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Review Article

Gut content, feeding behavior, and gut microbiome of *Pangasius nasutus* (Bleeker, 1863) in natural habitat and captivity environment: A review

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Abstract: *Pangasius nasutus* is a freshwater fish that has become one of the major economic sources for fish farmers in Pahang River, Malaysia. Effective aquaculture and conservation *P. nasutus* depends on the understanding of their gut composition, feeding habits, and gut microbiome. *Pangasius nasutus* in their natural habitat mostly consume a variety of food sources, including zooplankton, aquatic insects, and crustaceans. The advances in metagenomic sequencing technologies have made it possible to examine gut bacteria by examining hypervariable areas of 16S rDNA for prokaryotes and 18S for eukaryotes through cloning and transferring biologically produced DNA into a bacterial host. In recent times, significant attention has been directed towards nutritional manipulation and the modification of gut microbiota to align with the requirements of aquaculture, all the while aiming to preserve the health and welfare of the host. This paper intends to review the gut content and feeding behavior of *Pangasius* sp. that will be contributed to the local fish farmer for their breeding and production of high-quality *P. nasutus* in a short period of time.

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Introduction

Pangasius nasutus, known as Patin Buah, is a famous freshwater fish in the Pahang River, Pahang state, Malaysia. This species has become one of the major economic sources for fish farmers in Pahang state. However, there is a shortage of research on the feeding habits of *P. nasutus* from the larval to the adult stages which are crucial for understanding their growth. Additionally, local fish farmers encounter challenges in consistently obtaining high-quality wild *P. nasutus* throughout the year (Ghani et al., 2012). Hence, food intake analysis provides advantages for fish farmers aiming to breed and produce high-quality *P. nasutus* relatively quickly.

Feeding is a multifaceted behavior that encompasses various behavioral responses related to eating, including feeding methods and habits, food preference mechanisms, feeding frequency, and food detection (Volkoff et al., 2006). Feeding behavior in fish is influenced by several factors, including environmental factors and habitat. Environmental factors such as temperature and photoperiod affect feeding behavior and this is often closely related to reproductive season (Joshua et al., 2017). Structure of habitat, presence of predators, and energy associated with a given food type or item have also been observed to influence feeding behavior (Volkoff et al., 2006). Furthermore, analysis of food consumption is also important to fisheries management and it can help in increasing fish production, management of prey resources, and fish population to enhance fishing economy and activity (Kamler and Pope, 2001).

The Pangasiidae family consists of 28 species. *Pangasius hypopththalamus* is the most widely distributed catfish found in Southern Asia, including Palembang, Indonesia; Mekong, Vietnam, and Chao Phraya River, Thailand (Ferraris, 2007). Three other pangasiid species, including *P. nasutus, P. micronemus*, and *H. waandersii* have been found in Peninsular Malaysia (Tweedie, 1936; Herre and

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Myers, 1937; Roberts and Vidthayanon, 1991; Lim and Zakaria-Ismail, 1995). The largest pangasiid producer in Asia is Vietnam with a total production of 48.1%, followed by India (18.5%) and Bangladesh (15.6%) (FAO, 2020). IUCN Red List of Threatened Species (IUCN, 2013) listed several pangasiid populations such as *Pangasianodon gigas* (Hogan, 2011) as Critically Endangered (CR), whereas *P. hypophthalmus* (Sauvage, 1878; Hogan and Vidthayanon, 2011) is Endangered (EN). On the other hand, *P. nasutus* is rare, endangered, and under Least Concern (LC) (Vidthayanon et al., 2020). This paper intends to review the works done on gut content which includes gut microbiota and feeding behaviour of *P. nasutus* that will contribute to local fish farmers for breeding and improvement on the quality and quantity of *Pangasius* spp. specifically *P. nasutus.*

Biology: Family Pangasiidae is recognized by having two pairs of barbels (maxillary and mandibular), a lateral compressed body, and an inferior mouth, with the tooth band of the upper jaw entirely exposed when its jaw is closed. The jaw is also sharp and projected to effectively grip prey by extending it forward. This species is also able to swallow large food items due to this adaptation. It also has a projected snout and has a small eye diameter which is 14 times smaller in standard length compared to other species (Gustiano and Pouyaud, 2006). Meanwhile, this species has seven dorsal soft rays, two dorsal spines, and 26-30 anal soft rays (Froese and Pauly, 2018). There are 23 gill rakers on the lower arch, including the raker at the angle, and six on the upper arm of the first arch (Snovsky et al., 2012). They resemble combs and are found on the gill arches. These gill rakers assist in capturing and filtering minute particles from the water, such as plankton and debris. *Pangasius nasutus* also has palatal teeth with smaller palatine tooth bands and large median vomerine tooth patches (Baharuddin et al., 2014).

Pangasius nasutus can live up to 20 years and after two months during the breeding phase, it can reach up to 10-12 cm in length and up to 15 grams in weight (Waycott, 2015). After 10 years, *P. nasutus* weight in captivity can reach up to 25 kg (Waycott, 2015), while in its natural habitat, this species can reach up to 44 kg (Robert et al., 1991). The best harvesting period is when *P. nasutus* weighs between 800 and 1100 grams at six- to eight-month-old (Waycott, 2015). This species is famously used to make local dishes in Pahang state in Malaysia because it consists of a very fine, delicious, and completely white flesh (Hassan et al., 2011). Moreover, the Pahang River offers an essential ecosystem for the breeding and reproduction of a variety of commercial fish species which are essential for aquaculture (Baharuddin et al., 2014). Family Pangasiidae has high potential for aquaculture in Malaysia and in Southeast Asia (Hung et al., 2014) as *P. nasutus* can cost up to USD 25/kg in the market nowadays (Syazwani, 2020).

Distribution of *Pangasius nasutus*: *Pangasius nasutus* is one of Peninsular Malaysia's native freshwater species, it is most frequently found in the rivers of Pahang State, particularly in the Pekan and Maran Districts (DOF, 2005). *Pangasius nasutus* can be found in the Perak River (Baharuddin et al., 2014). While in Sumatra, this species can be found specifically in The Musi, Indragiri rivers, Barito, and Batang Rajang rivers (Gustiano et al., 2018). About 21 recognized species in the genus *Pangasius* are from India in the Southern Part of Asia up to the Indonesia Archipelago in Southeast Asia (Gustiano et al., 2018). *Pangasius nasutus* live in the middle or upper part of the major rivers (Gustiano et al., 2018).

Feeding habit of *Pangasius nasutus*: In freshwater fish, feeding categories can be divided into four types namely omnivorous, herbivorous, carnivorous, and plankton feeders. Fish that have more than 75% of the total gut content of plant material is considered herbivorous. Aquatic plants, unicellular algae, filamentous algae, as well as a small amount of sand or mud, are the main sources of food for herbivorous fish (German et al., 2006). Aquatic plants, unicellular and filamentous algae, rotifers, insects, insect larvae, and crustaceans are a few examples of the creatures and plants that omnivore fish ingest. A substantial proportion of animals, including insects, beetles, crustaceans, water bugs, dragonfly larvae, and small fishes, are consumed by carnivorous fish (Chang et al., 2010). By filtering the water through their gill rakers, plankton feeders eat phytoplankton and zooplankton. Juveniles of *P. nasutus* are omnivorous and consume various type of food such as animal which includes crustacean, fish, insects, and small plants (David, 1963). With an average of 60% protein requirement, feed formulations for juvenile *Pangasius* sp. catfish in captivity often contain high levels of fish meal (Hung et al., 2013). Furthermore, high-protein soybean meal is an alternate protein source for fish culture (Siddiqui et al., 2014). Striped catfish, or *Pangasius hypophthalmus*, is an omnivore fish that consumes a wide range of foods, including fish, crabs, vegetable waste, algae insects, zooplankton, and higher plants and adult fish may consume fruits and other fish species (Rainboth, 1996). Their feeding habit varies to their life stage because several studies have found evidence of cannibalism within the first week following hatching (Vu and Huynh, 2020). The Mekong River fishing relies heavily on the *P. hypophthalmus* species, which is also utilized extensively in aquaculture. The presence of free neuromasts in the fish's body indicates that *P. hypophthalmus* feeding behavior is often driven by chemo-sensing rather than visual sense (Mukai et al., 2010). It is an adaptable species that can be fed from a variety of food sources because of its omnivorous eating habits.

