BACHELOR IN NATURAL SCIENCES

NAT 6TH SEMESTER

Potential Benefits of Marine Copepods in Freshwater Aquaculture

Authors:
Erik Nielsen (62589)
Irida Gkouzou (62656)
Sofia Ramirez-Ekner (62648)

Supervisor:
Benni Winding Hansen

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RUC

Abstract

The current live feed commonly used in freshwater larviculture are not considered optimal choices as levels of polyunsaturated fatty acids (PUFAs) are poor. This report aimed to look into the idea of acclimating a euryhaline copepod species into freshwater environments to serve as a potential live feed for freshwater fish larvae. They are speculated of having rich biochemical profiles, and may specifically contain higher levels of PUFAs. A comparative analysis based on published literature was conducted comparing rotifers and *Artemia*, two common live feed types used in freshwater larviculture practices, with five euryhaline copepod species that exhibit high adaptability to environmental changes. The five copepods include the calanoid species *Acartia tonsa Acartia bifilosa*, *Eurytemora affinis*, *Pseudodiaptomus annandalei* and the cyclopoid *Apocyclops royi*. Salinity, temperature, and pH tolerances were compared between the different live feeds, as well as reported size range of their nauplii, swimming behavior, and three essential highly unstaurated fatty acids (HUFAs) (Docosahexaenoic acid (DHA), Eicosapentanoic acid (EPA), and Arachidonic acid (ARA)). DHA, EPA, and ARA were detected in four out of five copepods. Rotifers and *Artemia* were found to lack DHA, however, they showed higher levels when enriched through oil emulsions. If a euryhaline copepod species is able to be implemented as a live feed in freshwater larviculture practices, then it may be possible to eliminate enrichment procedures. This is a new area being explored, and thus requires more research on the matter. Factors such as other vital nutrients, cultivation of live feed for example must also be considered.

Preface

This project was conducted as a 6th semester bachelor thesis at Roskilde University in natural sciences. Due to the COVID-19 situation resulting in a lockdown of the university campus, our original plan of acclimating the cyclopoid copepod *Apocyclops royi* to lower salinities could unfortunately not be conducted. *A. royi* is speculated to be a promising candidate as a potential live feed alternative for freshwater larviculture practices, as it is a copepod species that exhibits high adaptability to salinity changes and has a nutritious biochemical profile. Although experiments were not able to be conducted, the idea of acclimating a euryhaline copepod species into a freshwater environment was still chosen to be explored. However, we decided to also look into four more euryhaline copepod species as potential live feed candidates for freshwater fish larvae. We chose to then conduct a literature review comparing chosen euryhaline copepods to the current live feeds used today.

We would like to thank Benni Winding Hansen, our supervisor, for guiding us and inspiring us with this bachelor thesis. We would also like to thank Rikke Guttesen, the laboratory technician at Roskilde University, for assisting with the set up of our original experiments.

Contents

1 Introduction

In a hatchery, several fish larvae species depend on live feed as an initial diet in order to survive and grow (Conceição et al., 2010). Copepods are an optimal diet choice for the initial feed, as they are considered highly nutritious in comparison to other live feed options utilized for larviculture (Payne et al., 2001; Wilcox et al., 2006; Støttrup, 2000). They are an attractive source of food for the larvae, and have an optimal biochemical profile necessary for development of the fish (Abate et al., 2016). Specifically, fish larvae require an external supply of polyunsaturated fatty acids (PUFAs) via the diet in order to facilitate healthy growth and optimize survival of the fish larvae. Marine copepods have been utilized as an additional food source in marine larviculture hatcheries in order to improve the production and reduce the development of deformities that may be observed in later stages of the fish life cycle due to deficiencies of nutrients (Wilcox et al., 2006; Støttrup, 2000). However, when it comes to freshwater aquaculture, it is not common for freshwater copepods to be utilized as an initial diet for larvae. This is mainly because freshwater copepods are not as rich in their biochemical profile as marine copepods, and thus, would not be enough in providing essential nutrients to the larvae (Højgaard et al., 2018; Ventura, 2006).

Artemia and rotifers are the typical live feed sources that are being utilized today as initial feed diets for freshwater fish larvae (Atsushi and Tatsuki, 2017; Ako et al., 2003). These live feed sources just like freshwater copepods, are lacking the appropriate amounts of certain nutrients, such as essential PUFAs needed by fish larvae. However, these are able to be supplemented by enriching rotifers and *Artemia* with different oil emulsions (Evjemo et al., 1997). Although this way of providing nutrients seems to be working, it is not an ideal scenario, as the use of oil emulsions in the aquaculture sector are exceeding sustainability limits due to the increasing demand (Naylor et al., 2000; Nasopoulou and Zabetakis, 2012). Development of deformities such as mal-pigmentation or an increased mortality rate may still occur during production (Højgaard et al., 2018). Therefore, exploring for an alternative live feed source that provides fish larvae with the appropriate fatty acids might improve freshwater aquaculture production.

A generally new research idea that has been explored is to acclimate euryhaline copepod species to withstand freshwater environments in order to use in particular their nauplii, as a possible initial live feed source for larvae. In theory, this could improve freshwater larviculture by providing a more nutritious diet compared to what is being used today. For example, in a study conducted by Højgaard et al. (2018), promising results were shown on the survivability of three euryhaline copepod species in low-salinity waters and was proposed as potential live feed sources for the freshwater Pikeperch larvae. Implementation of a euryhaline copepod species as a live feed for freshwater larvae could be possible as long as they are capable of exhibiting attractive swimming behaviour, which is vital for fish that rely on live feed (Turingan et al., 2007).

This research aims to compare five different euryhaline copepod species with each other based on their physiological traits in order to see if they may potentially survive. Moreover, we want to create a comparison between these copepods and the live feed that is currently being used today based on their PUFA content. These copepod species are the calanoids *Acartia tonsa Acartia bifilosa* , *Eurytemora affinis*, *Pseudodiaptomus annandalei* and the cyclopoid *Apocyclops royi*, referred to here as the 'Big five'. We hypothesize that one or more of the selected euryhaline copepod species can be adapted and utilized as live feed for freshwater fish larvae, providing nutritional advantages over the current live feed used today.

1.1 Problem Area:

This project explores, as far as theoretically possible, the proposal of using euryhaline copepods as a universal live feed for fish larvae. Five species that are already being investigated for this purpose were chosen as the subjects of this project, and their potential benefits are evaluated in the context of freshwater aquaculture practices.

The main research question is *Can a euryhaline copepod species be implemeted as live feed for freshwater larviculture practices?* Listed below, are the sub-questions that guide the research within the following different aspects:

1. Why is live feed important for fish larval development?
2. What are the current live feeds used in freshwater larviculture today?
3. What characterizes the five chosen copepod species?
4. How do they compare to current live feed regarding the following:

- Survival conditions such as salinity tolerance, temperature and pH range
- Size range of nauplii
- Swimming behaviour
- Fatty acid profile

2 Methods

The present is a literature review where different studies, articles and search materials were recruited, using the Roskilde University library, REX and Google Scholar data bases in order to access a variety of Journal publishing platforms. Some of the platforms accessed include the Journal of Plankton Research, Wiley online library, Science direct, World Register of Marine Species (WoRMS), and National Center for Biotechnology Information (NCBI).

Key words included in our search strings:
copepods, aquaculture, freshwater, larviculture, salinity, polyunsaturated fatty acids, eicosapentaenoic acid, docosahexaenoic acid, arachidonic acid, temperature, rotifers, live feed, *Artemia*, *Acartia tonsa*, *Acartia bifilosa*, *Eurytemora affinis*, *Pseudodiaptomus annandalei*, *Apocyclops royi*.

3 Theory

3.1 Aquaculture

Aquaculture also known as aquafarming, is defined as the culture of aquatic organisms under controlled or semi-controlled conditions (Boyd, 2013; Jørgensen et al., 2005). Aquaculture may include many different animal species such as fish, crustaceans, molluscs as well as aquatic plants and algae. However, for the purpose of this report, the focus will only be on aquaculture referring to fish production. Aquaculture is considered to be one of the world's fastest growing food producing sectors, offering efficient food supply and economic benefits. By using methods with low environmental impact, it is producing food with high quality protein for humans (Jørgensen et al., 2005). Other species may be commonly cultured for other commercial uses like bait, feeders, sports and even for ornamental purposes. Like all systems, aquaculture is in itself artificially constructed and to a various degree controlled aquatic ecosystems. It needs proper and continuous support to sustain the right culture conditions such as temperature, pH, proper oxygen levels, salinity and the removal of toxic waste (Boyd, 2013).

3.1.1 Freshwater aquaculture

Aquaculture can be categorised by the salinity of the water it contains into either marine, with salinity higher that 29, or freshwater, with salinity lower than 0.5 (Bimal and Rashid, 2017). Both use essentially similar ways of culturing, however for the purpose of this report, we will solely be focusing on freshwater aquaculture.

In freshwater aquaculture there are three main culturing methods. The most common is called extensive culturing, where a large body of water is enclosed, capturing wild species that are fed on natural food sources until harvesting. The second method is referred to as semi-intensive and works as the extensive culturing system, in an enclosed natural space. The difference is that in semi-intensive cultures the production and the growth of the captured species is increased by feeding them with supplementary feed, usually dry pellets. The third method is called intensive and involves ponds, cages or open-air concrete tanks. In this method the target species are domesticated and fed with dry pellets, that secure healthy growth and large yield in short time periods (Boyd, 2013; Jørgensen et al., 2005).

In freshwater aquaculture, ponds are the most common culturing system used mostly in Central and Eastern Europe. However, other flow-water systems such as net pens, cages and water recirculated aquaculture systems are also important (Boyd, 2013).

3.1.2 Larval rearing in freshwater aquaculture

A big part of freshwater fish aquaculture is the necessary starting point, the larviculture. The life cycle of fish consists of five stages. Aquaculture includes cultures of all stages making sure that the conditions are appropriate in order for the fish to develop into a healthy full grown fish. First is the egg stage, which is short, and their hatching usually depends on the temperature of the water in the hatcheries. When the eggs hatch, the embryos are called larvae and they are attached to a yolk sac. The larvae feed from this yolk sac, which provides them with the appropriate nutrients during the first stage of their life. A few days after hatching, the first feeding must take place in order to keep the larvae alive and healthy. After a couple of more days, and when the larvae consume the yolk sac, they move on to a sub-stage becoming fry or else "feeding larvae". In this sub-stage the fish is able to swim up to the surface of the water and get its prey. Later, through transformation called metamorphosis, the fry moves to the next stage, juvenile. Juvenile fish look like an adult but are smaller in size. In this stage, the fish is also called "fingerlings" since their body size is approximately the size of a human finger. In addition, the formation of scales is starting, the fin rays are fully developed, and the need for larger amount of food occurs. Fingerlings become adults when their sexual organs mature and are ready for reproduction (Miller and Kendall, 2009; Jakobsen et al., 2016). Figure 1 underneath shows a generalized overview of a fish life cycle. In this report the focus will be on the larvae and fry stages.

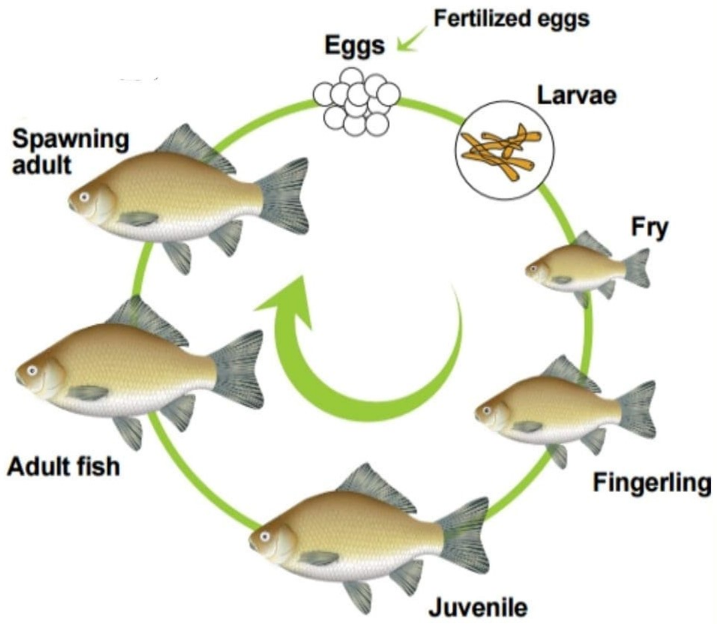

Figure 1: An illustration of the five stages of the fish life cycle. It starts as an egg and when the egg hatches it becomes a larva. Then it moves on to the sub-stage of fry and through metamorphosis, it becomes a smaller version of an adult fish called a juvenile or else a fingerling. When the sexual organs mature and the body size increases, the fish gets to its last life stage, adult, where it is ready to reproduce. Image modified by (RGJ Aquaponics, n.d.)

