Updating a new trend of horseshoe crab feeding behavior in captivity: towards a healthy practice of horseshoe crabs rearing

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Abstract. In the past few decades, the numbers of all four extant species of horseshoe crabs, namely *Limulus polyphemus, Tachypleus tridentatus, Tachypleus gigas* and *Carcinoscorpius rotundicauda* decline rapidly. Haemolymph is needed for rapid endotoxin detection kits for *Limulus/Tachypleus* Amoebocyte Lysate (LAL and TAL). Keeping the horseshoe crabs in their fittest condition prior to blood withdrawal in captivity needs ample knowledge about their feeding behavior and factors that affect their feeding rate. This review synthesizes the research available on horseshoe crab feeding behavior that relates to the consumption of food, and suggests possible feeding regimes compatible with hatchery management. Apart from that, conservation purposes triggered studies in developing alternative feeds for horseshoe crabs that are being discussed in terms of ingredient composition and feed additive characters to fit horseshoe crab alternative feeds in captivity. Since there were less available studies on nutrient requirements of horseshoe crabs in captivity, our references converged to the nearly equivalent various crustaceans species to suggest the optimal nutrient requirement needed by horseshoe crabs.
Key Words: alternative food, feeding factors, feeding regimes, food preferences, hatchery management.

Introduction. Horseshoe crabs are one of the oldest animals on earth and have survived for over 450 million years (Rudkin & Young 2009). They are slow-growing and bottom-dwelling animals. Their extant species, *Limulus polyphemus, Tachypleus tridentatus, Tachypleus gigas* and *Carcinoscorpius rotundicauda* need about 6 to 12 years to reach maturity depending on the species. They are opportunistic (Carmichael et al 2004) and their feeding behaviours are environmentally-dependent.

These millennial-lasting animals are experiencing extinction threats (John et al 2018; Vestbo et al 2018), such as habitat destruction, heavy metal pollution and are also harvested as food (John et al 2018; Joob & Wiwanitkit 2015). The discovery of horseshoe crabs amoebocyte for rapid endotoxin test in LAL and TAL kits many decades ago increased the demand for continuous supply, from 130000 to 250000 individuals since 1989 (ASMFC 1998) and had doubled its amount to 530797 individuals in 2013 (ASMFC 2013) with up to 15% post-bleeding mortality recorded (Rutecki et al 2004).

Prior to collecting blood samples for TAL and LAL research, wild horseshoe crabs are collected and kept in microenvironments (Noraznawati et al 2014). Conventionally, they are fed with various bivalves, their food within their niche, such as blood cockle, *Anadara granosa* and clams, like *Mercenaria mercenaria* (Botton 1984; John et al 2012; Mohd Razali et al 2017). Fit animals result from good diets and surrounding environments (Chen et al 2004). Natural food (bivalves), however, has fluctuations in minerals and nutrients (Padidela & Thummala 2015). This results from different culturing methods and environments (Padidela & Thummala 2015). Heavy reliance on natural food triggered the need for the development of alternative feeds (Chen et al 2004; Hu et al

2014). Ideal horseshoe crab alternative feed must mimic their natural food, being submerged in water column, easy to grip, with high digestibility and low immersion rate in the water column.

This review aims to discuss the horseshoe crab feeding behavior, diets in the wild and to suggest some optimal microenvironment conditions, including water quality and feeding regime for horseshoe crabs reared in captivity along with their ideal alternative food. Since there was scarce information available on the horseshoe crab nutrition, the review suggests the optimal horseshoe crabs nutrition characteristics partially based on previous studies on crustacean nutrition.

Horseshoe Crab Feeding Behavior. Horseshoe crabs use their chela, last pair of walking legs, and chelicerae, a pair of non-walking appendages, to direct the food to their mouths (Wyse 1971). Gnathobases are chitinous bristles serving as teeth, with the equivalent function of gripping the food, and are packed with chemoreceptors that trigger ingestion (Hayes & Barber 1982; Wyse 1971). Animal feeding behavior studies are essential to create the optimum hatchery management in terms of their micro-environmental conditions and feeding regime.

Horseshoe crab prey selection mechanisms. Horseshoe crab prey selection mechanisms in this review covered the niche of horseshoe crabs in their ecosystem. These hint their preferred feed in captivity. Apart from their body sensors that play a role in selecting prey, horseshoe crabs, like many other animals, have their internal anatomy and physiology that signals their sensors, such as digestive enzymes (Debnath 1992).

