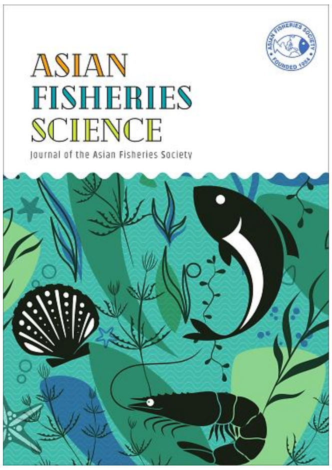

Size Matters: A Review of Live Feeds Used in the Culture of Marine Ornamental Fish

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ISSN: 0116-6514
E-ISSN: 2073-3720
https://doi.org/10.33997/j.afs.2020.33.2.007

Abstract

The marine ornamental fish trade generates over USD1.5 billion annually and continues to increase. However, only 35 fish species are thought to be commercially produced for sale currently, a small proportion of the 1800 species recorded within this trade. The limiting factor in marine ornamental fish production is the requirement for appropriately sized live food as a first feed. This is due to the small gape size of many fish species of interest to the trade. The need for suitable live feeds has therefore caused a bottleneck in the production of marine ornamental fish species and developments are needed to allow an expansion of this culture industry. This review considers the current usage of live feeds, including *Artemia*, rotifers, copepods, and ciliates and discusses the advantages and disadvantages of each when used to culture marine ornamental fish. Whilst success has been seen with these feeds for several commercially important marine ornamental fish species, the current lack of appropriately sized live feed items for higher value species, such as dwarf angelfish, remains a problem for the industry. Live feeds currently used often exceed the gape size of such species at the onset of exogenous feeding, resulting in limited commercial success. Future developments focussing on novel and existing live feeds used within the industry for these valuable species are explored. These developments will enable aquaculture, rather than the exploitation of wild populations, to meet future demand and will encourage progress in the aquaculture of marine ornamental fishes.

Keywords: *Artemia*, copepod, larviculture, nutrition, rotifer, gape size

Introduction

The tropical marine ornamental trade supplies live animals for an industry worth approximately USD1.5 billion annually (Biondo, 2017), dominated by the capture, transportation and sale of wild-caught fish from coral reefs and associated habitats (Wabnitz et al., 2003). Harvesting marine ornamental fish often uses indiscriminate techniques with detrimental effects on ecosystems, such as damaging physical structures of reefs when removing the desired specimens (Mak et al., 2005). Even if selective non-destructive fishing techniques are used, the demand for small-bodied, juvenile fish of specific sexes can lead to localised depletion of species and subsequent ecological change (Okemwa et al., 2016). Analysis of US customs import and export data shows that on average, 1800 fish species are traded annually (Rhyne et al., 2017). However, globally only 30–35 species are in commercial production (Biondo, 2017), highlighting the current dependence of this industry on wild-caught individuals to meet market demands. This presents a rare opportunity for the marine aquaculture sector to pursue financial gain while simultaneously aiding the conservation of wild populations. Cultured fish are also more attractive to consumers because they adjust more successfully to home aquaria (Olivotto et al., 2011), and avoid the known ecological impacts of harvesting on wild habitats (Alencastro, 2004). The need to culture marine fish is magnified when the potential collapse of coral reefs from climate change is considered (Hoegh-Guldberg et al., 2018). Marine culture specimens may also be the only living examples of their species and have a potential role in reintroduction and restoration of coral reefs (Obolski et al., 2016). However, one of the most significant bottlenecks currently limiting the culture of marine ornamental fish is the lack of appropriately sized live feeds for larvae at the onset of exogenous feeding (Olivotto et al., 2008; Moorhead and Zeng, 2010). This review aims to assess the efficiency and limitations of various live feeds currently used within the ornamental fish trade. Furthermore, new methods to produce live feeds are discussed to identify and overcome the bottleneck currently faced by the aquaculture industry. Hence, this paper presents the way forward to increase the culture of ornamental marine fish production.

Materials and Methods

This study conducted a detailed search of peer-reviewed published literature, reports from conferences and books (physical and electronic). Extensive information available from hobbyist forums was not used, as it could not be verified. Sources referring to commonly used live foods, appropriate rearing techniques and their use with marine ornamental fish were investigated, alongside reports of first successful reproduction or optimisation of ornamental species culture methods. Search terms included "live food", "marine ornamental aquaculture", "gape size", "*Artemia* nauplii", "rotifer", "copepod nauplii", "culture conditions", "larval diet", "fatty acid composition" and "enrichment" all of which were often combined with a host of species names of marine ornamental fish species. The sources referring to food fish were also analysed where appropriate, such as the techniques used to produce live foods in large aquaculture operations. Fish species were considered ornamental if a specific, or previous, source had referred to them as such or mentioned their presence in aquaria. Sources referring to wild populations of these species were used when referring to natural diets. However, only trials conducted in aquaria were used when analysing larval survival.

The Importance of Live Feeds

The capture, digestion and assimilation of live prey are necessary for the growth and development of early life stages of marine species (Olivotto et al., 2017b). Therefore, the use of live feeds is essential for the successful rearing of juveniles of nearly all cultured species. Fish larvae use movement to identify prey, with neuromasts on their body detecting the water motion and frequencies emitted by plankton, while eyes recognise appropriate movement patterns (Rønnestad et al., 2013). As a result, inert foods typically under-stimulate fish larvae. Live feeds are the crucial bridge between the end of endogenous yolk supplies and post-metamorphosis, when gastric glands have developed, allowing the digestion of artificial diets (Önal et al., 2008). Formulated feeds are, however, useful in achieving fast growth rates in juvenile fish, providing optimum nutritional values at a lower cost (Moorhead and Zeng, 2017).