Feeding behavior: Feeding behavior is influenced by a variety of factors, such as habitat and environment. The way fish consume food as well as the composition of their diet may influence their feeding behavior. Chemo receptors responding to nutrients detect olfactory gastronomic signals, which regulate feeding (Volkoff et al., 2006). Fish of different species use different sensory mechanisms. The majority of carnivorous fishes only use visual mechanisms to locate prey. Instead, many omnivorous fish, such as *Pangasius* sp., have precise feeding habits which means they search for non-living food via chemical stimuli. In the meantime, they use sight signals to prey on living food (Kam Foo, 2010). For example, the sensory organs of *P. hypophthalmus* are morphologically fresh when they hatch, but they quickly mature as the larva grows. Numerous taste buds could be detected on the larvae's barbels, head surface, and buccal cavity. At two days old larvae, other sensory organs and taste buds had also fully developed (Mukai et al., 2010). In the stage of juvenile and adult, gut content is fully developed and food such as molluscs, fishes, insects, and crustaceans can be digested well (Alikunhi, 1957).

Gut anatomy: A muscular tube enclosed by a mucous membrane of columnar epithelial cells can be defined as the gut (Switman et al., 2008). The head gut is the most anterior section, containing tissue origin of the two cavities, the oral (buccal), gill (branchial), and pharyngeal. The gut can be divided into the hindgut, foregut, and midgut. The mid and hindgut are the components of a fish gut. The intestine posterior to the pylorus, is the longest segment of the gut and may be coiled into complex loops, including in the mid gut. The esophagus, stomach, and pylorus are part of the foregut, which originates at the back edge of the gills. The beginning of the intestine in some fish is defined by an increase in gut diameter (Smiths, 1998)

As a carnivorous fish, the adult *P. nasutus* possesses a gut anatomy specifically adapted for efficient processing and digestion of prey. Among the various parts of the digestive tract, the pyloric stomach stands out with its notably thick muscularis (Sadeghinezhad et al., 2017). This muscular structure facilitates the relaxation needed to mix and grind food during the digestion process. Additionally, fish have a significant presence of goblet cells in their esophagus, which likely aids in the smooth transit of food to the stomach. These muscular contractions, combined with the secretion of digestive enzymes, play a vital role in breaking down prey items into smaller, digestible particles (Osman and Caceci, 1991; Domeneghini et al., 2005). This is necessary to absorb nutrients more quickly to have a more effective digestive system. Depending on their nutrition, habitat, and physiological requirements, different fish species may exhibit distinct specialized adaptations and variations in their gut structures.

Diet composition: Feeding behavior is another key factor affecting gastrointestinal microbial diversity.

The order of carnivores, omnivores, and herbivores was found to increase diversity in the GI microbial community (Ward et al., 2009; Larsen et al., 2014; Li et al., 2014; Miyake et al., 2015). Juvenile *P. nasutus* tends to be omnivorous, feeding on benthic organisms, hard seed or higher plants, and fishes (Bleeker, 1863). The adult *Pangasius pangasius* prefers molluscs and bottom-feeders called carnivores (David, 1963). As they grow, the adult *P. nasutus* tend to shift their diet become carnivorous based on their nutritional requirement and their digestive part becomes more complex compared to the juvenile *P. nasutus* stage (Chondar, 1999). Besides molluscs, fish, insects, crabs, and other organisms have also been observed in the intestines of adult *Pangasius* (Alikunhi, 1957; Menon et al., 1958). Juvenile *P. nasutus* consume various types of food such as crustaceans, fish, insects, and small plants (David, 1963). Most of the larvae stage feeding habit only focus on planktonic and small insects as their food source because their mouth and digestive part are too small to digest solid food. Live feed, such as small ants, amphipods, and copepods, is a major source of larval food consumption because it is rich in nutrients and easy to digest. Additionally, there is a lack of information from previous research that can be used to determine whether juvenile *Pangasius* is a carnivore or an omnivore. Therefore, more investigation is required to understand the dietary preferences of juvenile and adult *Pangasius* sp., both in the wild and in captivity.

Significance of interpreting dietary composition: Dietary intake affects gut flora through nutrient source and content. In general, plant-derived dietary proteins have been associated with a significant reduction in the variety of microbial flora (Desai et al., 2012) with an increase in the general abundance of *Pseudomonadales*, *Bacillales*, and *Lactobacillales* (Michl et al., 2017). Animal-derived proteins can promote the growth of bacteria such as *Bacteroidales, Clostridiales, Vibrionales, Fusobacteriales*, and *Alteromonadales* in the gut. Fish-fed diets based on synthetic casein exhibit greater diversity than fish-fed diets based on fish meals or soybean meals (Mansfield et al., 2017). Furthermore, the gut microbiome varies in composition depending on nutrition and diet (McDonald et al., 2012). Based on a recent study in Bangladesh, a comparison between farmed *Pangasius* sp. and wild *Pangasius* sp. revealed that wild *Pangasius* provides an optimal source of balanced essential amino acids and fatty acids compared to farmed *Pangasius* sp. (Chakma et al., 2022). Moreover, both wild and farmed *Pangasius* sp. in Bangladesh were safe for human consumption and met the daily EPA + DHA intake requirements recommended by the European Food Safety Authority (EFSA) (EFSA., 2010) for the prevention of primary cardiac diseases. Essential information can be extracted from dietary composition that assists in resolving more complex fish ecology problems. Dietary composition data provides in-depth insights into fish feeding ecology, resource availability, demand, potential competition, and other aspects of fish ecology, biology, consumption, or predation (Liao et al., 2001).

Microbes in Gastrointestinal (GI) tracts of *Pangasius nasutus*

Microbial composition of gut microbiome: Several studies have shown that the gut microbial community is shaped by nutrition and significant diversification that starts with the first feeding (Lauzon et al., 2010). New colonizing bacteria are obtained when the fish larvae start drinking water to regulate osmoregulation, and the microbiota then expands more through feeding (Hansen et al., 1999). It is recognised that the gastrointestinal (GI) tract microorganisms of fish play an important role in fish health (Begum et al., 2020), growth, and immunity (Perez et al., 2010). In addition, harmful microbes may enter through the gut, infect their host, and potentially reduce the population of fish species with high economic value (Begum et al., 2020). The presence of fish body nutrition of external and internal food sources would determine whether gut microbes will survive. In the meantime, many digesting enzymes can be secreted by gut microbiota, and they can produce nutrients like dietary sources during that activity (Bairagi et al., 2002).

The composition of gut microbes can be affected by feeding behaviour, lifestyle, and host genetic background (Yan et al., 2012). A recent meta-analysis data showed that the factors affecting the gut microbiota composition of fish are habitat, possibly host phylogeny, and trophic level (Sullam et al., 2012). Previous research in Bangladesh found that *Bacillus brevis, Staphylococcus gallinarum, Proteus* sp., *Aeromonas* sp., *Enterobacter* sp., *Klebsiella* sp. were found in the gut bacterial isolation from *P. hypopthalmus* collected from farm and market (Begum et al., 2020). Numerous studies have analyzed the gut microbial communities of various animal hosts over the past decade. However, most of them have focused on mammal gut microbiota (Colston et al., 2016), which represents less than 10% of vertebrate diversity. In contrast, fish exhibit the greatest species diversity among vertebrates (Nelson, 2006; Froese et al., 2019).

Previous research on *P. nasutus* collected from a local fish market in Pekan, Pahang, found *Lactococcus garvieae* in the stomach and gut content through co-isolation of Lactic Acid Bacteria (LAB) (Mohideen et al., 2023). This might also show the potential food sources the local farmers used to raise *P. nasutus*. Due to the high cost of fish meal, local farmers find that using chicken or broiler offal (necks, intestines, etc.) for fish feeding is more cost-effective. Eating this fish may have safety implications, as it is susceptible to ingesting these strains through its diet (Ismail et al., 2013). *Lactococcus garvieae* also have been reported in other aquatic species (Meyburgh et al., 2017) and are commonly associated with outbreaks of hemorrhagic sepsis in warm-water fish species like rainbow trout (Malek et al., 2019). Meanwhile, public health issues may arise if *P. nasutus* is consumed after being obtained commercially.

