The effect of dietary microalgae on the fatty acid profile, fecundity and population growth of the calanoid copepod *Pseudodiaptomus hessei* (Mrázek 1884) (Copepoda: Calanoida)

by

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General Abstract

This study compares the efficiency of different dietary microalgae on the fatty acid profile, especially the essential fatty acids Docosahexaenoic acid (DHA) and Eicosapentaenoic acid (EPA) of the calanoid copepod *Pseudodiaptomus hessei* (Mrázek 1884), a potential live food for finfish larvae in aquaculture. The effect of different dietary microalgae on fecundity and population growth was also investigated. Two mono-algal diets, the Tahitian strain of *Isochrysis galbana* (Parke) and *Rhodomonas salina* (Wislouch) and a 50:50 binary diet of the two were fed to copepods. Wild caught copepods were used as a baseline reference point. Copepods fed *I. galbana* had the highest DHA: EPA ratio and DHA content; although it was not significantly different from those fed the 50:50 binary diet, it significantly differed from those fed *R. salina*. The EPA content was similar for all three diets. Copepods were collected and preserved in 10 % buffered. The membrane sac was dissolved in a 5 % solution of sodium hypochlorite and gently agitated to dissolve the egg sac. Copepods fed *R. salina* produced the highest number of eggs per female (34.60 ± 5.97 eggs/female (mean ± standard error)), and were significantly different from those fed *I. galbana* (22.8 ± 5.44 eggs/female) and the 50:50 binary diet (23.30 ± 6.77 eggs/female). Copepods were counted under a microscope and each stage of development was identified. The highest population was obtained when the copepods were fed *I. galbana* (709 ± 92.23 individuals/treatment), and was significantly different from *R. salina* (433 ± 78.08 individuals/treatment) and the 50:50 binary diet (437 ± 40.02 individuals/treatment) populations. The results of this study show that the fatty acid composition of *P. hessei* can be altered by feeding a variety of dietary microalgae and that the copepod can accumulate fatty acids from their diet, especially DHA and EPA. It is also evident that diet has an effect on fecundity and population development. This makes *P. hessei* an ideal live food candidate for marine finfish larvae as its nutritional composition and productivity can be manipulated to suit the needs of marine finfish larvae. Based on this study, it is suggested that a 75: 25, *I. galbana* to *R. salina* treatment be tested in order to improve both fecundity and population gowth.

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Declaration

The following dissertation has not been submitted to any university other than the University of Fort Hare, Alice, South Africa. The work presented here is that of Oyama Siqwepu, student number 200806283. Aspects of this dissertation have been presented at the Aquaculture Association of Southern Africa (AASA) in 2013 and 2015 and at the Southern African Marine Science Symposium (SAMSS) in 2014.

Signature: Date: January 2016

List of Abbreviations

Abbreviations	Full text
ANOSIM	Analyses of similarities
ARA	Arachidonic Acid (20:4ω-6)
DHA	Docosahexaenoic Acid (22:6ω-3)
EPA	Eicosapentaenoic Acid (20:5ω-3)
EFA	Essential Fatty Acid
FAME	Fatty Acid Methyl Ester
FATM	Fatty Acid Trophic Marker
HUFA	Highly Unsaturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
NMDS	Non-metric Multidimensional Scaling
% TFA	Percentage Total Fatty Acid
PUFA	Polyunsaturated Fatty Acid
PCA	Principal Component Analysis
SFA	Saturated Fatty Acid
SIMPER	Similarity percentage

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Chapter 1: General Introduction and Literature Review