Larval fish must swallow prey items whole as teeth appear later in development (Rønnestad et al., 2013) and live prey must be of an appropriate size (Olivotto et al., 2017a). Consequently, larvae tend to select prey between 25–50 % of their gape size to avoid abrasion of the underdeveloped oesophagus (Yúfera and Darias, 2007). Larval fish fed oversized food within culture systems suffer high mortality rates as a result of not being able to ingest prey that is too large. For example, larvae of Linesnout goby (*Elacatinus lori* Colin, 2002) and Belize sponge goby (*Elacatinus colini* Randall and Lobel, 2009) fed *Artemia* nauplii from six days post-hatch, suffered higher mortality compared to those fed rotifers (Majoris et al., 2018). However, the same food item can become appropriate once gape size increases (Yúfera and Darias, 2007). *Elacatinus lori* and *E. colini* larvae when fed a combination of *Artemia* and rotifers, to 6 days post-hatching, performed significantly better than those fed only rotifers (Majoris et al., 2018). This reinforces that live foods must be employed at the correct age to avoid poor larval development. The diversity found on coral reefs translates into a range of gape sizes in ornamental marine fish larvae. However, this is a relative term as reef fish larvae have characteristically small mouths (Moorhead and Zeng, 2010). Consequently, live food must be of appropriate size for the species and its age. Generally, rotifers are used as a first feed, followed by *Artemia* nauplii and finally enriched *Artemia* as gape size increases (Wittenrich 2007; DiMaggio et al., 2017). The marine ornamental species that are currently cultured are able to ingest commonly used live feeds. The inability to identify a suitable live feed or correctly sized live feed has been highlighted as the barrier to further developing marine ornamental aquaculture (Moorhead and Zeng, 2010). Table 1 displays key species from popular ornamental fish families, their method of spawning, gape size upon hatching and common first live feed.

Artemia as Live Feed

Artemia are small crustaceans, with a pan-global distribution in brackish habitats (Kumar and Babu, 2015). There are multiple strains within eight species (Hou et al., 2006), although 90 % of the global trade in *Artemia* originates from the Great Salt Lake in Utah (Ruebhart et al., 2008). *Artemia* can produce cysts that remain dormant for long periods if dehydrated. However, hatching is easily initiated through hydration, exposure to light and heavy aeration for 24 hours (Bengtson et al., 1991). The ability to produce millions of individual *Artemia* on demand, without the infrastructure required to breed them (Bengtson et al., 1991) has made them the single most common live food in the aquaculture industry.

Table 1. Key groups of marine ornamental fish cultured on a variety of live feeds compatible to gape size recorded as close to hatching as possible.

Group	Fish species	Spawning method	Larval gape (µm)	First live feed used in protocol	Percentage survival to metamorphosis
Gobies	Neon goby (*Elacatinus figaro* Sazima Moura and Rosa, 199)	Demersal egg layer	350 (Shei et al., 2017)	Rotifers (*Brachionus plicatilis* Müller, 1786)	20 % (Shei et al., 2010)
Blenny	Forktail blenny (*Meiacanthus atrodorsalis* Günther, 1877)	Demersal egg layer	307 (Moorhead and Zeng, 2011)	Rotifer (*Brachionus rotundiformis* Tschugunoff, 1921)	74 % (Moorhead and Zeng, 2011)
Damselfish	False clownfish (*Amphiprion ocellaris* Cuvier, 1830)	Demersal egg layer	300 (Jackson and Lenz, 2016)	Rotifers (*Brachionus plicatilis*)	95 % (Avella et al., 2007)
Seahorses	Spotted seahorse (*Hippocampus kuda* Bleeker, 1852)	Brooder	260 (Chin, 2017)	Newly hatched *Artemia* nauplii and rotifers.	100 % (Dhamagaye et al., 2007)
Surgeonfish	Yellow tang (*Zebrasoma flavescens* Bennett, 1828)	Pelagic spawner	260 (Burgess and Callan, 2018)	Copepod nauplii (*Parvocalanus crassirostris* Dahl, 1894)	0.29 % (Burgess and Callan, 2018)
Cardinalfish	Two striped cardinalfish *Ostorhinchus fasciatus* (White, 1790)	Mouth brooder	160 (Saravanan et al., 2013)	Copepod nauplii (*Acartia erythraea* Giesbrecht, 1889,, *Oithona brevicornis* Giesbrechtt, 1891 and *Oithona rigida* Giesbrecht, 1896)	90 % (Saravanan et al., 2013)
Wrasses	Melanurus wrasse (*Halichoeres melanurus* Bleeker, 1851)	Pelagic spawner	125 (Barden et al., 2016)	Copepod nauplii (*Parvocalanus crassirostris*)	0.5 % (Groover, 2018)
Firefish	Purple firefish (*Nemateleotris decora* Randall and Allen, 1973)	Demersal egg layer	90–110 (Madhu and Madhu, 2014)	Ciliates (*Euplotes* sp.)	66 % (Madhu and Madhu, 2014)
Small groupers	Marcia's anthias (*Pseudanthias marcia* Randall and Hoover, 1993)	Pelagic spawner	76–80 (Anil et al., 2018)	Copepod nauplii (*Parvocalanus crassirostris*)	7.3 % (Anil et al., 2018)

Newly hatched Artemia

Artemia nauplii, known as newly hatched *Artemia* (Kumar and Babu, 2015) are the stage most commonly fed in the culture of marine ornamentals (Oliver et al., 2017). Newly hatched *Artemia* differ in size depending on species. However, they are typically between 400–500 µm (Conceição et al., 2010). Their size often limits their use as a first feed for most species of marine ornamental fish, as larval gape would have to be at least 800 µm. The lined seahorse, (*Hippocampus erectus* Perry, 1810) fed newly hatched *Artemia* displayed a significantly lower survival than conspecifics fed a nutritionally complete commercial diet (Vite-Garcia et al., 2014). Newly hatched *Artemia* are generally employed after larval gape size has increased. However, reduced survival is often experienced and could be linked to the fact that nauplii may represent a nutritionally incomplete diet for larvae. Sunrise dottybacks (*Pseudochromis flavivertex* Rüppell, 1835) fed newly hatched *Artemia* displayed 28 % lower survival than those fed enriched *Artemia* (Olivotto et al., 2006).

