

Adaptation and potential culture of wild amphipods and mysids as potential live feed in aquaculture: a review

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Live food such as phytoplankton and zooplankton are an important food source in the aquaculture fish industry. They are the dominant species of the benthic fauna with the highest diversity. Due to their small size, they are suitable for newly hatched larvae. *Artemia* and rotifer, are commonly live feed used in aquaculture, however; there is limited dietary value for each live feed which is not suitable for all of the cultured species. Currently, aquaculture depends on limited fisheries and feed with elevated n-3 LC-PUFA levels, but the development of more sustainable food sources is necessary. Even though *Artemia* is the best live feed due to its proficient nutritional quality, the cost is very expensive, which is about half of the production cost. A recent study suggests the use of amphipods and mysids as alternative live feed in aquaculture. High nutritional value is present in amphipods and mysids, especially proteins, lipids and essential fatty acids that required by fish larvae during early development. Amphipods and mysids are considered abundant in the aquatic ecosystem and have been used by the researcher in water toxicity studies. However, the culture of amphipods and mysids has been poorly studied. There is only a small-scale culture, under laboratory conditions for scientific research has been performed. Thus, further research is required to find a way to improve the mass culture of amphipods and mysids that can benefit the aquaculture industry. This review paper intended to provide the available information on the amphipods and mysids, including reproductive biology, culture method, nutritional value, feed enhancement and importance of them as potential live feed in aquaculture. This article is useful as a guideline for researcher, hatcheries operators and farmers.

Adaptation and potential culture of wild Amphipods and Mysids as potential live feed in aquaculture: a review

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Abstract

Live food such as phytoplankton and zooplankton are an important food source in the aquaculture fish industry. They are the dominant species of the benthic fauna with the highest diversity. Due to their small size, they are suitable for newly hatched larvae. *Artemia* and rotifer, are commonly live feed used in aquaculture, however; there is limited dietary value for each live feed which is not suitable for all of the cultured species. Currently, aquaculture depends on limited fisheries and feed with elevated n-3 LC-PUFA levels, but the development of more sustainable food sources is necessary. Even though *Artemia* is the best live feed due to its proficient nutritional quality, the cost is very expensive, which is about half of the production cost. A recent study suggests the use of amphipods and mysids as alternative live feed in aquaculture. High nutritional value is present in amphipods and mysids, especially proteins, lipids and essential fatty acids that required by fish larvae during early development. Amphipods and mysids are considered abundant in the aquatic ecosystem and have been used by the researcher in water toxicity studies. However, the culture of amphipods and mysids has been poorly studied. There is only a small-scale culture, under laboratory conditions for scientific research has been performed. Thus, further research is required to find a way to improve the mass culture of amphipods and mysids that can benefit the aquaculture industry. This review paper intended to provide the available information on the amphipods and mysids, including reproductive biology, culture method, nutritional value, feed enhancement and importance of them as potential live feed in aquaculture. This article is useful as a guideline for researcher, hatcheries operators and farmers.

Introduction

In the aquaculture industry, live food is typically used as a feed for larvae or fingerling with an emphasis on essential amino acids and fatty acids, as well as nutrients, vitamins, and minerals, to serve the inadequate amount of proteins and lipids needed for larvae growth (Hamre et al., 2013). Live feed such as *Artemia*, rotifer, copepod is mostly used in the aquaculture industry as a feed for larvae rearing. Marine aquaculture nowadays depends on this small number of live feed species in larvae rearing (Woods, 2009). However, these live feeds contain a limited dietary value which is not sufficient for all species cultures (Ostrowski & Laidley, 2001). There is also a need to find another suitable live feed to fulfil the dietary requirements of larval fish and crustaceans (Woods, 2009). Live feeds are planktonic, observable by naked eyes, and have a peculiar motion that draws larvae and crustaceans to eat and capture them (Samir & Banik, 2015). Live feed such as Amphipod has the potential to offer as substitute feed for aquaculture either it is still alive or dead (Baeza-Rojano, Hachero-Cruzado & Guerra-Garcia, 2014). They are small benthic crustaceans, commonly found in colonies and linked to a wide range of marine substrates throughout the world (Woods, 2009). They are the dominant species of the benthic fauna with the highest diversity (Cunha, Moreira & Sorbe, 2000a). Amphipod including crustacean characterized with substantial morphological modification to colonize various environments, as well as their resistance to a range of environmental conditions (Baeza-Rojano et al., 2014). Mysids are small shrimp-like crustaceans, a natural motile epibenthic invertebrate that is widely spread across marine environments, particularly in brackish, estuarine, coastal, and oceanic areas (Mauchline, 1980; Verslycke et al., 2004). Mysids are omnivorous and cannibal species (Berezina et al., 2019), which feed on the diatom and another small crustacean (Mauchline, 1980). They often prey on small aquatic species, such as phytoplankton, zooplankton, and detritus (Viherluoto, 2001). Mysids have been commonly used in the water toxicity studies as an indicator (Miller et al., 1990).

The use of live feed in aquaculture is essential to supply enough nutrients for newly hatched fish larvae. Marine aquaculture is mainly depending on the limited live food such as *Artemia*, rotifer, and copepod as feed for the larvae culture. Live food such as *Artemia*, rotifer, and copepod contain a limited dietary value that might not provide all the necessary nutrients to develop and reproduce all species culture (Ostrowski & Laidley, 2001). According to Shields et al. (1999), Rotifer and *Artemia* need to be enriched with a fatty acid, because they lack long-chain HUFA, especially DHA. Other than that, live food culture is generally expensive. The production costs of rotifer and *Artemia* are very high, ranging up to 79% of larval culture's total cost (Kolkovski, 2001). According to Awal, Andrew & Damien (2016), they have successfully cultivated amphipods using artificial substrates and confirmed that amphipods are higher in the population, growth, and survival than natural substrates. Though, there is still no report regarding people doing amphipod mass culture. Besides that, Drillet et al. (2011) has reported that the large-scale mysids culture has not been developed but currently is a very active area of research and development. Other than that, Mysids and amphipods are high in nutritional level required to

develop fish and crustaceans at the early stages (Eusebio, Coloso & Gapasin, 2010). This review is important to provide the available information on the amphipods and mysids, including nutritional value, reproductive biology, culture method, and feed enhancement for amphipods and mysids. Aquaculture could make good use of amphipods and mysids as an alternative food source. They are significant low trophic position organisms that aid in the transfer of nutrients from the ocean to the coastline and are crucial in the biological processing of algae inputs (Jimenez-Prada et al., 2021). They are also considered to be a significant source of food for fish species that are economically significant (Jimenez-Prada, Hachero-Cruzado, & Guerra-Garca, 2015; Lee et al., 2020).

