DETERMINATION OF TOTAL MICROORGANISMS COUNT AND *VIBRIO* SPECIES PROFILE IN FRESHWATER FISH

by

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LIST OF ABBREVIATIONS

spp.	species
FAO	Food and Agriculture Organization of the United Nations
IUCN	International Union for Conservation of Nature and Natural Resources
WHO	World Health Organization
ICMSF	International Commission on Microbiological Specifications for Foods
FDA	United State Food and Drug Administration
PUFA	Polyunsaturated Fatty Acids
BAM	Bacteriological Analytical Manual
TCBS	Thiosulfate Citrate Bile Salt Sucrose Agar
T_1N_1	Tryptone Salt Agar
T_1N_0	Tryptone Broth
T_1N_8	Trytone Salt Broth
SPSS	Statistical Package of Social Sciences

PENENTUAN JUMLAH MICKROORGANISMA DAN PROFIL *VIBRIO* DALAM IKAN AIR TAWAR

ABSTRAK

Ikan air tawar dan ikan air laut merupakan antara jenis ikan yang dikenali sebagai salah satu sumber protein utama dalam diet manusia. Ikan air tawar mempunyai rasa yang enak, kaya dengan nutrisi dan mempunyai banyak khasiat. Ikan air tawar berpotensi menjadi sumber penyebaran mikroorganisma *Vibrio* seperti *V. cholerae*, *V. parahaemolyticus* dan *V. vulnificus*. Walau bagaimanapun, penilaian tentang risiko mikroorganisma kurang diketahui terutamanya *Vibrio* dalam ikan air tawar disebabkan ketiadaan piawai mengenai had mikroorganisma *Vibrio* yang dibenarkan di Malaysia. Kajian mengenai penilaian risiko mikroorganisma dan kehadiran *Vibrio* dalam ikan air tawar telah dijalankan. Pelbagai spesis ikan air tawar (n=10) telah diperoleh dari pasar di sekitar Kota Bharu, Kelantan. Jumlah mikroorganisma telah ditentukan menggunakan tatacara *spread plate method* dan profil *Vibrio* dikenal pasti menggunakan agar *Thiosulfate Citrate Bile Salts Sucrose* (TCBS), agar *Tryptone Salt* (T_1N_1) untuk pengasingan mikroorganisma *Vibrio* dan ujian toleransi terhadap garam dilakukan untuk mengenal pasti spesis *Vibrio* tersebut. Jika dibandingkan dengan sampel ikan air tawar yang lain, keputusan kajian menunjukkan Tilapia Hitam (*Oreochromis mossambicus*) mempunyai jumlah mikroorganisma paling tinggi iaitu 3.34 10^6 cfu/g. Berdasarkan kajian yang telah dijalankan, jumlah mikroorganisma bagi 80% sampel ikan air tawar tidak melebihi piawai yang telah ditetapkan oleh Kementerian Kesihatan Malaysia di mana produk hasil ikan di Malaysia hanya boleh mengandungi jumlah mikroorganisma tidak melebihi 10^6 per gram. Kajian terhadap profil mikroorganisma *Vibrio* menunjukkan 20% sampel ikan air tawar mengandungi *V. cholerae* dan *V. parahaemolyticus*, manakala 80% daripada sampel ikan

air tawar tesebut hanya mengandungi *V. cholerae*. Kesimpulannya, mikroorgasnisma *Vibrio* berkemungkinan didapati dalam ikan air tawar. Justeru, pengendalian ikan air tawar yang betul mesti dipraktikkan bagi memastikan insiden penyakit bawaan makanan yang berpunca daripada mikroorganisma ini dapat dikurangkan. Penyelidikan lanjutan harus dilakukan bagi meningkatkan keselamatan makanan terhadap pengambilan ikan air tawar dan dapat membentuk piawai bagi mikroorganisma *Vibrio* di Malaysia.

DETERMINATION OF TOTAL MICROORGANISMS COUNT AND *VIBRIO* PROFILE IN FRESHWATER FISH

ABSTRACT

Fish consist of freshwater fish and marine fish are known as one of the main protein sources in human diet. Freshwater fish have a great taste, rich with nutritive values and many other health benefits. However, freshwater fish appealed to be a potential vector of *Vibrio* microorganisms such as *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*. Yet, very little information about the microorganisms risk assessments especially *Vibrio* in freshwater fish as there is no standard of permissible for *Vibrio* microorganisms in Malaysia. In this project, a study was conducted in order to assess the microorganisms risk and presence of *Vibrio* in freshwater fish. Different species of freshwater fish (n=10) were obtained from wet markets within Kota Bharu, Kelantan. The total microorganisms count was determined using spread plate method and *Vibrio* profile used Thiosulfate Citrate Bile Salts Sucrose (TCBS) agar, Tryptone Salt (T_1N_1) agar for purity and salt tolerance test for *Vibrio* spp. confirmation test. The results had shown that 'Tilapia Hitam' (*Oreochromis mossambicus*) had the highest total microorganisms count at 3.34 10^6 cfu/g as compared to the other freshwater fish samples. The limit for total microorganisms count in fishery products of Malaysia is at 10^6 per gram, and 80% of the freshwater fish samples in this study were within the permissible limit set by Ministry of Health Malaysia. For the *Vibrio* microorganisms profile, 20% of freshwater fish samples detected with both *V. cholerae* and *V. parahaemolyticus*, while 80% from the freshwater fish samples had only *V. cholerae*. In conclusion, it was suggested that *Vibrio* can be found in freshwater fish. Thus, proper handling of freshwater fish must be practiced in order to decrease the incidence of foodborne illness due to these microorganisms. Further research should be

done to enhance the food safety of freshwater fish consumption, hence able to set the standard of permissible for *Vibrio* microorganisms in Malaysia.