Highly unsaturated fatty acids (HUFA) should comprise between 1–2 % of the diet for marine fish larvae (Kanazawa, 2003) to enable correct development. Additionally, appropriate levels of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) in foodstuffs are required. Docosahexaenoic acid is crucial for the development of the central nervous system (Oberg and Fuiman, 2015). Eicosapentaenoic acid is responsible for the development of correct colouration as it regulates arachidonic acid (AA), which can result in malpigmentation (Copeman et al., 2002). Captive-bred juvenile Banggai Cardinalfish (*Pterapogon kauderni* Koumans, 1933) fed a diet deficient in HUFA showed 70.4 % higher mortality than those fed a HUFA enriched diet (Vagelli, 2004). In addition, those individuals fed a diet with low HUFA enrichment exhibited an increase in shock syndrome events, whereby a sudden stimulus (i.e. light exposure or water changes) caused severe shock. Several individuals perished after shock syndrome events when HUFA was low, or missing from the diet. Those fed a diet high in HUFA suffered no mortality after such events (Vagelli, 2004). A ratio of 2:1 DHA/EPA is generally used for larval diets as it replicates the ratios of marine species. However, optimum total fats and individual fatty acid values are species-specific (Hamre et al., 2013). Whilst unenriched *Artemia* nauplii are high in HUFA (2.6 %), they are almost entirely comprised of EPA with only a trace of DHA (Kenari and Mirzakhani, 2005). Newly hatched *Artemia* appear to lack the ability to fulfil the dietary requirements of many marine ornamental fish larvae.

Currently, newly hatched *Artemia* are frequently used for short periods, or in conjunction with other feeds, which provide appropriate nutrition. This method has been used successfully in the rearing of various ornamental species from relatively simple demersal spawners, such as clownfish (*Amphiprion* sp.) (Olivotto and Geffroy, 2017), cardinalfishes (*Apogonidae* sp.), gobies (*Elacatinus* sp.) and blennies (*Blennidae* sp.) (Wittenrich, 2007), to more complex species such as the flame angelfish (*Centropyge loriculus* Günther, 1874) (Laidley et al., 2008). These examples highlight the effectiveness of newly hatched *Artemia* in the culture of marine ornamental fish if used correctly. However, there remains a research need to identify ways to boost the nutritional composition of newly hatched *Artemia* to increase the range of species to which they can be fed. Where possible, larvae should be transitioned to enriched *Artemia*; which serves as a complete diet (Conceição et al., 2010).

Enriched Artemia

Enrichment of *Artemia* can only be undertaken when the nauplii are at the second developmental phase, Instar II, reached between 27 and 32 hours after cyst hydration (Sanders, 2008). Feeding begins at Instar II, and the nauplii can take on the diets nutritional profile (Ferreira de Sa, 2016). The benefit of using enriched *Artemia* is that enrichment products are bio-encapsulated in the *nauplii* (Sorgeloos et al., 2001), which provide the larvae with the desired nutrient composition. Importantly, this process allows enriched *Artemia* to be used independently of other live feeds. *Artemia* from the Great Salt Lake are approximately 660 μm after 12 hours of enrichment and 790 μm after 24 hours (Conceição et al., 2010). They can only be consumed by larvae with a gape size in excess of 1000 μm. Selective breeding has reduced the size of *Artemia fanciscana* Kellogg, 1906, nauplii by 12.4 % after 13 generations of culture (Sajeshkumar et al., 2014), which would enable the production of smaller enriched *Artemia*. However, upscaling to produce reduced sized enriched *Artemia*, may prove challenging.

Although enriched *Artemia* are not commonly used as a first feed in the culture of marine ornamentals, they are useful in the initial rearing of ornamental species without a larval phase, such as seahorses (Koldewey and Martin-Smith, 2010). The direct development to a juvenile phase allows the consumption of larger first food items such as enriched *Artemia*, as seen in the successful culture of the big-bellied (*Hippocampus abdominalis* Lesson, 1827) (Woods and Valentino, 2003), lined (*H. erectus*) (Vite-Garcia et al., 2014) and White's (*Hippocampus whitei* Bleeker, 1855) (Wong and Benzie, 2003) seahorse species. Although seahorses can be weaned onto dead prey successfully, enriched *Artemia* is essential until individuals can tolerate the rapid water currents needed to keep non-living items suspended (Woods and Valentino, 2003). Similarly, the Banggai cardinalfish (*P. kauderni*) mouth broods eggs until juveniles are released and these are large enough to accept enriched *Artemia* as a first feed (Vagelli, 2017).

Enriched *Artemia* are frequently used as the primary feed once larvae can ingest larger prey. The diets of appropriately sized larval clownfish (*Amphiprion* sp.), dottybacks (*Pseudochromidae* sp.), fairy basslets (*Gramma* sp.), comets (*Plesiopidae* sp.), jawfish (*Opistognathus* sp.), cardinalfishes (*Apogonidae* sp.), gobies (*Elacatinus* sp.) (Figure 1), blennies (*Blennidae* sp.) (Wittenrich, 2007), damselfish (*Dascyllus* spp.) (Shei et al. 2017), yellow tang (*Z. flavescens*) and Cuban hogfish (*Bodianus pulchellus*, Poey, 1860) (Holt et al., 2017) have included enriched *Artemia*. Even species with very small gape sizes such as the Pacific blue tang (*Paracanthurus hepatus* Linnaeus, 1766) are fed enriched *Artemia* towards the end of rearing protocols (DiMaggio et al., 2017). Despite their size, enriched *Artemia* still represent a critical live food for almost all marine ornamental larvae at some point in their culture.