Survey methodology

Google Scholar, Web of Science, Springer Link, and ScienceDirect resources were used to search articles from December 1965 to April 2023 that dealt with the use of alternative live feeds in aquaculture, enrichment diets, reproduction biology and the culture of amphipods and mysids as a potential live feed in aquaculture. This review included references to and excerpts from research and review articles written in English. We chose 118 articles in total to include in this review. The review did not include editorials, letters to the editor, or case studies.

Morphology characteristics and distribution: Amphipods and Mysids

Amphipods

Amphipoda (Crustacea, Malacostraca) is a major aquatic, estuarine, and terrestrial freshwater species. They live in pelagic and benthic compartments with various life patterns, ecosystem, environmental requirements, and ecological feeding (Podlesińska & Dabrowska, 2019). The previous study shows that amphipods widely use to test the marine and estuarine sediment quality due to their habitat and lifestyle. Amphipods are herbivores, detritivores or scavengers, omnivores, or parasites and are important compounds in marine ecosystems (Podlesińska & Dabrowska, 2019). Amphipods such as gammarids and corophids have laterally and dorsoventrally compressed bodies. According to Hyne (2011), sexual dimorphism and reproductive strategies vary within species. By looking at the presence of marsupium, a mature female can be identified. In contrast, the mature male can be identified by the emergence of genital papillae on the body's ventral side (Podlesińska & Dabrowska, 2019). In preparation for the next spawning, gravid females with embryos developing in the outer marsupial sac, oocyte maturation coincides within their ovaries. In exchange, amphipods bear their broods, which extend their breeding cycle after spawning (Hyne, 2011). Amphipods are commonly used as model organisms for determining the quality of marine and estuarine sediments due to their nature and lifestyle (Chapman, Wang & Caeiro, 2013; Podlesińska & Dabrowska, 2019). Epibenthic amphipods, such as gammarids, are abundant and ecologically important parts of marine benthic habitats since they exist in close interaction with sediment, are easy to manage

and culture in the laboratory (Hyne, 2011). As for Malaysian waters, the knowledge on amphipods has poorly been studied (Lim, Azman & Othman, 2010). Even though, eleven taxa including the new species of grammarian amphipods have been recorded by Azman & Othman (2013) at Pulau Tioman waters, it results in additional documented amphipod and provides new information on the range and distribution pattern of amphipods in the South China Sea. Figure 1 below shows a morphological diagram of adult amphipods.

Mysids

Mysids are generally small with lengths varying from 5 to 25 cm, also referred to as opossum shrimps, as oostegites form a ventral female marsupium to bear embryos in development (Verslycke et al., 2004). They are one of the most morphologically diverse classes of crustaceans (Tan & Rahim, 2018). Mysids species are omnivorous and cannibalistic (Berezina et al., 2017). The stomach of mysids gathered along the coast includes detritus, tiny crustacean bodies and appendages, and limited numbers of diatom shells (Herrera et al., 2011). Mysids are good candidates for determining endocrine disruption due to the abundance of information available on their endocrinology (Verslycke et al., 2004). Mysids have been used in regulatory toxicity studies for more than 20 years and it is responsible for certain organic emissions in the aquatic environment (Roast et al., 1998). Male mysids can be recognized by a presence of panels and secondary sexual characteristic while the female with marsupium presence (Ramarn, Chong & Hanamura, 2012). Mature females will hold broods of up to 25 eggs in their pouch and each newly hatched mysid is four times larger than *Artemia* nauplii (Hanamura, Siow & Chee, 2008). The new eggs will be produced as soon as after the pouch had been empty (Johnson, Stevens & Watling et al., 2001). Mysids are typically hyperbenthic, reside on or just above the sediment's surface, and usually move into the water column during night time. About 90% of the population is primarily marine water, but some live in brackish water and some exist in freshwater (Salemaa, Tyystjärvi-Muuronen & Aro, 1986). According to Tan, Azman & Othman (2014), Peninsular Malaysia waters recorded a total of 41 species of mysids and the most commonly found species are *Erythrops minute*, *Mesopodopsis orientalis*, *Acanthomysis longispina*, *Acanthomysis quadrispinosa*, *Lycomysis spinicauda*, *Pseudanchialina inermis*, and *Prionomysis aspera*. The first mysids species from Malaysian waters has been recorded by Tattersall (Tattersall, 1965), at northern region of the Malacca Straits. The details related to each species's geographical distribution from around the waters of Peninsular Malaysia can serve as a basic reference for future studies and provide an overview of the mysids shrimp species identified in these waters (Tan et al., 2014). Figure 2 below shows a morphological diagram of adult mysids.

Reproductive biology of Amphipods and Mysids

Amphipods

Amphipods has a direct development from juvenile to adulthood, which is after the juveniles are released from the females' brood chambers, they undergo several molts without metamorphosing