Functional potential of fish gut microbiome: The intestinal microbiome is formed up of the microbes that live in the intestinal lumen and are part of a microbial community. It is thought that this microbial community plays a crucial role in the host metabolism and immune role as well as in providing the recipient with nutritional benefits (Yukgehnaish et al., 2020). As a fish's diet changes from carnivorous to omnivorous to herbivorous, diversity typically increases (Liu et al., 2016). Additionally, the composition varies due to environmental factors. Freshwater fish guts are dominated by *Acinetobacter, Aeromonas, Flavobacterium, Lactococcus,* and *Pseudomona*s, as well as the obligate anaerobes *Bacteroides*, *Clostridium*, and *Fusobacterium* (Gómez and Balcázar, 2008). While, *Aeromonas, Alcaligenes, Alteromonas, Carnobacterium, Flavobacterium, Micrococcus, Moraxella, Pseudomonas,* and *Vibrio* are the dominant bacteria in the stomachs of marine fish (Gómez et al., 2008). Pure-culture experiments are essential for an accurate understanding of each population's functions, and it is crucial to isolate the physiologically dominant populations in a microbial community (Watanabe et al., 1998). The diversity of the gut's microbes is different depending on the species and range of physiological factors, including temperature, pH, the availability of nutrients, and environmental conditions. Physiological factors also impact the gut's microbes and immune system across every aspect of metagenomics, prebiotics, and probiotics (Yukgehnaish et al., 2020).

For the treatment of pathogen-induced bacterial diseases, various beneficial bacterial or probiotic strains have been produced, and their efficiency has been demonstrated (Verschuere et al., 2000). Some beneficial microbes can reduce or even eliminate inhibitory substances (Teplitski et al., 2009). A diverse microbial community that consists of harmless and helpful bacteria must be cultivated in an aquatic hatchery to maintain a good culture environment (Schulze et al., 2006). Due to the high density and proximity of resident bacteria in the gastrointestinal tract microenvironment, common microbiota components may transmit resistance genes via horizontal gene transfer (Navarrete et al., 2008).

Prebiotics are regarded as an environmentally beneficial feed supplement in aquaculture. Prebiotics are fermented by bacteria in the gastrointestinal tract, and reaction by-products are beneficial to the host's health. Therefore, prebiotics improve gastrointestinal conditions that promote those already-existing bacterial species boost growth efficiency, and decrease the susceptibility of the pathogen of the host organism, which appears to be an outstanding strategy that will help in the rapid growth of the aquaculture industry (Reverter et al., 2014). Prebiotics are an essential dietary supplement for enhancing growth and microbial activity in the digestive tract, which frequently improves the immune system and stress tolerance. Prebiotics are non-digestible substances that support good health by being digested by beneficial microbes like *Lactobacillus* and *Bifidobacterium*. Due to their ability to decrease the presence of intestinal pathogens and/or change the production of health-related bacterial metabolites, these bacteria have been considered to be advantageous to the host's growth and development (Roberfroid, 1993; Gibson and Roberfroid, 1995; Manning and Gibson, 2004). Common prebiotics established in fish are fructooligosaccharides (FOS) and inulin. Dietary Fructooligosaccharides, can influence the immunological function of fish and positively build intestinal microbiome communities. Multiple studies found that FOS could enhance fish humoral immune response (Mahious et al. 2006; Ring et al., 2014). Inulin, used in aquaculture and not a natural fiber in fish diets encourages normal bacterial flora in the stomach while also resisting infections and boosting the immune system (Possemiers et al., 2009). The use of inulin as a prebiotic in aquaculture encourages the growth of beneficial gut bacteria, inhibits harmful organisms, and improves immunological response. Several investigations examined the host's ability to survive after consuming prebiotics. Moreover, prebiotics typically promote weight gain of the host (Yukgehnaish et al., 2020).

The immunological systems of higher vertebrates, which include the innate and adaptive immune systems, are very similar to the fish. The innate immune system is assisted by the presence of antigens in monocytes and macrophages through cytokine and chemokine receptor profiles, allowing the adaptive immune system to obtain memory through the production of antibodies, while the antigen-containing bodies are prone to elimination. This provides immunity to a wide range of pathogenic microorganisms that are harmful to fish health (Gomez et al., 2008). Numerous biological components, including antimicrobial peptides, bile, mucosal layer, proteases, and stomach acids, are involved in the host's innate resistance (Huising et al., 2003). In addition to providing the host fish with some immunological benefits, several beneficial microbes that form in the gut microbiota of fishes also influence the fish's innate immune system by interacting with host NK cells, monocytes, and neutrophils (Yukgehnaish et al., 2020). Next, competition for nutrients, stimulating the host fish's nonspecific immune system, antagonism of pathogenic bacteria through excessive secretion of antimicrobial molecules, and competition for adhesion sites are all part of the mechanism through beneficial bacteria that help to modulate the immune response of the host fish to pathogenic bacteria. Lactic acid bacteria (LAB) like *L. rhamnosus, Clostridium butyricum, L. sakei,* and *L. lactis* boosted the levels of host fish immunoglobulin, and as a result, *Pseudomonas fluorescens*, a biological control agent that produces siderophores, was also successfully applied in the gut.

Probiotics have been proven to be bioactive, living microbial food/feed additives that enhance nutrition and disease resistance by positively affecting the microflora of the gastrointestinal tract (GIT) and digestion in general (Verschuere et al., 2000; Hoseinifar et al., 2016). Probiotics are live organisms that are consumed with food and have a variety of positive effects on the host (often bacteria, yeast, or a combination of both) (Fuller, 1989). A modified definition of probiotics in aquaculture has been proposed in considering the variances between the aquatic ecosystems and those of terrestrial organisms which is "A probiotic organism can be defined as a live, dead, or component of a microbial cell that is introduced via feed or to the rearing water that helps the host by improving health status, disease resistance, feed utilization, growth performance, general vigor or stress response, which occurs at least by improving the microbial balance of the ambient environment or host's microbial balance (Merrifield et al., 2010b). Several nations, including the USA and Canada, have limited the use of certain antibiotics in the aquaculture sector. In order to battle pathogenic agents through a variety of pathways as an alternative driving force of treatment with antibiotics, the use of probiotics together with food supplementation is a highly effective method (Bandyopadhyay et al., 2015; Wu et al., 2015). Probiotics also can contribute significantly to the productivity of aquaculture by offering pollution-free water sources and enhanced protection against non-specific diseases (Panigrahi et al., 2010; Nandi et al., 2018). The Food and Agricultural Organization (FAO) has now suggested using probiotics to enhance the quality of the aquatic environment by minimizing mortality (Subasinghe, 2005) or by improving resistance to potential host infections (Irianto and Austin, 2002). Interaction between host and probiotic is not limited to the digestive system but probiotic bacteria may also be present in the host's surroundings as well as on its gills or skin. It is because the culture environment and the host interact very closely in aquaculture, it implies that most probiotics are introduced via the culture environment rather than directly from feed (Fuller, 1989). The use of probiotics is one of the most important technologies that has been developed in response to issues with disease control (Browdy, 1998).

Application of probiotics in aquaculture: Probiotics function either directly or indirectly affect ways the body controls appetite and growth. Additionally, it appears that the host-associated microbiota is essential for the healthy growth and differentiation of gut components. Here, it appears that a healthy microbiota can influence epithelial cell growth, including the development of mucosal layers (Rawls et al., 2006; Bates et al., 2006). For example, rainbow trout gut that have probiotic *Pediococcus acidilactici* through their feed supplemented have enlarged their absorptive surface of the gut via an increased microvilli length in the proximal gut (Merrifield et al., 2009; Merrifield et al., 2009). Next, the most popular probiotic selections in aquaculture are *Bacillus* spp. and Lactic acid bacteria (LAB) (Soltani et al., 2019; Ringo et al., 2020;). The treatment of mixed probiotic spores of *Bacillus amyloliquefaciens* 54A, and *Bacillus pumilus* 47B raised antimicrobial peptides AMP levels in striped catfish *Pangasianodon hypophthalmus* (Thy et al., 2017). The mucosal epithelium is one of the oldest and most prevalent parts of the innate immune response. As a result, mucin glycoproteins create a physical barrier across the epithelium that inhibits pathogen adherence. In addition, the presence of probiotic *Bacillus mesentericus, Bifidobacterium infantis,* and *Bacillus coagulans* have been found in the gut of rosy barb (*Puntius conchonius*) via *in vivo* study and the number of pathogenic strains was reduced while the gut microflora's composition was significantly altered (Dhivya et al., 2012). Lactic acid bacteria (LAB) have several effects on the gut immune system that have been reported upon LAB administration for different fish species. For example, in Nile Tilapia, the administration of the probiotic *Lactobacillus rhamnosus* increases villous height in the mid-intestine and proximal along with increased intraepithelial lymphosytes number and acidophilic granulocytes (Pirarat et al., 2011). Meanwhile, based on previous research performed in rainbow trout, probiotic *Lactobacillus sakei*, *Lactococcus lactis* subsp.*, Leuconostoc mesenteroides* and *lactis* is the co-administration that results in enhanced phagocytic activity in gut leukocytes (Balcázar et al., 2006).