Issues with Artemia *use*

Although *Artemia* use in marine ornamental fish culture is ubiquitous, there are issues with its practical application. Of primary concern is the fact that as *nauplii* develop they metabolise enrichment, leading to increased size and reduced nutritional value. Lower temperatures can slow development and therefore preserve enrichment (Figueiredo et al., 2009). This is temporary and does not counter the inevitable growth of *nauplii*, meaning their value is time limited.

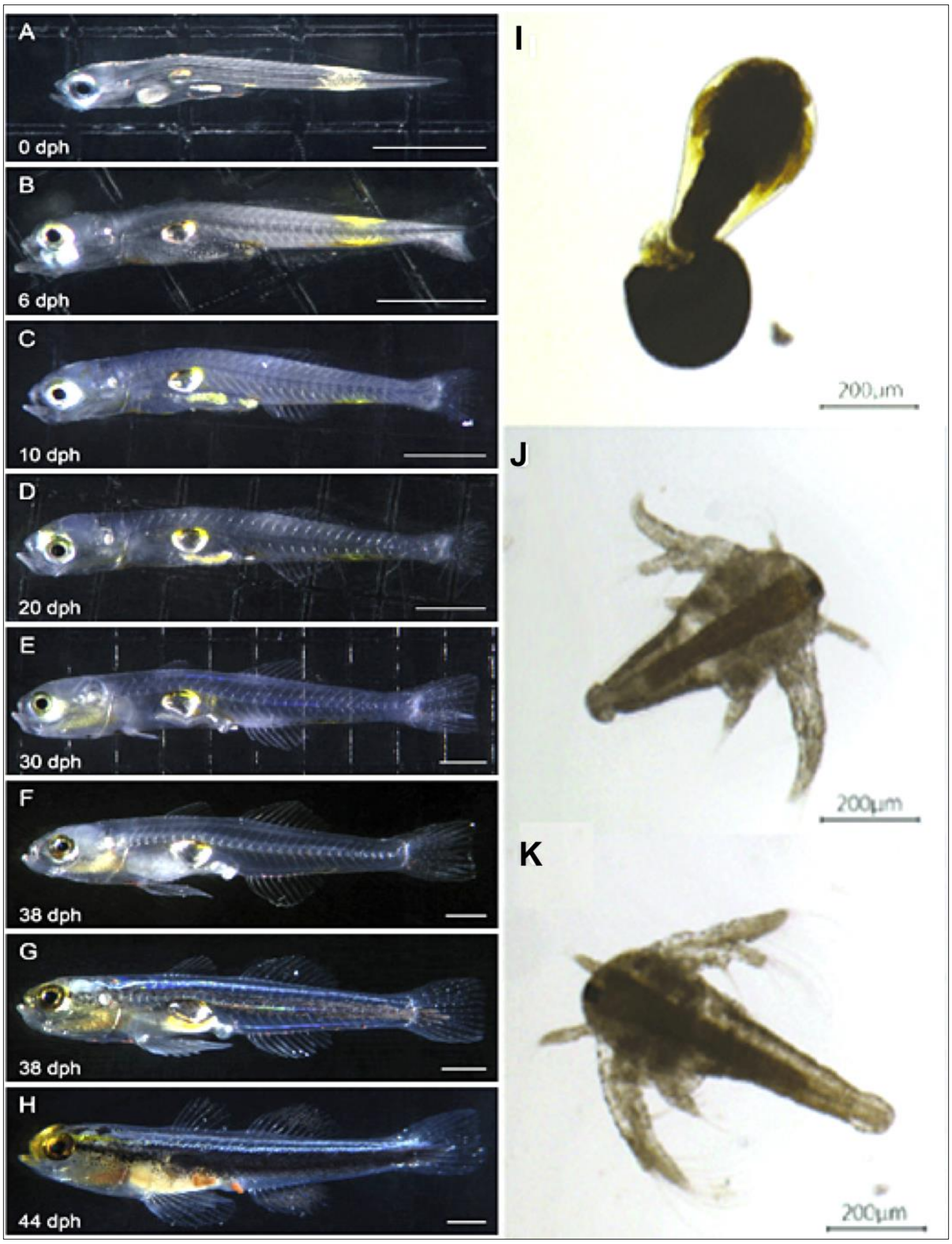

Fig. 1. *Elacatinus colini* larvae throughout the first 44 days post hatch (Images A-H - Scale bars = 1 mm) (Majoris et al., 2018). Size comparison with three stages of development of *Artemia franciscana*: (I) *Artemia* in the umbrella stage hatching from cyst, (J) *Artemia* nauplii Instar I, (K) *Artemia* nauplii Instar II (Adapted from: Lopalco et al., 2019).

In addition, cysts are harvested from wild populations, the majority of which originate from the Great Salt Lake (Ruebhart et al., 2008). Therefore, nearly all ornamental aquaculture projects are in some way reliant on wild populations. This has caused some issues with supply. In the mid 1990's a cyst shortage led to a sharp price increase (Dhont and Soregloos, 2002). As global climate change worsens, it is possible that large influxes of freshwater could enter the lake and limit future harvest by favouring predators, reducing food availability and affecting *Artemia* reproduction (Lavens and Sorgeloos, 2000). Wild harvest has caused ecological changes by removing floating cysts and increasing the number of less buoyant cysts. This negatively impacts nauplii survival, potentially causing further ecological damage and affecting future cyst supply (Sura and Belovsky, 2015).

Constant production of *Artemia* in aquaculture facilities also requires capital, labour and infrastructure (García et al., 2011) and decapsulating *Artemia* cysts can be a resource intensive process. The cysts shells are indigestible by larvae and can be vectors for bacterial introduction (Sorgeloos et al., 1977). Therefore, it is common practice to chemically remove this coating and store cysts in a brine solution (García et al., 2011). Coated cysts exist, which allow shells to be removed via magnets (Tagliafico et al., 2018), but it is unlikely these are completely effective or economical for large ornamental fish rearing facilities. More work is needed to develop a rapid and efficient process for cyst removal.