before becoming adults (Väinölä et al., 2008; Baeza-Rojano et al., 2010). The molt cycle can be divided into four periods which is post-molt, inter-molt, pre-molt, and ecdysis. The first stage, which starts at the final of the aged exoskeleton shedding, is known as an early post molt period. The second stage starts with the incremental coloring of the antennas and legs. During the late post-molting cycle, the calcareous concrete within the posterior caeca is then used to mineralize fresh skeleton. Next, there's the inter-molting process. At this stage, secretion of calcium carbonate and calcification of the last layer will continues to consolidates, which make up to 40% of crustaceans cuticle. When the new skeleton is gradually developed under the aged one, shows that it is entering the pre-molting period (Hyne, 2011). Molting and oogenic processes are closely related to malacostracan crustaceans with a high degree of fecundity and body development (Subramoniam, 2000). In preparation for the next spawning, fertile females with embryos undergoing development in the external marsupial pouch, and it happen concurrently within their ovaries during oocyte maturation. Also, amphipods bear their broods beyond spawning, extending their breeding period. The initiation of molting in these species is postponed until their juveniles are hatched and released (Sainte-Marie, 1991). The synchronization of the molting of the inflexible exoskeleton with the ovarian cycle allows the movement of the newly ovulated oocytes to the oviducts through the marsupium, while the existing exoskeleton is still strong enough to enable their passage (Hyne, 2011). According to Sainte-Marie (1991), Baeza-Rojano (2011), and Shahin et al. (2023a), amphipods such as *Grandidierella halophila*, *Pontoporeia affinis*, *Orchestia mediterranea*, *Caprella grandimana*, and *Gammarus palustris* have iteroparous, semiannual, multivoltine life histories that produce multiple broods in a brief lifespan. The characteristics of tropical amphipods can be defined by their maturation time, size and length of brood, fecundity rate, and juveniles' development (Cunha et al., 2000b; Grabowski et al., 2007; Baeza-Rojano et al., 2013b; Xue et al., 2013; Shahin et al. According to Wang et al. (2009), a female of *Grandidierella japonica* can produce 13 to 17 juveniles from four broods in their lifespan. Previous study has documented that among different species of amphipod, the number of embryos per broods are varies. As an example, in average, *Cymadusa filosa* produce 20 juveniles, *Parhyale hawaiiensis* produce 13 juveniles, *Elasmopus pecteniscrus* produce 7 juveniles, *Elasmopus levis* produce 22 juveniles, and *Eogammarus possjeticus* produce from 48 to 16 juveniles in their lifespan (Borowsky, 1986; Aravind et al. Understanding the potential fecundity of amphipods depends on a variety of aspects of their reproductive biology (Wang et al., 2009). Figure 3 below shows a general life cycle of amphipods.

Mysids

Numerous species of tropical mysids have been found to reproduce continuously throughout the year (Hanamura et al., 2008, 2009; Biju & Panampunnayil, 2010; Biju et al., 2010; Ramarn et al., 2011). Mysid reproductive biology resembles that of marsupials, including an embryonic stage, a nauplioid stage, and a post-nauplioid stage (Wittmann, 1981; San-Vicente et al., 2014). Mysid female marsupium has three distinct but sequential stages of development from oviposition to

early embryonic stages (Wortham-Neal & Price, 2002). At some point during her ecdysis, the mother will release her young before laying another clutch of eggs in the marsupium. Oocytes undergo a secondary vitellogenic phase beginning on day two of the molten stage (Verslycke et al., 2004). In crustaceans, including amphipods, isopods, and decapods, secondary vitellogenesis is cyclical and closely associated to the molten stage, serving as an example of a two-type form for the control of concurrent gonadal and somatic development (Verslycke et al., 2004). Therefore, the average percentage of mysids in ovigerous females might be used to estimate the duration of the embryonic, nauplioid, and post-nauplioid phases (Mauchline, 1973a; Wortham-Neal & Price, 2002). The nauplioid stage, the embryonic stage, and the post-nauplioid stage occur in progressively shorter succession as development proceeds (Mauchline, 1980; Delgado et al., 2013). The results showed that nauplioid-stage brooding females were much more common than those in any other developmental stage. The *Rhopalophthalmus hastatus* breeding females' widely varying mean body lengths are consistent with the general trend of increasing mysid body size through time (Yolanda et al., 2023). Another species exhibited very similar behaviour, and researchers concluded that this was due to the inter-moult development phase of the abdomen (Mauchline, 1973b; Fenton, 1994). While the number of offspring in a given brood may vary greatly amongst mysid species, in general, tropical mysids are smaller overall and have fewer broods than their temperate counterparts, who are bigger overall and have more broods (Mauchline, 1980; Fenton, 1992; Mees et al., 1994). *Rhopalophthalmus hastatus* may lay as many as 17 eggs at a once. *Rhopalophthalmus indicus*, *Rhopalophthalmus mediterraneus*, and *Rhopalophthalmus tattersallae* are all in the same genus and each have between 13 and 31 larvae (Grabe, 1989; Baldó et al., 2001; Biju et al., 2010). Brood loss or abortion during data collection, storage, and experimental processing accounts for differences in brood size, as stated by Murtaugh (1989) and Paul et al. (2016). In contrast, the brood limits for temperate mysids like the *Archaeomysis articulata*, *Gastrosaccus spinifer*, *Orientomysis japonica*, and *Neomysis integer* are 93, 162, 102, and 88, respectively (Hanamura, 1999; Rappé et al., 2011; Akiyama et al., 2015). Little is known about brood size, although a study on *R. hastatus* by Yolanda et al. (2023) indicated a substantial correlation between the size of the brood and the length of the brooding females. This indicates that a bigger marsupial mother is capable of producing and caring for a larger brood (Saltzman, 1996). Figure 4 below shows a general life cycle of mysids.

Culture method

Amphipods

Despite the fact that Amphipods are frequently found in their natural habitats with high numbers, it is necessary to develop a good culture practice to produce a consistent supply of food for fish species (Baeza-Rojano et al., 2013a). There is presently little information available on the use of amphipod culturing methods in aquaculture. A deeper knowledge of reproductive biology at the species level is essential to maximize mass production. The life history features that influence productivity are a crucial issue to investigate in order to access amphipod

reproductive potentials and develop culture techniques. Some of these features may be used to identify possible new sources of aquaculture feed (Jiménez-Prada et al., 2018; Shahin et al., 2023a; 2023b). According to some studies, amphipods have a short life span, reproduce continuously throughout the year in temperate waters and they have multiple broods throughout the cycle. These qualities make amphipods suitable for culture in controlled environments. The successful culture of amphipods on small scale has been reported by Baeza et al. (2013a), Soucek, Dickinson & Major (2016) and Awal et al. (2016), and intensive gammarids amphipod culture has only been performed under laboratory conditions for scientific research (Delgado, Guerao & Ribera, 2011), however, commercial-scale culture has not been reported. The culture method used by Awal et al. (2016) is using a different type of substrate which are seaweed and rope. Because of the habitat of amphipods, which can commonly be found on benthic and live their whole lives by being bound to firm substrates (Oliver et al., 2020), this culture methods are vital for the reproduction of amphipods. As for the study by Soucek et al. (2016), successfully culture amphipod *Hyaella azteca* in a small scale by laboratory has been found by manipulating the amphipod's food. The use of different food combination manages to provide details on the growth, survival, development, and reproduction of the amphipod *H. azteca*.