Fish larvae are incredible organisms. They are among the smallest vertebrates that exist and their growth potential is extraordinary. However, larval fish cultures are one of the most risky and sensitive phases of fish cultures, but are considered to be the most profitable type of culture (Yúfera, 2018; Ludwig, 1999). In order to reduce the risk of elevated mortality in this vulnerable stage, special planning is enforced. Appropriate culturing facilities are set in a way that larvae, fry and fingerlings are allowed to grow and develop into healthy adult fish. Important parameters include: optimal temperature, pH, oxygen saturation, but also large quantities of quality food. In nature, fry usually consume small zooplankton species like copepod nauplii, rotifers, and cladocerans (Yúfera, 2018; Ludwig, 1999). Therefore, some zooplankton species are strongly suggested to be the first feed of larvae in cultures since they contribute to faster growth, and their morphological and behavioural characteristics influence the larvae positively (Bruno et al., 2018). When hatched, the size of fish larvae may vary from 2 to 15 mm depending on the species. For example, the Northern pike is 7.5-10 mm right after hatching, see Figure 2 (Cooper, 2016). Some zooplankton species become bigger in size than freshly hatched larvae as they become adults, and therefore, there is a high probability that they prey upon them. This means that the first feed type must be smaller than the larvae species that is cultured (Ludwig, 1999; Conceição et al., 2010). For fish larvae like Sunshine bass, the only type of zooplankton small enough are the rotifers and nauplii from copepods. However, for bigger fry, rotifers are too small and are not worth the effort and energy to chase, since they do not provide enough nutrients (Ludwig, 1999). In aquaculture, rotifers and *Artemia* are the most common and preferred feed because of their low cost-effective protocols for their mass production (Yúfera, 2018; Conceição et al., 2010).

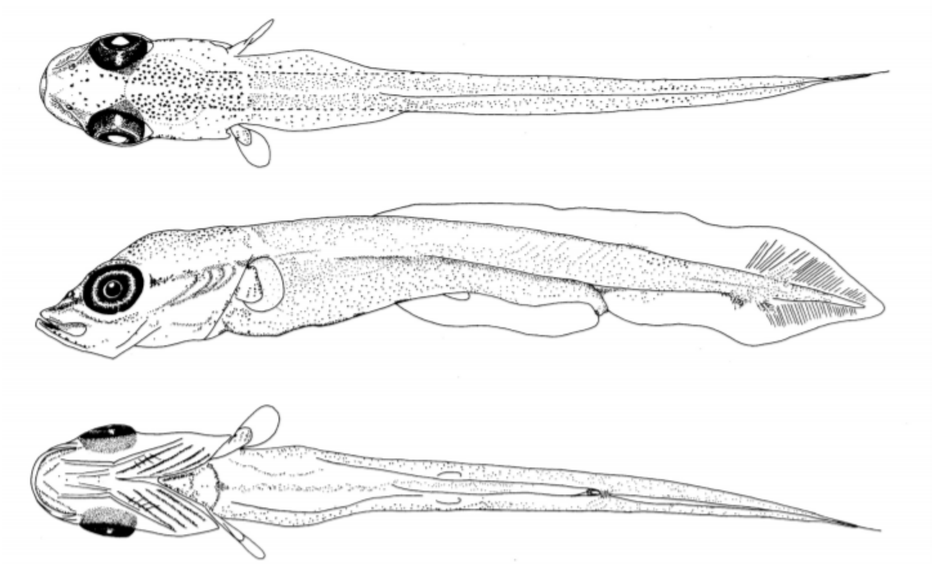

Figure 2: Dorsal, lateral and ventral drawing of Northern Pike larva. (Cooper, 2016)

Live feed is the main diet in a fish larviculture (Yúfera, 2018). During the first feeding, the digestive system of the fry is not fully developed. The stomach is still absent and most of the digestion process takes place at epithelial cells. This means the fry is incapable of fully processing formulated diets like pellets, and its growth and survival is stunted. Despite the progress in technology providing the inert diets for larvae fish, they still depend on live feed during their early larvae stages. In addition, live feem.d is always available, since it can be detected and preyed upon by the predatory fish larvae. This is due to their characteristic swimming movements triggering the larval hunting behavior (Conceição et al., 2010). Formulated feeds, such as pellets, usually aggregate and can float to the surface or sink to the bottom of the water after a while. Most larvae are "visual predators" learning to attack moving prey in nature, adapting to their prey's swimming behaviour (Conceição et al., 2010; Bruno et al., 2018). Furthermore, the dry, hard pellets are more difficult to process by the fry's mouth compared to when manipulating the thin exoskeleton of the live feed.

3.1.3 The importance of nutrients derived from the live feed diet

An important aspect of providing larvae with the appropriate live feed is due to the different dietary requirements. Fish larvae are dependent on several different classes of compounds that they must receive via the diet. These different classes include proteins, lipids, carbohydrates, vitamins and minerals. Although all are essential components for larval development, this report will have a specific focus on the essential fatty acids from the lipids class. (Ako et al., 2003).

3.1.4 Essential PUFAs

Lipids are an important component in fish, as they for example, act as a source of metabolic energy and are essential components of cell membranes. In particular, fatty acids are a kind of lipid that are vital for the survival and development of fish larvae. Many fatty acids are known to be produced naturally in the fish, however, polyunsaturated fatty acids (PUFAs) belonging to the n-3 or n-6 series are not able to be synthesized. Therefore, it is important for these to be supplied via the diet (Das et al., 2012). Being deficient in PUFAs may lead to higher mortality rates of the larvae, or developed deformities that appear in later life stages of the fish.

Specifically, three kinds of PUFAs that are also considered highly unsaturated fatty acids (HUFAs) are vital for ensuring proper growth of fry (Ako et al., 2003). Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) are HUFAs from the n-3 series, and are known to lead to deformities or increased mortality when there is an insufficient supply offered from the live feed. Having an adequate ratio of the two, has been said to lead to better growth, pigmentation, and better stress resistance (Reitan et al., 1994; Mourente et al., 1993). Another HUFA that is said to be vital for fish larvae is arachidonic acid (ARA) from the n-6 series. ARA is also said to improve growth of fish larvae and improve pigmentation. Providing a feed high in fatty acids is necessary for larval rearing, and the amounts vary depending on the different fish species (Ako et al., 2003; Koven et al., 2001).

3.2 Current feed in freshwater aquaculture

As stated before, appropriate nutritional content provided by the live feed diet is vital for the survivability of fish larvae. The current live feed used as initial diet may vary depending on the fish species, however, two common live feeds used today are rotifers and *Artemia*. These will be described further and used for a comparative analysis.

3.2.1 Rotifers

Rotifers are considered one of the favorable live feed options used in both marine and freshwater larviculture practices. Rotifers are small, microscopic aquatic animals belonging to the phylum Rotifera (Rafferty, n.d.). They are also commonly referred to as "wheel animals" due to their small cilia resembling a rotating wheel-like structure located around the mouth, which assists with filter feeding. Figure 3 is an image of the rotfier *Brachionus angularis* exibiting the common pot-shaped lorica, the major body portion of rotifers. There are about 2,000 different species of rotifers that can be found in freshwater, brackish, as well as marine environments. In general, they can range in size anywhere from 0.1 mm to 0.5 mm in length. They are known to reproduce asexually by parthenogenesis, but some species can also reproduce sexually (Rafferty, n.d.). Females are able to produce up to around 15-25 eggs in their lives, and the eggs are able to hatch within several hours (Das et al., 2012). Rotifers that are used as live feed in the freshwater larvae cultures are commonly from the genus *Brachionus* (Lim and Wong, 1997; Ogata and Kurokura, 2012; Lubzens et al., 1987).

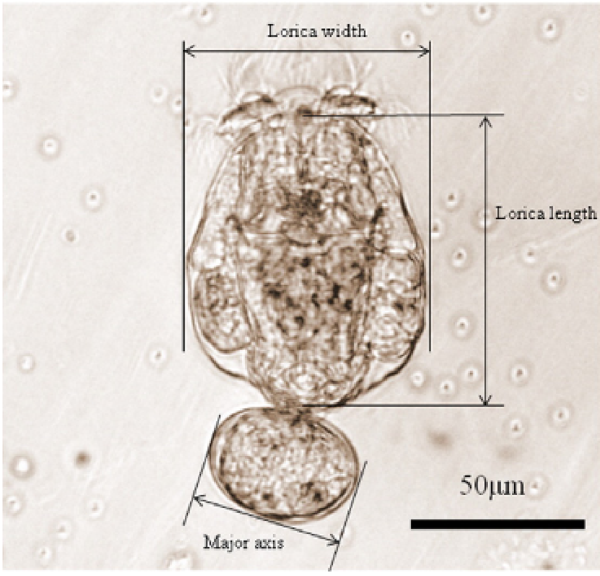

Figure 3: Microscopic image of the rotifer *Brachionus angularis.* Lorica length and width indicated in image, as well as the length of the major axis where parthenogenic eggs are located. (Ogata et al., 2011)

Their suitability as a live feed for larvae has to do with a variety of characteristics. Their small size range fits perfectly for the mouth opening of several fish larvae species, since the feed has to fit whole in the mouth of the larvae (Conceição et al., 2010; Dhont et al., 2013). Rotifers are also able to feed on several different sources, such as different microalgae as well as yeast cells (Dhont et al., 2013; Lim and Wong, 1997). However, their feed source is an essential factor that plays a role in their nutritional composition (Dhont et al., 2013; Lim and Wong, 1997; Whyte and Nagata, 1990). Yeast cells are not an optimal choice of feed for rotifers, as their nutritional composition lacks the appropriate amount of HUFAs, essential for the survival of the fish larvae (Dhont et al., 2013; Whyte and Nagata, 1990). However, it is possible to provide a combination of yeast cells with microalgae particles, which is more nutritionally optimal in order to have enough essential nutrients. It is also common to enrich rotifers through different forms of supplementation before they are given to the fish larvae, in order to provide the essential nutrients that were missing (Dhont et al., 2013).

3.2.2 *Artemia*

Another common live feed used in many hatcheries all over the world is *Artemia* (Das et al., 2012). *Artemia* is a kind of zooplankton, that is also commonly referred to as brine shrimp. They belong to the phylum Arthropoda, subphylum Crustacea, and the order Anostraca (Criel and Macrae, 2002a). They have a segmented body structure that is divided into the head, thorax and abdomen with leaf like appendages attached (Criel and Macrae, 2002b). The typical size of adult *Artemia* can range from approximately 8 mm to 12 mm (Criel and Macrae, 2002b). Adults are too large in size to be utilized as live feed for larvae. Therefore, nauplii are exclusively used instead, as they are significantly smaller in size (< 0.55 mm approx.) (Das et al., 2012; Lim et al., 2003). *Artemia* are great osmoregulators and have been reported to tolerate salinities anywhere from around < 60 to 340 g/L (Gajardo and Beardmore, 2012; Sui et al., 2014). They are thus, commonly found in many hypersaline environments all over the world. These hypersaline regions provide a safe environment in order to avoid predators (Dhont et al., 2013). Reproduction of *Artemia* can either result in ovoviviparity or oviparity (Dhont et al., 2013; Criel and Macrae, 2002c). Ovoviviparity occurs either through fertilization or as a result of parthenogenesis, and results in free swimming nauplii. Oviparity is when the embryos reach only up to the gastrula stage, resulting in dormant eggs, known as cysts. Cysts are usually a result of unfavorable environmental conditions, but they can hatch when conditions become more suitable (Dhont et al., 2013).