Rotifers as Live Feed

Rotifers are small metazoans extensively used in aquaculture (Le et al., 2017) which, like *Artemia*, are popular due to the ability to maintain high densities of individuals in a limited space. Unlike *Artemia*, they are not hatched on demand but maintained in live cultures (Lawrence et al., 2012). By using advanced techniques, cultures can achieve 160,000 individuals.mL^{-1} (Yoshimura et al., 2003). Identification of the exact rotifer used in aquaculture is complex - it was previously thought that all rotifers were various strains of *Brachionus plicatilis*. However, in 1995 it was found that *B. plicatilis* is more likely a species complex, with *Brachionus rotundiformis* being newly identified (Dhont et al., 2013). Consequently, aquaculture uses a method of differentiation based on three sizes, large, small and super small (Le et al., 2017).

Large and small rotifers

Large (130–340 µm) and small (100–120 µm) rotifers are all *B. plicatilis*, differentiated by body size (Hagiwara et al., 2014; Le et al., 2017). The size variation of *B. plicatilis* makes them a suitable first feed for a host of marine ornamental fish with varying gape sizes. Larvae with very small gapes are often transitioned to rotifers after smaller prey has been used (DiMaggio et al., 2017), indicating that the role of *B. plicatilis* may become more significant with industry progression. *Brachionus plicatilis* are routinely used as a first feed for the false percula clownfish (*A. ocellaris*) in commercial operations (Avella et al., 2007). When combined, the percula clownfish (*Amphiprion percula* Lacepède, 1802) and *A. ocellaris* were the fifth most imported species into the United States in 2005 (Rhyne et al., 2012), signifying the importance of *B. plicatilis* to the industry. *Brachionus plicatilis* is responsible for the commercial production of other popular marine ornamental species, such as damselfish, blennies, gobies and dottybacks (Olivotto et al., 2017a). However, this is where the limit of *B. plicatilis* lies. It is an appropriate first food for many demersal spawners, but too large for smaller larvae produced by pelagic spawners (Olivotto et al., 2017a), such as butterflyfish, angelfish, groupers (Olivotto et al., 2011) and dwarf angelfish (Leal et al., 2016). Despite the traditional role of *B. plicatilis* as a feed for small-mouthed larvae (Dhont et al., 2013), its use has prevented the culture of ornamental species with very small gape sizes. To diversify the species of marine ornamental fish that are cultured, smaller prey are required (Calado et al., 2017).

Super small rotifers

Super small rotifers (90–110 µm) are currently classified as *B. rotundiformis* (Hagiwara et al., 2014), although they are sometimes referred to as part of the *B. plicatilis* species complex (Dhont et al., 2013; Le et al., 2017). They have been identified as being particularly useful for small-mouthed larvae (Wullur et al., 2009) and have been used to successfully culture food fish such as groupers (Ostrowski and Laidley, 2001). However, there is little reference to *B. rotundiformis* in ornamental marine aquaculture, potentially because it appears unsuitable for some larvae. *Brachionus rotundiformis* fed to larval red snapper (*Lutjanus argentimaculatus* Forsskål, 1775) were excreted live, indicating they were not being digested (Schipp et al., 1999). Poor results were seen when *B. rotundiformis* was offered to some marine ornamental species. Rusty angelfish (*Centropyge ferrugata* Randall & Burgess, 1972) larvae showed low (<11.5 %) survival (Hagiwara et al., 2014) while semi-circle angelfish (*Pomacanthus semicirculatus* Cuvier, 1831) larvae did not survive beyond 7 days post-hatch (Leu et al., 2009). *Brachionus rotundiformis* was combined with *B. plicatilis* and *Paramecium* sp. in a diet fed to the red head goby (*Elacatinus puncticulatus* Ginsburg, 1938). Although this was the best performing diet of the trial, it is not representative of *B. rotundiformis* alone and no post-metamorphic juveniles were produced (Pedrazzani et al., 2014). Presently, evidence from the scientific literature suggests the lack of digestibility of this species is likely to make it unsuitable for marine ornamental aquaculture.

Proales similis *de Beauchamp, 1907*

Proales similis, a euryhaline rotifer with a typical body size of approximately 83 µm long and 40 µm wide (Wullur et al., 2009), is a relatively new addition to aquaculture feeds after being discovered in 2004 (Hagiwara et al., 2014). *Proales similis* have been used successfully to raise *C. ferrugata*, a species from a family that have initial gape sizes of approximately 160 µm (Hagiwara et al., 2014). When compared to the theoretical maximum prey size, *P. similis* is at the upper tolerance for a first feed. However, survival on day 6 was high (38 %), indicating this is a useful first feed for *C. ferrugata* (Hagiwara et al., 2014). Despite the positive result from a highly prized ornamental family (Baensch, 2017), the wider application of *P. similis* appears under researched and work with higher value ornamentals, including species of dwarf angelfish, should be undertaken to explore the use of this species to the industry.

Issues with rotifer use

Rotifers are maintained live prior to larval feeding and can be easily managed, but require resources and can have potential issues. Cultures require regular water changes to maintain ammonia below 1 mg.L^{-1} to avoid low population growth (Lawrence et al., 2012). Samples of rotifer cultures must be counted regularly to monitor population levels and to calculate the correct volume for larval feeding (Dhont et al., 2013). Additionally, when used for culturing marine ornamental fish, rotifers must be fed the correct diet. Unenriched rotifers lack vitamins C and E and contain between 7–13 % total lipid content, with maximums of 13.8 % EPA and 13.7 % DHA (Hamre, 2016). Therefore, rotifers must be supplemented with microalgae or other enrichment products to produce nutritionally complete individuals (Hamre, 2016). Rotifer enrichment is a simple process as they can consume microalgae, which provides adequate levels of fatty acids if the correct species are used (Thepot et al., 2016). However, production of microalgae is expensive (Conceição et al., 2010) and although prepared feeds for rotifers that provide correct nutritional values exist (Hamre, 2016), they are more expensive than lower quality feeds such as yeast. Further, the amount of feed given is crucial. Too much will reduce growth rates as sufficient digestive enzyme production for nutrient extraction is not maintained, while low feed densities prevent population growth through lack of ingestion (Dhont et al., 2013).