Mysids

The Mysids culture has been recorded in which they study small-scale production of mysid shrimp in a static water system. Then further study has been made on the effect of density and temperature on production, survival, and growth of mysids in a static water system. According to Lee et al. (2020), he found that temperature is an important environmental factor for the mysid's marsupial development. Later, culturing mysids in a flow-through system has been studied. Through all the study, they manage to get a lot of information on the method for culturing mysids. Higher culture density can increase the growth of mysids, however, there will be a greater risk of cannibalization between the adult and the juvenile when they are too crowded and less space to hide (Lee et al., 2020). Furthermore, the density will also affect the reproductive behaviour in which a higher number of females with empty broodstock appear in the denser population. The time required for mysids to reach the stage of maturity and the first brood release is related to the culture water temperature. An increase in the temperature will shorten the breeding duration, producing more youth and shortening the maturation cycle. The rapid growth and improved hatchling survival at higher temperatures improve the economic efficiency of rearing mysids as food for marine species cultured. The previous study shows that the large-scale mysids culture has not been developed, but currently is a very active area of research and development. Table 1 below shows the water quality parameters of amphipod and mysids culture that have been studied by Baeza et al. (2013a) and Lee et al. (2020). To achieve successful culture, it is important to maintain optimum range of water quality.

Nutritional value of Amphipods and Mysids

In order to increase the production of aquaculture, the knowledge on the biochemical profile of marine organisms is vital for the discovery of new live feed that can be used effectively. According to Leaver et al. (2008), fish larvae required essential fatty acids (EFAs) such as polyunsaturated fatty acids and highly unsaturated fatty acids for sustainable growth and survival. In addition, both amphipods and mysids also shows adequate characteristics for use as live feed in aquaculture, such as, suitable optimum sizes (0.3 – 2.5 cm), digestible, adequate amount of lipids (10 – 15%) and protein (~40%) (Jiménez-Prada et al., 2018; Lee et al., 2020; Jiménez-Prada et al., 2021).

Nutritional value of Amphipods

Baeza-Rojano et al. (2014), examined the lipids of various types of amphipods. The percentage of saturated fatty acids for each specimen range between 16.9 to 24, monounsaturated fatty acids for each specimen range between 10.6 to 24.2, and polyunsaturated fatty acids for each specimen range from 8.51 to 17.7 for 20:5(n-3), 0.8 to 13.9 for 22:6(n-3) and 1.7 to 5.8 for 20:4(n-6) according to Baeza-Rojano et al. (2014) (Table 2). Through the study done by Suontama et al. (2007), the nutritional value of Arctic amphipod (*Themsto libellula*) can to support the growth and feed utilization of Atlantic halibut when meal from arctic amphipod was used to substitute fish meal in fish feed partially. According to the Moren et al. (2006) amphipods of genus *Gammarus* have a high level of protein and beneficial polyunsaturated fatty acid docosahexaenoic acid (DHA (22:6(n-3)), eicosapentaenoic acid (EPA (20:5(n-3))) (Moren et al., 2006) and it can provide the nutritional requirements for the protein and lipids for marine larvae fish (Kanazawa, 2003). Woods (2009) stated that, the amphipod caprellids contain moderately high amounts of beneficial polyunsaturated fatty acids, including DHA (22:6n-3) and EPA (20:5n-3). Promising results have been obtained by previous studies when they explored amphipods as alternative protein and lipids sources in experimental diets for farmed fish (Harlioğlu and Farhadi 2018).

Nutritional value of Mysids

According to the Table 3 below, its showed that the total lipids percentage and fatty acids content of mysids are comparable with other live feed used in industry such as enriched *Artemia*, copepods, rotifer and cladocera. Based on the Table 3, the total lipid content of mysids (10.6 ± 0.1) is higher than enriched *Artemia* (4.1±1.1). The percentage of saturated fatty acids C16:0 of mysids (26% of FAME) higher than enriched *Artemia* (15.5% of FAME). The percentage of monounsaturated fatty acids C18: 1n-9 of mysids (7.8% of FAME) lower than enriched *Artemia* (25.2% of FAME). The percentage of polyunsaturated fatty acids 20:5 (n-3), 22:6 (n-3), and 20:4 (n-6) of mysids is higher than enriched *Artemia* according to Eusabio et al. (2010; Table 3). Eusebio et al. (2010) stated that, mysids contain a high nutritional value such as protein, lipid, and beneficial fatty acid DHA (22:6 (n-3), EPA (20:5 (n-3) compare to enriched *Artemia*. Since live food such as *Artemia*, rotifer, and copepod contain a limited dietary value which might not

provide all the essential nutrients for growth and survival of all species cultures (Otrowski & Liadley, 2001).

Potential feed enhancement for Amphipods and Mysids

Research on marine amphipods and mysids has been stimulated by the need to find substitute live feed for aquaculture stocks (Shahin et al., 2023b). Live food such as *Artemia*, rotifer, and copepod are common species that are used in marine aquaculture, however, to fill in the lack of long-chain HUFA, especially DHA in the rotifer and *Artemia*, they need to be enriched with a fatty acid (Rasdi & Qin, 2016). The same also goes for the Amphipods and Mysids, even though they already contain high nutritional value as being reported by Woods et al. (2009) and Eusebio et al. (2010), they still need to be fed with microalgae or zooplankton, which can help in increasing growth and population size. A nutritional enrichment of *Phronima pacifica*, a type of Amphipoda microcrustacean has been studied by Herawati et al. (2020) by enrich them with two species of microalgae which is *Chlorella vulgaris* and *Chaetoceros calcitrans*. Their study has found that *P. pacifica* mass-cultured with *C. vulgaris* resulted in highest biomass, growth and also proteins and fats of *P. pacifica*. Gammaridan amphipod, *Gammarus insensibilis* was proven by Jiménez et al. (2018) to possess all the characteristics for supplementing formulated diets in aquaculture due to its good biochemical composition such as proteins, lipids and amino acids for feed (live or dry), however they are still need to be enriched with a fatty acid. Through the research by Awal et al. (2016) on a different type of feed for caprellids amphipods, reveals that the caprellids fed on the *Phaeodactylum tricornutum* showed massive development and reproductive performance, resulting in an increase in population size. Other than microalgae and zooplankton as feed to amphipod, a study by Jiménez-Prada et al. (2020) has found that waste product such as detritus to be useful for amphipod culture and provide with desirable biochemical profile. The use of detritus as waste product to feed amphipod is an interesting topic due to its advantages of being cheap to produce.