CHAPTER 1: INTRODUCTION

1.1 Background of the Study

Fish supply has become the great concern because it occupies the highest tropic level, one of the major economic sources, represents a valuable source of protein and nutrients in humans diet. Globally, fish provided 6.7% of all protein consumed by humans, as well as offering a rich source of omega-3 fatty acids, vitamins, calcium, zinc and iron (Food and Agriculture Organization of the United Nations, 2016a). In Malaysia, fishery and aquaculture industry is one of the important income sources, thus significantly influence the nation economic development. The aquaculture industry is expected to increase and produce 800,000 tonnes of fish in year 2017 (News Straits Times, 2012a). It is known that about 78% Malaysians consume fish at least twice per week, either one or one-half per day (Nurul Izzah *et al.*, 2016). Therefore, the food safety pertaining fish consumption is crucial to ensure the food security to the community.

Freshwater fish is one of the constituent of fish consumed. Freshwater fishes are the species that lives all or have a critical part of its life in either freshwater inland or brackish estuaries (International Union for Conservation of Nature and Natural Resources, 2015). Worldwide, there are various species of freshwater fish such as carps, eels, salmon, archer fish and gobies. As there are highly demand on fish consumption, the growth of freshwater fish cultured system also expanded despite of their natural habitat. According to Jabatan Perikanan Malaysia (2015), freshwater fishes in Malaysia are cultured using pen culture, freshwater pond, ex-mining pool, freshwater cage, cement tank and canvas tank. This study also stated that the freshwater pond recorded the total highest freshwater fish production (47,536.81 tonnes) and the most type of fish cultured is freshwater catfish (50,683.12 tonnes).

Generally, people consumed fish as it has high protein content, little or no carbohydrate and acceptable fat values, contains essential fatty acids, vitamins and minerals (Roos *et al.*, 2007; Sanjee and Karim, 2016). This is applied in freshwater fish as it is known for its nutritive values, has a great taste and potential health benefits. Freshwater fish such as 'Haruan' is believed to help in wound healing, promote tissue growth, reduced postoperative pain and discomfort (Song *et al.*, 2013). As a result, 'Haruan' is commonly consumed among women who recently giving birth (Song *et al.*, 2013).

Apart from the benefits of freshwater fish consumption, the freshwater fish may act as a potential vector of pathogenic microorganisms which is either comes from the natural aquatic environment or contaminated from the surroundings, responsible in the outbreak of communicable disease namely foodborne illness. It will affect the food safety issue and pose a considerable public health threat. Foodborne illness is defined as a disease which is due to ingestion of foodstuffs contaminated with microorganisms or chemicals (World Health Organization, 2016). Foodborne illness is a condition whereby a person experienced diarrhea, vomiting, abdominal cramps, nausea, fever or bloody stool (Nsoesie *et al.*, 2014).

Foodborne illness has been reported worldwide. In France, the incidence rates that have been reported to be 1210 cases per 100,000 populations, 2600 cases per 100,000 populations in the United Kingdom and more than 25,000 cases per 100,000 populations in Australia and the United States (Teisl and Roe, 2010). Commonly, these foodborne illness caused by fish may be occurred due to consumption of contaminated, raw or undercooked fish and fish products. These microorganisms related to freshwater fish foodborne illness can be generally divided into two groups, those native to natural freshwater environment and those associated with water pollution such as *Vibrio* spp.,

Listeria monocytogenes, *Salmonella serovars*, *Clostridium botulinum, Escherichia coli* and *Yersinia* spp. (Novoslavskij *et al.*, 2015). It has been implemented that the set of standard for total microorganisms count in fresh and frozen fish is ranged from 10^5 to 10^7 cfu/g (International Commission on Microbiological Specifications for Foods, 1986). Meanwhile, the microbiological standard regulated by Malaysia for fish and fish products are the total microorganisms count is at most at 10^6 per gram and 5 10 per g ram for the permissible limit of coliform (Ministry of Health Malaysia, 2014). There are also various factors affected the presence of these microorganisms in freshwater fish, including its natural habitat, feeding type, inadequate cooking, cultural practices, environmental conditions, processing and distribution of products (Noorlis *et al.*, 2011; Zamri-Saad *et al.*, 2014; Novoslavskij *et al.*, 2015). Thus, the microorganisms detected in the freshwater fish reflected the condition and safety of freshwater fish consumption.