Probiotics can improve the feed's digestibility or supply microbial metabolites such as cofactors, vitamins, or vital fatty acids, which can boost the feed's nutritional value (Wuertz et al., 2021). Next, in aquaculture, water quality is important to maintain the quality of stocks and to prevent disease. Due to the capacity of probiotics to take part in the turnover of organic nutrients in aquaculture, probiotics can help in the improvement of water quality (Wang et al., 2007; Wang and Wang, 2008). Probiotics are recommended to be kept at high levels in production ponds to reduce the organic carbon load, improve the water quality, and promote the health of the fish since they are more effective at converting organic matter to CO_2 (Wuertz et al., 2021). Additionally, probiotic agents are essential for the host's improvement in nutrient absorption. The rise in levels of crude lipid, total protein, and body weight in Nile tilapia (*Oreochromis niloticus*) fed probiotic *Lactobacillus* sp. strain has been reported (Hamdan et al., 2016). Probiotic microorganisms also help aquatic animals' gastrointestinal (GI) tracts in both the digestion of dietary components and the production of energy. Lactic acid bacteria LAB preparations are the most often used probiotics for this purpose (Ringø et al., 2018). Probiotics may also increase the surface area of the host GIT, according to a few recent studies, which were based on quantitative changes in histological measurements of the area of the microvillus, enterochromaffin cells, and intestinal fold (Zhou et al., 2010). Probiotic *Vibrio* sp. and *Pseudomonas* sp. in aquaculture can against viral pathogens such as 'infectious hematopoietic necrosis virus' (IHNV) (Sahu et al., 2008). The lymphocystis disease virus (LCDV) is resistant to *Paralychthys olivaceus* fed a diet containing Sporolac (*Lactobacillus* sp.). Similar studies have demonstrated that grouper fish given the probiotic strain of *Bacillus subtilis* E20 developed higher viral resistance (Liu et al., 2012). Since probiotics have positive effects on all age groups, from larvae to adults, probiotic regulation of the gut microbiota is not constrained to fish age and maturity (Merrifield and Carnevali, 2014).

Challenges and biases in gut content analysis of *Pangasius nasutus*: *Pangasius nasutus* is challenging to find in the market because its availability depends on wild catches, which have been reduced due to pollution in the area (MDC, 2015). The main economic activities in Maran, Pahang are focused on agriculture, farming, and fishing, which may be the main sources of pollution in this area. Maran is surrounded by isolated forests and oil palm plantations (MDC, 2015). The extraction of high-quality colony DNA from the microbial populations is a significant problem because the gut secretes and absorbs a variety of enzymes while carrying out activities, including physically and chemically processing the food and extracting and absorbing minerals. For instance, the presence of complex polysaccharides and bile salts in the stomach limits PCR amplification's downstream activities (Schrader et al., 2012). Another limiting aspect of DNA barcoding of stomach contents is DNA degradation during digestion which impacts the lack of study on gut content analysis (Kresset et al., 2015; Sakaguchiet et al., 2017). Since stomach contents will still be digesting (Chipps et al., 2007) after fish is dissected, it is important to preserve fish or remove guts as soon as possible to avoid losing resolution. Different species digest at different rates (Sutela et al., 2000; Kim et al., 2001).

There is still a lack of research about the feeding habits and microbial communities of *P. nasutus* which is important for their survival rate in the population. Therefore, studies on feeding habits and microbial communities are important to determine their feeding habits and food sources that can be used to increase stock culture of *P. nasutus*. To obtain a deeper knowledge of the diet of *P. nasutus* or other fish species, researchers must realize and take into consideration these challenges and biases when interpreting the outcomes of stomach content analysis.

Future perspectives in stomach content analysis of *Pangasius nasutus*: Detailed studies of the gut microbial genetic diversity are required to offer significant insight into their functional potential that has limited composition for fish gut microbiome. These analyses can be made easier by sequencing and analyzing the cultured isolates' genomes, as has been done in other niches of research (Sangwan et al., 2015). The symbiotic and antagonistic interactions between bacteria and eukaryotes, such as fish, crustaceans, and molluscs, can be better understood with the application of metagenomics. The information obtained from the extracted DNA can be associated with host or environment-specific host species (Suttle, 2007; Gianoulis et al., 2009). Additional evidence on the microbial diversity in aquaculture facilities could be discovered through metagenomics. We are currently able to understand the broad range of these microbes by examining hypervariable areas of 16S rDNA for prokaryotes and 18S for eukaryotes (Not et al., 2009; Hugerth et al., 2014). Furthermore, the taxonomic resolution of prey can be enhanced through the emerging technology of DNA metabarcoding from stomach-content samples. This barcoding strategy is a valuable addition to visual approaches, such as presence-absence and relative-fullness methods, despite some technological challenges. It is worth noting that additional diet-tracing methods, including stable isotopes and biomarkers like fatty acids, are also in widespread use (Nielsen et al., 2018).

A new technology called the 'minimal gut genome' has found wide applications in humans and plays a role in maintaining the ecosystem's homeostasis. While the concept of the 'minimal gut genome' is relatively new, there is limited knowledge and research on the gut ecosystems of vertebrates, such as fish. Functional analyses are needed to explore the metabolic and genetic potential of the gut microbiome in animals like fish. This concept refers to studying more about genes that are essential for gut bacteria to compete in the gut environment (such as adhesion to the host epithelium) (Qin et al., 2010). The characterization of this basic gut genome might enhance our understanding of how evolution has produced successful gut-colonizing bacteria. It is expected to be present in most or all gut bacterial populations. These research projects will help us better understand how the microbiome influences changes in fish physiology and growth (Ghanbari et al., 2015).

The goal of metagenomic analysis is to identify probiotic candidates for commercially and nutritionally significant fish. This is achieved by altering the process by which bacteria accumulate their metabolic products, and also helps the host to perform better in terms of physiologically and immunologically. More studies are being carried out in metagenomic analysis on the gut of the fish such as the physiological effect of gut microbiome on the host fish and factors influencing the composition of the gut microbiome. Metagenomics analysis can also be used to study nutritional patterns, ecological influences such as trophic levels, pollutants, reef settlement, and the behavior of host fish species. These factors were investigated in relation to their potential impact on shaping the composition of fish gut microbiota, which was hypothesized to play a role in regulating the host fish (Baldo et al., 2015; Estruch et al. 2015; Brown-Peterson et al., 2015; Miyake et al., 2015; Eichmiller et al., 2016; Liu et al., 2016). Furthermore, metagenomics also can determine the genetic factor of the host fish on the impact of the target fish's gut microbiota, physiological change to the gut microbiota, and vice versa (Li et al., 2013; Smith et al., 2015). Metagenomics analysis on stomach content analysis is to determine the primary microbiota that inhibits fish species (Roeselers et al., 2011; Star et al., 2013; Parris et al., 2016). By studying metagenomics, we can understand the huge range of fish gut microbes by examining hypervariable areas of 16S rDNA for prokaryotes and 18S for eukaryotes (Not et al., 2009; Hugerth et al., 2014). According to the goals of the research, functional metagenomics or sequence-based metagenomics, also known as shotgun metagenomics, can be used to assess antibiotic resistance in gut bacterial communities (Schmieder and Edwards, 2012). Shotgun metagenomic methods may be helpful in the clinical detection of viral infections in fish, however, they have usually been used to explore genomic diversity (Rosario et al., 2009; Bibby et al., 2011). Next, cloning and transferring biologically produced DNA into a bacterial host are examples of functional metagenomic, which is used to determine the functions of genes that may not be fully understood by analyzing their sequences (Schmieder and Edwards, 2012). Metagenomics showed greater effectiveness and accuracy in the detection of multiple genomes when compared to other methods, such as PCR or microarrays (Yozwiak et al., 2012).

Conclusion

Previous research indicates that *P. nasutus* has been a significant economic resource for local fish farmers due to the high aquaculture potential of Pangasiidae in Malaysia and Southeast Asia. A comparison of stomach content analysis between wild fish and those in captivity can help identify variations in food consumption, ultimately determining their feeding habits. The composition of gut microbiota is influenced by feeding behavior, lifestyle, and host genetic background. By employing DNA extraction for bacterial genomic (gDNA) and metagenomic analysis, we can analyze stomach contents. Fish metabolism is influenced by their gut microbiota, which in turn affects their immune response against harmful bacteria. Further research focusing on the application of bacteria in the fish GI tracts is necessary. Lastly, studies on feeding habits and stomach content are essential to determine their dietary preferences and identify high-nutrient food sources that can enhance the growth and stock of *P. nasutus* in aquaculture.