Vision. Each sense is developed differently to fit their ecological role within their niche and ecosystem (Pallas 2017). Horseshoe crabs have two lateral compound eyes, which consist of independent complex ommatidia (Barlow 2009). It is widely reported that the function of ommatidia is for mate hunting (Saunders et al 2010), but there is no reported studies justifying the function of ommatidia in foraging for food. However, it is believed that horseshoe crabs do not neglect their sight in food hunting. Therefore, it is assumable that vision is a secondary sense and apart from that, other senses had developed for hunting (Fahrenbach 1979, 1977; Wyse 1971).

Chemosensory system. The chemosensory system had been developed as the primary sense of horseshoe crabs in prey hunting. In the brain of *Limulus polyphemus*, Corpora pedunculata or mushroom bodies are linked to 1 million fine chemosensory fibers in gnathobases (Fahrenbach 1977) and are able to discriminate food from non-food (Hayes & Barber 1982; Wyse 1971). Chemoreceptors are sensory cells that respond to chemical stimuli. They are densely found on the celae, gnathobases and claws of horseshoe crabs. These structures are heavily used during feeding, suggesting chemoreception as a primary sense in food foraging (Mohd Razali & Zaleha 2018b). This is further supported with an in-lab experiment using *L. polyphemus* (Wyse 1971). The horseshoe crabs only responded to food solutions. Prey and non-prey discrimination lies on their ability to detect the stimulant properties, i.e. amino acids and their different perception in the amino acids make the food attractive in their own way (Kasumyan & Morsi 1996). Other than the types of amino acid present, the concentration of amino acids is also important (Jobling et al 2012). However, horseshoe crabs were less sensitive (0.01 to 0.1 M of amino acid) compared to other crustaceans (0.001 M of amino acid). It is suggested that the different needs of protein as an energy source between slow-growing horseshoe crabs and actively moulting crustaceans is a contributing factor.

Mechanosensory mechanism. Crustaceans such as lobsters and mud crabs rely partially on their mechanosensory mechanism in preying (De Oliveira et al 2015). The direct contact of the appendages, chelicerae and pedipalps with food triggers and initiates the feeding action in holding tanks (Mohd Razali & Zaleha 2018a). However, laboratory tests showed poor mechanosensory response to stimuli (Wyse 1971). This suggests that chemoreception and mechanosensitivity work independently during food foraging and cues from various senses altogether provide "food images" or prey discrimination from non-food.

Digestive enzymes. Other than the listed senses, horseshoe crabs also depend on their chelicerae to grip and gnathobases to chew their food, due to the absence of true jaws and teeth (Botton 1984). However, most of the grinding action is done in the gizzards, chitinous folded ridges in the guts (Debnath 1992). The concentration of digestive enzymes suggests the level of nutrients assimilated in animal's body (Table 1)(Pavasovic 2004). Therefore, by the digestive enzyme profile, the nutrient requirements can be determined, and this would help future research in designing the alternative feed formulation for horseshoe crabs. This could provide more precision than using growth rates and survival rates (Hu et al 2018, 2014). One of the few studies on horseshoe crab digestive profile was conducted by Debnath (1992), but no continuation was found afterwards.

Sand is reported to be present at around 7% in the gut of wild-caught horseshoe crabs (John et al 2012). Despite being undiscussed, the presence of sand is not believed to be accidental. Sand particles act as an abrasive agent. Friction generated by passive rubbing of sand and food particles is reported in avian species (Gionfriddo & Best 1996). Therefore, future food development for microenvironment-kept horseshoe crabs should take this into account for ideal alternative food criteria.

Table 1

The concentration of the most important digestive enzymes present in four different gut areas of *Tachypleus gigas* (mean±SE)

Enzyme assayed	*Esophagus*	*Gizzards*	*Intestine*	*Hepatopancreas*
Acid protease (mg tyrosine)	0.192±0.015	0.679±0.061	0.756±0.113	0.642±0.098
Alkali protease (mg tyrosine)	0.406±0.053	0.172±0.059	0.273±0.035	0.296±0.034
Eaterase (mg B-naphtol)	0.500±0.055	0.260±0.031	0.273±0.035	0.296±0.034
Cellulase (mg glucose)	10.944±1.061	11.529±1.037	7.736±0.618	9.351±0.912
Invertase (mg glucose)	3.462±1.209	4.653±0.601	5.899±0.612	6.059±0.557
Amylase (mg glucose)	13.587±1.290	15.640±1.801	15.658±1.733	16.608±2.011

Note: source - Debnath (1992).

Horseshoe crabs as environmental and dietary generalist species. Many slow growing animals showed the same life histories patterns: being environmental and dietary generalist species (Tuomainen & Candolin 2011), and horseshoe crabs are no exception (Debnath 1992). They tolerate a wide range of water salinity (8.2 to 31.9 ppt) and temperature (20 to 35^{o}C) (Debnath 1992) (Figure 1).