Aquaculture is rapidly developing and growing in many parts of the world (Figueiredo *et al.* 2009). It plays a significant role in poverty alleviation, with fish being one of the most merchandised food commodities (FAO 2014). World per capita fish consumption has increased from 15.4 kg in the year 2010 to 19.2 kg in 2012 (FAO 2014). According to Stickney (2005), at least 20 % of the fish and shellfish in the world are produced by aquaculturists and there is an increasing demand for aquaculture products and an expected increase in the percentage of production globally. The annual production of aquaculture products have also continued to increase in South Africa over time (DAFF 2012). This increase is due to production by fish farms operational in different parts of South Africa. The Western Cape has 20 operating farms, Eastern Cape has six, North West has three and KwaZulu Natal has 1 (DAFF 2012). Different species of fish, molluscs, crustaceans, and aquatic macrophytes and algae have been successfully cultured. Examples of these species cultured in 2011 are abalone (*Haliotis midae* Linnaeus 1758), oyster (*Crassostrea gigas* Thunberg 1793), mussels (*Mytilus galloprovincialis* Lamarck 1819 and *Choromytilus meridionalis* Krauss 1848), finfish (*Argyrosomus japonicus* Temminck & Schlegel 1844 and *Seriola lalandi* Valenciennes in Cuvier & Valenciennes 1833) and algae *Ulva* sp. and *Gracilaria* sp. (DAFF 2012). The increasing production of fish, shellfish and other products for commercial use in aquaculture hatcheries requires certain culture techniques and production of live food organisms for feeding the developing larvae (Lavens and Sorgeloos 1996).

The marine finfish of interest in aquaculture such as snapper (*Pagrus aurutus* Forster 1801) and grouper (*Epinephelus* sp.*)* produce small pelagic eggs whose larvae need to be fed on live organisms. The current live food; rotifers (*Branchionus plicatilis* Müller 1786) and brine shrimp (*Artemia* sp.) have low survival rates in some species (Rippingale and Payne 2001). Examples of species that produced poor growth are *Hippoglossus hippoglossus* (Linneaeus 1758) when fed enriched *Artemia,* compared to those fed wild copepods (McEvoy *et al.* 1998), and halibut larvae that showed better survival and pigmentation when fed wild copepods (Shield *et al.* 1999). Larval Mahimahi fed copepods also resulted in better survival than larvae fed *Artemia* (Kraul *et al*. 1993). The first feeding larval stages are crucial for the survival and growth of the fish and may in turn affect the cost of production in commercial hatcheries (Rippingale and Payne 2001, Fleeger 2005). Lack of suitable nutritional live food or the inability to culture balanced nutritional live food prey for first feeding larvae of marine finfish is a limiting factor in aquaculture (Rippingle and Payne 2001, Fleeger 2005, Marcus 2005).

Wild zooplankton which is the natural source of food for larvae is unreliable due to its seasonal availability and may introduce pathogens and diseases to larvae therefore it is rarely used in rearing of fish larvae (Lavens and Sorgeloos 1996, Figueiredo *et al.* 2009). There is a clear need to find and culture suitable live prey which will allow growth, reproduction and survival of the larvae (Rippingale and Payne 2001).

There are certain nutritional properties that zooplankton must have in order to be optimal prey for fish larvae. Algal diets of zooplankton are a source of these nutritional properties: amino acids, vitamins and fatty acids. Fatty acids are one of the essential nutrients, especially of polyunsaturated fatty acids namely docosahexaenoic acid, DHA (22:6ω-3), eicosapentaenoic acid, EPA (20:5ω-3) and arachidonic acid, ARA (20:4ω-6) which are important to the growth of marine fish larvae (Watanabe 1982, Watanabe 1993, Brown *et al.* 1997).