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References

Alikunhi K.H. (1957). Fish culture in India. Farmers Bulletin of Indian Council of Agricultural Research, 20: 1-144.

Baharuddin H., Rizman-Idid M., Muniandy S., Zakaria M. (2014). The Occurrence of *Pangasius polyuranodon* Bleeker, 1852 (Teleostei: Pangasiidae) in Peninsular Malaysia with Remarks on the Comparative Morphology with *Pseudolais micronemus* (Bleeker, 1847). Sains Malaysiana, 43: 1707-1714.

Balcázar J.L., Vendrell D., de Blas I., Ruiz-Zarzuela I., Gironés O., Muzquiz J.L. (2006). Immune modulation by probiotic strains: Quantification of phagocytosis of *Aeromonas salmonicida* by leukocytes isolated from gut of rainbow trout (*Oncorhynchus mykiss*) using a radio labelling assay. Comparative Immunology Microbiology Infectious Diseases, 29(5): 335-43.

Baldo L., Riera J.L., Tooming-Klunderud A., Alba M.M., Salzburger W. (2015). Gut microbiota dynamics during dietary shift in eastern African cichlid fishes. PLoS ONE, 10(5).

Bandyopadhyay P., Mishra S., Sarkar B., Swain S.K., Pal A., Tripathy P.P., Ojha S.K. (2015). Dietary *Saccharomyces cerevisiae* boosts growth and immunity of Indian Major Carps *Labeo rohita* (Hamilton.) juveniles. Indian Journal of Microbiology, 55(1): 81-87.

Bates J.M., Mittge E., Kuhlman J., Baden K.N., Cheesman S.E., Guillemin K. (2006). Distinct signals from the microbiota promote different aspects of zebrafish gut differentiation. Developmental Biology, 297: 374-386.

Baumgartner G., Nakatani K., Gomes L., Bialetzki A., Sanches P. (2004). Identification of spawning sites and natural nurseries of fishes in the upper Paraná River, Brazil. Environmental Biology of Fishes, 71(2): 115-125.

Begum M.A., Punom N.J., Eshik M.M.E., Begum M.K., Khan T., Saha M.L., Rahman M.S. (2020). Pathogenic gut microbiota associated with striped catfish, *Pangasianodon hypophthalmus* cultured in Bangladesh and their antibiotic sensitivity pattern. Dhaka University Journal of Biological Sciences, 29(1): 61-73.

Belton B., Karim M., Thilsted S., Murshed-E-Jahan K., Collis W., Phillips M. (2011). Review of aquaculture and fish consumption in Bangladesh. In: Studies and reviews, The World Fish Center. 53 p.

Bibby K., Viau E., Peccia J. (2011). Viral metagenome analysis to guide human pathogen monitoring in environmental samples. Letters in Applied Microbiology, 52: 386-392.

Bleeker P. (1846). *Siluroideorum bataviensium* species nuperrime detectae. Natuur Geneeskunde Archives Nederland, 3: 284-293.

Bleeker P. (1847). Nieuwe bijdrage tot de kennis der Siluroiden van Java. Verhandelingen Bataviaasch Genootschap, 21: 1-12.

Bleeker P. (1852). Zesde bijdrage tot de kennis der ichthyologische fauna van Borneo. Visschen van Pamangkat, Bandjermassing, Braboekarta en Sampit. Natuur Tijdschrift NederlaIndsch, 3: 407-442.

Bleeker P. (1858). Zesde bijdrage tot de kennis der vischfauna van Sumatra. Visschen van Padang, Troessan, Priaman, Sibogha en Palembang. Acta Societiarum Scientiarum Indo-neerlandicae, 3: 1-50.

Bleeker P. (1863). Description de trois espèces nouvelles de Siluroïdes de l'Inde archipélagique. Verslagen Akademie Amsterdam, 15: 70-76.

Browdy C. (1998). Recent developments in penaeid broodstock and seed production technologies: improving the outlook for superior captive stocks. Aquaculture, 164: 3-21.

Brown-Peterson N.J., Krasnec M., Takeshita R., Ryan C.N., Griffitt K.J., Lay C.R., Mayer G.D., Bayha K.M., Hawkins W.E., Lipton I., Morris J.M., Griffitt R.J. (2015). A multiple endpoint analysis of the effects of chronic exposure to sediment contaminated with Deepwater Horizon oil on juvenile Southern flounder and their associated microbiomes. Aquatic Toxicology, 165: 197-209.

Chakma S., Rahman M.A., Siddik M.A.B., Hoque M.S., Islam S.M.M., Vatsos I.N. (2022). Nutritional Profiling of Wild (*Pangasius pangasius*) and Farmed (*Pangasius hypophthalmus*) *Pangasius* Catfish with Implications to Human Health. Fishes, 7(6): 309.

Chatchavalvanich K., Marcos R., Poonpirom J., Thongpan A., Rocha E. (2006). Histology of the digestive tract of the freshwater stingray *Himantura 545ignifier* Compagno and Roberts, (*Elasmobranchii, Dasyatidae*). Anatomy and Embryology, 211: 507-518.

Chipps S.R., Garvey J.E. (2007). Assessment of food habits and feeding patterns. In: C.S. Guy, Brown M.L. (Eds.). Analysis and interpretation of freshwater fisheries data. American Fisheries Society, Bethesda. pp: 473- 514.

Chondar S.L. (1999). Biology of Finfish and Shellfish. SCSC Publishers, India. pp: 1-514.

Clark G.W., Ackerman S.H., Tillier E.R., Gatti D.L. (2014). Multidimensional mutual information methods for the analysis of covariation in multiple sequence alignments. BMC Bioinformatics, 15: 157.

Clarke K.R., Gorley R.N. (2015). Getting started with PRIMER v7. PRIMER-E: Plymouth, Plymouth Marine Laboratory. Department of Fisheries. 2017. Annual Fisheries Statistics. Available from http://www.dof.gov.my.

Clarke K.R., Somerfield P.J., Gorley R.N. (2008). Testing of null hypotheses in exploratory community analyses: similarity profiles and biota-environment linkage. Journal of Experimental Marine Biology and Ecology, 366(1-2): 56-69.

Colston T.J, Jackson C.R. (2016). Microbiome evolution along divergent branches of the vertebrate tree of life: what is known and unknown. Molecular Ecology, 25(16): 3776-800.

David A. (1963). Fishery biology of the Schilbeid catfish, *Pangasius pangasius* (Hamilton) and its utility and propagation in culture ponds. Indian Journal of Fisheries, 10: 521-600.

Desai A.R., Links M.G., Collins S.A., Mansfield G.S., Drew M.D., Van Kessel A.G., Hill J.E. (2012). Effects of plant-based diets on the distal gut microbiome of rainbow trout (*Oncorhynchus mykiss*). Aquaculture, 350-353: 134-142.

Dhayalan A., Velramar B., Govindasamy B., Ramalingam K.R., Dilipkumar A., Pachiappan P. (2022). Isolation of a bacterial strain from the gut of the fish, *Systomus sarana*, identification of the isolated strain, optimized production of its protease, the enzyme purification, and partial structural characterization. Journal of Genetic Engineering and Biotechnology, 20(1).

Divya K.R., Isamma A., Ramasubramanian V., Sureshkumar S., Arunjith T.S. (2012). Colonization of probiotic bacteria and its impact on ornamental fish *Puntius conchonius*. Journal of Environmental Biology, 33(3): 551-5.

Eichmiller J.J., Hamilton M.J., Staley C., Sadowsky M.J., Sorensen P.W. (2016). Environment shapes the fecal microbiome of invasive carp species. Microbiome, 4: 44.

Ellerbroek L., Johne R. (2012). PCR inhibitors—occurrence, properties and removal. Journal of Applied Microbiology, 113: 1014-1026.

Erzini K., Gonçalves J.M.S., Bentes L., Lino P.G. (1997). Fish mouth dimensions and size selectivity in a Portuguese longline fishery. Journal of Applied Ichthyology, 13(1): 41-44.