1.2 Problem Statement

Foodborne illness is one of the communicable diseases, a growing public health concern as the food consumed can be contaminated through the environment including the water, soil and sediment. This foodborne illness also can worsen the condition of people with non-communicable disease. As there are various causal factor of non-communicable disease, the fish consumption may arise an issue as there are many different species of fish and could be contaminated with microorganisms, thus concerning the food safety among healthy and unhealthy individuals. In Malaysia, foodborne illnesses are common. Ministry of Health Malaysia (2012), estimated that the incidence of cholera, typhoid, Hepatitis A and dysentery has shown a declining trend from year 2000 to 2011. However, the incidence of cholera had increased from 1.57 per 100,000 populations in 2010 to 2.05 per 100,000 populations in 2011, with 11 deaths (Ministry of Health Malaysia, 2012). In addition, World Health Organization (2014) stated that the diarrheal disease has contributed 3% mortality globally and therefore should be a concern issue about diarrhea caused by *V. cholerae*.

Usually, the foodborne illness cases had been under reported and limited study on the causative effect of *Vibrio* spp. was done in Malaysia (Bilung *et al.*, 2005). It is known that *V. cholerae* O1 is the cause of cholera outbreak as the patient had most likely been infected while handling and preparing fish imported from Nigeria (Senderovich *et al.*, 2010). This distressing issue then supported by Nakaguchi (2013) that claimed *V. parahaemolyticus* is often associated with molluscan shellfish, however a high prevalence of these bacteria is also observed in fish, and the pathogens detected in more than 50% of fish samples in Vietnam, Malaysia and Indonesia. In Malaysia, the presence of *Vibrio* spp. and *V. parahaemolyticus* in catfish and red tilapia has been proven (Noorlis *et al.*, 2011). Meanwhile, previous study by Senderovich *et al.* (2010) has found the presence

of *V. cholerae* at 5 10^3 per gram in the fish intestine of *Sarotherodon galilaeus*. Chitov *et al.* (2009) also stated that one of the species of *Vibrio*, *V. mimicus* was the cause of foodborne outbreak related to ingestion of dishes containing freshwater fish and seafood prepared by a food catering establishment. This showed that the *Vibrio* spp. may contaminate the freshwater fish and concerning the safety of freshwater fish consumption.

As the main protein source in human diet, freshwater fish appealed to be a potential vector for microorganisms related foodborne illnesses and other vulnerable risk factors of microorganisms contamination. Hence, the occurrence and prevalence of microorganisms in the freshwater fish must be accessed to determine the potential source of human pathogenic bacteria, gained better understanding on distribution of microorganisms in the food chain and ensure the food safety. Unfortunately, the research regarding the occurrence and prevalence of *Vibrio* spp. in freshwater fish is limited as the *Vibrio* spp. is mostly found in the crustaceans and might not be relatively true for other species of fish. As there is no set of standard of *Vibrio* spp. in fish and fish products at Malaysia also influence the limited study of these microorganisms. Therefore, the aim of this research is to assess the microorganisms risk by determine the total microorganisms count and *Vibrio* spp. profile in different species of freshwater fish.

1.3 Study Objectives

1.3.1 General Objective

To determine the total microorganisms count and *Vibrio* spp. profile in freshwater fish.

1.3.2 Specific Objectives

1. To compare the total microorganisms count in the different species of freshwater fish.

2. To compare the total microorganisms count between the different species of freshwater fish with the permissible limit in Malaysia that set by Ministry of Health Malaysia.

3. To compare the *Vibrio* spp. profile in the different species of freshwater fish.

1.4 Research Questions

1. What is the difference of total microorganisms count between the different species of freshwater fish?

2. What is the difference of total microorganisms count between the different species of freshwater fish with the permissible limit in Malaysia that set by Ministry of Health Malaysia?

3. What is the association between *Vibrio* spp. profile with the different species of freshwater fish?

1.5 Hypotheses

1) **Null Hypothesis:** There is no significant difference between total microorganisms count and the different species of freshwater fish.

   Alternative Hypothesis: There is a significant difference between total microorganisms count and the different species of freshwater fish.

2) **Null Hypothesis:** There is no significant difference of total microorganisms count between the different species of freshwater fish with the permissible limit in Malaysia that set by Ministry of Health Malaysia.

   Alternative Hypothesis: There is a significant difference of total microorganisms count between the different species of freshwater fish with the permissible limit in Malaysia that set by Ministry of Health Malaysia.

3) **Null Hypothesis:** There is no significant association between *Vibrio* spp. profile and the different species of freshwater fish.

   Alternative Hypothesis: There is a significant association between *Vibrio* spp. profile and the different species of freshwater fish.

1.6 Significance of the Study

There is limited research regarding the microorganisms risk assessment in freshwater fish especially in *Vibrio* spp. This is due to numerous different species of freshwater fish available in the markets. The quality of freshwater fish consumed are also cannot be guaranteed as the microorganisms contamination may occurred due to various factors and stages of handling processes. Thus, the concerning issue of food safety that involving freshwater fish consumption is unable to be justify.