As a live culture, they are also vulnerable to contamination, especially by ciliates, which compete with rotifers for feed and limit the available harvest (Reguera, 1984). Contamination of cultures by different rotifer species is also probable since culture techniques are not strain specific (Dhont et al., 2013). Effectively separating different species or strains from contaminated cultures seems unlikely.

Copepod Nauplii as Live Feed

Copepods are the most abundant animals in ocean environments (Humes, 1994). Their nauplii are the natural prey of most wild fish larvae (Hunter, 1981), making copepod nauplii a more appropriate diet than rotifers or *Artemia* nauplii for culturing fish. (Figueiredo et al., 2009). Copepod nauplii are an attractive prey item to larval fish as their erratic "zig-zag" motion provides a visual stimulant to foraging animals (Barroso, et al., 2013). Copepod nauplii are also considered excellent live food since no enrichment is required, due to the levels of fatty acids already provided by their diet. The fatty acid composition of copepods varies depending on the feed used (Arndt and Sommer, 2014). Even when fed a monoalgal diet of *Isochrysis galbana*, Parke 1949, the calanoid species *Pseudodiaptomus hessei* Mrázek, 1894 attained DHA/EPA ratio of 2:1 (Siqwepu et al., 2017). Copepods offer other nutritional benefits beside fatty acid provision, containing 700 times more iodine than *Artemia*. This aids in the production of thyroid hormone, helping to regulate metamorphosis in fish (Alajmi and Zeng, 2013).

Calanoid copepods have received particular interest in fish culture as they are entirely pelagic and some have the ability to produce eggs which can be stored (Støttrup, 2006). Rapidly changing abiotic conditions put eggs in a state of quiescence, which can be reversed to initiate hatching in favourable conditions (Jørgensen et al., 2019), thereby removing the need for culture by facilities. Diapause eggs are laid by some copepod species in poor abiotic conditions, but hatch after a compulsory refractory period (Hammervold et al., 2015). Egg storage and hatching have been demonstrated in *Centropages hamatus* Lilljeborg, 1853, however, this particular species is unsuitable for marine ornamental larvae as it cannot tolerate temperatures over 25 °C (Marcus and Murray, 2001). Alongside calanoid copepods, there are species of harpacticoid and cyclopoid copepods which have been used (Støttrup, 2006), by the aquaculture industry showing the most interest in species with very small nauplii.

Euterpina acutifrons *Dana, 1847*

Although classified as harpacticoid, *E. acutifrons* is an atypical harpacticoid since it is distributed in the plankton (Støttrup, 2006; Camus and Zeng, 2012). Despite this, Gopakumar and Santhosh (2009), state that adults were associated with the bottom of the tank, but the nauplii were present in the water column, a characteristic of some "true" harpacticoid species (Støttrup, 2006). There also appears to be some debate over the size of this copepod. Freshly hatched nauplii are recorded at 50–60 µm long and 40–45 µm wide (Gopakumar and Santhosh, 2009). Conversely, other papers suggest this species to be larger. Guisande et al. (1996) record the smallest nauplii length to be 89µm and Goswami (1976), states the length as 107 µm. *Euterpina acutifrons* nauplii have been reportedly used to successfully rear a range of marine ornamental fish. When *E. acutifrons* nauplii were fed in combination with rotifers to rear the barber goby (*Elactinus figaro* Sazima et al., 1997), larvae exhibited enhanced growth rates compared to those fed rotifers alone (Côrtes and Tsuzuki, 2011), suggesting benefits can still be gained from supplemental feeding of copepod nauplii. *Euterpina acutifrons* nauplii were used in the rearing of the blue striped angelfish (*Chaetodontoplus septentrionalis* Temminck and Schlegel, 1844). Larval gape was measured at 293–437 µm and first feed size calculated at between 68–170 µm. *Euterpina acutifrons* nauplii were found in the gut at three days post hatch indicating its suitability as a first feed for this commercially valuable species (Leu et al., 2015). *Euterpina acutifrons* has also been used as a first feed for the three-spot damselfish (*Dascyllus trimaculatus* Rüppell, 1829), the humbug damselfish (*Dascyllus aruanus* Linnaeus, 1758) and the blue damselfish (*Pomacentrus caeruleus* Quoy and Gaimard, 1825) (Gopakumar and Santhosh, 2009). *Euterpina acutifrons* was used, in conjunction with larger *Pseudodiaptomus serricaudatus* Scott, 1894, nauplii, to rear these species to at least 20 days post-hatch (Gopakumar and Santhosh, 2009). Larval gape sizes were recorded between 150–200 µm. Using 20–50 % of the smallest larval gape to calculate the maximum sized feed for these species suggests they can predate on items between 30–75 µm, indicating *E. acutifrons* is in the smaller size range. References are also made of the use of *E. acutifrons* in the early rearing of the particularly valuable *P. hepatus* (Olivotto et al., 2017a). This species is known to have a very small gape size and successful rearing to metamorphosis was achieved by offering copepod nauplii under 75 µm as first foods (DiMaggio et al., 2017). There is some contradiction in the size of *E. acutifrons* nauplii or at least large variation. As a consequence, there is a need for further work to establish if there are cryptic species or "dwarf" morphotypes, which may be useful in aquaculture.