In terms of feeding, animals have in general the ability to switch their feeding behavioral mode, which is environmental-dependent (Rosen & Trites 2002; Smith et al 2013). Despite being good environmental generalist species, horseshoe crabs are not necessarily good diet generalists, as they showed biases in food preferences (Carmichael et al 2009; Rutecki et al 2004; Zhou & Morton 2004). They can switch their mode from being diet generalists to specialists when food is abundant or when the environment is more hospitable (Carmichael et al 2009). Horseshoe crabs experience metabolic depression upon starvation (Hu et al 2010). Despite being in a metabolically depressed-mode, there were no significant changes in body weight between starved and fed *T. tridentatus* and *C. rotundicauda*, probably due to the ability to refill the empty cavity from the loss of muscle with surrounding water (Hu et al 2010, 2011). However, better and direct parameters (blood parameters) indicate that horseshoe crabs store and utilize energy such as glucose and lipids within their body in unconducive environments (Hu et al 2010).

Food abundance is seasonally influenced (Botton 1984; Chatterji et al 1992; John et al 2012). Food scarcity force horseshoe crabs to prey on plant-based materials such as algae (John et al 2012). Therefore, horseshoe crab feeding is interchangeable throughout the year.

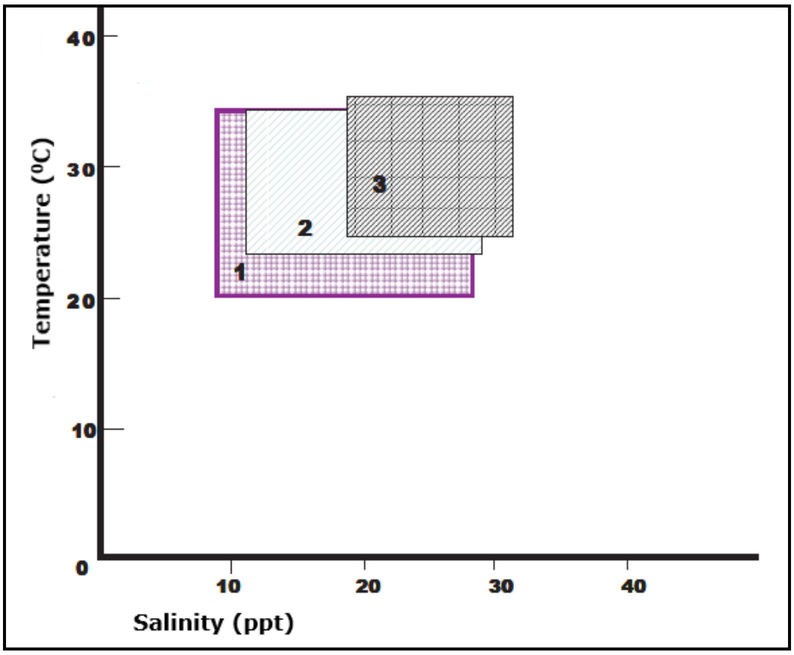

Figure 1. The climatograph of water salinity and temperature range where *T. gigas* was found around India. 1 - estuarine Sudarbans; 2 - estuarine Dharma and Mahanadi Delta; 3 - Digha and Chandipur seashore. Source: Debnath (1992).

Horseshoe crab hatchery management. Wild horseshoe crab feeding behavior hints to possible hatchery management characteristics in terms of microenvironment in the holding tanks and feeding regime. This helps in determining the optimal feed consumed by the horseshoe crabs in the holding tanks. Asian horseshoe crabs (*T. tridentatus, T. gigas* and *C. rotundicauda*) behaviors were less studied. The behavior of *L. polyphemus*, on the other hand, was widely studied (Chabot et al 2016; Chabot & Watson 2010; Saunders et al 2010; Watson et al 2008). The increasing research using horseshoe crabs as a model system for artificial insemination (Sheikh et al 2019) and alternative feeding (Razali et al 2020) had gained attention in studying the feeding behaviour of horseshoe crabs in-laboratory (Mohd Razali & Zaleha 2018a, 2018b).

Highlighting the horseshoe crab feeding regime and environmental conditions from previous studies is important in suggesting and encouraging the healthy rearing practice in captivity. The parameters and other details observed in previous studies are as follows: feeding should take place at 5 P. M. (Hu et al 2018); temperature should be maintained between 28 and 30^{o}C (Wang et al 2016); the salinity should be between 30 and 35 ppt (McGaw 2006); dissolved oxygen levels should range from 6 to 8 mg O_2 L^{-1} (Tzafrir-Prag et al 2010); ammonia levels should be maintained under 0.05 mg L^{-1} (Hu et al 2014); the water circulation should maintain a flow rate from 0.8 to 1.5 L min^{-1} (Kwan et al 2016).