By understanding the occurrence of total microorganisms count and *Vibrio* spp. profile in the freshwater fish, researchers will be able to assess the microorganisms risk in the freshwater fish, relationship between those microorganisms with foodborne illness and later capable in developing new prevention steps that can be applied to enhance the food safety of freshwater fish consumption. Therefore, the Department of Fisheries Malaysia could take further effort in regulating the quality of freshwater fish consumption by implementing better policies in managing the freshwater fish cultured system and penalty the fish farmers that do not meet the appropriate aquaculture practices.

The fishmongers and food handlers must also be aware in order to avoid the poor hygiene and prevent mishandling practices that lead to microorganisms related foodborne illness. This had been proven that proper hand washing, adequate cleaning and good sanitation procedures can reduce the risk of cross-contamination that lead to foodborne illness (Sneed *et al.*, 2004). Besides, the consumers are able to compare and choose the better freshwater fish in context of quality and the cleanliness of trade environment. As result, the microorganisms risk assessment can be identified, the exposure to the microorganisms such pathogenic bacteria of *Vibrio* spp. can also be avoided, reducing the risk of foodborne illness as well as decreasing the mortality rate due to communicable diseases.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction of Freshwater Fish

Among vertebrae animals, fish occupies the largest category. Fish play an important role as one of the main protein sources throughout the world. Fish can be finfish, shellfish (molluscs and crustaceans), or any other form of marine or freshwater animal life that can be used for human or domestic animal consumption (Begum *et al.*, 2010). Fish is rich with nutritive value such as high protein content, with little or no carbohydrate and has acceptable fat value (Sanjee and Karim, 2016). Fish is also a valuable source of vitamin A, vitamin B, iodine and oils containing polyunsaturated fatty acids (Ross *et al.*, 2007; Adebayo *et al.*, 2012). However, there is difference in fish nutritive value as it is generally recognized that polyunsaturated fatty acids (PUFA) composition might vary among species of fish (Nur Airina and Jamaludin, 2012).

In Malaysia, the online database has records of 640 species of freshwater fish (FishBase, 2014). Freshwater fish is a typical term used to describe fishes that are found in inland streams, rivers, lakes and regions of weak brackish water (Helfman *et al.*, 2009). Primarily, the freshwater fish occupy habitats that include streams, rivers, lakes, ponds, paddy fields, peat swamps and other freshwater swamps (Chong *et al.*, 2010). It is known that the freshwater habitats in Malaysia are typically affected by two monsoon seasons, namely the Northeast Monsoon that peaks between October to February which has frequent and intense rainfall, and Southwest Monsoon that prevails from April to August (Ng *et al.*, 2017). Besides the natural habitats, freshwater fish is also been cultured. Jabatan Perikanan Malaysia (2015) reported that the freshwater fish is cultured using pen culture, freshwater pond, ex-mining pool, freshwater cage, cement tank and canvas tank. Freshwater pond recorded the total highest freshwater fish production (47,536.81 tonnes)

and the most species cultured is freshwater catfish (50,683.12 tonnes) (Jabatan Perikanan Malaysia, 2015).

These freshwater fish has significant impact either on economic, social or political as it mostly consumed by human and export to the other country such as Europe and United State. Based on the recent data, it is revealed that the current production of freshwater fish is increasing to meet the high demand of population growth in the country and also for exportation. The production of fish is expected to be changes from 1.3 million tan matric in year 2013 to 1.9 million tan matric in year 2020 (Utusan Malaysia, 2013). The export value of freshwater fish is expected to be changes from RM2.4 billion (478,245 tonnes) in year 2009 to RM7.0 billion (727,300 tonnes) in year 2015 also boost freshwater fish production which is in concurrence with continuous government support (Utusan Malaysia, 2013). Another factor that influence the increasing number of freshwater fish production is due to awareness of consumer health consciousness as the freshwater fish consumption may benefits people, thus the freshwater fish consumption is implemented at the school hostel (Utusan Malaysia, 2013).

2.2 Freshwater Fish Commonly Consumed

There are various species of freshwater fish available in Malaysian freshwater fish industry. The examples of freshwater fish found are giant snakehead, black tilapia, grass carp, goby, giant catfish, snakeskin gourami and freshwater sea bass. Nurul Izzah *et al.* (2016) stated that in recent study on the freshwater fish consumption shows that Malays consumed the highest amount (175±143 g/person/day), followed by the Chinese (152±133 g/person/day) and the least amount by the Indians (136±141 g/person/day). The same research also claimed that about 78% of Malaysians consumed fish at least twice per week (Nurul Izzah *et al.*, 2016).

In this study, several species of freshwater fish that commonly found at wet markets in Kota Bharu, Kelantan are studied. The selected freshwater fish are freshwater catfish, red tilapia, river catfish, black tilapia, feather back, river carp, snakehead, swamp eel, climbing perch and blue-spot mullet. These freshwater fishes are determined based on the types of freshwater fish commonly consumed among different ethnicities in Peninsular Malaysia (Nurul Izzah *et al.*, 2016).