Estruch G., Collado M.C., Penaranda D.S., Tomas Vidal A., Jover Cerda M., Perez Martınez G., Martines-Llorens S. (2015). Impact of fishmeal replacement in diets for gilthead sea bream (*Sparus aurata*) on the gastrointestinal microbiota determined by pyrosequencing the 16S rRNA Gene. PloS ONE, 10(8): e0136389.

European Food Safety Authority. EFSA Panel on Dietetic Products, Nutrition, and Allergies (NDA) (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol; European Food Safety Authority: Parma, Italy. 1461 p.

FAO. Fishery and Aquaculture Statistics. Global Production by Production Source (2020). 1950-2018 (FishtatJ) [Online]. Rome: FAO Fisheries and Aquaculture Department; www.fao.org/fishery /statistics/software/fishtatj/en. Accessed February 22, 2021.

Ferraris C.J. (2007). Checklist of catfishes, recent and fossil (Osteichthyes: *Siluriformes*), and catalogue of Silutiform primary types. Zootaxa, 1418(1): 628.

Froese R., Pauly D. (2019). FishBase: Species list: World

Wide Web electronic publication.

Froese R., Pauly D. (2018). *Pangasius nasutus* Bleeker, 1863. FishBase. Available: https://www.fishbase.de/summary/*Pangasius-nasutus*.html (August 2018).

Froese R., Pauly D. (2021). *Pangasius nasutus* summary page. FishBase. Retrieved December 2021, from https://www.fishbase.de/summary/pangasius-nasutus.html.

Fuller R. (1989). Probiotics in man and animals. Journal of Applied Bacteriology, 66: 365-78.

German D.P., Horn M.H. (2006). Gut length and mass in herbivorous and carnivorous prickleback fishes (Teleostei: *Stichaeidae*): ontogenetic, dietary, and phylogenetic effects. Marine Biology, 148: 1123-1134.

Ghanbari M., Kneifel W., Domig K.J. (2015). A new view of the fish gut microbiome: Advances from next-generation sequencing. Aquaculture, 448: 464-475.

Ghani A.A., Chang C.K., Leow C.S., Zakaria N.A. (2012). Sungai Pahang digital flood mapping: 2007 flood. International Journal of River Basin Management, 10(2): 139-148.

Gibson G.R., Roberfroid M.B. (1995) Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. The Journal of Nutrition, 125: 1401-1412.

Gomez G.D., Balcazar J.L. (2008). A review on the interactions between gut microbiota and innate immunity of fish. Federal European Microbiology Societies Immunology and Medical Microbiology, 52(2): 145-154.

Gupta S. (2016). *Pangasius pangasius* (Hamilton, 1822), a threatened fish of Indian Subcontinent. Journal of Aquaculture Research and Development, 07(02): 2-3.

Gustiano R., Pouyaud L. (2006). Diversity of Pangasiid catfishes from Sumatra. Bulletin Plasma Nutfah, 12: 83-88.

Gustiano R., Prakoso V.A., Ath-thar M.H.F. (2018). Asian catfish genus *Pangasius*: Diagnosis and distribution. Indonesian Fisheries Research Journal, 24(2): 99.

Hamdan A.M., El-Sayed A.F., Mahmoud M.M. (2016). Effects of a novel marine probiotic, *Lactobacillus plantarum* AH 78, on growth performance and immune response of Nile tilapia (*Oreochromis niloticus*). Journal of Applied Microbiology, 120(4): 1061-1073.

Hansen G., Olafsen J. (1999). Bacterial interactions in early life stages of marine cold water fish. Microbiology Ecology, 38: 1-26.

Hassan A., Ambak M.A., Samad A.P.A. (2011). Crossbreeding of *Pangasionodon hypopthalmus* (Sauvage,1878) and *Pangasius nasutus* (Bleeker, 1863) and Their Larval Development. Journal of Sustainability Science and Management, 6(1): 28-35.

Herre A.W.C.T., Myers G.S. (1937). A contribution to the ichthyology of the Malay Peninsula. Bulletin of the Raffles Museum, 13: 5-75.

Hoseinifar S.H., Ringø E., Masouleh A.S., Esteban M.A. (2016). Probiotic, prebiotic and synbiotic supplements in sturgeon aquaculture: a review. Review Aquaculture, 8: 89-102.

Hugerth L.W., Muller E.E.L., Hu Y.O.O., Leburn L.A.M., Roume H., Lundin D., Wilmes P., Andersson F. (2014). Systematic design of 18S rRNA gene primers for determining eukaryotic diversity in microbial consortia. PLoS ONE, 9: e95567.

Huising M.O., Stolte E., Flik G., Savelkoul H.F.J., Verburg-van Kemenade B.M.L. (2003). CXC chemokines and leukocyte chemotaxis in common carp (*Cyprinus carpio L.*). Developmental and Comparative Immunology, 27(10): 875-888.

Hungin A.P.S., Mulligan C., Pot B., Whorwell P., Agreus L., Fracasso P., Lionis C., Mendive J., Philippart de Foy J.-M., Rubin G., Winchester C., de Wit N. (2013). Systematic review: Probiotics in the management of lower gastrointestinal symptoms in clinical practice – an evidence-based international guide. Alimentary Pharmacology and Therapeutics, 38(8): 864-886.

Hussin M.H., CheLah N.A. (2020). Heavy metal and water turbidity impact on corroded patin fish cages in Temerloh River, Pahang, Malaysia. International Journal of Engineering and Advanced Technology, 9(3): 674-679.

Hyslop E.J. (1980). Stomach contents analysis: a review of methods and their application. Journal of Fish Biology, 17: 411-429.

Irianto A., Austin B. (2002). Probiotics in aquaculture. Journal of Fish Diseases, 25(11): 633-642.

Ismail S., Kamarudin M.S., Ramezani-Fard E. (2013). Performance of commercial poultry offal meal as fishmeal replacement in the diet of juvenile Malaysian Mahseer, *Tor tambroides*. Asian Journal of Animal and Veterinary Advances, 8: 284-292.

IUCN. (2013). IUCN Red List of Threatened Species. Version (2013).2. Accessed on 18 March 2014.

Joshua O., Adogbeji P., Bussa N., Campus A., Buss N. (2017). Factors Affecting Feed Intake in Cultured Fish Species, 14: 2697-2709.

Kam Foo Ng (2010). Feeding and swimming behavior of Patin, *Pangasius hypopthalmus* Larvae Under Dim Light Condition. Universiti Malaysia Sabah (Unpublished Thesis).

Kamler J.F., Pope K.L. (2001). Nonlethal methods of examining fish stomach contents. Reviews in Fisheries Science, 9(1): 1-11.

Karachle P.K., Stergiou K.I. (2008). The effect of season and sex on trophic levels of marine fishes. Journal of Fish Biology, 72(6): 14631487.

Karachle P.K., Stergiou K.I. (2010). Intestine morphometrics: A compilation and analysis of bibliographic data. Acta Ichthyologica et Piscatoria, 40(1): 45-54.

Karpouzi V.S., Stergiou K.I. (2003). The relationships between mouth size and shape and body length for 18 species of marine fishes and their trophic implications. Journal of Fish Biology, 62(6): 1353-1365.

Larsen A.M., Mohammed H.H., Arias C.R. (2014). Characterization of the gut microbiota of three commercially valuable warmwater fish species. Journal of Applied Microbiology, 116: 1396-1404.

Lauzon H.L., Gudmundsdottir S., Petursdottir S., Reynisson E., Steinarsson A., Oddgeirsson M., Biornsdottir R., Gudmundsdottir B.K. (2010). Microbiota of Atlantic cod (*Gadus morhua L.*) rearing systems at pre-and post-hatch stages and the effect of different treatments. Journal of Applied Microbiology, 109: 1775-1789.

Li J., Ni J., Wang C., Li X., Wu S., Zhang T., Yu Y., Yan Q. (2014). Comparative study on gastrointestinal microbiota of eight fish species with different feeding habits. Journal of Applied Microbiology, 117: 1750-1760.

Li X., Yan Q., Xie S., Hu W., Yu Y., Hu Z. (2013). Gut microbiota contributes to the growth of fast-growing transgenic common carp (*Cyprinus carpio L.*). PLos One, 8(5): e64577.

Liao H., Pierce C.L., Larscheid J.G. (2001). Empirical assessment of indices of prey importance in the diets of predacious fish. Transaction of the American Fish Society, 130(4): 583-591.

Lim K.K.P., Zakaria-Ismail M. (1995). The occurrence of the catfish *Helicophagus waandersii* (Pisces: *Pangasiidae*) in Peninsular Malaysia. Malayan Nature Journal, 49: 37-40.