1.1 Importance of ω-3 Highly Unsaturated Fatty Acids (HUFA) in the diet of marine finfish larvae

Lipids are high energy nutrients; fatty acids and triaclyglycerols are known as simple lipids. Fatty acids can be categorized into saturated fatty acids (SFA, no double bonds), polyunsaturated fatty acids (PUFAs >2 double bonds) or highly unsaturated fatty acids (HUFAs >4 double bonds) (Craig and Helfrich 2002). Polyunsaturated fatty acids are produced by desaturation and elongation of oleate (18:1ω9), linoleate (18:2ω6) and linolenate (18:3ω3) (Bell *et al*. 1986). However, marine finfish species are unable to undergo the elongation and desaturation reactions because they lack the necessary enzymes (Watanabe 1982). The highly unsaturated fatty acids, DHA, EPA and ARA, are considered essential fatty acids (EFAs) because marine finfish cannot synthesize them *de novo* (Watanabe 1982, 1993). They are required by marine finfish larvae for many physiological functions and as cellular membrane components, their presence is required for growth, survival and development (Watanabe 1982, 1993). Deficiencies in ω-3 highly unsaturated fatty acids lead to retarded fish growth, high mortality rates and a poor ability to handle stress (Izquierdo 1996). The requirements of these HUFAs differ from species to species (Watanabe 1993). Some finfish species require high concentrations while others require lower ones, varying from 0.5 to 2.0 % (Watanabe 1993). Watanabe (1993) discussed the HUFA requirements of a number of species; the larval red seabream requirement is 0.5 %, while 2.0 % is required for juvenile yellowtail and flounder, 0.8 % for turbot, 1.8 % for striped jack and 1.0 % for seabass and gilthead seabream. The ratio of DHA to EPA significantly affects the survival of marine finfish larvae (Nanton and Castell 1998). Increasing the DHA to EPA ratio in the diet of larvae increases the growth and survival

of larvae (Nanton and Castell 1998). The DHA:EPA ratio of 2.0 in the yolk of wild marine finfish eggs was quoted by Ohs *et al.* (2013) translating to at least 2:1 required DHA:EPA ratio. Bell (1985) found that the survival of juvenile turbot significantly increased when the DHA: EPA ratio was increased from 0.1 to 0.5 in the diet.

The ω-3 series of the polyunsaturated fatty acids is considered essential for marine finfish while the ω-6 series requirements were previous unclear (Bell *et al*. 1986). Castell *et al.* (1994) found that the ω-6 HUFA 20:4ω6 is involved in the production of eicosanoids that play a role in the functioning of gills, kidneys and intestines of marine fish. Another ω-6 HUFA, 18:2ω6 was found to have a growth promoting function (Castell *et al*. 1994). ARA also improves survival of larval fish after handling (Koven *et al.* 2001). DHA and EPA are found in high concentrations in the eggs of marine finfish and are depleted quickly during development (Watanabe 1993, Izquierdo 1996). DHA has been found to be the most important essential fatty acid for marine finfish larvae and juveniles (Watanabe 1993). Because of their importance in marine fish, DHA and EPA are preserved during starvation at the expense of other fatty acids (Izquierdo 1996).

Docosahexaenoic acid is an essential fatty acid found in carnivorous species of fish. It has a unique role in vertebrate rod function (Bell *et al.* 1995). It is found in high concentrations in neutral tissues, in the retina, brain and testes of marine fish (Bell *et al.* 1995, Harel *et al.* 2002). Dietary DHA is reputed to be superior to EPA as an essential fatty acid for marine finfish larvae (Watanabe 1993). It also gives higher growth rates to marine finfish larvae and has different physiological functions to EPA (Watanabe 1993, Curé *et al.* 1996). Total length growth in marine fish fed DHA was found to be higher than in fish fed EPA (Watanabe 1993).

Curé *et al.* (1996) found that in marine fish larvae, DHA deficiencies affect visual development and lead to lower survival and growth potential. Both the total ω-3 highly unsaturated fatty acids and high DHA content in the diet of marine finfish larvae in some species (for example, gilthead sea bream) resulted in better growth (Mourente *et al.* 1993).

Eicosapentaenoic acid is a precursor for eicosanoid generation, a selection of hormones that are responsible for certain physiological functions (Sargent *et al*. 1999). It is the dominant prostanoid in fish (Bransden *et al.* 2004). Eicosanoid is a term referring to prostaglandins, thromboxane and leukotrienes (Koven *et al*. 2001). They are responsible for certain immune and neural functions, fluid and electrolyte fluxes, cardio vascular and reproductive systems,

osmoregulation and controlling stress responses (Sargent *et al*. 1999, Koven *et al.* 2001). There is a positive correlation in the dietary content of EPA and pigmentation of fish (Villalta *et al.* 2008).