2.2.1 Freshwater catfish

The freshwater air breathing catfish or *Clarias batrachus* is from family *Clariidae*. It is commonly known as walking catfish, freshwater catfish or 'Keli' that widely distributed throughout Asian countries. International Union for Conservation of Nature and Natural Resources (IUCN) (2015) claimed that *C. batrachus* inhabits lowland fresh, brackish waters and can be found in the mud as they lie concealed for hours. The diagnostic feature of *C. batrachus* is elongate body, large and depressed head with small

eyes, has large terminal mouth and the common size ranged from 22 to 24 cm (Food and Agriculture Organization of the United Nations, 2016b).

2.2.2 Red tilapia

Red tilapia or *Oreochromis niloticus* comes from family *Cichlidae*. It is also known as 'Tilapia Merah' or Nile tilapia. *O. niloticus* usually lives in warm, freshwater and brackish water. This omnivore red tilapia can be easily found at Africa, Japan, Taiwan, Nigeria and Southeast Asia (International Union for Conservation of Nature and Natural Resources, 2015). The red tilapia has compressed body and interrupted lateral line and can have the size up to 36 cm (Food and Agriculture Organization of the United Nations, 2016b).

2.2.3 River catfish

Pangasius hypophthalamus, river catfish or commonly called 'Patin' is part of family *Pangasiidae*. According to Nur Airina and Jamaludin (2012), *P. hypophthalamus* have shark headed features and also known as iridescent shark. *P. hypophthalamus* lives in main channels, floodplains of large rivers and usually found in tropical country. The geographical distributions of *Pangasius* in South and Southeast Asia, actually indicated that it favors warm water (22 to 26°C) and cannot endure temperature under 14°C for extended time due to declined growth rate and diseases-resistant ability (McGee, 2015). River catfish is an omnivore which feeding primarily on algae, plants, zooplankton, insects, fruits, crustaceans and fish (International Union for Conservation of Nature and Natural Resources, 2015). The diagnostic feature of *P. hypophthalamus* is latterly flattened long body with no scales, relatively small head, mouth broad with small sharp

teeth on jaw and relatively large eyes (Food and Agriculture Organization of the United Nations, 2016b).

2.2.4 Black tilapia

The omnivore *Oreochromis mossambicus* is from family *Cichlidae* and known as black tilapia or 'Tilapia Hitam'. *O. mossambicus* can be found at Africa, Japan and Southeast Asia. This black tilapia is categorized as omnivore because they mainly feed phytoplankton or benthic algae, but also relying more heavily on plants (International Union for Conservation of Nature and Natural Resources, 2015). This black tilapia lives in wide variety of freshwater habitats. According to Food and Agriculture Organization of the United Nations (2016b), *O. mossambicus* has compressed body, interrupted lateral line and the size can up to 36 cm.

2.2.5 Feather back

Chitala borneensis, feather back or locally called as 'Selat' is part of family *Notopteridae*. International Union for Conservation of Nature and Natural Resources (2015) revealed that this feather back inhabits freshwater environment, and distributed throughout Asia including Sumatra in Indonesia and Malaysia (Sarawak, Sambas, Barito and Peninsular Malaysia). The maximum length observed in the feather back is 39.3 cm (Food and Agriculture Organization of the United Nations, 2016b).

2.2.6 River carp

River carp or 'Lampam' is from family *Cyprinidae*. The scientific name is *Puntius schwanenfeldii*. International Union for Conservation of Nature and Natural Resources (2015) stated that *P. schwanenfeldii* consuming aquatic macrophytes, submerged land plants, filamentous algae, and occasionally feed on insects, small fishes, worms and crustaceans. The river carp is natively establish at Brunei, Cambodia, Malaysia, Singapore, Thailand and Vietnam, mostly found in rivers, streams, canals and ditches (International Union for Conservation of Nature and Natural Resources, 2015). The diagnostic feature for river carp is comprise of one dorsal fin, has skin of lower lip separated from the lower jaw by a shallow groove and the length size can up to 40.5 cm (Food and Agriculture Organization of the United Nations, 2016b).

2.2.7 Snakehead

The freshwater snakehead *Channa striata* is from the family *Channidae*. It is commonly known as snakehead or 'Haruan'. Based on Song *et al.* (2013), who claimed this snakehead has a wide range of habitats ranging from rivers, swamps, ponds, canals, lakes and land of paddy fields. This fish are distributed across Asian countries. *C. striata* has elongate body, broad and flattened head, the top and sides of head covered with scales, large mouth with small teeth on palate, the eyes located at the anterior part of head and the usual size is relatively small (Food and Agriculture Organization of the United Nations, 2016b). However, the length of *C. Striata* can reach up to a maximum length of 100 cm and has maximum weight of 3000 g (Nur Airina and Jamaludin, 2012).

2.2.8 Swamp eel

Monopterus albus or also known as swamp eel is from family *Synbranchidae*. This swamp eel is recorded available at India, throughout Southeast Asia, including Bangladesh, China, Japan, Malaysia and Thailand. *M. albus* inhabits muddy ponds, swamps and rice fields, but sometimes found in gently flowing streams (International Union for Conservation of Nature and Natural Resources, 2015). The eating habits of *M. albus* is known as nocturnal predator devouring fishes, worms, crustaceans, other small aquatic animals and also feeds on detritus (International Union for Conservation of Nature and Natural Resources, 2015). The diagnostic feature of swamp eel is with no scales, no pectoral and pelvic fins, and the common length is 40 cm (Food and Agriculture Organization of the United Nations, 2016b).