Parvocalanus crassirostris (*Dahl, 1894*)

The calanoid copepod *P. crassirostris* have small nauplii, with recorded sizes of 62 µm long and 38 µm wide (McKinnon et al., 2003) and 68 µm long and 56 µm wide (Burgess and Callan, 2018), making this species potentially suitable for larvae with the smallest gape sizes. This has led *P. crassirostris* nauplii to be partly responsible for a series of industry firsts. *Parvocalanus crassirostris* nauplii use led to the first successful culture of the flame angelfish (*C. loriculus*) (Laidley et al., 2008). Larvae consumed copepod nauplii between 60–70 µm at first feeding (Baensch, 2017). It is suggested that *P. crassirostris* may be suitable for the mass rearing of many other dwarf angelfish species, which are the most heavily traded ornamental angelfish (Baensch, 2017). The nauplii of *P. crassirostris* were central to another high-profile aquaculture achievement, the larval rearing of the yellow tang (*Zebrasoma flavescens*). Larval *Z. flavescens* preferentially selected *P. crassirostris* nauplii from wild plankton samples and performed significantly better when offered only this species (Burgess and Callan, 2018). Initial gape was measured at approximately 260 µm long by 126 µm wide, but after 6 days larvae could consume larger items (Burgess and Callan, 2018). *Parvocalanus crassirostris* nauplii were used to similar effect in the first confirmed captive metamorphosis of *P. hepatus*. *Parvocalanus crassirostris* nauplii were offered from days 3–11, with larger prey items introduced afterwards (DiMaggio et al., 2017). All three species are significant members of the trade and therefore the contribution, and future potential, of *P. crassirostris* nauplii cannot be underplayed. It is a crucial live feed for very small-mouthed larvae prior to transitioning onto larger feeds.

Issues with copepod nauplii

The benefits of using copepod nauplii in culturing marine ornamental fish are obvious. However, smaller nauplii might not sustain larger larvae as they grow. Therefore, copepod species should be appropriately selected. The largest obstacle to their incorporation into mainstream aquaculture is their practical application. Unless there is access to wild plankton populations, or eggs, a live culture must be maintained to provide a constant supply of nauplii. Live cultures exhibit very low densities in captivity (Olivotto et al., 2017a), rarely exceeding 2 adults or 10 nauplii per millilitre. Exceptional densities are measured in hundreds of individuals (Ajiboye et al., 2010). When compared to the density of rotifer cultures, it is evident that significantly larger cultures are required for equivalent output, resulting in higher maintenance time, consumables and expenditure for infrastructure. However, maintaining copepods in increased culture densities may not improve production rates. *Apocyclops panamensis* Marsh, 1913, can live at densities of up to 5,120 adults.L^{-1} of culture water, however nauplii production per female decreases when the density exceeds 2,560 adults.L^{-1} (Phelps et al., 2005). Therefore, cultures should be kept below maximum density to optimise output.

Additionally, most calanoid copepods perform optimally when fed phytoplankton (Dhont et al., 2013). Phytoplankton production is itself costly and complex (Conceição et al., 2010), limiting feasibility for smaller facilities. Algal pastes given to *P. crassirostris in lieu* of live phytoplankton during trials were able to sustain the culture. However, egg production, hatching success, naupliar and copepodite survival, post-embryonic development time and population growth were all substantially higher in those given live algae (Alajmi and Zeng, 2013). Therefore, the long-term viability of prepared algae feeds is questionable. Continually breeding the best performing individuals from cultures fed prepared feeds could potentially create more tolerant strains of copepods. Selective breeding has already proven successful in copepods and less demanding strains would be more popular with aquaculture facilities (Alajmi et al., 2014).

The process of removing nauplii from the culture is labour intensive. Sieving can be used to harvest the nauplii of calanoid species, however, it is difficult and time consuming for harpacticoids, which have nauplii living in close proximity with adults (Støttrup, 2006). Compared to the harvesting methods of rotifer or *Artemia* nauplii where the whole population is fed, the increased workload is apparent. Removal of the nauplii alone may affect population dynamics of the culture. Overharvesting is likely to deplete the population (Cutts, 2003), potentially leading to a crash. A controlled abiotic environment and a known population may optimise this technique. It could allow for maximum harvest whilst maintaining the integrity of the culture. However, the exact parameters of such a system remain unknown.

Copepod cultures are prone to crashing through contamination. Although copepod species can dominate wild zooplankton populations (Barroeta et al., 2017), contamination is a serious risk in hatcheries. Not only are copepod cultures vulnerable to ciliate infestations, they are frequently contaminated by rotifer species (Conceição et al., 2010) which are likely kept on site. Copepod cultures are unlikely to outcompete invaders, such as ciliates, as they rapidly consume available food (Drillet and Dutz, 2013).

Euplotes *sp.*

Ciliates themselves have proven to be useful in the aquaculture of marine ornamentals, although they have received less attention than other groups. They have characteristics of an effective live food, reproducing rapidly, tolerating dense cultures and consuming a variety of food items, allowing enrichment (Côrtes et al., 2013) and are used in aquaculture. However, due to the lack of species identification, their exact size varies from 20 × 30 μm to 135 × 100 μm (Lee et al., 2018). They are smaller than, or at least comparable to copepod nauplii.

Alongside copepod nauplii, *Euplotes* sp. were found in the gut of three-day old *C. septentrionalis* during feeding trials (Leu et al., 2015), indicating they were a suitable live food. The treatment combining ciliates and copepod nauplii gave the highest survival, but ciliates alone did not outperform copepod nauplii and rotifers indicating they are best used in conjunction with other feeds. However, some species actively select ciliates. For example, the reef butterflyfish (*Chaetodon sedentarius* Poey, 1860) has been shown to selectively predate *Euplotes* sp. in preference to *P. crassirostris* nauplii (Lee et al., 2018).