Mysids are frequently used by aquarium hobbyist or in laboratory setting as a food source for variety aquatic species such as cuttlefish, seahorses, and fish. They are also considered as alternative live food for the culture of marine species (Oliveira et al., 2023). In mysids culture, *Artemia* nauplii usually used as feed however, due to the expensive cost of *Artemia* cyst, the less expensive feed need be found to produce mass culture. Table 4 below shows the major nutritional components and example of live feed enrichment diets that were used by other researcher and farmers as guidelines to find suitable diet for live feed culture and enrichment. Therefore, to meet nutritional requirement of fish/prawn larvae, it is crucial to study the nutritional composition of enrichment diets so that it will contribute to the healthy development of fish during their early-life stages. Species dependent is one of the factors for the growth performance of fish larvae to live feed enriched with important nutrients (Ma & Qin, 2014). Enrichment diets provides live feeds with adequate amount of nutritional value necessary for

survival, growth and stability. Achieving natural live feeds including amphipods and mysids with higher growth rates, stress tolerance and good nutritional quality after enrichment are goals in aquaculture industry in order to produce better quality of fish larvae. Numerous studies have focused on establishing methods for enhancing the nutritional quality of live feed with nutrients for example protein, lipid and fatty acid (Kandathil et al., 2019). In aquaculture, enriched zooplankton is essential for improving the nutritional value of fish and shellfish.

Potential use of Amphipods and Mysids as alternative live feed in aquaculture

Amphipods and mysids have been recognized to be one of the crucial natural preys to various marine species. Studies on the nutritional effects of their partial substitution for fish meal in fish diet have produced promising results. Amphipods and mysids have been reported to be essential components of aquatic food webs in the wild because they act as conduits of nutrients and energy to higher trophic level (Shahin et al., 2023a). Hence their potential use for aquaculture species under captivity and laboratory conditions is ecologically acceptable. Live feeds including amphipods and mysids provide suitable initial feed for fish/shrimp larvae than fish pellet due to their ability to swim in the water that stimulates a feeding response in larvae, high in digestibility and provide adequate nutrient (Kandathil et al., 2020). Protein and lipid are crucial for the better growth, development, and survival of fish/shrimp larvae. A study on the use of the amphipod meal as a fishmeal substitute on grey mullet has been taken by Ashour et al. (2021). Their result shows that amphipod meal with 50% partial replacement with fishmeal has benefited on growth performance, feed utilization and histological and economic status of grey mullet (*Mugil cephalus*) fry. Table 5 below shows a comparison on the proximate biochemical compositions of fishmeal and amphipod meal based on the study by Ashour et al. (2021). According to their results, fishmeal has high amount of crude protein, while amphipod has produced high amount of ether extract and crude fiber than fishmeal.

The dependence on *Artemia* as a feed for newly hatched larvae greatly impacts on the aquaculture industry. *Artemia* is essential to the marine fish and ornamental industry due to its high nutritional content to satisfy the need for fish culture. A recent study introduces a new live feed such as amphipods and mysids to solve this problem. According to Baeza-Rojano et al. (2010), one of the obstacles in culturing cuttlefish on a large-scale would be the live feed that is costly to culture. Therefore, less expensive live feed is needed to minimize production costs and allow a large-scale culture (Domingues et al., 2008). According to Woods (2009), amphipods such as gammarids and caprellid are among the most flexible species throughout the world. The production cost will be cheaper because of their opportunistic feeding, rapid growth, and menstrual cycle (Domingues et al., 2008). Successful culture of amphipod in small scale able to provide information which is required by the researcher for further study on this aspect (Baeza-Rojano et al., 2013b; Soucek et al., 2016). Research by Baeza-Rojano et al. (2013b), the first to be able to grow *caprellid* amphipods in cultivation tanks, big enough to achieve adequate

densities for possible use as live feed for fish with a maximum density of 10,460 individuals m^{-2} and an average of 3625 individuals m^{-2} on the artificial substrates which were dominated by juveniles. Furthermore, the result also shows that larger individuals were produced from this culture compared to the size in natural conditions in southern Spain. In this study, Baeza-Rojano et al. (2013b) uses a different type of substrate to test the effect on population growth. The type of substrate used was a plastic mesh with different sizes of folding. In natural environments, structural properties and the substrate's structure on which amphipods live can be crucial in hiding from predators (Corona & Soto, 2000). *Caprella scaura* tends to become aggressive during the mating period, which leading to fighting each other, resulting in mortality (Baeza-Rojano et al., 2013a) and cannibalism between adult and juvenile. Applying substrate into the culture may help the juvenile to seek shelter and prevent direct contact with each other but that is not the only factor. An inadequate amount of feed also leads to cannibalism between each organism. The rate of cannibalism reduces when the food supply is sufficient and not limited (Baeza-Rojano et al., 2013b). On the other hand, Awal et al. (2016) also studied the different species of amphipod, *Caprella penantis*. This study was to observe the substrate selectivity and food preference of the *C. penantis*. For the substrate selectivity test, *Sargassum* sp. act as a natural substrate and for artificial substrate, the rope was used. *C. penantis* attached to the substrate surface for its whole life (Awal et al., 2016), as a method of survival (Guerra-García, Corzo & García-Gómez, 2002). The use of natural substrate turned out to be unfit for agriculture due to the deterioration that then polluted the water by increase the ammonia level (Awal et al., 2016). Artificial substrate proves to be suitable in for this species because of its ability to harbour colonizing algae that result in increased colonization of *C. penantis* (Hosono, 2010). For the feed preference test by *C. penantis*, a different type of feed such as *Phaeodactylum tricornutum*, *Dunaliella tertiolecta*, and *Cylindrotheca Fusiformis* were given. The highest growth, reproduction, and population size observed when it is fed by *P. tricornutum* (Awal et al., 2016). It is evident that caprellids can acquire food from the substrates by scraping the diatom that attaches at the substrate (Guerra-García et al., 2002) and catch detritus (Cook, Willis & Lozano-Fernandez, 2007) by scraping of the substratum.