2.2.9 Climbing perch

Climbing perch is parts of family *Anabantidae*. Climbing perch is locally known as 'Puyu' or scientifically named as *Anabas testudineus*. The air breathing climbing perch is most commonly found in South and Southeast Asia and inhabits fresh and brackish waters mostly in rivers, canals, lakes, ponds, swamps and paddy fields (Mukherjee *et al.*, 2002; International Union for Conservation of Nature and Natural Resources, 2015). Morphologically, *A. testudineus* has laterally compressed body, anterior mouth, slightly longer lower jaw and possesses a special accessory air breathing organ that situated just above the gills in a large extension on the upper part of each gill chamber (Marimuthu *et al.*, 2009). This climbing perch contains very high amount of available iron and copper essentially needed for hemoglobin synthesis, possess easily digestible fat of very low melting point, has many essential amino acids, believed to have medicinal properties such

as disease prevention and capable in slowing down the ageing process for females (Patowary and Dutta, 2012).

2.2.10 Blue-spot mullet

The blue-spot mullet is a fish belonging to the *Mugilidae* family. Scientifically known as *Moolgarda seheli* and locally named as 'Belanak'. The mullets are found worldwide in coastal temperate and tropical water (Al-Asous and Al-Harbi, 2016). It is also stated by International Union for Conservation of Nature and Natural Resources (2016) that the blue-spot mullet are observed at South Africa, Hawaii, India, China, Malaysia, Australia and Germany, and inhabits brackish, freshwater and marine environment. The mullet have diagnostic features such as cylindrical and robust body, broad head, have two dorsal fins and the common size ranged from 35 to 50 cm (Food and Agriculture Organization of the United Nations, 2016b).

2.3 Factors Influence Consumption of Freshwater Fish

Particularly, at least one fish consumed per day by the Malaysians. The amount of fish eaten is either one or one-half per day, and about 78% Malaysians consumed fish at least twice per week (Nurul Izzah *et al.*, 2016). Commonly, people like to eat fish due to the nutritive values, potential health benefits and influences of traditional eating culture to eat cooked rice with protein source (Nurul Izzah *et al.*, 2016). According to Sanjee and Karim (2016), fish contains higher nutritive value such as high quality protein, little or no carbohydrate and acceptable fat value. The study conducted by Sidhu claimed that fish contains unsaturated essential fatty acids, minerals and vitamins (Sidhu, 2003). It is also supported by Salihu *et al.* (2012) that fish is known for its high nutritional quality, relative

low fat content, saturated fat, cholesterol and high levels of poly unsaturated fatty acids, proteins and minerals such as calcium, phosphorous, sodium, potassium and magnesium.

In addition, people tend to consume freshwater fish due to their quality, taste and flavour even their price can be incredibly high (Dhanaraj *et al.*, 2009). This is supported by Roselan (2012) that the wild freshwater fish is tastier compared to the cultured freshwater fish. According to Song *et al.* (2013), the freshwater fish is preferred as they have recuperative and medicinal properties. As evidence, *C. striata* is consumed for its postoperative medicinal application to enhance wound healing, promotes tissue growth, reduce postoperative pain and discomfort (Song *et al.*, 2013). Another research stated that *A. testudineus* is well known for their taste, high nutritive value, and recuperative and other medicinal qualities (Marimuthu *et al.*, 2009). Besides, the climbing perch is favoured as they rich with omega-3 fatty acids (News Straits Times, 2012b). The omega-3 fatty acids from the freshwater fish also could reduce cholesterol level, the incidence of heart disease, stroke, preterm delivery and mood stabilizer among female (Nurul Izzah *et al.*, 2016).

2.4 Microorganisms Commonly Found in Freshwater Fish

Fish is the main protein source for human consumption in Malaysia. However, fish has high possibility to become one of the vectors for human pathogenic bacteria due to the contaminated aquatic environment, mishandling and poor hygiene practices. The microorganisms that commonly found within the fish can be divided into two groups, gram negative such as *Vibrio* spp., *Escherichia coli* and *Salmonella* spp. and gram positive bacteria such as *Listeria monocytogenes*. These microorganisms may worsen the

condition of individuals with non-communicable diseases and act as the indicator of environmental pollution.

In research field, the microorganisms associated with the fish only can be further analysis for the characterization by using the basic and standard total plate count. The plate count is prepared by either using pour or spread plate method and widely accepted measure of the general degree of microbial contamination. It have been found that the microbial load on the skin, gills and intestines of fish living in clear waters is usually high, and muscles are assumed to be sterile (Pamuk *et al.*, 2011). However, Budiati *et al.* (2015) revealed that the catfish has the mean aerobic plate count values ranged from 5.30 to 6.84 log per gram and 5.77 to 9.12 log per gram for tilapia. These findings are worrying as the International Commission on Microbiological Specifications for Foods (ICMSF) recommended total aerobic bacteria in fresh and frozen fish, and cold-smoke is ranged from 10^5 to 10^7 cfu/g (International Commission on Microbiological Specifications for Foods, 1986). Meanwhile, the microbiological standard in Malaysia regulate the permissible limit of the total microorganisms count at 10^6 per gram and 5 10 per gram for coliform in the fish and fish products (Ministry of Health Malaysia, 2014).