Horseshoe crabs are usually administered feed in the evening, at 5 P. M. (Hu et al 2014). Traditionally, this practice has subscribed to the belief that horseshoe crabs present nocturnal behavior (circadian-influenced). However, studies found the opposite. For example, in one study, of all *L. polyphemus* tested, 40% of them were synchronized with tidal rhythm and only 10% of them showed light-dark rhythm, and some of them were arrhythmic (Chabot & Watson 2010). Even though the nocturnal behavior is disputed, night feeding regime practice probably mimics the calmer and quieter environment found at night. Their feeding activity is higher at night (from 5 pm to 8 am) than in day time (from 8 am to 5 pm), which supports the calmer night time environment theory (personal observations).

Sea water temperature in the holding tanks needs to be maintained between 28 and 30^{o}C (Chen et al 2010; Hu et al 2010), as it accelerates the feeding activity and, indirectly, the growth (Chen et al 2010; Wang et al 2016) (Figure 2). Temperature and feeding rate and linked. A lower gut retention time, or faster gut emptying, signals the hunger response and the need for more food, increasing the ingestion rate (Wang et al 2016). Dissolved oxygen needs to be higher than 6 mg L^{-1}, as the feeding activity, including foraging efforts, consume much oxygen (Hu et al 2011). The hypoxic condition is severe and can discourage feeding (Carmichael et al 2004). In many hatchery practices, the waters are aerated 24 hours daily.

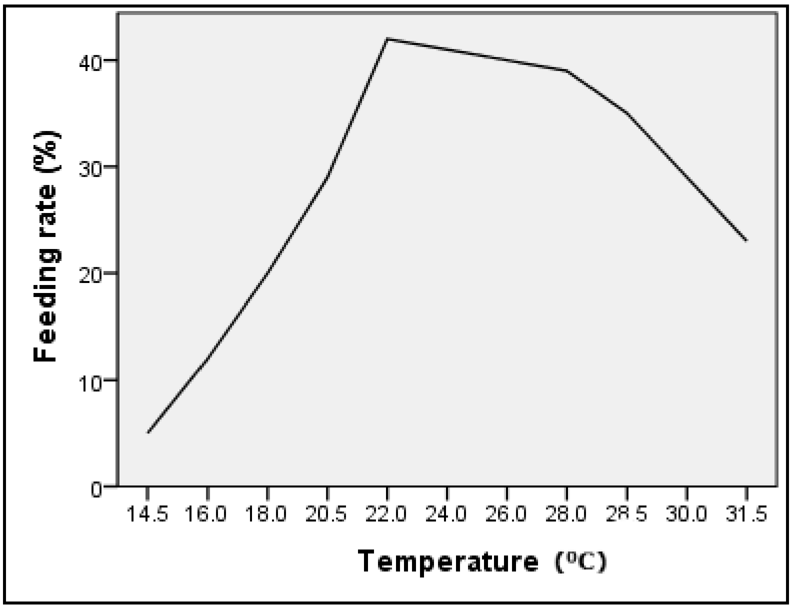

Figure 2. The relationship between *Tachypleus* spp. feeding rate and temperature. Feeding rate increased as temperature increases, before it declines rapidly after 28°C. Since *Tachypleus* spp. are mostly found in Asian tropical countries with high temperatures, the preferable water temperature for optimum feeding intake is also high (FAO 1990).

The feeding rates of horseshoe crabs were sparsely reported. This is probably because of the lack of conservation efforts of horseshoe crabs, especially the adults in captivity. In recent decades, there has been an amplification of conservation efforts for horseshoe crabs, especially for juveniles, to restore their population in the wild. One of the most important aspect in animal feeding regime is the feeding rate, which provides information for overcoming underfeeding and overfeeding, as both deteriorate the health of the horseshoe crabs and their environment (Mohd Razali & Zaleha 2018b). Horseshoe crabs are slow-growing and slow-feeder animals. They do not need much food. Previously, some feeding rates of horseshoe crabs were reported, like 1.5% of body weight (BW) for juveniles (Schreibman & Zarnoch 2009), 3% BW for juveniles (Tzafrir-Prag et al 2010), 7% BW for juveniles (Kwan et al 2016), or *ad libitum* feeding for adults (Hu et al 2011). Tzafrir-Prag et al (2010) reported that 3% of BW fed daily produced the highest weight gain and width gain in juveniles, while Mohd Razali & Zaleha (2018b) stated that the adults of *T. gigas* need a lower percentage (1.8% BW per day) of food.