Arachidonic acid (ARA) is found in smaller amounts in the tissues of some fish compared to the presence of DHA and EPA, suggesting it may not be in demand as much as DHA and EPA (Castell *et al.* 1994, Nanton and Castell 1998, Koven *et al.* 2001). In the European sea bass, dietary ARA was found to increase growth even when there were high amounts of dietary DHA and EPA (Atalah *et al*. 2011). It was also found to decrease stress due to handling in marine fish (Koven *et al*. 2001, Harel *et al.* 2002, Atalah *et al.* 2011). Larvae fed higher amounts of dietary ARA exhibited larger body weights compared to those fed lower amounts of dietary ARA (Atalah *et al*. 2011). However, in the common sole (*Solea solea* Linnaeus 1758), there was no significant relationship between growth, survival, and abnormal eye migration and dietary ARA (Lund *et al.* 2007). But it was significantly related to pigmentation (Lund *et al.* 2007). This further indicates how different species of fish have different requirements of EFAs (Watanabe 1982, Watanabe 1993).

Authors such as: Koven *et al*. (2001), Lund *et al.* (2007), Atalah *et al.* (2011), have investigated the effects of EFAs on marine fish. However, because different marine fish species have different requirements for EFAs (Watanabe 1982, Watanabe 1993) studies to investigate EFA requirements for marine fish are still necessary, especially for species of interest in aquaculture.

1.2 Use of microalgae in aquaculture

In the mariculture food web, phytoplankton is at the base of the food web and in addition to other uses, it is fed to cultured zooplankton, which is then fed to larvae during the rearing process (Lavens and Sorgeloos 1996, Brown *et al*. 1997, Ianora 2005). Fatty acids from microalgae may be transferred from the microalgae to higher trophic levels such as fish larvae using zooplankton such as copepods (Watanabe *et al.* 1983).

Microalgae used in the production of zooplankton in commercial aquaculture are chosen based on mass-culture potential, cell size, digestibility, and overall nutritional value provided to the predator (Lavens and Sorgeloos 1996). Algal species are not equal in nutritional value and thus are not equally successful in supporting the growth, reproduction and survival of zooplankton, some may give high yields of the zooplankton while others give low yields (Lavens and Sorgeloos 1996, Puello-Cruz *et al.* 2009, Ohs *et al.* 2010a, Camus and Zeng 2012). Each species of microalgae has different conditions which give it its optimal growth, for example,

nutrient quantity and quality, light, pH, turbulence, salinity and temperature (Lavens and Sorgeloos 1996). The species of algae selected should match the salinity and temperature tolerance of the species being cultured (Ohs *et al.* 2010a).

A species of microalgae may be superior to another in terms of nutritional value or one may lack a nutrient that another has; in this case a combination of two or three different species is used to provide the adequate nutrients to the cultured species of zooplankton (Lavens and Sorgeloos 1996, Ohs *et al.* 2010a, Puello-Cruz *et al.* 2009, Camus and Zeng 2012). Many researchers have used mixtures of different microalgae to find optimum culture conditions for copepod species of interest (Knuckey *et al.*2005, Puello-Cruz *et al.* 2009, Ohs *et al.* 2010a, Camus and Zeng 2012). Optimum culture conditions are species specific; one species of zooplankton may thrive when fed a specific mono-algal diet, whereas another may require a binary or tri-algal diet (Lavens and Sorgeloos 1996, Puello-Cruz *et al.* 2009, Ohs *et al.* 2010a, Camus and Zeng 2012). For example, *Pseudodiaptomus pelagicus* (Herrick 1884) fed on a mono-algal diet of Tahitian *Isochrysis galbana* (Parke) or a 50:50 or 25:75 binary diet of T-Iso/*Thalassiosira wessifloggi* (Stachura-Suchoples & Williams) resulted in good growth (Ohs *et al.* 2010a); on the other hand T-Iso was not found to be a good mono-algal diet *Acartia sinjiensis* (Mori 1949) (Knuckey *et al.* 2005). High yields of *Pseudodiaptomus euryhalinus* (Johnson 1939) were obtained when fed a mono-alagal diet of *Chaetoceros muelleri* as opposed to binary and tri-algal diets (Puello-Cruz *et al*. 2009). Camus and Zeng (2012) found that a binary diet of *Chaetoceros muelleri* (Lemmerm) + *Tetraselmis chuii* (Butcher) and resulted in high numbers of the copepod *Euterpina acutifrons* (Dana 1848). Thus each species cultured has its specific dietary requirements which need to be met by the appropriate cultured algae (Brown *et al*. 1997, Puello-Cruz *et al.* 2009).