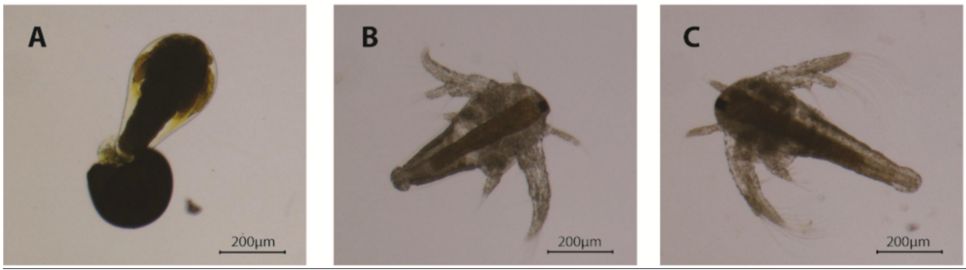

Figure 4: Microscopic image of *Artemia franciscana* nauplii hatching from a cyst. A-C indicate three different development stages. A) Hatching from the cyst. B) The first nauplius stage called Instar I. C) Second nauplius stage called Instar II. (Lopalco et al., 2019)

Cysts produced by *Artemia* are considered a great convenience in the freshwater aquaculture production, as they can be stored for long periods of time, and only take 24 hours to hatch when placed in the appropriate conditions (Dhont et al., 2013; Das et al., 2012). It is most common to utilize freshly hatched *Artemia* nauplii (Instar I stage) as live feed, since this is when they have the highest energy reserves, are smallest in size, and exhibit attractive swimming behaviours. Figure 4 shows an image of *Artemia* nauplii hatching from a cyst. Several hours after hatching, *Artemia* nauplii become less suitable as live feed as they grow a bit larger, exhibit "too" fast of swimming behaviour, and have less free amino acid content, another nutrient vital for fish larvae development (Sorgeloos et al., 2001). Although *Artemia* is one of the most common sources of live feed in larviculture today, there are some negative qualities about them as well. The overall nutritional value of *Artemia* is not considered suitable for larvae surviveability (Dhont et al., 2013). However, just like rotifers, nutrient emulsions are also possible in order to improve PUFA content (Dhont et al., 2013; Das et al., 2012; Sheikh-Eldin et al., 1997; Sorgeloos et al., 2001). Another negative aspect of using *Artemia* in freshwater larviculture is that they will typically die after entering a freshwater environment after approximately one hour. Therefore, it is common for *Artemia* to be fed to larvae every few hours intermittently for freshwater fish larvae (Lim et al., 2003).

3.3 Exploring the idea of utilizing copepods as a live feed

Within the last years, the larviculture producers use rotifers and *Artemia* as the basic feed for fry. This led to a continuous supply of larvae fish which increased the total fish farming production (Yúfera, 2018). However, copepods have also been used in marine aquaculture although not as often as other live feeds. When copepods have been incorporated as live feed in marine larviculture, improved results were yielded in terms of larvae performance when compared to the previous mentioned types of live feed (Conceição et al., 2010). This report will describe copepods in general and their use in marine aquaculture as well as the characterisics of five marine species that can potentially be used in freshwater larviculture.

3.4 Copepods

Copepods are considered the dominant member of the zooplankton community, and are actually the most numerous multicellular organisms existing in the world (Mauchline et al., 1998). The Copepoda form a subclass under the phylum Crustacea (Mauchline et al., 1998; Encyclopaedia Britannica, 2019). These organisms are in general, small aquatic animals, typically ranging in size from 0.5 to 2 mm in length (Encyclopaedia Britannica, 2019). There are ten orders of Copepoda, where the three dominant orders include Calanoida, Cyclopoida, and Harpacticoida (Mauchline et al., 1998; Johnson et al., 2012). Copepods that will be referred to later on in this report belong either to the orders Calanoida or Cyclopoida.

Copepods are primarily found in marine environments, although they are also commonly found in brackish water environments as well as freshwater locations (Mauchline et al., 1998). Copepods can be found at all depths and latitudes, but a vast majority is known to be located in pelagic waters, where they feed on phytoplankton (Mauchline et al., 1998). These herbivorous copepods play an important role in aquatic food webs by serving as an essential link between phytoplankton and higher trophic level organisms (Mauchline et al., 1998). Other types of copepods are known to be carnivorous, feeding on various kinds of mesozooplankton for example. Feeding behaviours vary from the different copepod species. Suspension feeding is common in those who rely on microalgae present in the water column, and raptorial feeding when hunting for prey items. The benthic copepods can be either grazers or browsers on benthic microflora and/or fauna (Johnson et al., 2012).

Copepod body structures vary amongst the different species, however in general, are divided into two main parts; The prosome and the urosome (Johnson et al., 2012). The prosome is the term that refers to the head fused to the thoracic segments. A long pair of antennae can be found on this half of the body, which can be used for swimming. The hairy structure on their antennae called setae, is used as sensory organs. They serve the purpose of detecting their food and sense other organisms close to them but also as chemoreceptors detecting dissolved chemical substances in the water. Copepods also have a single eye (Johnson et al., 2012). The other half of the body known as the urosome, refers to the narrow posterior portion. The urosome typically lacks appendages, but contains the genital segment of the copepod. Egg sacs present on certain female species would be located on this portion of the body. A simplified overview of the copepod body structure is depicted in Figure 5.

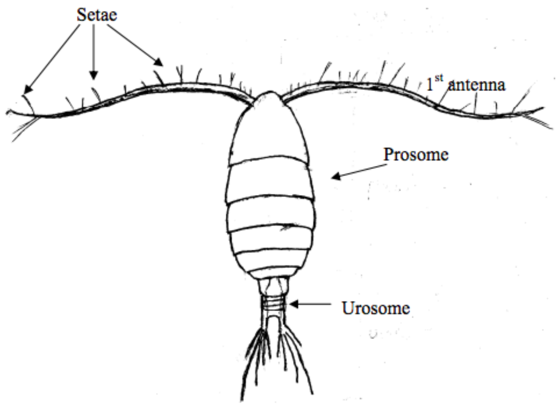

Figure 5: Illustration of a calanoid copepod body structure. The 1st antenna containing setae is located on the prosome part of the body, the anterior portion. The posterior portion of the body is referred to as the urosome. (Vanderlugt and Lenz, 2009)

Reproduction for copepods is sexual, and a single event of copulation typically results in multiple clutches containing a number of eggs (Johnson et al., 2012). Eggs can either be attached to a female in egg sacs, or freely spawned into the water column. The typical life cycle of copepods can be visualized from Figure 6. It starts from eggs hatching into nauplii. These nauplii proceed their lifecycle into six naupliar stages and molts before becoming a copepodite. The copepodite form consists of another five stages, where it begins to undergo physical structural changes leading to the sixth stage, the adult stage (Johnson et al., 2012).

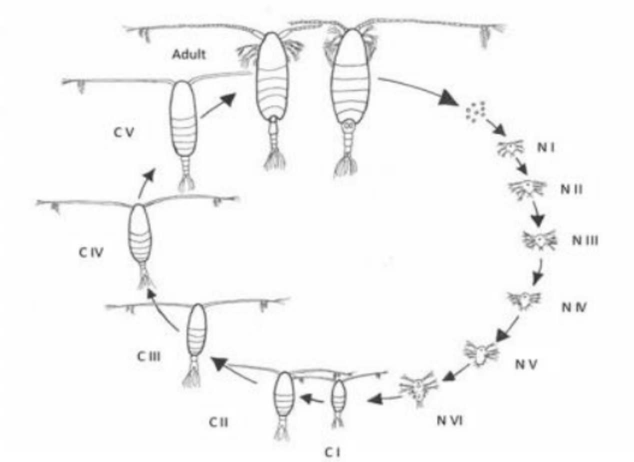

Figure 6: General illustration of the lifecycle of copepods. Eggs are followed by the six nauplliar stages represented as NI- NVI in the diagram. CI-CV indicates the different copepodite stages. Last stage is the adult stage of the copepod lifecycle. (Mauchline et al., 1998)

3.5 The use of copepods in marine larviculture

Marine copepods have been utilized as a live feed source for improving marine larviculture purposes (Payne et al., 2001; Wilcox et al., 2006; Støttrup, 2000). This is because they are considered as an optimal diet source due to their several qualities. Their small size ranges are an appropriate fit for the mouth size of many fish larvae, making them consumable. Copepods also exhibit attractive swimming patterns vital for fish larvae to recognize as a food source (Turingan et al., 2007). Copepods are also nutritionally superior to other typical live feed diets utilized in larviculture as a result of their biochemical profile. Because of their marine origin, they have naturally high levels of n-3 HUFAs, EPA, as well as DHA content (Imsland et al., 2006). Providing the right nutritional content to the fish may lead to less deformities observed at later stages (Bell et al., 2003).

3.6 The implementation of euryhaline copepods as live feed in freshwater larviculture

In contrast, freshwater copepods are not commonly utilized as a source of live feed for freshwater larvae, as they are not considered an optimal food source. Freshwater copepods, of course, also exhibit the same attractive swimming behaviour and are similar in size. However, they are not nutritionally optimal (Højgaard et al., 2018). Their biochemical profile is not optimal for fish larvae, and could potentially lead to deformities such as mal-pigmentation observed in the later stages of the fish life cycle such as what is observed in some cases with the current live feed options used (Højgaard et al., 2018). Instead, acclimating a euryhaline copepod species to a freshwater environment is being tested and researched, as this could be a solution to improving and optimizing live feed in freshwater larviculture production.

In the following sections, descriptions of five selected copepod species will be provided. These copepod species were chosen as candidates for a comparative analysis in order to see what qualities may benefit a freshwater larviculture system. All copepods to be mentioned are euryhaline species and are in theory, potentially able to be acclimating to survive in a low saline environment. Therefore, a literature comparison based on their documented qualities is necessary for determining a potential candidate that is most suitable for improving freshwater larviculture production.

3.7 The five euryhaline copepod species

The five copepod species that are presented below are from environments that may exhibit fluctuating conditions. Therefore, these species may exhibit high adaptability to different changes. This may mean that they could possibly be acclimated and used in the fresh water larviculture. The five copepod species that will be presented include *Acartia tonsa, Acartia bifilosa, Eurytemora affinis, Pseudodiaptomus annandalei* and *Apocyclops royi.*

3.7.1 *Acartia tonsa*

Acartia tonsa Dana is a copepod belonging to the order Calanoida. Their distribution has been seen worldwide in coastal boreal and subtropical waters. The species is usually found in warm coastal areas, estuaries and brackish waters where temperatures and salinity can fluctuate to a big degree. *A. tonsa* has a relatively short abdomen and body width. Females can get slightly bigger in size than males, measuring 1.2 - 1.5 mm compared to 1.0-1.1 mm in length. A female and male *A. tonsa* is shown in Figure 7. *A. tonsa* usually dominates the zooplankton community, especially when the water temperature is optimal, around 15 – 20°C (Mauchline et al., 1998). While most calanoids are characterized by their single or double egg-sac(s), *A.tonsa* females are free spawners (Mauchline et al., 1998; Johnson et al., 2012). They produce diapause eggs that can hibernate if the conditions are not optimal. This is one of the reasons why they are considered to be so adaptable to different environments. Furthermore, *A. tonsa* is separated from other copepod species by its body structure. Copepods belonging to this species have a joint between their fifth and sixth body segment, long first antennae and branched second antennae (Mauchline et al., 1998).

Figure 7: Adult male (left) and female (right) copepod of the species *Acartia tonsa.* (Alchetron.com, 2019)

While its optimal salinity ranges from 15 to 22, it has been reported that *A. tonsa* is able to survive and hatch eggs in lower or higher salinities such as 5, 10 or 30 (Højgaard et al., 2018). Moreover, *A. tonsa* inhabits environments with neutral or slightly alkaline pH (7-8), while it has also been reported that it is able to survive in slightly acidic conditions (Mauchline et al., 1998; Aguilera et al., 2020). Its osmoregulation depends on the environment that it lives in. Primarily, *A. tonsa* is a marine copepod, therefore it would be an osmoconformer, conforming to the surrounding environment by excretion of salt and intake of water for salt balance. Further studies have also shown that marine/ euryhaline species also exhibits osmoregulation on a cellular level, where they keep an ionic balance. In Mauchline et al. (1998), is indicated that the ionic composition is regulated to match the surrounding environment and medium (Greve et al., Unpublished). Their main food source is phytoplankton and they need a stable light/dark photoperiod.

Depending on their food source, *A. tonsa's* PUFA content will vary. For example, food such as the algae species *Rhodomonas salina* would yield a good amount of DHA, but lower amounts of EPA in *A. tonsa* (Veloza et al., 2006). Another example is that when *A. tonsa* is feed on *Thalassiosira weissflogii* it will have a lower content of DHA than when it is feed on *Isochrysis galbana* and *Heterocapsa triquetra.* The last two algae types give the copepods less amount of EPA when compare to *Thalassiosira weissflogii.* However, all three types of algae provide similar amounts of total fatty acid content.