As with copepod nauplii, ciliates are only a first feed and are likely to be outgrown within the first few days of larval development. At present, there is not a clear consensus as to the efficacy of *Euplotes* sp. when compared to copepod nauplii. If ciliates are comparable, it may negate the need to culture some of the more demanding copepod species. Burgess and Callan, (2018) suggest ciliates are effective as larvae can consume them easily whilst hunting for harder to capture prey. Similarly, Leu et al. (2015) suggest that ciliates play an important role in wild larval survival rates as they bridge the gap between the end of yolk supplies and encountering more elusive prey, such as copepod nauplii. This would justify their inclusion into aquaculture diets as it may act as an ideal first feed

Summary of Live Feeds Used to Date

The ontogenetic shift in gape size of marine ornamental species means no single live food species is suitable for all aquaculture fish species. This results in complex rearing plans encompassing different feeds (Wittenrich 2007; DiMaggio et al., 2017). Each existing live food has advantages and disadvantages when used in the production of marine ornamental fish species (Table 2), but what is clear is that further research is required to identify novel live foods if a wider range of species are to be commercially cultured.

Conclusion

The current preferred protocol for feeding marine ornamental fish larvae of most commercially cultured species, by transitioning from rotifers to *Artemia* nauplii and then to enriched *Artemia*, has limited wider application to enable a shift in future aquaculture development. In order to diversify the number of species cultured within the marine ornamental fish trade, there is a need to increase the availability of a wider range of small sized live feeds. Copepod nauplii are an excellent choice of live feed before transitioning to rotifers. However, the current limitations of their culture need to be overcome. Therefore, consideration should be given to developments necessary to overcome the bottlenecks currently being experienced within the commercial ornamental fish aquaculture trade.

The most crucial area lacking data is the initial gape dimensions of larvae. It would be pertinent to record the first gape dimensions of any spawned species in a communal database to allow live foods to be appropriately sized to maximise the chances of successful rearing. Further investigations should focus on the replacement, or at least supplementation, of both rotifer and *Artemia* with copepod nauplii of varying sizes hatched from diapause eggs. This would be the most significant advancement in the culture of marine ornamental fish. In addition, significantly more work is required to develop approaches for creating and storing diapause eggs from copepods. In order to achieve this, further research is needed to determine which species produce diapause eggs. Progression in storage techniques is required to allow the integration of this food source into mainstream aquaculture and to diversify species cultured. The use of diapause copepod eggs would allow utilisation of a more effective live food, without the resources and risks associated with current feeds.

The use of ciliates should also be further investigated, as these naturally occurring preys might be an excellent accompaniment to copepod nauplii and aid in the rearing of fish with small gape sizes. Further feeding trials of known ciliate densities in conjunction with other small feeds, such as copepod nauplii, would identify their potential in culturing marine ornamental species.

Alongside these developments, there exists a need for optimising currently used live feeds. Aquaculture facilities need to be more specific in the species and strain of rotifer used, potentially leading to an improvement in survival of larvae through stricter controls of diets provided, rather than a wide range of prey sizes. These factors combined would optimise and advance the culture of marine ornamental fish and subsequently aid the protection of natural ecosystems.

Table 2. A summary of the advantages and disadvantages of the use of *Artemia*, rotifer and copepods utilised in the rearing of ornamental fish species.

Live food type	Advantages	Disadvantages
Artemia nauplii	Storage; no live culture needed as cysts are dormant (Ruebhart et al., 2008). Ease of production; exposure to light and air initiates hatching (Bengtson et al., 1991). Nutritional manipulation; bioencapsulation allows delivery of specific nutrients to larvae (Sorgeloos et al., 2001). Transitional feed; bridges gap between smaller feeds and prepared diets (DiMaggio et al., 2017).	Large size; first stage nauplii between 400 – 500 µm (Conceição et al., 2010). Nutritionally incomplete; comprised almost entirely of EPA unless enriched (Kenari and Mirzakhani, 2005). Enrichment issues; nauplii increase in size (Bengtson et al., 1991). Supply concerns; cysts harvested from wild populations (Bengtson et al., 1991). Decapsulation; cysts casings are vectors for disease transmission and are indigestible by larvae (Sorgeloos et al., 1977).
Rotifer	Dense populations; large numbers of prey produced in small volume. (Yoshimura et al., 2003). Size; suitable for larvae of many demersal spawners (Olivotto et al., 2017a). Smaller species (under 100µm) also exist (Hagiwara et al., 2014). Nutritional manipulation; bioencapsulation allows delivery of specific nutrients to larvae (Lawrence et al., 2012).	Kept in live cultures; this requires resources and may be contaminated (Reguera, 1984). Classification; difficult to identify exact species used (Le et al., 2017) Nutritional content; enrichment required to complete nutritional profile (Hamre, 2016). Size; not small enough for some larvae from pelagic spawning fish (Olivotto et al., 2017a).
Copepod nauplii	Natural prey; the first food of many larval fish (Hunter, 1981). Attractive; larval fish stimulated by "zig-zag" motion (Barroso, et al., 2013). Nutritionally balanced; correct levels and ratios of DHA and EPA (Siqwepu et al., 2017) alongside other micronutrients (Alajmi and Zeng, 2013). Storable eggs; eggs in a state of diapause can be stored and hatched a later date (Marcus and Murray, 2001) Size; nauplii as small as 68µm exist which make them suitable first feed for small pelagic spawning fish (Burgess and Callan, 2018).	Live culture; storable eggs only exist for few species and thus must be kept in live culture which may be contaminated (Ajiboye et al., 2010). Low density; copepods cannot be kept at high density, leading to large volumes of culture water (Ajiboye et al., 2010), Live feed; most copepod species perform optimally when fed live algae, which is expensive to produce (Conceição *et al.*, 2010). Harvesting; sieving is required to obtain just the nauplii from a whole population (Støttrup, 2006).

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