There has been a record of a few people using amphipods as a feed for larvae rearing. According to Vargas-Abundez, Simões & Mascaró (2018), they have already experimented by feeding the amphipods to the lined seahorse *Hippocampus erectus*. Baeza-Rojano et al. (2010) also reported that they have used the amphipods as alternative prey to culture cuttlefish *Sepia officinalis* hatchlings. Both of this experiment has shown the positive result, that the amphipods can successfully help in the growth development and susceptibility by the lined seahorse *Hippocampus erectus*. Vargas-Abundez et al. (2018) and culture cuttlefish *Sepia officinalis* hatchlings (Baeza-Rojano et al., 2010). González, Pérez-Schultheiss, & López (2011), had done a test in feeding amphipod *Jassa marmorata* to the "Baby octopus" *Robsonella fontaniana* paralarvae. Based on the research that has been done on amphipods, it shows that a suitable substrate and feed play an important role in order to successfully culturing amphipods. Different

species of amphipods need a different type of substrate and feed according to their habitat. It shares the same concept as the zooplankton such as copepod where the availability of various food sources is essential to enhance the nutritional value of live feed culture in hatcheries (Yuslan et al., 2022b).

Other than that, mysids also show great potential as an alternative live feed. Mysid is one of the major constituents in coastal area and estuary that is important in transporting nutrient in the form of energy in the trophic levels from low to high level (Yolanda et al., 2023). Mysids are also regarded to be the primary source of food for fish and crustaceans (Oh et al., 2001; Link et al., 2002; Tomiyama et al., 2013). Mysids culture has been recorded by Wittmann, (1984), Domingues et al. (1998) and Domingues et al. (1999). Their study has provided a lot of information on the method for culturing Mysids. A study of the culture of *Mysidopsis almyra* in the static water system shows an advantage where mysids can focus the energy on growth and reproduction due to no water current, but it also leads to the poor quality of water owing to the build-up of biological waste at the bottom (Domingues et al., 1998). Generally, poor water quality is related to the excessive or harmful nitrogen component concentrations in water (Rahman et al., 2023). So, a daily water change is important to prevent water deteriorate which causing increases in ammonia and nitrate level. Large-scale of mysid cultivation, that used a shallow tray with a broad area in proportion to volume tend to be successful. Reproduction occurred at levels of ammonia-nitrogen and nitrate-nitrogen as high as (1.5 mg l⁻¹ and 0.250 mg l⁻¹), respectively, and pH values as low as (7.6), but reproduction suddenly stopped and mortality rose dramatically when the pH level decreased to (7.38) (Domingues et al., 1998). This shows the important role of pH level in determining the rate of reproduction and mortality in mysids culture, especially for large scale culture. Apart from that, temperature also influence maturity time of mysids male and females (Yolanda et al., 2023). According to Yolanda et al. (2023), at Malaysia waters, male of adult mysids in estuary are matured earlier than female. While, at the coastal area female are matured earlier than male. Although temperate and tropical mysids differ in growth, maturity, and life duration, however, their productivity performance and maturation still can be seen clearly, and temperature is one of the key determinants that will influence their maturity. At 17°C, male *Acanthomysis mitsukurii* developed sooner than females, while, at 23°C female was reaching maturity earlier than male (Yamada and Yamashita, 2000). Study by Sudo et al. (2011) has showed that, *Orientomysis robusta* male was matured in 13 to 32 days in the spring, 10 to 14 days in the summer, and 40 to 70 days in the fall and winter. While, female was defined matured in 15 to 35 days in the spring, 10 to 17 days in the summer, and 57 to 86 days in the fall and winter (Sudo et al., 2011).

Previous studies pointed out the problem in the culture of mysids is the use of *Artemia* nauplii as a feed that is expensive and not economically sustainable for commercial culture. Aquaculturists and hatcheries are currently struggling with the cost of producing cysts for commercial hatchery operations, as well as the labour and infrastructure costs associated with producing live *Artemia*

to operate hatcheries. As a result, alternative zooplankton options are required for hatchery propagation (Aziz et al., 2023). In finding the solution to this problem, a studied on alternative diet for mysids have been done. The test was run on the flow-through system, and the rotifer was used as a substitute feed for the *Artemia* nauplii. Feeding 100% rotifer to mysids gives a poor result on the production, growth, and survival of mysids, but by using mixed diet composed of rotifer and *Artemia* nauplii it produces a better result on the growth, and survival of juvenile mysids compared to *Artemia* nauplii alone (2000). It is suggested by Domingues et al. (2000), in order to minimize the costs of production, rotifers diet the mysids for the first 2-3 weeks and then feed a mixed diet of rotifers with *Artemia* nauplii just before sexual maturity is achieved to ensure that mysids become sexually mature and reproduced. This feeding regime was suggested in order to get the lowest production cost. Domingues et al. (2000) stated that, replacement of 50% of *Artemia* by rotifer would reduce feeding costs two-fold during culture. Thus, to ensures the effectiveness of aquaculture activities, scientists are concentrating their efforts on the development of low-cost live feed alternatives (Suhaimi et al., 2023). Both amphipods and mysids show great potential as an alternative live feed in the aquaculture industry. The study on these two live feeds is too little, further research on the method of large scale, the effect of water parameter on the reproduction, feed enhancement, and species that suitable for culture needed to be done for a better understanding.

Conclusions

This review concludes by highlighting the possibility of raising amphipods and mysids for use as a potential live feed in aquaculture. Both amphipods and mysids have adequate amount of protein, lipid and essential polyunsaturated fatty acids like DHA, EPA, and ARA, which make them both very promising as a live feed that can provide the nutrients needed for fish and crustacean growth in their infancy. However, to develop a technique for mysid and amphipod mass culture, additional research on its nutritional compositions is necessary. The aquaculture industry will be greatly impacted by the ability to mass produce amphipods and mysids because it will increase the amount of usable live feed available and possibly lower production costs in aquaculture hatcheries.

