrimp. Currently, the most cultured species in shrimp aquaculture is the whiteleg shrimp (Poornima et al., 2012).

In 2019, *L. vannamei* accounted for 52% of the global crustacean aquaculture production (FAO, 2021). The shrimp aquaculture industry was transformed by the introduction of cultivated and genetically enhanced, also known as specific pathogen free (SPF) *L. vannamei* has replaced *P. monodon* as the preferred shrimp species for cultivation due to its higher resistance to white spot syndrome virus (Liao and Chien, 2011). Additionally, the availability of specific pathogen free (SPF) broodstock and post-larvae facilitated shrimp farmers' transition to *L. vannamei* culture (Ravichandran, 2012). Their tolerance for a wide range of salinity and temperature allowed them to be cultured inland and in a multitude of seasons (Zhang *et al*, 2006). *L. vannamei* also can be cultured at higher densities, enabling farmers to cultivate them intensively (Tantu *et al*., 2020).

The shrimp farming industry in Malaysia likewise displayed the same trend as the worldwide shrimp aquaculture industry. *L. vannammei* culture was introduced to Malaysia in 2001 (Manan *et al*, 2015). After a few cycles of cultivation, culture output was stated to have increased significantly; in 2005, whiteleg shrimp production was 11497 tonnes, but increased to 18601 tonnes in 2006, surpassing black tiger shrimp production (Kua *et al*., 2018). According to a report from Malaysia Department of Fisheries (DOF) (2010), whiteleg shrimp harvest totalled 69,084 tonnes in 2010, more than 50 times that of black tiger shrimp, showing that whiteleg shrimp is a more suitable shrimp species to culture. *L. vannamei* was observed to grow faster than *P. monodon*, and to have a higher survival rate (Liao and Chien, 2011). Three or four crops a year are generally achievable since each crop takes just 80-90 days to reach harvest size (Sandifer *et al.*, 1987).

Unfortunately, a few years after the introduction of *L. vannamei* in Southeast Asian shrimp culture industry, viral infection from Taura syndrome virus (TSV), infectious hypodermal and haematopoietic necrosis virus (IHHNV) and infectious myonecrosis virus (IMNV) were reported from shrimp farms in Indonesia, Vietnam and Thailand and soon other neighbouring countries (Flegel, 2012). Besides that, a few years after the outbreak of viral disease, a new shrimp disease initially known as early mortality syndrome (EMS) began affecting *L. vannamei* farms. This disease was first detected in China in 2009 (Sriurairatana *et al*, 2014). Affected shrimps would appear to be lethargic, anorexic and have slow growth and eventually mortalities. A study found out that the causative agent for this disease was a distinctive strain of *Vibrio parahaemolyticus*, which could produce toxin. The toxin released by this bacterium would cause massive sloughing of the epithelial cells in shrimp's hepatopancreatic tubule. Due to this frequent symptom observed on infected shrimps, the condition was now commonly referred as acute hepatopancreatic necrosis disease (AHPND) (Tran *et al.*, 2013). *L. vannamei* farms in several states in Malaysia begin to report the occurrence of AHPND in 2011, and a study revealed that the infected shrimps showed presence of *Vibrio* species, particularly *Vibrio parahaemolyticus* as well as *Photobacterium damselae* (Kua *et al.*, 2018).

Throughout the years, the pattern in worldwide aquaculture industry is for a severe, unknown disease to emerge every three to five years or so, spread quickly, and then result in significant yield loss (Bondad-Reantaso, 2016). As shown in the epidemic situation for AHPND, it often took a prolonged period, usually years for the animal health experts and the industry to study the disease, identify and confirm the related pathogen and develop a risk management measures; before severe mortality cases or severe loses were observed in the field (Bondad-Reantaso, 2016). Currently, the problem of stunted growth shrimp in *L. vannamei* culture is displaying the possibility of following such pattern.