2.4.1 *Vibrio* spp.

Vibrio spp. is abundant in aquatic environment, found free living in water or with plankton, inhabits marine, estuarine, brackish and coastal areas, and also been observed on the parts of fish and shellfish. Thus, the study of *Vibrio* spp. in freshwater fish is crucial as there are evidences of foodborne illness outbreak due to *Vibrio* spp. and suggest that fish are possible reservoirs of these microorganisms.

Vibrio encompasses more than 63 species, and a number of these microorganisms are known to be commonly associated with outbreaks of gastrointestinal and wound infections in humans (Thompson *et al.*, 2004; Austin, 2010; You *et al.*, 2016). The pathogenic species such as *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. fluvialis* and *V. mimicus* are the common aetiology of vibriosis worldwide and have been reported to cause mortality and severe economic loss (Chowdhury, 2004; You *et al.*, 2016). *Vibrio* spp. is characterized as gram negative, rod shaped bacteria that are fermentative, catalase and oxidase positive, motile by polar flagella, halophilic, sensitive to the vibriostatic agent and mostly have a requirement for sodium chloride (Farmer *et al.*, 2005; Noorlis *et al.*, 2011). *Vibrio* microorganisms grow well at neutral and alkaline pH values up to pH 9.0, therefore the pH values of both selective and enrichment media are generally 8 to 8.8 and all species are acid-sensitive (Igbinosa and Okoh, 2008). *Vibrio spp.* most frequently found in marine fish and shellfish, however these pathogens also observed in freshwater fish. This is evident by Abdellrazeq and Khaliel (2014) that found positive result of *Vibrio* spp. in 32% of the farm water fish.

The number of *Vibrio* spp. also increase with temperature as they favour higher water temperature like summer season. Austin (2002) claimed that bottom-feeding fish, especially those that consumed mollusks and crustaceans as well as plankton-feeding fish contained high population of *V. vulnificus*, 10^8 bacterial 100 per gram and 10^5 bacterial 100 per gram respectively. The earlier study also had proven the occurrence and prevalence of *Vibrio* spp. in aquatic animals such as fish (Schmidt *et al.*, 2000; Messelhausser *et al.*, 2010), shrimp (Reboucas *et al.*, 2011) and mussel (Lhafi and Kuhne, 2007) has been mentioned, although bacterial contamination in freshwater fish is less

studied. Therefore, the freshwater fish may be immediate reservoirs of these microorganisms and act as the potential vector of vibriosis.

Based on recent data, *V. cholerae* and *V. vulnificus* are present in the water, sediment, gills and intestine of tilapia examined (Pakingking *et al.*, 2015). Freshwater fish samples studied by Saad *et al.* (2015) revealed the incidence of *V. alginolyticus* (2%), *V. damsela* (4%), *V. fluvialis* (4%), *V. furnissi* (4%), *V. mimicus* (4%), *V. vulnificus* (4%), while *V. cholerae* and *V. parahaemolyticus* failed detected biochemically. Scharer *et al.* (2011) reported the incidence of *V. cholerae* and *V. alginolyticus* in the freshwater fish fillet samples. Another study conducted by Begum *et al.* (2010) revealed that the *Vibrio* spp. is found in freshwater fish samples from the different markets. The study by Nakaguchi (2013) found that *V. parahaemolyticus* is often associated with molluscan shellfish, however a high prevalence of these bacteria is also observed in fish, and the pathogens detected is more than 50% of fish samples in Vietnam, Malaysia and Indonesia. In contrast, the study by Al-Harbi and Uddin (2005) revealed that there is no *V. cholerae* isolated in the gills of tilapia.

Moreover, Scharer *et al.* (2011) identified the presence of *Vibrio* spp. can be characterized with smooth, either green (presumptive *V. parahaemolyticus*, *V. vulnificus* or *V. mimicus*) or yellow (presumptive *V. cholerae*, *V. alginolyticus* or *V. fluvialis*) using Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar. International Commission on Microbiological Specifications for Foods (1986) has established the limit of *V. parahaemolyticus* in fresh and frozen fish, and cold-smoke is at ranged 10^2 to 10^3 cfu/g. Meanwhile, Food and Drug Administration (FDA) has stated that the fishery products must be free of *V. cholerae* and *V. vulnificus* (Food and Drug Administration, 2016). Thus, the presence of *Vibrio* spp. in freshwater fish samples indicates poor quality of fish

due to bad aquaculture practices, mishandling and unhygienic practices, occurrence of cross contamination, and may cause food borne illness due to consumption of insufficiently heat-treated fish.