3.7.2 *Acartia bifilosa*

Acartia bifilosa, as seen in Figure 8, is also a calanoid copepod belonging to the genus Acartia, like the previous mentioned copepod. It usually dominates parts of the Northern Baltic sea close to coastal areas, bays and estuaries where the salinity and temperature are not stable. In these areas, the currents can be strong and the water is well mixed (Katajisto, 2003). Its body structure is similar to the rest of the calanoid copepods, and its size differs depending on gender. Females tend to be slightly larger, reaching 1.1 mm, while males typically grow up to 1 mm in length. However, like *A. tonsa*, this copepod is also an exception in its order by being a free spawner, releasing its eggs directly in the water column where a certain fraction may reach the sediment. They can hatch residing in both compartments when they are ready. Eggs do not need light to trigger the hatching process and a nauplius take around 16 days to mature and become an adult (Viitasalo and Katajisto, 1994). After the 20th day, the females are able to produce eggs (Yoon et al., 1998).

Figure 8: Adult calanoid copepod of the species *Acartia bifilosa*. (Vehmaa et al., 2013)

It inhabits environments with high salinity, 28-30, but it can also function in lower salinities such as 5.7. It is mostly found in areas with temperature around 13 – 19°C and pH of 7.6 (Vehmaa et al., 2013). In addition, it has been reported that *A. bifilosa* is able to hatch eggs in extremely low salinity of 0, however under salinity 7, the number of the hatching eggs starts to decrease (Højgaard et al., 2018).

Information on *A. bifilosa's* biochemical composition was not able to be found and my therefore indicate a lack of data on the matter.

3.7.3 *Eurytemora affinis*

Eurytemora affinis, which can be seen in Figures 9 and 10, is also a calanoid copepod capable of living in environments with shifting temperature and salinity. It can live in low salinities and dominate the zooplankton community in oligohaline environments. In some regions, it accounts for 90-99% in abundance of the crustaceans throughout year. Due to its dominant role and its production and population dynamics, different aspects about its surrounding environment can be analyzed (Peitsch, 1995). Studies have been conducted in the Seine estuary in France, that found specimens that were able to migrate up-stream during summer to environments closer to freshwater. This was thought to be because *E. affinis* wanted to avoid predators and competition found in the areas with higher salinities. Therefore, *E. affinis* was able to alter its location by moving upstream or downstream in the estuary and enhance its fitness (Souissi et al., 2014).

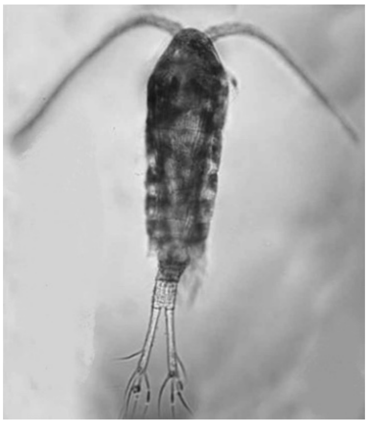

Figure 9: Calanoid copepod, *E. affinis*. Picture modified from (Alekseev and Souissi, 2011)

Figure 10: Picture of an ovigerous female copepod, *Eurytemora affinis*, carrying eggs in its single egg-sac. (Lee, 2016)

E. affinis is able to reproduce in a wide range of salinities including extreme conditions such as 0 and 30. Its temperatures tolerance fluctuates from $10-30°C$ throughout the year, whereas the pH is around 7-9.5 (Hansen et al., 2017; Højgaard et al., 2018). Regarding the temperature, even if they can reach 30 degrees, *E. affinis* is more abundant at regions with lower temperature (Gonzalez and Bradley, 1994). In a study by Souissi et al. (2016), *E. affinis* was tested in 24°C temperature and salinity 25 as stress factors relative to the environments they collected the copepods. The species demonstrated good overall plasticity and fitness to salinity and temperature change due to acclimation to the environment.

Looking at the biochemical profile of this species, studies indicate that they are a good candidate for larvae food, because they are rich with fatty acids, free amino acids and micro nutrients. Due to this, it is believed that they induce higher larvae survival rates, and their fatty acid composition helps fish development and growth performance (Souissi et al., 2014).

3.7.4 *Pseudodiaptomus annandalei*

Pseudodiaptomus annandalei, as it can be seen Figure 11, belongs to the calanoid order, like the species above. It dominates Taiwanese aquaculture ponds and inhabits the brackish waters of the Indo-Pacific region (Blanda et al., 2015, 2017; Rayner et al., 2015). It is an egg-carrying species with a body structure similar to the rest of the calanoid copepods, with females reaching the size of 1.36 mm while males are smaller, reaching 1.09 mm in length (Dur et al., 2010).

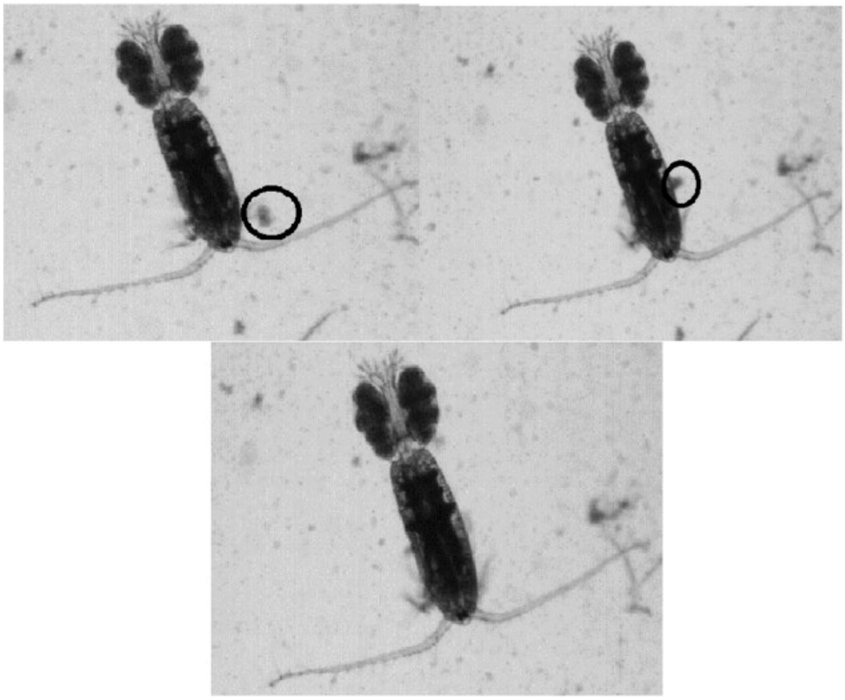

Figure 11: Pictures of the adult female Calanoid copepod *Pseudodiaptomus annandalei* spotted pursuing and ingesting the rotifer species *Brachionus rotundiformis.* (Dhanker et al., 2012)

In nature *P. annandalei* can tolerate big scale changes in its environment. Blanda et al. reports data from two field studies in Taiwan in 2015 and 2017, where the productivity of the aquaculture ponds were re-analysed. It was reported that *P. annandalei* has no problem adapting to extreme hypoxia, low PUFA concentrations in their diet, and in general low quality water conditions. Low quality living conditions in the ponds occur because some of the abiotic factors change based on the seasons of the year. Thus, this copepod species must be also highly adaptable, since its natural environment changes every couple of months. In the ponds, the surface temperature can reach 32°C in the summer and drops to 19°C during winter. Salinity changes from 23 to 15 during the year and can sometimes abruptly drop even lower to 9 (Blanda et al., 2015). The pH alters from 8 to 8.7 and the surface oxygen levels drop and rise from 7.9 ± 2.6 to 16.3 ± 4.2 mgL^{-1} with some exceptions that show that it can drop down to 3.81 mgL^{-1} depending the weather. However, the ideal cultivation conditions is a salinity of 15-20, pH around 8 and the temperature approximately 25°C ± 5 with provided algae (Pan et al., 2016; Drillet et al., 2011). It has been showed that *P. annandalei* reduces its reproductivity based on the space that is available in the ponds. The maximum population density is around 385 $indL^{-1}$ with reproduction rate decreasing after the number increases ≥270 $indL^{-1}$ (Blanda et al., 2017). The optimal salinity for *P. annandalei* is 15 to 20. It can survive big ranges of salinity 5-30, without experiencing high stress levels. However, in extreme salinities (0 and 35) the adults can survive but their egg production reduces significantly, and the eggs can barely hatch (Chen et al., 2006).

In their majority, calanoid copepods are unable to produce high amounts of HUFAs. However, *P. annandalei* is a calanoid that is considered to have the ability of biosynthesizing n-3 HUFAs in big amounts when fed with relatively HUFA low microalgae or even fed simple bakers yeast. One of the several experiments reported by Rayner et al. (2017), showed that when *P. annandalei* is fed with the algae species *Tetraselmis chuii*, known for its poor DHA content, can still produce those fatty acids in high amounts. As it is mentioned before, *P. annandalei* inhabits Taiwanese aquaculture ponds where the algal and seston quality is low. This works as a selective force on the copepod's ability to increase its HUFA profile. *P. annandalei* is a potential candidate as live feed in the aquaculture world because of its biochemical composition and ability to modify its fatty acid composition (Rayner et al., 2017).

3.7.5 *Apocyclops royi*

The species *Apocyclops royi* belongs to the order Cyclopoidea, one of the three main orders of free-living copepods (Pan et al., 2016). This order includes hundreds of species, which inhabit freshwater, brackish, and marine locations. Cyclopoids can usually be distinguished from the other orders by their first antennae, which are shorter than the length of their body. Other characteristics include: a dorsal median eye and an abdomen separated in four segments that lacks appendages, as it is shown in Figures 12 and 13. Cyclopoids are also egg carriers and usually carry two egg sacs, as it can be seen in Figure 14, and they can hatch 10-15 nauplii per day (Hickman et al., 2017; Jepsen et al., Unpublished). *A. royi* is smaller in size than the previous mentioned copepods and its size is based on gender and its living conditions, with males reaching around 0.95 mm and females 0.71 mm in length (Dhanker and Hwang, 2013).

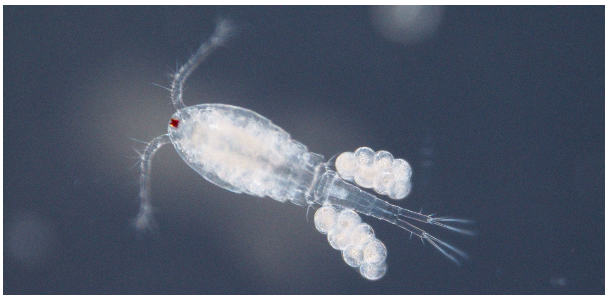

Figure 12: Cyclopoid copepod, *Apocyclops royi*. The copepod is characterized by its small anntennae, single eye and its abdomen that is separated in four segments, lacking appendages. (Cirino, 2019)
(Picture taken by Hans van Someren Gréve)

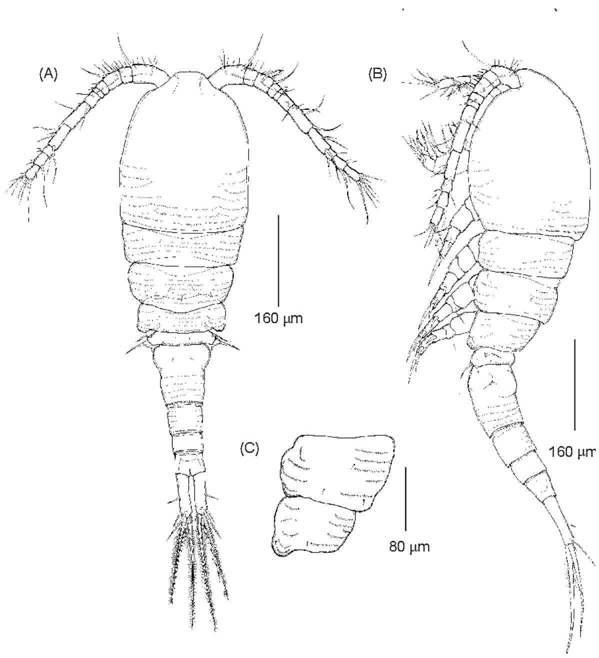

Figure 13: Body structure of *A. royi*. Drawing (A) illustrates the dorsal view of the copepod. Drawing (B) shows the left lateral side, while drawing (C) shows the part tht connects the 3rd and 4th segment. (Chullasorn et al., 2008)

Figure 14: Close picture of Cyclopoid copepod, *A. royi*, with the characteristic small anntennae and the two egg-sacs. (Nygaard et al., 2018)

This cyclopoid species is a tropical copepod that can be found in estuaries and brackish aquaculture ponds in tropical and subtropical regions like Taiwan, where it is used as live food for fish larvae (Pan et al., 2017; Blanda et al., 2017). This means that *A. royi* experiences the same extreme environmental conditions, hypoxia, poor-quality water and available food particles, as the before mentioned copepod *P. annandalei*. It survives and thrives at the same temperatures (32 – 19°C), salinity (23-15), pH (8-8.7) and oxygen (7.9 ± 2.6 to 16.3 ± 4.2 mgL^{-1}) changes in its natural environment (Blanda et al., 2015, 2017). *A. royi* is considered to be one of the copepod species that can survive and function in various salinities. Environmental biologists have conducted several experiments acclimating *A. royi* in different salinities testing mortality, fecundity, growth and egg hatching rate. Experiments showed that *A. royi* could survive and function in extreme salinities (0 and 35), although their population and nauplii production was significantly low. The optimal salinity is 20 since the recorded numbers of individual reproduction and population development were high. In middle salinities, for example 10 and 15, *A. royi* was affected resulting in lower population growth, when compared to the salinity of 20 (Pan et al., 2016). Similar results with Pan et al. (2016), about the salinity tolerance, can be seen in the experiments by Hansen et al., Unpublished.