In recent years, there have been an increasing reports of smaller shrimp sizes in production, causing large size variation of shrimps in one crop. According to a review from Anderson *et al.* (2019), the proportion of small-sized shrimp in harvest yield rose from 27% in 2010 to 48% in 2017. While the percentage of small counts declined to 37% in the most recent study, it remains higher than the percentages recorded prior to 2011 (Anderson et al., 2019). Growth retardation in shrimp development was seen as an effect from pathogenic agents such as bacterial *Vibrio* causing Acute Hepatopancreatic Necrosis Disesase (AHPND), infections of *Enterocytozoon hepatopenaei* (EHP) parasites or viral pathogens (Aranguren *et al*., 2017; Fang *et al*, 2019). However, affected farms reported very few mortality cases and shrimps with this condition survive until harvest, leading to economic losses (Kooloth-Valappil *et al*., 2021). Despite causing concerns to shrimp farmers since it affects the production yield causing profit loss, there are currently few studies that reported detailed information on the features of the affected *L. vannamei*. The study on its growth or length and weight analysis in relation to bacterial loads should be gathered and processed in order to do an in-depth analysis.

The occurrence of stunted growth was recently linked to stunted growth affected by the *Enterocytozoon hepatopenaei* (EHP) parasite (Tang *et al*., 2015). However, possible association of *Vibrio* and viruses was also thought to contribute upon the occurrence of stunted growth shrimp. The *Vibrio* spp. is a common bacteria found in shrimp and are known to cause diseases in shrimp (de Souza Valente and Wan, 2021). It is well reported that the pathogen responsible for vibriosis was highly associated with acute hepatopancreatic necrosis disease (AHPND), a disease that can possibly cause slow growth to shrimp (Hong *et al*., 2016). In other cases, viral infections were previously discovered leading to the slow or stunting growth of *Penaeus* species (Flegel, 1997; Flegel *et al*., 2004). In our case, stunted shrimp did not show diseased conditions related to viral infections upon inspection. Therefore, the first information that should be retrieved in this case is the bacterial variation of stunted growth *L. vannamei* that needs to be further studied since literature was limited.

Despite causing concerns to shrimp farmers and creates problems in their farming operations, there are currently few studies that reported detailed information on morphology and features of the affected *L. vannamei*, as well as the study on its growth or length and weight analysis. Such data should be gathered and processed in order to do an in-depth analysis. Following that, further studies into the causative agents may be done and to identify solutions and preventative measures.

Currently, information on the bacterial variation of stunted growth *L. vannamei* is scarce and thus need to be studied more. This research aims to establish a clear distinction between normal and stunted shrimp in terms of weight and length, as well as to determine and compare the length-weight relationship of normal and stunted shrimp, and to investigate the bacterial aspects, specifically the variation of *Vibrio* species in stunted growth shrimp and its comparison to normal shrimp. This study's results would also contribute to our understanding of the characteristics of stunted growth shrimp in *L. vannamei* culture.

1.2 Objectives of research

The objectives of this study are:

1. To determine the size difference between normal and stunted growth whiteleg shrimp
2. To determine the length-weight relationship of normal and stunted growth whiteleg shrimp
3. To evaluate the *Vibrio* species variation in normal and stunted growth whiteleg shrimp

1.3 Scope of research

This study was basically focused on:

1. Collecting baseline data of growth of normal shrimp in one production cycle (0 day after stocking to 105 days after stocking)
2. Analysing the length-weight relationship of normal shrimp and stunted shrimp (33 days after restocking to 94 days after restocking)
3. Analysing the total bacteria count and total *Vibrio* count in normal and stunted shrimp

4. Identifying *Vibrio* species associated with normal and stunted shrimp using 16s rRNA gene sequencing

1.4 Expected advantages

This study was expected to provide the baseline data for growth of pond-cultured *L. vannamei* beginning from day of restocking until harvest. Information on the length-weight relationship of normal and stunted *L. vannamei* can be used by the community in shrimp industry to assess the occurrence of stunted shrimp in their culture production. More details about stunted shrimp were to be recorded and reported; such as the characteristics of stunted growth *L. vannamei* in terms of length and weight, as well as the *Vibrio* variation in stunted growth shrimp and its comparison with normal shrimp.

CHAPTER 2

LITERATURE REVIEW

2.1 External morphology of *L. vannamei*

L. vannamei has 19 pairs of body segments: 13 pairs of the segments are located in the cephalothorax, which is covered by an exoskeleton called carapace, which is referred normally as the head of shrimp (Dugassa and Gaetan, 2018). The abdomen or body part of the shrimp contains the last six pairs of body segments: five pairs of swimming legs or pleopods are located on the first to fifth abdominal segment, tail fan which consists of 2 pairs of uropods and the telson are connected at the end of the sixth abdominal segment (Dugassa and Gaetan, 2018). Figure 2.1 shows the external morphology of *L. vannamei*.