Liu H., Guo X., Gooneratne R., Lai R., Zeng C., Zhan F. Wang W. (2016). The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. Scientific Reports, 6: 1-12.

Liu C.H., Chiu C.H., Wang S.W., Cheng W. (2012). Dietary administration of the probiotic, *Bacillus subtilis* E20, enhances the growth, innate immune responses, and disease resistance of the grouper, *Epinephelus coioides*. Fish and Shellfish Immunology, 33(4): 699-706.

Liu H., Guo X., Gooneratne R., Lai R., Zeng C., Zhan F., Wang W. (2016). The gut microbiome and degradation enzyme activity of wild freshwater fishes influenced by their trophic levels. Scientific Reports, 6(1).

Mahious A.S., Gatesoupe F.J., Hervi M., Metailler R., Ollevier F. (2006). Effect of dietary inulin and oligosaccharides as prebiotics for weaning turbot, *Psetta maxima* (Linnaeus, C. 1758). Aquaculture International, 14: 219–229.

Malek A., De la Hoz A., Gomez-Villegas W.I., Nowbakht C., Arias C.A. (2019). *Lactococcus garvieae*, an unusual pathogen in infective endocarditis: case report and review of the literature. BMC Infectious Diseases, 19(1): 301.

Manko P. (2016). Stomach content analysis in freshwater fish feeding ecology. University of Presov. 114 p.

Manning T.S., Gibson G.R. (2004). Prebiotics. Best Practice and Research Clinical Gastroenterology, 18(2): 287-298.

Mansfield G.S., Desai A.R., Nilson S.A., Van Kessel A.G., Drew M.D., Hill J.E. (2010). Characterization of rainbow trout (*Oncorhynchus mykiss*) intestinal microbiota and inflammatory marker gene expression in a recirculating aquaculture system. Aquaculture, 307: 95-104.

McDonald R., Schreier H.J., Watts J.E. (2012). Phylogenetic analysis of microbial communities in different regions of the gastrointestinal tract in *Panaque nigrolineatus*, a wood-eating fish. PLoS One, 7: e48018.

MDC. (2015). Official Portal of Maran District Council. Accessed at http://www.mdmaran.gov.my/. 23 February 2015.

Menon M.D., Chacko P.I. (1958). The food and feeding habits of some freshwater fishes of Madras State. Journal of Bombay Natural History Society, 55: 117-124.

Merrifield D.L., Carnevali O. (2014). Probiotic modulation of the gut microbiota of fish. In: D.L. Merrifield, E. Ringø (Eds.), Aquaculture Nutrition: Gut Health, Probiotics and Prebiotics,). Oxford, UK: Wiley Blackwell Publishing. pp: 185-222.

Merrifield D.L., Dimitroglou A., Foey A., Davies S.J., Baker R.T., Bøgwald J., Castex M., Ringo F. (2010b). The current status and future focus of probiotic and prebiotic applications for salmonids. Aquaculture, 302: 1-18.

Merrifield D., Bradley G., Harper G., Baker R., Munn C., Davies S. (2009). Assessment of the effects of vegetative and lyophilized *Pediococcus acidilactici* on growth, feed utilization, intestinal colonization and health parameters of rainbow trout (*Oncorhynchus mykiss Walbaum*). Aquaculture Nutrition, 17: 73-79.

Merrifield D.L., Harper G.M., Dimitroglou A., Ringã E., Davies S.J. (2009). Possible influence of probiotic adhesion to intestinal mucosa on the activity and morphology of rainbow trout (*Oncorhynchus mykiss*) enterocytes. Aquaculture Research, 41: 1268-1272.

Meyburgh C.M., Bragg R.R., Boucher C.E. (2017). *Lactococcus garvieae*: an emerging bacterial pathogen of fish. Diseases Aquatic Organisms, 123(1): 67-79

Michl S.C., Ratten J.M., Beyer M., Hasler M., LaRoche J., Schulz C. (2017). The malleable gut microbiome of juvenile rainbow trout (*Oncorhynchus mykiss*): Diet-dependent shifts of bacterial community structures. PLoS One, 12: e0177735.

Miyake S., Ngugi D.K., Stingl U. (2015). Diet strongly influences the gut microbiota of surgeonfishes. Molecular Ecology, 24: 656-672.

Mohideen N.H.R.H., alias N.S., Mansur N.N., Hamid T.A. (2023). The Co-isolation of lactic acid bacteria (LAB) and a related pathogenic strain from *Pangasius nasutus*. International Journal of Life Sciences and Biotechnology, 6(2): 143-154.

Mukai Y., Tuzan A.D., Lim L.S., Yahaya S. (2010). Feeding behavior under dark conditions in larvae of sutchi catfish *Pangasianodon hypophthalmus*. Fisheries Science, 76(3): 457-461.

Nandi A., Banerjee G., Dan S.K., Ghosh K., Ray A.K. (2018). Evaluation of *in vivo* probiotic efficiency of *Bacillus amyloliquefaciens* in *Labeo rohita* challenged by pathogenic strain of *Aeromonas hydrophila* MTCC 1739. Probiotics and Antimicrobial Proteins, 10(2): 391-398.

Navarrete P., Mardones P., Opazo R., Espejo R., Romero J. (2008). Oxytetracycline treatment reduces bacterial diversity of intestinal microbiota of Atlantic salmon. Journal of Aquatic Animal Health, 20(3): 177-83.

Nelson JS. (2006). Fishes of the World:4th Edition, John Wiley and Son, Hoboken. 601 p.

Not F., del Campo J., Balague V., de Vargas C., Massana R. (2009). New insights into the diversity of marine picoeukaryotes. PLoS ONE, 4: e7143.

Panigrahi A., Kiron V., Satoh S., Watanabe T. (2010). Probiotic bacteria *Lactobacillus rhamnosus* influences the blood profile in rainbow trout *Oncorhynchus mykiss* (Walbaum). Fish Physiology and Biochemistry, 36(4): 969-977.

Perez T., Balcazar J.L., Ruizzarzuela I., Halaihel N., Vendrell D., De Blas I., Muzquiz J.L. (2010). Host-microbiota interactions within the fish intestinal ecosystem. Mucosal Immunology, 3(4): 355-360.

Pirarat N., Pinpimai K., Endo M., Katagiri T., Ponpornpisit A., Chansue N., Maita M. (2011). Modulation of intestinal morphology and immunity in Nile tilapia (*Oreochromis niloticus*) by *Lactobacillus rhamnosus* GG. Research Veterinary Science, 91(3): 92-7.

Possemiers S., Grootaert C., Vermeiren J., Gross G., Marzorati M., Verstraete W., de Wiele T. (2009). The intestinal environment in health and disease – recent insights on the potential of intestinal bacteria to influence human health. Current Pharmaceutical Design, 15: 2051-2065.

Prim P., Lawrence J.M. (1975). Utilization of marine plants and their constituents by bacteria isolated from the gut of echinoids (Echinodermata). Marine Biology, 33(2): 167-173.

Rainboth W.J. (1996). Fishes of the Cambodian Mekong. FAO species identification field guide for fishery purposes. Food and Agriculture Organization, Rome. 265 p.

Rawls J.F., Mahowald M.A., Ley R.E., Gordon J.I. (2006). Reciprocal gut microbiota transplants from zebrafish and mice to germ-free recipients reveal host habitat selection. Cell, 127: 423-433.

Reverter M., Bontemps N., Lecchini D., Banaigs B., Sasal P. (2014). Use of plant extracts in fish aquaculture as an alternative to chemotherapy: Current status and future perspectives. Aquaculture, 433: 50-61.

Rezende C.F., Caramaschi E.P., Mazzoni R. (2008). Fluxo de energia em comunidades aquáticas, com ênfase em sistemas lóticos. Oecologia Brasiliensis, 12(4): 626-639.

Ringø E., Dimitroglou A., Hoseinifar S.H., Davies S.J. (2014). Prebiotics in finfish: An update. In: D. Merrifield, E. Ringø (Eds.), Aquaculture Nutrition: Gut

Health, Probiotics and Prebiotics. Wiley-Blackwell Publishing, Oxford, UK. pp: 360-400.

Ringø E., Van Doan H., Lee S.H., Soltani M., Hoseinifar S.H., Harikrishnan R., Song S.K. (2020). Probiotics, lactic acid bacteria and bacilli: interesting supplementation for aquaculture. Journal of Applied Microbiology, 129(1):116-36.