A. royi is exceptional because it can tolerate a wide range of salinities, and also because it can produce fatty acids without needing to feed on PUFA-rich algae. In few words, this species has the capacity to produce n-3 PUFA in high quantity (Nielsen et al., 2019; Pan et al., 2017; Rayner et al., 2015). *A. royi* has been found to possess the ability to biosynthesize n-3 PUFAs, when fed different types of algae (Nielsen et al., 2019). Copepods fed with PUFA rich and PUFA poor algae showed, that even after DHA-starvation for two generations, produced a high amount of DHA. This indicates that *A. royi* has highly active n-3 PUFA biosynthesis and ability to produce DHA when fed with poor or rich PUFA diet.

4 Comparative analysis

In this section of the report, a comparative analysis will present the information gathered from different research articles on the different characteristics regarding the five copepod species (*Acartia tonsa, Acartia bifilosa, Eurytemora affinis, Pseudodiaptomus annandalei* and *Apocyclops royi*). They will be compared to two common live feed types that are used today (rotifers and *Artemia*). The purpose of this analysis is to take the reported information on the characteristics of each copepod species and analyze the potential advantages or disadvantages they may bring as live feed in freshwater larviculture practices. There are many factors that contribute to the success of an organism as a live feed type, however, we will limit this analysis and focus on only a few.

4.1 Comparison of survival conditions

In a hatchery, it is essential to have optimal conditions in order to limit the number of factors that could negatively affect the development of fish larvae. Such conditions may include maintaining the appropriate salinity, temperature, pH, oxygen levels etc. As certain freshwater larvae rely greatly on live feed for survival, these conditions must prove to be acceptable for the live feed as well. Table 1 compares a few of the different survival conditions that each type of potential life feed organisms can tolerate. Survival conditions for each of the 'Big five' are provided as well as survival conditions of two common rotifer species, used as live feed (*Brachionus plicatilis* and *Brachionus calyciflorus*) for freshwater larviculture, and *Artemia*.

Types of live feed	Salinity tolerance	Temperature range (◦C)	pH range	References
Brachionus plicatilis	1- 97	25 - 35	8	(Lubzens, 1987)
Brachionus calyciflorus	2-5	24 - 32	7	(Park et al., 2001) (Bailey et al., 2004)
Artemia	< 60 - 340	5 - 40	7 - 8	(Gajardo and Beardmore, 2012) (Sui et al., 2014)
Acartia tonsa	5 - 30	15 - 20	7 - 8	(Højgaard et al., 2018) (Mauchline et al., 1998) (Aguilera et al., 2020)
Acartia bifilosa	0 - 30	13 - 19	7.6	(Vehmaa et al., 2013) (Højgaard et al., 2018)
Eurytemora affinis	0 - 30	10 - 30	7 - 9.5	(Højgaard et al., 2018) (Hansen et al., 2017)
Pseudodiaptomus annandalei	5 - 30	18 - 32	7.9 - 8.6	(Chen et al., 2006) (Blanda et al., 2017)
Apocyclops royi	3 - 30	18 - 32	7.9 - 8.6	(Pan et al., 2016) (Blanda et al., 2017)

Table 1: Comparison of two species of rotifers, *Brachionus plicatilis*, *Brachionus calyciflorus* with *Artemia* and the 'Big Five', based on their salinity tolerance, temperature range and pH values.

As can be seen in Table 1 for each of the live feed types, the range of salinity that they can tolerate vary greatly. The rotifer *B. plicatilis* is recorded to be able to tolerate salinities from 1-97, and *B. calyciflorus* tolerates a lower range of 2-5. *Artemia* on the other hand, have been recorded to tolerate a range considered as hypersaline from < 60 to 340. Salinity 60 was recorded to be optimal for *Artemia*, however, no values lower than 60 were mentioned. For the copepod species, both *A. tonsa* and *P. annandalei* are recorded to tolerate salinity levels as low as 5. Both *A. bifilosa* and *E. affinis* seems to be able to tolerate the lowest salinity level of 0 compared to the other copepod species and common live feed types. *A. royi* was recorded to tolerate salinity as low as 3.

Temperature ranges for each type of live feed item can also be seen on Table 1. According to the data gathered, both *B. plicatilis* and *B. calyciflorus* have similar temperature ranges, they can tolerate; 25-35◦C and 24-32◦C respectively. *Artemia* show the greatest array of temperatures tolerating a range from 5◦C to 40◦C. *A. tonsa* exhibits tolerance to temperatures of 15-20◦C, and *A. bifilosa* a range of 13-19◦C. *E. affinis* can tolerate the lowest temperature out of all the mentioned copepod species of 10◦C and all the way up to 30◦C. The Taiwanese species *P. annandalei* and *A. royi* tolerate the same temperatures, 18-32◦C.

The last condition presented on Table 1 is the pH tolerance. For all types of live feed the reported ranges are very similar. *B. plicatilis* tolerates pH of 8 while *B. calyciflorus* tolerates a pH of 7. *Artemia* also can tolerate pH levels of 7-8 pH. Between the five different copepods, the lowest pH tolerance is 7, and the highest tolerance of 9.5 is seen from *E. affinis*.

4.2 Comparison of size ranges

The current live feed that is being used today in freshwater larviculture exhibits the appropriate size range for consumption by the larvae. As mentioned in Section 3.1.2, the live feed provided for fish larvae must be small enough to fit into the mouths of fish larvae. Fish larvae of different species however, range in different body sizes reflected also in mouth sizes, and may therefore require a different food size range. Moreover, during ontogeny a given fish larvae change food size successively. It is more common to utilize nauplii as a feed source, as the adults are too large for larval consumption. Adult rotifers however, can be small enough to consume. Table 2 lists common size ranges of the common live feed, as well as the size ranges of the 'Big five'.

Types of live feed	**Size range of Nauplii**	**References**
Brachionus angularis	(Lorica length) 86 - 127.8 μm	(Ogata and Kurokura, 2012) (Ogata et al., 2011)
Brachionus plicatilis	(Lorica length) 212 - 375 μm	(Ogata et al., 2011)
Artemia nauplii	< 550 μm	(Lim et al., 2003)
Acartia tonsa nauplii	70 - 193 μm	(Marcus and Wilcox, 2007) (Lonsdale et al., 1979)
Acartia bifilosa nauplii	98 - 266 μm	(Veloza et al., 2006)
Eurytemora affinis nauplii	132 - 202 μm	(Titelman and Kiørboe, 2003)
Pseudodiaptomus annandalei nauplii	107 - 114 μm	(Pan et al., 2016)
Apocyclops royi nauplii	75 - 120 μm	(Pan et al., 2017)

Table 2: Comparison of two species of rotifers, *Brachionus angularis* and *Brachionus plicatilis* with *Artemia* and the 'Big Five' based on their nauplii body size ranges. Adult size ranges are shown for the two rotifers.

Due to data availability, information on another common rotifer used as live feed for freshwater larviculture, *Brachionus angularis*, is presented in Table 2. The reported length of the lorica (greatest portion of the body structure) has been reported to range from 86 - 127.8 μm for this species. *B. plicatilis* is reported to be a bit larger with a length ranging from 212 - 375 μm. *Artemia* on the other hand, may exhibit a greater body length than the rotifers reported at <550 μm. *A. tonsa* nauplii was recorded to have a body size range of 70- 193 μm. *A. bifilosa* nauplii has a size range of 98 - 266 μm. *E. affinis* nauplii is reported to range from 132 - 202 μm. *P. annandalei* nauplii has a recorded size range of 107 - 114 μm and *A. royi* nauplii ranges from 75 - 120 μm.

4.3 Comparison of swimming behaviour

An important characteristic of the live feed used in freshwater aquaculture is the attractiveness of the swimming behaviour exhibited. Fish larvae that rely on live feed are dependent on the swimming motions exhibited by the prey items as mentioned in Section 3.1.2. Table 3 compares general descriptions of swimming behaviours for rotifers and *Artemia* and each of the five copepod species (excluding *A. bifilosa* due to data availability).

Types of live feed	**Swimming description**	**References**
Rotifers	Slow swimming velocity and ciliary movements	(Lim et al., 2003) (Clément, 1987)
Artemia nauplii	Fast swimming behaviour and "jerky swimming type	(Sorgeloos et al., 2001) (Anufriieva and Shadrin, 2014)
A. tonsa nauplii	jump/ sink	(Bruno et al., 2018)
A. bifilosa	n.d	
E. affinis nauplii	jump/sink in NI-II and smooth swimming in NIV-V	(Titelman and Kiørboe, 2003)
P. annandalei nauplii	jump/ sink and slow motion swimming	(Wu et al., 2011)
A. royi nauplii	jump/ sink and whirling (fast helicoidal swimming or swimming in circles)	(Wu et al., 2011)

Table 3: Comparison of rotifers, *Artemia* and the 'Big five' based on their swimming behaviour. This table reports the description of the common swimming bahavior/ patterns reported when observing each organism. Information on *A. bifilosa* is not reported due to no available data (indicated as n.d). NI - NII for *E. affinis* refers to the first two naupliar stages. NIV - NV refers to the third and second last naupliar stages.

The description presented in Table 3 on the general swimming behaviour of rotifers is reported as typically having slow swimming speeds or velocity due to their use of cilia for movement. Quite the contrary, *Artemia* swimming behaviour of nauplii has been described as being generally fast, with an overall jerky movement pattern. *A. tonsa, E. affinis* in naupliar stages one and two, *P. annandalei*, as well as *A. royi* all have exhibited the jump/ sink swimming behaviours in the naupliar stages. *E. affinis* in naupliar stages NIV and NV exhibits a smoother swimming pattern as it matures. Nauplii of *P. annandalei* also may exhibit slow motion swimming in some cases. *A. royi* may also exhibit a faster swimming pattern and a whirling or circling swimming behaviour.

4.4 Comparison of fatty acid profiles

The importance of fulfilling dietary requirements of live feed ensures the survival and development of fish larvae. Section 3.1.4, highlighted the importance of fatty acids in the diets of freshwater fish larvae. There are many kinds of fatty acids that are vital for larvae to consume, but for simplicity, a focus on the levels of three of the most important fatty acids found in live feed items were compared. These three fatty acids that have been mentioned earlier are Docosahexaenoic acid (DHA), Eicosapentanoic acid (EPA), and Arachidonic acid (ARA).

The results gathered on Table 4 come from a study conducted by Ako et al. (2003), where the fatty acid profiles measured in rotifers and *Artemia* samples, were compared when they have undergone enrichment procedures or not. In this table, measurements on DHA, EPA, ARA and total fatty acids present in mg/100mg of dry weight. Both unenriched rotifers and *Artemia* showed no levels reported of DHA, but showed an increase following an enrichment procedure. Levels of both EPA and ARA for both rotifers and *Artemia* were increased as well in the enriched samples. Specific rotifer species were not mentioned in the study.

Fatty acids	Rotifers		*Artemia*					
	Unenriched (mg/100mg dw)	Enriched (mg/100mg dw)	Unenriched (mg/100mg dw)	Enriched (mg/100mg dw)				
DHA	-	0.42	-	0.5	0.25	2.33	0.16	0.11
EPA	0.11	0.52	0.44	1.02	0.59	1.83	0.82	0.68
ARA	0.05	0.08	0.08	0.20	0.19	0.11	0.10	0.15
Total	6.27	6.34	5.97	11.55	12.60	14.50	11.10	8.66

Table 4: Fatty acid profile of rotifers and *Artemia*. Their DHA, EPA and ARA as well as the total fatty acid content values are shown in the table, categorized based on if the live feed received any enrichment procedure. Each column shown under enriched *Artemia* represents values from different enrichment methods. The values are measured in mg/ 100mg dw (Ako et al., 2003).