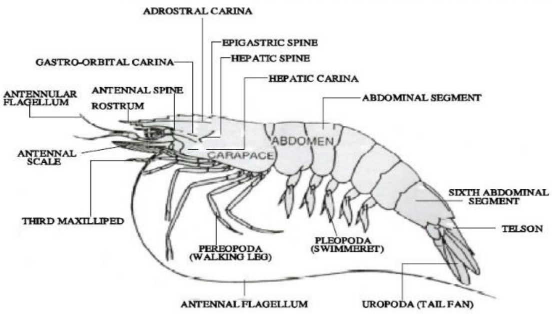

Figure 2.1 : External morphology of *L. vannamei*
Source : Dugassa and Gaetan (2018)

Mid gut of shrimp consists of the stomach, hepatopancreas and the intestine in the abdominal segments, meanwhile the hindgut is the terminal part of the digestive tract of the shrimp (Figure 2.2). The stomach and hepatopancreas of whiteleg shrimp are located in the cephalothorax (Abrunhosa and Melo, 2008).

Hepatopancreas is the main organ in the digestive system of shrimp and it functions as a digestive gland, performing synthesis and secretion of digestive enzymes, absorption of nutrients, metabolism of lipid and carbohydrate and calcium absorption (M*anan et al*, 2015; Dugassa and Gaetan, 2018).

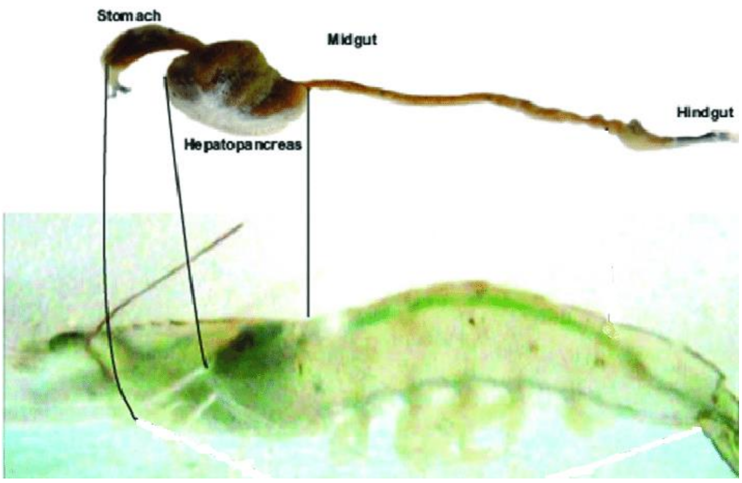

Figure 2.2 : Digestive system of *L. vannamei*
Source : Ozório *et al.* (2015)

2.2 Life cycle of *L. vannamei*

Penaeid shrimp like has an interesting life cycle (Figure 2.3). *L. vannamei* lives in tropical marine waters. Adult shrimps live in the ocean but upon maturity, berried females will migrate to offshore areas for spawning of eggs, which are fertilized externally (Dugassa and Gaetan, 2018). After hatching, larvae undergo several metamorphosis in different larval stages (nauplius, zoea, mysis) before developing into postlarvae and then juvenile stage, while simultaneously migrating nearer to inshore waters (Menon, 1965). Therefore, shrimp larvae and juveniles are normally found in estuaries or coastal areas and as they are developing into adults, they migrate back to the offshore areas (Gambil *et al.*, 2015).

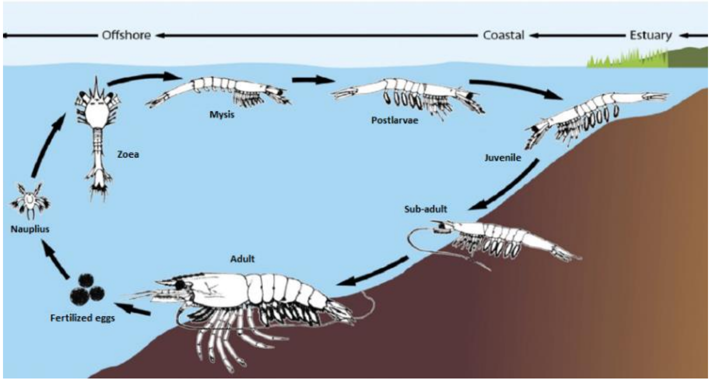

Figure 2.3 : Natural life cycle of *L. vannamei*
Source : Gambill *et al.* (2015); Wei *et al.* (2014)