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Competing Interests

The author(s) declare(s) that there is no conflict of interest regarding the publication of this article.

Author Contributions

- Muhammad Irfan contributed to the writing of the original draft, review outline and preparation.
- Hidayu Suhaimi contributed in writing and reviewed drafts of article, prepared figures and tables.
- Aisyah Ashaari contributed in writing and reviewed drafts of article, prepared figures and tables.
- Nadiah W. Rasdi contributed to conceptualization, the main idea of this research work, visualization, reviewed drafts of the article and approved the final draft.

Data Availability

The following information was supplied regarding data availability:
This is a literature review.

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Figure 1

Morphological diagram of adult Amphipods

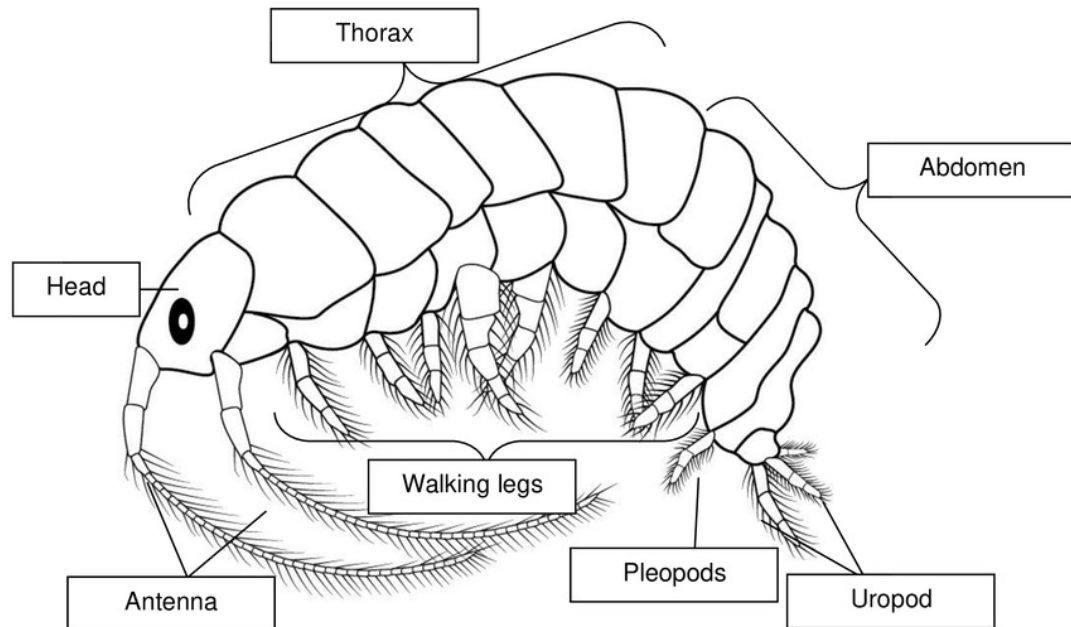


Figure 1 Morphological diagram of adult Amphipods

Figure 2

Morphological diagram of adult Mysids

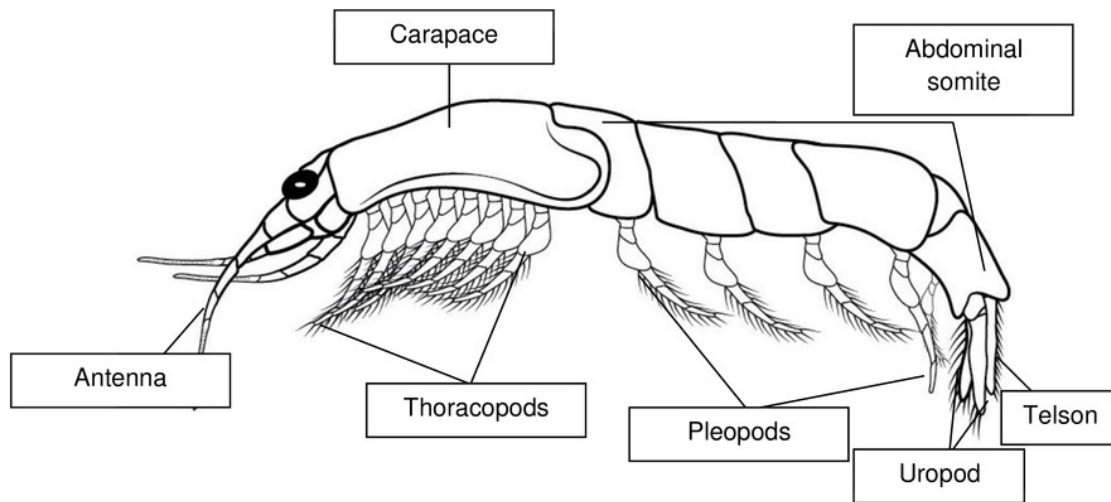


Figure 2 Morphological diagram of adult Mysids

Figure 3

General life cycle of Amphipods (modified from Birmingham et al., 2005)

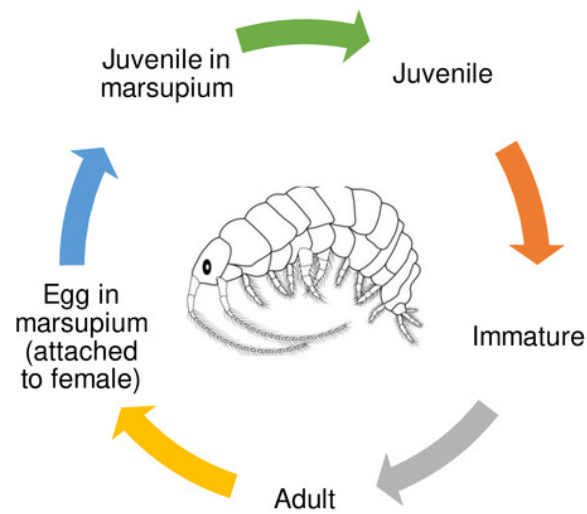


Figure 3 General life cycle of Amphipods (modified from Birmingham et al., 2005)