Due to the differences in reported measurements and units on fatty acids in copepods, two tables have been created, Tables 5 and 6, in order to divide the information gathered for simplicity. Each of the tables report the measurements of DHA, EPA, ARA and the total reported PUFA content, depending on the food source provided for the copepods. Information on *A. bifilosa* has not been acquired, since to the best of our knowledge these data do not exist and is therefore not included in either of these tables.

Fatty Acids	Species	Food source				
		Rhodomonas baltica	*Thalassiosira weissflogii*	*Heterocapsa triquetra*	*Isochrysis galbana*	Unspecified
DHA	*A. tonsa*	28.5	28.5	41.6	30.3	-
	E. affinis	n.d	n.d	n.d	n.d	23.94
EPA	*A. tonsa*	14.2	23.3	6.6	6.8	-
	E. affinis	n.d	n.d	n.d	n.d	16.76
ARA	*A. tonsa*	0.6	2.3	0.5	0.8	-
	E. affinis	n.d	n.d	n.d	n.d	1.43
Total PUFAs	*A. tonsa*	68	60.7	64.3	54.9	-
	E. affinis	n.d	n.d	n.d	n.d	50.36

Table 5: The values of the DHA (22:6 n-3), EPA (20:5 n-3) and ARA (20:4 n-6) as well as the total PUFA content of the two calanoid species *A. tonsa* nauplii, and *E. affinis* adults, based on different food source. The copepods fed with different algae species: *Rhodomonas baltica*, *Thalassiosira weissflogii*, *Heterocapsa triquetra*, *Isochrysis galbana* and unspecified food source and their fatty acid profile was analyzed. Values are measured in g/100g of fatty acids for *A. tonsa* and in % of μg mg $^{-1}$ of dry weight for *E. affinis*. Values for *E. affinis* are a calculated average of male and female adults. 'n.d' stand for 'no data'. Also, the species *A. bifilosa* is missing from the table because of data availability about its fatty acid profile (Støttrup et al., 1999; Cabrol et al., 2015).

Table 5, shows the reported values of fatty acids for the two species *A. tonsa* and *E. affinis*. The values present for *A. tonsa* referred to the nauplii, but values for *E. affinis* were only about the adults.

The amount of DHA reported in *A. tonsa* was highest when fed with the algae *Heterocapsa triquetra* at 41.6g/100g. The study on fatty acids for *E. affinis* did not specify the food source, but showed a presence of DHA at 23.94 % of μg mg $^{-1}$. Values were reported for both EPA and ARA in these two copepod species as well as indicating their presence.

Table 6, shows the values for the last two copepod species *P. annandalei* and *A. royi* also only found in the adult stages. DHA, EPA, and ARA are found in varying levels in both species, depending on the food source provided as can be seen in the table. One of the food sources present in the table is Baker's yeast, a typical PUFA poor source. However values were recorded for the species *A. royi* for each fatty acid and total fatty acid content when fed on this source. In addition, the values of DHA and EPA for *P. annandalei* when fed with bakers yeast are extremely low. This is because the copepods cannot survive more than a couple of days consuming only yeast cells.

Fatty Acids	**Species**	**Food source % (ind $^{-1}$)**			
		Rodomonas salina	*Dunaliella tertiolecta*	*Tetraselmis suecica*	Bakers yeast
DHA	*P. annandalei*	56.99 ± 4.27	29.82 ± 7.14	5.4	0.25 ± 0.16
	A.royi	48.8 ± 18.27, 30.48 ± 9.54	21.61 ± 2.69, 28.34 ± 9.67	29.08 ± 2.24	35.30 ± 3.97
EPA	*P. annandalei*	12.57 ± 0.31	2.86 ± 0.65	n.d	0.22 ± 0.11
	A.royi	11.17 ± 2.0, 13.41 ± 2.71	3.15 ± 0.26, 1.81 ± 0.44	7.11 ± 0.13	1.16 ± 0.34
ARA	*P. annandalei*	0.80 ± 0.06	4.07 ± 0.20	n.d	n.d
	A. royi	0.10 ± 0.14, 0.98 ± 0.22	0.56 ± 0.09, 0.92 ± 0.30	1.80 ± 0.12	0.44 ± 0.08
Total PUFAs	*P. annandalei*	85.98 ± 1.40	77.30 ± 7.94	n.d	n.d
	A. royi	81.05 ± 10.48 80.01 ± 11.47	55 ± 1.19 69.19 ± 13.64	53.37 ± 1.82	42.47 ± 3.17

Table 6: The values of the DHA (22:6 n-3), EPA (20:5 n-3) and ARA (20:4 n-6) fatty acids as well as the total PUFA content of the two tropical species *P. annandalei* and *A. royi* based on different food sources. The copepods fed with different algae species: *Rhodomonas salina, Dunaliella tertiolecta, Tetraselmis suecica* and bakers yeast and their fatty acid profile was analyzed. 'n.d' stands for 'no data' (Nielsen et al., 2019; Nygaard et al., 2018; Nielsen et al., Unpublished a.; Nielsen et al., Unpublished b.)

5 Discussion

5.1 Comparison of current live feeds with the 'Big five'

Members of the 'Big five' were compared to rotifers and *Artemia* in this report. In the following sections, the information provided in the Comparative analysis section will be further elaborated on, and interpretations on the data will be discussed.

5.1.1 Survival conditions

Amongst the different survival conditions that were compared, salinity is the most important to consider. As mentioned in Section 3.1.1, freshwater aquaculture is typically defined as having a salinity around 0.5 or less. For a euryhaline copepod species to be considered as a live feed for freshwater aquaculture, it must first be able to survive for a given amount of time, a typical feed out situation, at lower salinity levels.

In the Comparative analysis section of this report, the ranges of salinity tolerances on the 'Big five' were compared to two rotifers that are common live feed items for freshwater fish larvae. Both *Brachionus plicatilis* and *Brachionus calyciflorus* can tolerate lower salinities as low as 1- 2. Hence, one reason as to why they are considered good live feed items for freshwater aquaculture. According to the data gathered however, *Acartia bifilosa* and *Eurytemora affinis* were both shown to tolerate 0 salinity, even lower than what has been found for the two rotifer species. This is a good indication that these two copepods may also survive long enough to be utilized as live feed. *Apocyclops royi* would be the next best candidate from the 'Big five' according to salinity tolerance as it was recorded to tolerate salinity as low as 3. However, as stated before, freshwater aquaculture is typically defined as having a salinity of around 0.5, and therefore, neither *A. Royi* nor any of the other copepods compared (excl. *E. affinis* and *A. bifilosa*), may survive long enough according to the gathered data.

Artemia, on the other hand, is also commonly used as live feed in freshwater aquaculture practices and was found to tolerate a salinity of < 60 (exact value for the lower end of the range was not found. Salinity 60 is the optimal salinity). This is still far beyond freshwater aquaculture conditions. It was stated by Lim et al. (2003), that in many cases *Artemia* can survive in freshwater for up to 2 hours at a time, and is therefore intermittently supplied as a live feed to the fish larvae. Every member of the 'Big five' was able to tolerate lower salinity levels compared to that of *Artemia*, and could also therefore, potentially be utilized as an intermittent feed as well. However, in order for this to be possible, more experiments need to be conducted to find out the period each copepod species could survive for at lower salinities. If able to survive for around 1-2 hours like *Artemia* nauplii can, then perhaps copepod nauplii may then be considered as a live feed in freshwater larviculture practices. Results by Højgaard et al. (2018), actually showed promise for *A. tonsa* nauplii survival at a salinity of 0. The nauplli were first hatched at a salinity of 5, and then transferred to a salinity of 0. A high fraction of the nauplii that were transferred were mobile for up to two hours, which may be a long enough window for feeding by larvae.

Temperature and pH are also important conditions to consider in freshwater aquaculture practices following salinity. They are typically kept at optimal conditions for the freshwater fish larvae species, but the live feed must also be able to tolerate them to a certain extent. *B. plicatilis* and *B. calyciflorus* are able to tolerate temperatures of $25-35°C$ and $24-32°C$, respectively. *E. affinis, Pseudodiaptomus* *annadalei* and *A. royi* are all able to tolerate the same temperature ranges as the two rotifer species. *A. tonsa* and *Acartia bifilosa* on the other hand, have lower temperature ranges compared to the rotifers with 15 – 20°C and 13 – 19°C, respectively. *Artemia* has the widest range of temperatures it can tolerate from 5 – 40°C. Ultimately, the temperature that is set in a freshwater aquaculture practice depends on the species of fish larvae that is being produced. This will be further elaborated in the upcoming Section 5.3.2. The pH tolerance of each live feed option is generally around 7-9. Both of the rotifers, *Artemia*, and the 'Big five' do not exhibit big differences pertaining to their pH tolerance. However, this is again dependent on the type of fish species being produced.

5.1.2 Size range

The different live feed species varied in their range of sizes from each other. Rotifer species such as *Brachionus angularis* and *B. plicatilis* are suitable live feeds for many fish larvae species due to their wide size range availability. It is common for rotifers to be categorized according to their size and labeled as either SS, S, SM, M, L etc. Each of the letters represents a specific size selection (SS being the smallest, and L representing the largest) and rotifers of each species can be placed according to what size ranges they fulfill (Atsushi and Tatsuki, 2017). One species can be categorized as several different labels. For example, the data gathered on *B. plicatilis* revealed it's size range of 212 - 375 μm. This size range refers to three different size categories of *B. plicatilis*; SS, S, and L (Ogata et al., 2011). Depending on the fish larvae species, the suitable size range is then bred specifically on a larger scale. This is a great advantage that rotifers are able to be distinguished into such a large array of sizes.

From the comparative analysis section, it was shown that copepod nauplii of the 'Big five' could also occur in an array of sizes like rotifers. Our analysis revealed that the *A. tonsa* and *A. royi* species were found to be smaller than both rotifer species presented in the table, at 70 μm and 75 μm in length, respectively. These may be suitable for fish larvae species that have mouth gaps that cannot consume the size class SS of rotifers. SS indicates the smallest size class of rotifers, and for some species of fish larvae that were unable to feed on this size range, other initiatives such as body modification by chemical treatment by use of hormones on rotifers has been applied in some cases. However, it is not ideal and shows only temporary effects. Therefore, species such as *A. tonsa* and *A. royi* may present as potential live feed items where fish species have smaller mouth sizes for example. It has also been discussed that some fish species are unable to feed on rotifers as they need a live food item between two size class categories. Breeding of rotifers by selection has relieved this issue; however, utilizing the 'Big five' could be another potential solution as the naupliar lifecycle could be taken advantage of here. The naupliar life cycle of copepods consists of six stages before becoming a copepodide. As they progress from one naupliar stage to the next, they increase slightly in size. Copepods could be sieved through different mesh sizes, in order to isolate the appropriate size range. Of course, depending on the size range needed for fish larvae, the targeted size range in this case, could be isolated without the use of artificial breeding methods.

Artemia on the other hand, was found to occur in a size range up to 550 μm in length or less. Although, the lower end of the size range was not found for this study, this was the highest reported size in comparison to any of the 'Big five' and rotifers. According to these findings, any member of the 'Big five' could thus, be used in the placement of *Artemia* as none of them would be too large for the mouth size of the majority of fish larvae. However, live feed may also be considered as unsuitable for fish larvae consumption if considered too small. Therefore, some of the copepod nauplii that were considerably smaller than *Artemia* was reported to be, might not work as a substitute in these cases.

5.1.3 Swimming behaviour

Swimming behaviour descriptions were summarized in Table 3 of the Comparative analysis section. Rotifers in general, are considered slow swimmers and remain in a constant swimming pattern at all times as their movement depends on ciliary action. It is stated that this may be advantageous in larviculture practices as the behaviour is considered attractive for fish larvae due to the constant movement, and fish larvae do not need to expend an incredible amount of energy to catch their prey. The swimming patterns of both *E. affinis* and *P. annandalei* also were recorded to exhibit a constant slow swimming pattern that may, to some extent, be comparable to that of rotifers. However, copepods in the naupliar stages on the other hand, are said to be faster than rotifers, which is also attractive for fish larvae but requires more energy to catch. Another swimming pattern shown in four of the 'Big five' (data for *A. bifilosa* was not provided) was the jump/ sink swimming pattern. This type of swimming behaviour has been speculated as being both advantageous as well as a disadvantage. The long swimming "breaks" where copepod nauplii are sinking in the water column, may be seen as a negative swimming pattern, as they are less detectable in this state. However, copepod nauplii are said to have great escape responses from predators compared to rotifers, and therefore with the presence of fish larvae, this may trigger the jumping response of the copepod nauplli (Buskey et al., 1993). Further experiments where rotifers and copepod nauplii are present at equal amounts as a live feed, can hopefully lead to whether or not fish larvae have a preference.