2.4.2 Others Microorganisms

Apart from that, the prevalence of others microorganisms are also recorded by the researcher as they may promote the foodborne illness. Previous study Thi *et al.* (2014) showed that frozen fillets from pangasius processed in Vietnam can be contaminated with *E. coli*, *Staphylococcus aureus* and even *V. cholerae*. This is proven by Kaktcham (2017) that found the *E. coli*, *Salmonella* spp. and *Vibrio* spp. are the most dominant bacteria of the microorganisms in the pond water and fish intestines. Meanwhile, Pakingking *et al.* (2015) observed total coliform in the gills and intestine of *O. niloticus* is ranging from 10^5 to 10^6 cfu/g and 10^7 to 10^8 cfu/g respectively. Sanjee and Karim (2016) also revealed that the fecal coliforms (presumptive *E. coli*) ranged from 3 MPN/g to 8.3 MPN/g in the freshwater fish samples. *E. coli* is also the most common contaminant and encountered in high numbers in the tilapia samples except muscle tissues (Thampuran *et al.,* 2005). In addition, Binsi *et al.* (2015) unable to detect the presence of *E. coli* from the muscle of catfish sample. This *E. coli* microorganisms could cause E. coli infection or hemorrhagic colitis (Food and Drug Administration, 2016).

Apart from that, Begum *et al.* (2010) recorded the presence of *Salmonella* spp. in almost all tilapia samples either from local markets or super shop markets. Another study also found highest numbers of *Salmonella* spp. are isolated from catfish, tilapia, milkfish, rohu, mackerel and mirgal (Elhadi *et al.,* 2004). The presence of *Salmonella* spp. is a concern as it may cause typhoid fever or Salmonellosis (Food and Drug Administration,

2016). Lastly, *L. monocytogenes* that causal factor of Listeriosis has also been found in both freshwater and saltwater fish samples (Novoslavskij *et al.*, 2015). Based on Yucel and Balci (2010), *L. monocytogenes* (44.5%) and *L. welshimeri* (31.2%) are the most commonly isolated species in freshwater fish samples and *L. monocytogenes* particularly favoured the gills.

2.5 Factors Related to Microorganisms Found in Freshwater Fish

Commonly, the microorganisms found in the freshwater fish can be divided into two groups which is those native to the aquatic environments such as *Vibrio* spp. and those associated with various types of water pollution, derived from deposition of sewage, human or animal contaminated water such as *Salmonella* spp. (Novoslavskij *et al.*, 2015; Sanjee and Karim, 2016). It has been found that aquatic environments such as freshwater rivers, brackish water, estuarine and marine environments with optimal salinity and temperature may support the growth of *Vibrio* spp. (Noorlis *et al.*, 2011). The study by Huehn *et al.* (2014) stated that *Vibrio* spp. is more frequently found in warm and lower water salinity. Another study found that *L. monocytogenes* and *Salmonella* spp. detected in both freshwater and marine fish due to city sewage system and higher prevalence during monsoon season (Novoslavskij *et al.*, 2015). Apart from that, Budiati *et al.* (2015) identified that the land and type of water used such as stream water and hold water in Malaysian earthen ponds and mining pools might be contaminated by coliform and associated with the coliform density.

Another main factors influence the presence of microorganisms in the freshwater fish is due to the mishandling and unhygienic practices. The introduction of microorganisms into the freshwater fish occurred during various stages such as

harvesting, transporting and trade. According to the recent study, the usage of contaminated ice to cover the catfish and red tilapia on the display bench at the market and mishandling lead to temperature abuse, thus favoured the growth of *Vibrio* spp. (Noorlis *et al.*, 2011). It is supported by Berkel *et al.* (2004) that in the high ambient temperatures of the tropics, fresh fish will spoil within 12 hours. Notably, physical properties of fresh fish such as firmness and appearances of the skin are greatly influenced by storage time due to cellular flaking of autolytic and microbial changes (Abbas *et al.*, 2008; Martinsdottir *et al.*, 2009). Besides, a study conducted show a positive result of *V. cholerae* in iridescent shark in Mekong Delta whereas the water is highly contaminated with human sewage (Scharer *et al.*, 2011). Mishandling and poor hygiene practices also may cause cross contamination either with ready to eat foods or raw materials at the same environment, thus introduced the presence of *Vibrio* spp., *L. monocytogenes* and *E. coli* (Novoslavskij *et al.*, 2015). Meanwhile, poor environmental sanitation such as poor hygienic standard of harvesting management, high stocking density and introduction of contaminated water into the culture system may increase the risk of streptococcal infection and salmonellosis (Zamri-Saad *et al.*, 2014).

The state of freshwater fish itself may be contributed to the presence of microorganisms and posed threat to human consumption. This state can be divided into fresh, chilled or live freshwater fish available at the markets, and the ways of fish been consumed. Budiati *et al.* (2015) claimed that the coliform level is relatively higher in fresh or chilled tilapia compared to live catfish. This is evident with the risk of *L. monocytogenes* contamination in the chilled fresh catfish fillet (Novoslavskij *et al.*, 2015). Besides, the fish such as tilapia is keeping alive prior to consumption is due to the factor that the fish has particular large pool of nitrogenous extractives, thus more prone to raid spoilage (Shinkafi and Ukwaja, 2010).