Horseshoe crab diets in the wild. Diets of horseshoe crabs in the wild were widely studied by analyzing their gut contents (John et al 2012; Mohd Razali et al 2017; Zhou & Morton 2004). However, this method is controversial since biases occur as the gut contents are only 'snapshots', as only leftovers shown (Alexander et al 1996). It would not represent the whole diet preferences, as the food items might have been fully digested before the gut analysis was carried out. Gaines et al (2002) started to use new methods in studying *L. polyphemus*, using the content of nitrogen (N) and carbon (C) stable isotopes in horseshoe crabs, while Kwan et al (2018) analyzed the fatty acids content. This promising, more reliable and precise methods had unraveled the true diets of horseshoe crabs, because they did not only showed the food consumed by horseshoe crabs, but also the assimilated food (Kwan et al 2015). However, direct gut content analysis is still reliable (Saikia 2015).

Species-specific diet preference. Extensive studies on feeding were previously conducted on *L. polyphemus* (Carmichael et al 2004). They exhibited a strong preference for thin-shelled clams compared to highly abundant *Gemma gemma* clam in their environment. More recent studies showed a higher variety in the diet of adult *L. polyphemus*, from particulate organic matter (POM) to crustaceans and polychaetes (Carmichael et al 2004). Bivalves, on the other hand, were mostly found in the guts of *T. gigas* in Balramgari, in the Indian coasts. However, no specific species could be identified because of the small, broken pieces of shells. *T. gigas* found in Pahang, Malaysia, had consumed echinoderms during the open sea phase. These differences are attributed to geographical factors that affect the abundance and diversity of prey in each location. Surprisingly, both *L. polyphemus* and *T. gigas* forage most of their feed outside the spawning ground (Carmichael et al 2004; Mohd Razali et al 2017). The stable isotope studies with N and C of Carmichael et al (2004) show that hypoxic spawning grounds probably repel horseshoe crabs from scavenging the area.

C. rotundicauda on the other hand, forage their food in their spawning ground, and prefer gastropods and polychaetes the most, followed by bivalves, despite the higher abuncance of bivalves in Pahang coast, Malaysia (John et al 2012). Apart from the water quality in the spawning ground, no other reasons were found to explain the species-specific behavior. This species specific factor that drives the difference in food preferences is better understood by using sympatrically living horseshoe crabs to identify the interspecific competition that may play a role in their niche (John et al 2012; Mohd Razali et al 2017).

Life stage specific diet preference. The food selection of juvenile and adult horseshoe crabs is different (Carmichael et al 2009; Zhou & Morton 2004). Prey size is discussed in many studies (Carmichael et al 2009, 2004; John et al 2012). Juveniles of *T. tridentatus* and *C. rotundicauda* exhibit a clear desire for macrofauna, such as insect larvae and polychaete (*Chironomous* sp.) (Zhou & Morton 2004), whereas adult *T. gigas* and *C. rotundicauda* strongly avoid meiofauna in high concentrations. However, this might be inaccurate, or disputed, as the bivalves consumed were identified only from past shell debris that could not be excreted. Unlike small meiofaunal organisms, they were easily digested and probably fully digested upon catching.

However, an overlooked point, nutritional needs, should be important in answering the food preference at different stage of horseshoe crabs. Carmichael et al (2009) found that growing juveniles of *L. polyphemus* shifted their diets from algae and POM to crustaceans and polychaetes. Despite being rarely reported in horseshoe crab studies, the feeding patterns in juveniles, sub-adults and adults in crustaceans are linked with nutritional values needed prior to moulting (Sugumar et al 2013). By being unbiased towards the size of food, it is suggested that horseshoe crab prey selection in wild is nutrient-induced to maximize growth rate.

Sex-specific diet preference. No sex-linked differences in diets were observed (John et al 2012). Males and females prefer the same kind of food. However, Mohd Razali et al (2017) noted a greater diversity of food composition in the gut of females *T. gigas* ($P<0.05$). Also, the differences in intensity of feeding, determined from the gastro-somatic index (GSI) value were significant between sexes ($P<0.05$) (Mohd Razali et al 2017). Horseshoe crabs usually migrate inshore prior spawning with a full gut content. Higher gut fullness during post-spawning period (John et al 2012; Mohd Razali et al 2017) and longer gut transit time (Mohd Razali & Zaleha 2018b) were observed in males, suggesting restricted accessibility to food during spawning, the crab being in an amplexus position. Apart from this, certain studies suggested that lower levels of digestive enzymes in males aid in the mechanisms (John et al 2012; Mohd Razali et al 2017; Smith et al 2013).