Roberfroid M. (1993). Dietary fibre, inulin and oligofructose: a review comparing their physiological effects. CRC Critical Reviews in Food Science Technology, 33: 103-148.

Roberts T.R., Vidthayanon C. (1991). Revision of the tropical Asian catfish family Pangasiidae with biological observations and descriptions of three new species. Proceedings of the Philadelphia Academy of Natural Sciences, 143: 97-144.

Roeselers G., Mittge E.K., Stephens W.Z., Parichy D.M., Cavanaugh C.M., Guillemin K., Rawls J.F. (2011). Evidence for a core gut microbiota in the zebrafish. The International Society for Microbial Ecology Journal, 5(10): 1595-1608.

Rosario K., Nilsson C., Lim Y.W., Ruan Y., Breitbart M. (2009). Metagenomic analysis of viruses in reclaimed water. Environmental Microbiology, 11: 2806-2820.

Sadeghinezhad J., Rahmati-Holasoo H., Mirzaei A., Mousavi H.E., Zadsar N. (2017). Histomorphological and mucin histochemical study of the alimentary canal of pangas catfish, *Pangasius pangasius* (Hamilton 1822). International Journal of Aquatic Biology, 5(3): 208-217.

Sahu M.K., Swarnakumar N.S., Sivakumar K., Thangaradjou T., Kannan L. (2008). Probiotics in aquaculture: importance and future perspectives. Indian Journal of Microbiology, 48(3): 299-308.

Sangwan N., Lambert C., Sharma A., Gupta V., Khurana P., Khurana J.P., Sockett R.E., Gilbert J.A., Lal R. (2015). Arsenic rich Himalayan hot spring metagenomics reveal genetically novel predator-prey genotypes. Environmental Microbiology Reports, 7: 812-823.

Schmieder R., Edwards R. (2012). Insights into antibiotic resistance through metagenomic approaches. Future Microbiology, 7: 73-89.

Schulze A., Alabi A.O., Tattersall-Sheldrake A.R., Miller K.M. (2006). Bacterial diversity in a marine hatchery: Balance between pathogenic and potentially probiotic bacterial strains. Aquaculture, 256(1-4): 50-73.

Sengupta S., Homechaudhuri S. (2011). Comparison of trophic niche and digestive enzymes of four species of catfishes of the Punarbhaba River in India. Indian Journal of Fisheries, 58: 79-85.

Siddiqui M.I., Khan M.A., Siddiqui M.W. (2014). Effect of soybean diet: Growth and conversion efficiencies of fingerling of stinging catfish, *Heteropneustes fossilis* (Bloch). Journal of King Saud University - Science, 26(2): 83-87.

Smith C.C., Snowberg L.K., Gregory Caporaso J., Knight R., Bolnick D.I. (2015). Dietary input of microbes and host genetic variation shape among-population differences in stickleback gut microbiota. The International Society for Microbial Ecology Journal, 9(11): 2515-2526.

Snovsky G., Golani D. (2012). The occurrence of an aquarium escapee, *Pangasius hypophthalmus* (Sauvage, 1878), (*Osteichthys, Siluriformes, Pangasiidae*) in Lake Kinneret (Sea of Galilee), Israel. BioInvasions Records, 1(2): 101-103.

Soltani M., Ghosh K., Hoseinifar S.H., Kumar V., Lymbery A.J., Roy S., Ringo E. (2019). Genus *Bacillus*, promising probiotics in aquaculture: aquatic animal origin, bio-active components, bioremediation and efficacy in fish and shellfish. Reviews in Fisheries Science and Aquaculture, 27(3): 331-79.

Star B., Haverkamp T.H., Jentoft S., Jakobsen K.S. (2013). Next generation sequencing shows high variation of the intestinal microbial species composition in Atlantic cod caught at a single location. BioMed Central Microbiology, 13: 248.

Subasinghe R.P. (2005). Epidemiological approach to aquatic animal health management: opportunities and challenges for developing countries to increase aquatic production through aquaculture. Preventive Veterinary Medicine, 67(2-3): 117-124.

Sullam K.E., Essinger S.D., Lozupone C.A., O'Connor M.P., Rosen G.L., Knight R., Kilham S.S., Russel J.A. (2012). Environmental and ecological factors that shape the gut bacterial communities of fish: a meta-analysis, 21: 3363-3378.

Syazwani N. (2020). Harga RM339.20 Sekilogram, Tips Dan Potensi Menternak Ikan Patin Air Tawar – Rentas Asia. Rentas Asia. Available https://rentas.asia/harga-rm339-20-sekilogram-tips-dan-potensi-menternak-ikan-patin-air-tawar/.

Teplitski M., Wright A.C., Lorca G. (2009). Biological approaches for controlling shellfish-associated pathogens. Current Opinion in Biotechnology, 20: 185-

190.

Thy H.T.T., Tri N.N., Quy O.M., Fotedar R., Kannika K., Unajak S., Areechon N. (2017). Effects of the dietary supplementation of mixed probiotic spores of *Bacillus amyloliquefaciens* 54A, and *Bacillus pumilus* 47B on growth, innate immunity and stress responses of striped catfish (*Pangasianodon hypophthalmus*). Fish and Shellfish Immunology, 60: 391-399.

Tweedie M.W.F. (1936). A list of the fishes in the collection of the Raffles Museum. Bulletin of the Raffles Museum, 12: 16-28.

Verschuere L., Rombaut G., Sorgeloos P., Verstraete W. (2000). Probiotic bacteria as biological control agents in aquaculture. Microbiology and Molecular Biology Reviews, 64: 655-671.

Vidthayanon C., Ng H.H. (2020). *Pangasius nasutus*. The IUCN red list of threatened species (2020): e.T180981A89815740.

Volkoff H., Peter R.E. (2006). Feeding behavior of fish and its control. Zebrafish, 3(2): 131-140.

Vu N., Huynh T. (2020). Optimized live feed regime significantly improves growth performance and survival rate for early life history stages of *Pangasius* catfish (*Pangasianodon hypophthalmus*). Fishes, 5(3): 20.

Wang D., Yao L., Yu J., Chen P., Hu R. (2021). Response to environmental factors of spawning ground in the Pearl River Estuary, China. Journal of Marine Science and Engineering, 9(7): 763.

Ward N.L., Steven B., Penn K., Methe B.A., Detrich W.H. (2009). Characterization of the intestinal microbiota of two Antarctic notothenioid fish species. Extremophiles, 13: 679-685.

Watanabe K., Teramoto M., Futamata H., Harayama S. (1998). Molecular detection, isolation, and physiological characterization of functionally dominant phenol-degrading bacteria in activated sludge. Applied and Environmental Microbiology, 64(11): 4396-4402.

Waters D.S., Kwak T.J., Arnott J.B., Pine W.E. (2004). Evaluation of stomach tubes and gastric lavage for sampling diets from blue catfish and flathead catfish. North American Journal of Fisheries Management, 24(1): 258-261.

Waycott B. (2015). *Pangasius* farming: water quality and biosecurity. *Pangasius* Farming: An Overview. The Fish Site. Available https://thefishsite.com/articles/pangasius-farming-an-overview

Wu Z.Q., Jiang C., Ling F., Wang G.X. (2015). Effects of dietary supplementation of intestinal autochthonous bacteria on the innate immunity and disease resistance of grass carp (*Ctenopharyngodon idellus*). Aquaculture, 438: 105-114.

Wuertz S., Schroeder A., Wanka K.M. (2021). Probiotics in fish nutrition—Long-Standing household remedy or native nutraceuticals? Water, 13(10): 1348.

Yan Q., Van Der Gast CJ., Yu Y. (2012). Bacterial community assembly and turnover within the intestines of developing zebrafish. Plos One, 7(1): e30603.

Yozwiak N.L., Skewes-Cox P., Stenglein M.D., Balmaseda A., Harris E., DeRisi J.L. (2012). Virus identification in unknown tropical febrile illness cases using deep sequencing. PLoS ONE, 6: e1485.

Yukgehnaish K., Kumar P., Sivachandran P., Marimuthu K., Arshad A., Paray B.A., Arockiaraj J. (2020). Gut microbiota metagenomics in aquaculture: factors influencing gut microbiome and its physiological role in fish. Reviews in Aquaculture, 12(3): 1903-1927.

Zhou Q.C., Buentello J.A., Gatlin D.M. (2010). Effects of dietary prebiotics on growth performance, immune response and intestinal morphology of red drum (*Sciaenops ocellatus*). Aquaculture, 309(1-4): 253-257.