Figure 4

General life cycle of Mysids (modified from McKenney, 2005)

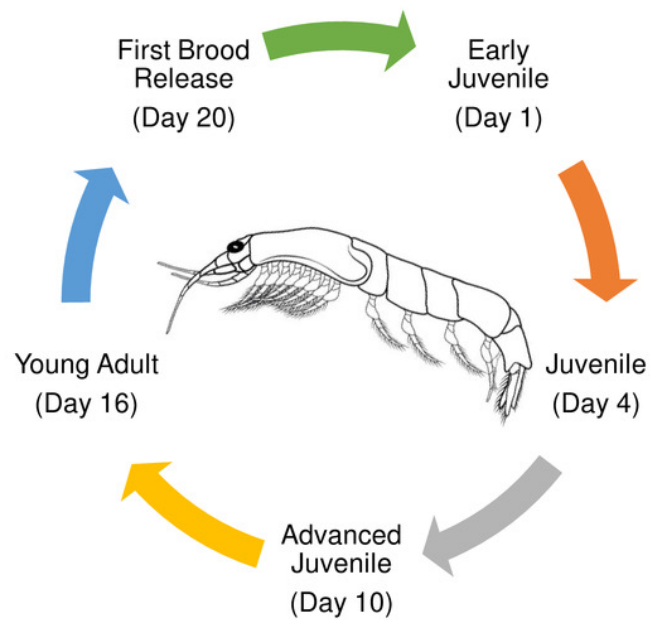


Figure 4 General life cycle of Mysids (modified from McKenney, 2005)

Table 1 (on next page)

Optimum water quality parameters for the culture of amphipods and mysids

Table 1:
Optimum water quality parameters for the culture of amphipods and mysids

Parameters	Amphipods	Mysids
Temperature	18-23°C	20°C
Dissolved oxygen (DO)	5-9 ppm	6.6-7.2 ppm
Salinity	37-39 ppt	30 ppt

Sources: Baeza et al. (2013a); Lee et al. (2020).

Table 2 (on next page)

Percentage of fatty acid composition in the gammaridea and caprellidea from the Strait of Gibraltar. AA: Arachidonic acid; EPA: Eicosapentanoic acid; DHA: Docosahexanoic acid

1 **Table 2:**

2 Percentage of fatty acid composition in the gammaridea and caprellidea from the Strait of
3 Gibraltar. AA: Arachidonic acid; EPA: Eicosapentanoic acid; DHA: Docosahexanoic acid

	<i>Hyale perieri</i>	<i>Caprella penantis</i>	<i>Echinogammarus</i>	<i>Caprella equilibra</i>	<i>Caprella grandimana</i>	<i>Elasmopus rapax</i>	<i>Jassa</i>	<i>Caprella dilatata</i>
Saturated								
16:0	24.83	21.56	16.98	20.98	21.98	20.65	19.71	17.79
Monounsaturated								
18:1(n-9)	13.77	12.57	24.23	10.63	10.66	17.79	12.27	10.84
Polyunsaturated								
20:4(n-6)	5.43	3.48	2.36	1.75	5.87	2.80	2.14	4.48
20:5(n-3)	8.90	15.87	8.52	17.69	16.02	16.10	17.67	17.14
22:6(n-3)	2.08	13.98	0.86	12.14	4.53	8.81	11.84	13.57

4 Source: Baeza-Rojano et al. (2014).

5

Table 3(on next page)

Comparison of total lipids (% of Dry Matter (DM)) and fatty acid profiles (% of Fatty Acid Methyl Esters (FAME)) of live prey organisms as food for developing fish and crustaceans' larvae

Table 3:
Comparison of total lipids (% of Dry Matter (DM)) and fatty acid profiles (% of Fatty Acid Methyl Esters (FAME)) of live prey organisms as food for developing fish and crustaceans' larvae

	Enriched <i>Artemia</i>	Mysids	Copepods	Rotifer	<i>Moina</i> spp.
Total lipids	4.1±1.1	10.6±0.1	14.45±1.78	ND	9.84±2.46
Fatty acids					
C16:0	15.5±1.3	26.5±4.7	19.40±0.15	1.38±0.34	10.53±0.01
C18:1n-9	25.2±2.4	7.8±2.5	4.55±0.37	5.61±0.43	6.91±0.04
C20:4n-6	3.3±0.3	6.4±1.3	3.86±0.08	2.38±0.56	4.20±0.73
C20:5n-3	5.5±0.6	15.3±1.6	14.62±0.33	3.53±0.31	8.78±0.01
C22:6n-3	0.0	13.2±1.8	9.79±0.25	5.05±0.79	1.53±1.21

¹ND: No data available

²Source: Eusebio et al. (2010); Yuslan et al. (2022a); Suhaimi et al. (2022); Rocha et al. (2017); Singh et al. (2019).

Table 4(on next page)

Major nutritional components and example of live feed enrichment diets (%)

Table 4:

Major nutritional components and example of live feed enrichment diets (%)

	Protein	Lipid	Carbohydrate	Ash
<i>Artemia</i>	52.2	18.9	14.8	9.7
<i>Chlorella vulgaris</i>	51.45	12.18	11.86	9.50
Yeast	49.63	4.64	31.55	7.98
Rice bran	10.74	11.42	42.87	8.23
Palm kernel cake (PKC)	17.6	5.5	50.4	6.1
Soybean meal	39.24	30.31	5.08	4.61

Sources: Zarei et al. (2012); Shalaby et al. (2013); Onofre et al. (2017); Bayero et al. (2018).

Table 5(on next page)

Proximate biochemical compositions of the fishmeal and amphipod meal (% of dry matter)

Table 5:

Proximate biochemical compositions of the fishmeal and amphipod meal (% of dry matter)

	Fishmeal	Amphipod meal
Dry matter (%)	92.03	93.15
Crude protein (%)	60.17	40.02
Ether extract (%)	12.50	14.21
Crude fiber (%)	0.62	1.50
Ash (%)	15.40	19.82
Nitrogen free extract	11.50	24.51
Gross energy (Kcal kg⁻¹)	3930.14	3364.05

Sources: Ashour et al. (2021).