Alternative Feeding Experiments with Horseshoe Crabs. What Do We Need to Know? There are many aspects needed to be taken care of in developing alternative feed for aquatic animals. Other than the abovementioned feeding regime, feeding behavior and diets of the horseshoe crabs in the wild, the accessibility and attractiveness of the feed is also important. Alternative feed must be submerged within the water column as the horseshoe crabs, specifically adults, are benthic feeders (Zhou & Morton 2004). The alternative feed must have a low leaching rate as horseshoe crabs take several hours to approach their food with a maximum of 16 hours of feeding time (Hu et al 2014). In terms of feed size, it must be approximately the size of the mouth opening of crabs. Feed size matters in preventing any food avoidance; for example, *L. polyphemus* rejects larger bivalves (Botton 1984).

Apart from the feeding rate and the holding tank environment that affects the feeding activity of the horseshoe crabs, the nutritional composition also plays a role and may affect the culturing cost of the horseshoe crabs in captivity. Previously, the nutritional requirements were widely studied in other crustaceans, such as crabs (Catacutan 2002; Genodepa & Failaman 2016), lobsters (Rodríguez-Viera et al 2017; Smith et al 2003) and shrimps (Kureshy & Davis 2002; Lee & Lee 2018). Three major nutrients that were widely discussed are proteins, lipids and carbohydrates (Nafikov & Beitz 2007). These nutrients varied greatly with the feeding mode (carnivores, omnivores and herbivores) and life stages (juveniles, sub-adults and adults) (Lee & Lee 2018). Food for cultured animals alone attribute up to 60% of the total hatchery management cost (Ghiasvand et al 2012). Therefore, a particular research should be conducted to measure the optimal nutrient requirements for specific animals at specific life stages.

Protein. Protein is the most expensive ingredient in developing alternative feeds (Watanabe 2002). Therefore, thorough research must be conducted to minimize the general feed cost, such as substitution of expensive protein with the cheaper options (Hu et al 2018). Proteins are utilized by animals in many ways, for muscle forming, growth, energy and others. Since many animals are able to spare protein from their diets and utilize other non-protein elements (such as lipid and carbohydrates) to fuel the daily activities, it is very crucial to study and measure the minimal total protein content that is needed (Holme et al 2008).

The total protein content required by crustaceans varies according to species and life stage, but usually ranges from 30 to 40% (Holme et al 2008). However, the majority of studies used crustacean juveniles and larvae such as crabs as model systems, as they were reared for commercialization purposes. Adult crustaceans are usually cultured for broodstock (Ikhwanuddin et al 2018). Information on adult crustacean total protein requirement was sparsely documented (Ikhwanuddin et al 2018). We suggest that actively moulting juveniles and larvae need more proteins than the adults, as they utilize more protein prior to moulting for muscle building. Recent artificial insemination and spawning of horseshoe crabs studies used adult individuals. Therefore, the studies on the protein requirement of adult horseshoe crabs held in captivity is crucial (Razali et al 2020).

Horseshoe crab culture studies first experimented with juveniles *L. polyphemus* and endagered *T. tridentatus* (Chen et al 2010; Schreibman & Zarnoch 2009). The efforts were focusing on the microenvironment to increase the survival rate of juveniles to restock the wild population. Later, the efforts were continued with nutrition studies and it was reported that juvenile horseshoe crabs need 40% total digestible protein. Adult horseshoe crabs needs at least 26% total protein content in their feeds (Razali et al 2020). Therefore, the horseshoe crab culture and the food depend on the life stages and purposes for culturing horseshoe crabs. Table 2 shows the summary of the total protein requirement of various species and life stages of crabs.