Earthen ponds are frequently utilised in the shrimp aquaculture industry for cultivating domesticated *L. vannamei* stocks (Ren *et al*., 2020). Broodstocks are kept in maturation tanks and unilateral eyestalk ablation on each female is usually performed to promote repeated maturation and spawning (Chamberlain and Lawrence, 2009). According to a guide from FAO, the procedures of shrimp culture are as follows (Figure 2.4): 1) mature females ready for spawning are transferred into spawning tank overnight to release their eggs, 2) eggs are collected on the following day and transferred to hatching tanks, 3) hatched eggs released nauplii, 4) nauplii collected and transferred into larval rearing tank until developed into postlarvae, 5) postlarvae harvested and transferred to grow-out pond or sold off to farmers and cultivated until ready for harvest.

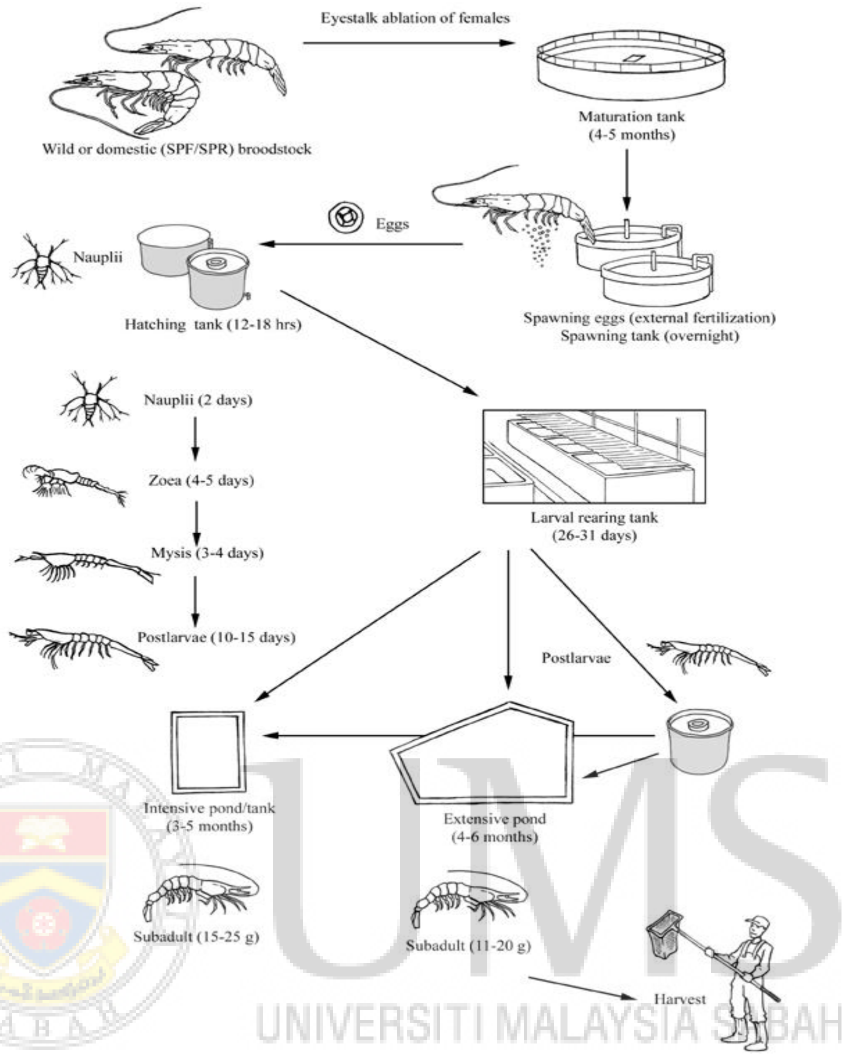

Figure 2.4 : Life cycle of *L. vannamei* in culture condition
Source : FAO (2006)

2.3 Culture system in *L. vannamei* farming

There are three commonly practised systems for *L. vannamei* culture: extensive, semi-intensive, and intensive, which correspond to low, medium, and high stocking densities (Tacon *et al*., 2002). The categorization is mostly based on pond facilities, stocking density, food availability, water management, yield and other significant input (Table 2.1).