Artemia swimming behaviours are more comparable to copepod swimming behaviour than the ciliary rotifers. They are both considered muscular, fast swimmers in general. *Artemia* are said to exhibit a jerky swimming pattern in their naupliar stages, which is said to be attractive for fish larvae. Although they are faster swimmers than rotifers, they have already proven as a suitable live feed and are used for many species of fish larvae. This may mean that the extra expenditure of energy needed to capture their prey in comparison to rotifers does not matter. Therefore, copepod nauplii supplemented as a live feed may also not affect fish larval consumption based on swimming behaviour. Højgaard et al. (2018), observed no significant difference in consumption rates of Pikeperch larvae when offered equal amounts of *Artemia* and *A. tonsa* nauplii. These results show a good indication that swimming behaviour between these two live feed types is not very important for that particular species of larvae.

One important consideration when analyzing swimming behaviour of live feeds, is to realize that the different studies that reported the swimming descriptions, were not recorded under stressful conditions. They were recorded under the optimal conditions of each live feed item. As this study aimed to utilize euryhaline copepods that can be acclimated to a freshwater environment, this may change completely how the organism's swimming behaviour is recorded under such stressful conditions. Experiments would need to be conducted in order to see the duration of how long each copepod species could survive for in a freshwater environment, as well as to see whether their swimming behaviours become less attractive for fish larvae.

5.1.4 Fatty acid profile

The presence of three essential fatty acids (Docosahexaenoic acid (DHA), Eicosapentanoic acid (EPA), and Arachidonic acid (ARA)) as well as the total amount of fatty acids present in each of the live feed items were compared. Although rotifers may be considered as a suitable live feed for various reasons, their biochemical profile does not quite contribute to this. Typically they do not contain adequate levels of PUFAs necessary for fish larvae survival, and as mentioned in Section 3.2.1, undergo nutrient emulsion procedures in order to make up for this. This is one of the main reasons of potentially acclimating euryhaline copepod species into a freshwater environment as they, by nature, have very rich biochemical profiles in comparison to the current live feed. As was seen in the Comparative analysis, rotifers lacked DHA in their composition unless supplied with supplements. In a study conducted by Wilcox et al. (2006), it was shown that the survival rates of marine fish larvae increased when rotifers were supplied together with copepods. In another study conducted by Hansen (2011), *A. tonsa* provided as a live feed for Atlantic cod larvae resulted in better survival rates and less deformities developed later than when provided with rotifers. If euryhaline copepods are able to be acclimated to a freshwater environmental setting, the enrichment procedures used for rotifers could be eliminated.

Artemia, like rotifers, also naturally do not have a rich biochemical profile and require the additional supplementation. Their DHA levels were also not present like the rotifers according to the data obtained, and may therefore be seen as inferior to copepods based on this.

The individual nutritional needs vary from each fish species, but it is said that an adequate ratio of DHA:EPA is vital for survival. Euryhaline copepod nauplii were shown to be richer in certain vital lipids in this analysis section, however there are many other lipids that need to be considered, as well as other nutrients that play important roles. Further experiments that take all of these into consideration is necessary.

5.2 Comparison of the 'Big five' to each other

In the previous section, the 'Big five' were compared to the current live feed items. In this section, the 'Big five' will be compared to each other. This is to see if any of the 'Big five' may be considered more suitable when compared with each other according to their different characteristics.

5.2.1 Survival conditions

As mentioned above, looking at survival conditions in regard to freshwater aquaculture, salinity is of great importance. One point to look at here could be that, if the copepods show a good overall adaptation to salinity this would mean that they could be administered to a wide range of fish species. When looking at the Comparative analysis, all copepod species showed tolerance to a wide range of salinity. When looking at conditions in freshwater aquaculture, as is also mentioned in Section 3.1.1, the salinity is around 0.5. Therefore from the data gathered, *A. bifilosa* and *E. affinis* show most promise because they can both tolerate a salinity of 0. (Højgaard et al., 2018; Souissi et al., 2016). Especially for *E. affinis* which shows even more promise due its migration to freshwater, as stated in Souissi et al. (2016). *A. bifilosa* on the other hand, was shown to tolerate such low salinity under experimental conditions. This may have an effect on the duration of survival. *E. affinis* would most likely survive for a longer amount of time than *A. bifilosa* in a freshwater environment.

A. royi has been reported to tolerate a salinity range of 3-30. As mentioned in Section 3.7.5, experiments have shown that *A. royi* is able to survive in extremely low salinities, such as 0, but with severe negative effects. However this salinity is not included in Table 1 due to the fact that its population growth and nauplii production were significantly low (Pan et al., 2016). Even if this is the case, one could argue that if the species is administered and the fish can get the right nutrients in the time of the feeding, then it would not pose a problem, as long as the live feed could survive long enough for the fish larvae to hunt and eat it. In regard to the last two species of the 'Big five', *A. tonsa* and *P. annandalei* were found to also tolerate a wide range of salinity concentrations, however, are not seen to tolerate salinities under 5.

Pertaining to temperature, *A. tonsa* and *A. bifilosa* inhabit environments with temperature around 20°C, while *E. affinis* is able to tolerate a bigger range of temperatures, $10-30$°C. *P. annandalei* and *A. royi* are copepods found in tropical or subtropical environments with higher temperatures. In additon, almost all of the copepods from the 'Big five' are found in environments with neutral to basic pH. (see Table 1). However, the temperatures and pH of the water depends on the fish species that is cultured, as it will also be elaborated later in Section 5.3.1.

5.2.2 Size range and Swimming behaviour

The nauplii of the 'Big five' exhibit a vast range of sizes. However, the size of the copepod nauplii that will be used in aquaculture, again depends on the mouth size of the fish species. The bigger the larvae are in size, the bigger prey they will need. When the fish eggs hatch, larvae are typically small in size and every approaching day, they grow slightly larger. Therefore, the size of feed provided should be administered according to their size, and increase in size as the larvae grow. For example, for some fish species, it is common to administer different sizes of rotifers as live feed for the first few days. Within time, the fish larvae are too large in size, and rotifers are considered too small to fulfill their size requirements. Rotifers are then commonly replaced with *Artemia*, as they are larger in size (Lim et al., 2003).

Among the five copepod species, *A. tonsa* and *A. bifilosa* exhibit wider size ranges of their naupliar stages, and could thus be utilized as live feed for various phases of larval growth. Copepod nauplii could be sieved to isolate the desired size. By having a wider size range this means that the species could also be utilized for an array of different fish species. On the other hand, *E. affinis*, *P. annandalei* and *A. royi* have narrower size ranges, and might not be able to be utilized as live feed for as many fish larvae species, or for further fish larvae phases.

Swimming behavior differs from copepod to copepod. However copepods in nauplii stage share some similarities. Data for the species *A. bifilosa* on this matter was not able to be found. As mention in Section 5.1.3 above, four out of five of the chosen copepods exhibit the jump/ sink swimming behaviour at some point in their nauplii stages. Both *E. affinis* (NIV-NV) and *P. annandalei* nauplii were described as having smooth swimming and slow motion swimming patterns, respectively. These two descriptions of swimming behaviours may be comparable swimming types, as both of these types refer to a swimming pattern of moderately swimming in a forward motion at a given pace (Titelman and Kiørboe, 2003; Wu et al., 2011). *A. royi* nauplii on the other hand, was the only one to exhibit a whirling or circling swimming behaviour. Whirling refers to a fast helicoidal swimming pattern in the water column.

Wu et al. (2011) argued that the whirling/ circling swimming behaviour of *A. royi* is a more visible and conspicuous swimming behavior in comparison to a smooth gliding swimming type, which may be comparable to those of *E. affinis* and *P. annandalei*. Fish larvae may have an easier time detecting cyclopoids like *A. royi* as this whirling behavior is commonly observed within Cyclopoida. Wu et al. (2011) also states that the jump/sink swimming type may also be comparable to the whirling/ circling swimming pattern, in the sense that they both are considered visible and attractive swimming patterns (Buskey et al., 1993). The high frequent whirling swimming pattern was specifically observed when food was present, and therefore, it is not certain that this swimming pattern would also be triggered in the presence of fish larvae. This swimming pattern is speculated to allow *A. royi* to search for food particles over a larger area. *A. royi* also exhibits the jump/ sink method which is considered attractive to fish anyways.

In Bruno et al. (2018) however, it was reported that different swimming behaviours of copepod nauplii, does not really matter when it comes to larval fish feeding. Therefore, it was suggested that when choosing a copepod species as a live feed.

5.2.3 Fatty acids

When looking at the nutritional aspect of the 'Big five' we conducted comparisons of the copepods and their fatty acid profile depending on specific food items. *A. bifilosa* was again, not included in this comparison due to data availability.

Copepods fed on different found sources may exhibit different PUFA results. When *A. tonsa* nauplii were fed on four different algae species, it showed different DHA, EPA and ARA values. However when the total amount of PUFAs was measured in the copepod for each algae species, the values were similar ranging from 54.9 to 68 g/100g of fatty acids. In a report by Støttrup et al. (1999), it was indicated that *A. tonsa* produces higher amounts of PUFAs in their early nauplii stages compared to when they are adults. This is relevant, as nauplii are the targeted live feed for fish larvae.

Results for *E.affinis* were not conclusive because the data available was limited to only unspecified food sources. Nevertheless, *E.affinis* was found to have all three fatty acids (DHA, EPA, and ARA), however it is unclear which food items may yield the best results (Cabrol et al., 2015). In addition, whether or not these fatty acids are in the nauplii stages of *E. affinis* when fed to fish larvae is not mentioned, however, according to the results of *A. tonsa*, we can perhaps speculate that nauplii of *E. affinis* may also have adequate levels of PUFAs. Further experiments can test this assumption. While the total fatty acid production in copepods depends to a big degree, on the food source provided, copepod species *P. annandalei* and *A. royi*, proved to be capable of biosynthesizing large amounts of PUFAs when fed on PUFA poor food sources (Nielsen et al., 2019; Rayner et al., 2017). This could be beneficial in larviculture practices, since these copepods could be fed on cheaper and low maintenance food sources, like bakers yeast.

5.3 Do any of the 'Big five' fit the survival conditions of different freshwater fish species?

As stated in Section 3.1.2, maintenance of larvicultures are considered to be extremely risky. Fish larvae are only a few millimeters in size after hatching, making the larvae phase one of the most vulnerable phases in a fish's life cycle. In order to reduce the risk of high mortality, hatcheries must maintain specific abiotic conditions, such as salinity and temperature, to allow the larvae to develop into healthy adults. In the above sections the 'Big five' were compared to each other based on survival conditions, swimming behaviour and PUFA content. However, the salinity or temperature tolerance of the 'Big five' is hard to apply if their ranges do not fit the survival conditions of the fish which varies depending on the species. The purpose of the following paragraphs, will be to provide information on five species of freshwater fish, in order to show that there may be differences in their required optimal rearing conditions. Further, we will make a hypothetical comparison of salinity and temperature tolerances of the 'Big five' and correspond them to the five selected fish species. This is to create a simplistic idea of where each copepod could hypothetically be implemented as a live feed.

5.3.1 Different freshwater fish species require different survival conditions

The Morone hybrid, the Siberian sturgeon, the Eurasian perch, the Pikeperch and the Northern pike are some examples of some cultured freshwater fish. Almost all of these fish species can tolerate lower salinities from 0 to 10, except the Northern pike which, is also able to tolerate higher salinities up to 25. Each type of fish can live in different ranges of temperatures, with most of them preferring cool waters, around 20°C. Morone hybrid can survive in temperature range of 12 – 24°C but the optimal temperature has being reported to be around 20°C. Siberian sturgeon has the biggest temperature range (1 – 18°C), with its optimal temperature around 13 – 15°C. Eurasian perch and Northern pike's temperature range is narrow, being around 22 – 24°C and 21 – 26°C, respectively. Pikeperch on the other hand, prefers lower temperatures 8 – 15°C, with its optimal at 10°C (Cooper, 2016; FAO, 2019). The salinity and the temperature of the water are two of the most important conditions for the survival of the larvae. However, these are not the only conditions that contribute to their survival. The survival conditions , as well as the accumulation of nutrients through the diet, have to stay stable during the larval stage in order to ensure health and avoidance of deformities. (Yúfera, 2018).

Table 7 below, shows the ranges of the most important survival conditions, salinity and temperature that the five selected fish can survive in. The table also shows the size range of the fish larvae in length right after hatching.