Table 2

The total protein requirement by various aquatic animals

Species	*Life stage*	*Dietary proteins*	*Source*
Crab			
Scylla serrata	Juvenile	32-40 %	Catacutan (2002)
	Juvenile	45-55%	Truong (2008)
Portunus trituberculatus	Juvenile	45%	Unnikrishnan & Paulraj (2010)
	Juvenile	51.5%	Jin et al (2013)
Fish			
Lutjanus argentimaculatus	Juvenile	43%	Abbas & Siddiqui (2013)
Sebastes schlegeli	Juvenile	42%	Lee et al (2002)
Lobster			
Panulirus ornatus	Juvenile	61%	Williams (2003)
	Juvenile	47.4-53.3%	Smith et al (2003)
Panulirus cygnus	Post-puerulus	50%	Glencross et al (2001)
Shrimp			
Litopenaeus vannamei		25-33%	Ayisi et al (2017)
	Juvenile	34% + AA	Nunes et al (2019)
	Juvenile	34.5% + supp	Lee & Lee (2018)
	Juvenile	30-36% + supp	Li et al (2017)
	Sub-adults	35.6% + supp	Lee & Lee (2018)
	Adults	32.2% + supp	Lee & Lee (2018)
Lysmata wusdermanni	Post-larvae	34-40%	Díaz-Jiménez et al (2019)
Horseshoe crab			
Tachypleus tridentatus	Juvenile	43 %	Hu et al (2014)
Carcinoscorpius rotundicauda	Juvenile	43 %	Hu et al (2014)
Carcinoscorpius rotundicauda	Adult	26 %	Razali et al (2020)

Note: supp - supplementation; AA - amino acid.

In the past decades, there has been an evolution trend in substituting conventional fishmeal and seafood meal with reusable waste with high sustainability from plant-based proteins, as the main ingredients in aquafeed studies (Floreto et al 2000; Holme et al 2006). Since horseshoe crabs are omnivorous (John et al 2012), their ideal food should be composed of mixed animal and plant proteins to reduce manufacturing costs. Hu et al (2014) formulated feeds from fish, rapeseed and soybean meals, whereas an extension of their studies (Hu et al 2018) utilized cheaper poultry waste meals, such as blood meal, meat and bone meal and poultry by-product meal. Only certain waste meals showed promising results (high survival rate and better growth performance), such as poultry by-product and meat and bone meals (Hu et al 2018) This fishmeal substitution had reduced the general cost, while improving the nutrient content of the feeds up to 25% (Hu et al 2014, 2018; Mente 2006). The plant-based proteins are not only cheap, but also act as sustainable and continuous sources (Dersjant-Li 2002).

Most common plant-based protein ingredients are soybean meal (Floreto et al 2000) and rapeseed meal (Hu et al 2014). Other than the growth performance of the animal, the general acceptability and absorption of the feed after the incorporation of the plant-based proteins in the aquafeed must also be taken into account. This is because the plant-based proteins confer anti-nutritional factors and deterrent properties that might affect the general feeding and nutrient absorption by the animals (Floreto et al 2000).

This caused thorough studies of the usage of plant-based proteins with other external stimulants, such as external amino acids (Floreto et al 2000). Only partial replacement (less than 50%) of animal proteins can deliver the best results (Floreto et al 2000).

Lipids. If the proteins aid in providing the amino acid for muscle growth, lipids play a major role in providing energy (Holme et al 2008). Total lipid concentrations needed by many crustaceans range from 6 to 12%. No studies were conducted, to our knowledge, to measure the optimal total lipids required by horseshoe crabs. With the same trend as proteins, the quantity of lipids required is species and life stage specific (Holme et al 2008) (Table 3).

Protein sources such as fishmeal usually contain lipids in small amounts (about 10%) (Mente 2006; Padidela & Thummala 2015). Usual alternative feeds are fortified with external lipid sources, such as fish oil (Han et al 2018; Hu et al 2014), since the lipids were usually gained externally from diet and not naturally produced within the body (Tocher 2010).

Table 3

Total lipid requirement by some crustaceans at different life stages

Species	*Life stage*	*Lipid level (%)*	*Source*
Lobster			
Jasus edwardsii	Juveniles	13.5	Williams (2003)
Panulirus ornatus	Juveniles	6-10%	Smith et al (2003)
Panulirus cygnus	Post-puerulus	10%	Glencross et al (2001)
Crab			
Portunus trituberculatus	Juveniles	10.47%	Han et al (2018)
Scylla serrata	Juveniles	6-12%	Catacutan (2002)
	Sub-adult	10%	Alava et al (2007)
Shrimp			
Lysmata wusdermanni	Post-larvae	7-8%	Díaz-Jiménez et al (2019)
Litopenaeus vannamei	Post-larvae	11.8-12.4%	Xie et al (2019)

Carbohydrates. Carbohydrates were given less attention by many feed developers in comparison to proteins and lipids (Wang et al 2016). This was probably because of the limited use of protein-sparing energy sources (Moon 2001; Wang et al 2016). Carbohydrate is a cheap source of instant energy for animals (Pavasovic 2004). The role of carbohydrates was tested in many aquatic animals, such as fish and crustaceans with promising results. Some of the tested and reviewed animals are fish (Hemre et al 2002), including grass carp (*Ctenopharyngodon idella*) (Gao et al 2010), tilapia (*Oreochromis niloticus*) (Azaza et al 2015) and crab (*Scylla serrata*) (Balito-Libo-On & Traifalgar 2017). No studies on horseshoe crabs were conducted for determining the optimum level of carbohydrates needed. Since amylase is present at high concentration in the digestive lining of horseshoe crabs (Debnath 1992), these results assume a high acceptance level of horseshoe crabs towards carbohydrates. Apart from sparing protein to provide the energy, carbohydrate also helps in better utilizing proteins and lipids (Catacutan et al 2003).

Most of the studies note that aquatic animals need between 20 and 30% of carbohydrate content (Holme et al 2008). The values vary because carbohydrate is present in many forms, such as starch, cellulose, glucose and sucrose, each of them having different physicochemical properties (Alcázar Alay & Meireles 2015) and different levels of digestibility and acceptance rate (Balito-Libo-On & Traifalgar 2017). Hence, not all types of carbohydrates deliver the same results (Balito-Libo-On & Traifalgar 2017). Early concerns of the incorporation of carbohydrate sources was with using plant-based materials as part of the aquafeeds. The majority of animals are carnivores and omnivores, with no or very little concentrations of amylase to digest carbohydrates. It was found that the degree of enzymatic activities is changeable depending on the different concentration of nutrients within the feed (Pavasovic 2004). Table 4 shows the optimal total carbohydrate level needed by various crustaceans.

Carbohydrates do not only provide energy, but it also gelatinizes the alternative feed mixture, preventing heavy leaching of the nutritional content (Partridge & Southgate 1999). Carbohydrate sources, such as starch, act as binders and bind the loose particle arrangement of the protein source (Alcázar Alay & Meireles 2015). Too little starch will result in poor bounding in the feed, promoting heavy leaching from the pellets, whereas too much starch will result in too hard pellets, despite the low leaching rate. Likewise, different types and compositions of the carbohydrate sources produce different outcomes in the palatability, water stability and texture of the alternative feeds (Apper-Bossard et al 2013; Fagbenro & Jauncey 1995).

Table 4

Summary of the total carbohydrate requirement needed by various crustaceans

Species	*Stages*	*Carbohydrate level*	*Source*
Lobster			
Panulirus argus	Intermoult (Juveniles)	20%	Rodríguez-viera et al (2017)
Jasus edwardsii	Juvenile	27%	Williams (2003)
Shrimp			
Shrimp	-	20-30%	Cuzon et al (2000)
Litopenaeus vannamei	-	15-20%	Li et al (2017)

Additives. Additives are used to enhance the attractiveness or to increase the shelf life of the alternative feeds (Santana et al 2015). Additives such as stimulants, binders and emulsifiers are usually incorporated in many alternative feed. Stimulants are used to increase the feeding activity in aquatic animals (Floreto et al 2000). The information about the need of stimulants for horseshoe crabs is generally absent. However, stimulants such as specific amino acids have been proven to stimulate feeding in lobster (*Homarus americanus*) (Floreto et al 2000). Other than that, emulsifiers such as soybean lecithin is incorporated to homogenize the ingredients and mould inhibitor is used to prevent the formation of mould, increasing the shelf life of alternative feeds.

Binders. Good binders are equivalently important as the other feed ingredients. Depending on the type of feed and the target species, binders can alter the characteristics of the feed. Possessing the same function as starch, binders keep the homogenized ingredients within feed intact, reducing the space between particles (Partridge & Southgate 1999). There are several commercial types of binders (natural and synthetic), that provide different functions and suit alternative feeds differently (Valverde et al 2008; Volpe et al 2014, 2012). The most common binders used are gelatins. To date, no prior research had studied the efficiency of different binders on the acceptability of horseshoe crabs, to our knowledge. Hu et al (2014) used gelatin in their alternative feed for juvenile *C. rotundicauda* and *T. tridentatus* without necessarily affecting the nitrogen and energy absorption in juvenile horseshoe crabs (Hu et al 2014, 2018).

Conclusions. Microenvironment-kept horseshoe crabs in hatcheries for research purposes need to have a fit condition. This is also applicable for juvenile horseshoe crabs for restocking purposes. Since overfeeding decelerates growth, formulated diets for horseshoe crabs must undergo some tests to search for the best formulation and ingredient composition. Natural food such as bivalves alone do not serve the needs of horseshoe crabs in farms. Rather, the development of alternative feeds and customizing the nutrients upon the daily requirement to obtain better diets for horseshoe crabs are minimizing the mortality rate of horseshoe crabs reared in captivity and improve the fitness.

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