Fish species (scientific name)	Fish species (common name)	Salinity tolerance	Temperature tolerance ∘C	Larvae size mm
Morone chrysops* x *Morone saxatilis	**Morone hybrid**	3-10	12-24	4-7
Acipenser baerii	**Siberian Sturgeon**	0.5 - 10.5	1-28	10-12
Perca fluviatilis	**Eurasian Perch**	0- 4	22-24	5-5.4
Sander lucioperca	**Pike- Perch**	0-10	8-15	4-5.5
Esox lucius	**Northern Pike**	3-25	21-26	7.5-10

Table 7: The salinity and temperature tolerance (incl. optimal temperature) of five selected freshwater fish species that are cultured in freshwater aquaculture and depend on live feed during their larvae stage (optimal salinity is not indicated). Size ranges in mm of each fish species is also provided (Cooper, 2016; FAO, 2019).

All larvae fish are small right after they hatch. Morone hybrid, Eurasian perch and Pikeperch larvae are the smaller of the five, reaching a maximum of 5.5 - 7 mm in length. Siberian sturgeon and Northern Pike larvae a few millimeters bigger, averaging around 10 mm. The size of larvae in the larvae stage is a factor that determines the type of food required. As stated in Section 3.1.2, the live feed that will be provided to the fish has to be small enough in order to fit whole in their mouth (Conceição et al., 2010; Dhont et al., 2013). However, it is not possible to determine which nauplii of the 'Big five' has the right size to be a live feed for the selected fish species, unless if mouth sizes of the larvae are known. Mouth sizes were unable to be found for each of the five fish species for this report.

5.3.2 Corresponding the survival condition of the 'Big five' with the five fish species

Since fish larvae are dependent on live feed, and the 'Big five' showed tolerance in low salinities, Table 8 was created in order to show which of the 'Big five', hypothetically correspond to the selected fish species. The survival conditions of the 'Big five' has to "fit", or at least "semi-fit" the range of the survival conditions of the fish, in order to be provided as their food. The table is based only on the two main survival conditions, salinity and temperature. The term "fit" refers to a complete match of survival conditions of the copepods with the survival conditions of the fish species. The term "semi-fit" refers to a scenario where adjustments need to be made of either the fish or the copepod's survival conditions, in order to create a potential match. "Semi- fit" however, may not necessarily be possible in all cases.

In order to achieve the "semi-fit", and address the high mortality rate of the 'Big five' due to these conditions, selective breeding could be used for low-salinity or temperature tolerance in each of the 'Big five'. However, this process requires time and substantial effort. Alternatively, the survival of the copepods can be ameliorated by adjusting the salinity and the temperature in the water of fish larvae, to the salinity and temperature range of the copepods, if this is possible. Højgaard et al. (2018) reflected on a study conducted by Ložys (2004), where Pikeperch growth increased when salinity was raised in the larval feeding tanks, in order to "fit" the salinity tolerance of the copepod *A. tonsa*. This process is difficult but if it is possible to do, survival of the live feed for a longer amount of time could occur.

Fish species / Copepod species		**Morone hybrid**	**Siberian sturgeon**	**Eurasian perch**	**Pike-perch**	**Northern pike**
A. tonsa	**Salinity**	S	S	X	S	S
	Tem.	S	S	X	S	X
A. bifilosa	**Salinity**	P	P	P	P	P
	Tem.	S	S	X	S	X
E. affinis	**Salinity**	P	P	P	P	P
	Tem.	P	S	P	S	P
P. annandalei	**Salinity**	S	S	X	S	S
	Tem.	S	S	P	X	P
A. royi	**Salinity**	P	S	S	S	P
	Tem.	S	S	P	X	P

Table 8: A hypothetical check list of which copepods of the 'Big five' can be utilized as a live feed on the cultures of five freshwater fish species based only on salinity and temperature ranges. P= Perfect fit; S= semi-fit; X= does not fit

For example, *A. tonsa* can be used as live feed for almost all five fish types but only after certain adjustments. This means that in cultures of Morone hybrid, Siberian sturgeon and Pikeperch the salinity has to be higher than 5 and the temperature between 15 – 20°C. However, it might not be able to be used as live feed in Eurasian perch and Northern pike cultures, due to salinity and temperature restrictions. *A. bifilosa*, may be able to be used in almost all of the five fish cultures, due to its broad salinity range. However its temperature range does not fit the temperature ranges of Eurasian perch and the Northern pike making it an unsuitable candidate.

E. affinis survival conditions fit perfectly in the living conditions of Morone hybrid, Eurasian perch and Northern pike. In the case of Siberian sturgeon and Pikeperch the salinity ranges also fit perfectly but both fish species have a relatively big range of temperature tolerance (1 – 28°C and 8 – 15°C) and *E. affinis* can tolerate temperatures only from 10 – 30°C. Therefore, the temperature in the fish culture has to be anywhere between the range of 10 – 28°C and 10 – 28°C, respectively.

P. annandalei is a good candidate as live feed for Morone hybrid, Siberian perch and Northern pike cultures. Its salinity and temperature ranges do not fit perfectly in the range of the fish, but those conditions could be adjusted to the survival range of the copepod. On the other hand, in the case of Eurasian perch and Pikeperch, *P. annandalei* may not be a good choice, due to salinity and temperature restrictions, respectively. Finally, *A. royi* can be utilized in almost all the five fish types, since its salinity and temperature range fit those of the fish. Pikeperch is the only fish that may not be able to get *A. royi* as live feed, due to temperature restrictions. This is because, Pikeperch lives in relatively cold waters (8 – 15°C) and *A. royi* is a tropical copepod living in warmer waters (18 – 32°C).

5.4 Further considerations for the live feed

The comparative analysis of this report, only considered a few factors of live feed that contribute to the survival of freshwater fish larvae. In the following sections, other considerations that are important to assess will be discussed briefly in order to recognize some of these other factors.

5.4.1 Culturing of the live feed

It is common for live feed to be kept in culture batches in large quantities before being supplied as feed for fish larvae. Rotifers specifically, are competent in this area as they are able to be cultured in high densities and have high reproductive rates. Densities reported of 10,000-30,000 ind. ml^{-1} are common in rotifer cultures. A group in Japan cultured rotifers at even higher densities of 160,000 ind. ml^{-1}. This is able to be done when proper oxygen levels are kept, by removing toxic waste products often, and through overall maintenance of the cultures (Yoshimatsu and Hossain, 2014).

Copepod cultures on the other hand, is said to need further improvements in order to be comparable to culturing of current live feeds, like rotifers (Drillet et al., 2011). Unlike rotifers, high densities of some copepod species are not always possible. Harpacticoids and Cyclopoids have already been established in high density culturing systems; densities around a few thousand ind. ml^{-1} or more are common. However, Calanoid copepods are not able to be cultured at such high densities, as they are said to be more sensitive to this factor. For example, *A. tonsa* has shown a maximun culturing denisity of approximately 4000 ind L^{-1}. *P. annandalei* has not been able to exceed the density of 385 $indL^{-1}$, even less of that then reported for *A. tonsa* (Drillet et al., 2011; Blanda et al., 2017).

Artemia are typical live feeds used in aquaculture, and are utilized mainly due to the convenience of being able to store their cysts. Commonly, they are cultured in natural sources, such as the Great Salt Lake in the United States, and their cysts are distributed to many places all over the world. They can be stored for long periods of time, and hatched whenever needed. Although proven to be useful in the aquaculture sector, it has been stated that *Artemia* cysts production can fluctuate due to shortages of natural supply, which can have a negative effect on aquaculture production. Shortage of cysts could be due to production at unsustainable levels or even due to environmental changes.

Copepods from environments with fluctuating conditions, have also been found to produce resting eggs, similar to *Artemia* cycts. Holm et al. (2017) presented a list of 42 copepod species that have shown to be able to produce these resting eggs. *A. tonsa*, *A. bifilosa* and *E. affinis* were amongst these 42 copepod species presented. This could be a potential solution for copepods as live feed instead of culturing methods.

Culturing and the production of eggs, are also important factors to consider for the success of euryhaline copepods being utilized as a live feed in freshwater larviculture practices.

5.4.2 Potential disturbance of surrounding ecosystems

The idea of using euryhaline copepods in freshwater aquaculture shows promise. If euryhaline copepods are introduced as live feed in freshwater aquaculture, on top of the natural challenges that exist, an additional concern must be addressed. Naturally the 'Big five' can be found in estuaries in the Northern sea and ponds in Taiwan (Blanda et al., 2015; Katajisto, 2003; Mauchline et al., 1998). However, aquaculture exists all around the world, meaning that copepods need to be transported and used in places outside their natural environments. This brings up the question: What if any copepod escapes the aquaculture facilities? Would it disturb the surrounding ecosystem?

As mention in Section 3.1.1, there are three methods of culturing. The intensive, which involves ponds, cages and concrete tanks under controlled conditions. The extensive and semi-intensive is where a large body of water is enclosed, and the conditions are semi-controlled (Jørgensen et al., 2005). When the last two methods are used in larviculture, it is extremely risky due to lack of biosecurity. It is possible for the copepods to escape the facility, contaminate the surrounding environment and become an invasive species, if they are able to increase in number by reproducing. As an example, when some copepods reach the adult stage, they are able to prey upon other smaller zooplankton species which leads to a disturbance of the surrounding ecosystem. On the other hand, the first method is safer and ensures that the copepods cannot escape. As a conclusion, the 'Big five' can be used as live feed in aquaculture intensive practices without consequences to any natural environment.

6 Conclusion

Freshwater aquaculture is an increasing sector worldwide. One of the most risky steps in this production is the larval rearing stage, where maintenance and care of fish larvae is vital in order to ensure their growth and survival. Various species of fish larvae rely greatly on live feed at this stage. Rotifers and *Artemia* are two common live feeds administered to fish larvae as initial diets. However, they are not considered optimal due to their relatively poor biochemical profiles. Specifically, they lack the appropriate amounts of essential fatty acids, vital for the growth of fish larvae. Thus, they must undergo enrichment procedures through oil emulsions. This report aimed to look into the alternative idea of acclimating euryhaline copepods into freshwater environments in order to potentially be utilized as a live feed in freshwater aquaculture.

Euryhaline copepods are suspected of having richer biochemical profiles, specifically in regards to their polyunsaturated fatty acids (PUFAs), than the current live feeds, rotifers and *Artemia*. Therefore, they may be considered as a more optimal food choice for fish larvae. Five copepods were chosen to be compared to current live feed as they are suspected to exhibit high adaptability to changes in their environment i.e low salinity. The five copepods include the calanoid species *Acartia tonsa Acartia bifilosa*, *Eurytemora affinis*, *Pseudodiaptomus annandalei* and the cyclopoid *Apocyclops royi*, referred to as the 'Big five'. The comparative analysis that was conducted analyzed a few characteristics of each of the 'Big five' and compared it to the characteristics of the rotifers and *Artemia* based on published literature. These characteristics included different survival conditions, such as the salinity, temperature, and pH tolerances of each species. The common size ranges, swimming behaviors, and content of selected essential fatty acids (Docosahexaenoic acid (DHA), Eicosapentanoic acid (EPA), and Arachidonic acid (ARA)) of each species were also compared.

The aim of the comparative analysis was to create a simplistic overview of the characteristics of each copepod, and how they may be comparable to rotifers and *Artemia*. This is a good first step to see whether or not euryhaline copepods may be suitable live feeds. Our analysis revealed many similarities and differences with the current live feed. This makes it seem possible for some members of the 'Big five' to be utilized as live feed in freshwater larviculture practices. It was seen that all members of the 'Big five' exhibited the presence of the three essential fatty acids (DHA, EPA, ARA), whereas the common live feed items lacked DHA. Based on these findings, the potential implementation of euryhaline copepods as live feed for larviculture practices could lead to the assumption that, elimination or reduction of enrichment through oil emulsions of live feed may be possible. However, these results are only meant to shed light on the idea and can thus, only conclude the need for further research and experiments to be conducted in the area.

To answer our hypothesis directly, several of the selected euryhaline copepod species could potentially be utilized as a live feed for freshwater fish larvae, as long as they are able to survive for a long enough time to show attractiveness to the larvae. Implementation of a euryhaline copepod could provide nutritional advantages by possibly leading to elimination or reduction of oil emulsion procedures, as they show reported levels of specific essential fatty acids. All of our findings are however, very dependent on the specific species of fish. Conditions will vary in the production of a specific species of fish larvae, that may have an effect on euryhaline copepod survivability.

This report highlighted a few factors that are essential to consider when analyzing the idea of possibly implementing euryhaline copepods as live feed. However, there are several other factors to consider such as cultivation of the live feed, other essential nutrients needed for fish growth, as well as the potential disturbance of a nonindigenous species into surrounding habitats when live feed is utilized where it doesn't originate from.

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