Some classes of algae have higher values of essential fatty acids than others; diatoms such as *Chaetoceros muelleri* (Lemmerm), and cryptophytes such as *Rhodomonas salina* (Wislouch) have relatively high values of EPA whereas some prymnesiophytes like *Isochrysis galbana* (Parke) and *Pavlova lutheri* (Green) have relatively high values of DHA (Brown *et al.* 1997).

It is necessary to investigate the effect of different combinations of microalgae on different aspects of copepod culture. It is also necessary to evaluate new species of microalgae for different species of copepods, as there are species specific differences between copepods, these have not been done for many species of copepods and microalgae.

The microalgae used in this study also vary in their fatty acids, cell sizes and carbon content.

Table 1.1: Microalgae: *Isochrysis galbana* and *Rhodomonas salina* used in the experiment.

Microalgae	Cell size (µm)	Cell weight (pg)	Carbon content (pg C/cell)	DHA (%TFA)	EPA (%TFA)
Isochrysis galbana	6 – 4	41 – 83	7	5 – 20	≤ 0.2
Rhodomonas salina	5 – 12	100	40.7	5 – 20	5 – 20

1.3 Use of rotifers and *Artemia* as live feed

In aquaculture hatcheries, larvae are mostly fed live rotifers (*Brachionus* sp.) followed by brine shrimp, *Artemia* spp nauplii (Rippingale and Payne 2001, McKinnon *et al.* 2003, Fleeger 2005). Rotifers (*Brachionus* sp.) are a preferred first feed due to their small size, planktonic nature, ability to be mass cultured with ease (high growth rate and reproductive success) and their slow swimming speed (Lavens and Sorgeloos 1996, Kostopoulou *et al.* 2012). The nauplii of *Artemia* are used due to their ability to form cysts which are available year round. They are not labour intensive to culture, however their nutritional profile must often be improved by placing in an enriched solution with the nutrients that they lack (Lavens and Sorgeloos 1996, Figueiredo *et al.* 2009).

There have been numerous studies conducted to optimise the nutritional value of rotifers and brine shrimp in order to improve the nutritional profile, growth and survival of fish larvae which prey on them (Alver *et al.* 2007, Figueiredo *et al.* 2009, Kostopoulou *et al.* 2012, Woolley *et al.* 2012). Enrichments to improve the nutritional profile of *Artemia* such as the addition of ARA, EPA, and DHA are important in larval growth because they cannot be synthesized by the larvae itself (Lavens and Sorgeloos 1996, Figueiredo *et al.* 2009). However, rotifers and *Artemia* may not be suitable feeds for the larvae of some species; they may be too large and lack the required nutritional value to support the growth and survival of the larvae even when enriched (Lavens and Sorgeloos 1996, Rippingale and Payne 2001, Marcus 2005). *Artemia* catabolise essential fatty acids such as DHA very quickly resulting in a low DHA: EPA ratio (Sorgeloos *et al*. 2001, Ritar *et al*. 2004). Marine fish larvae with a small mouth gape are unable to consume *Artemia* because of their size. Enriched *Artemia* are larger in size mainly because they can only be enriched at instar stage 2, because their alimentary tract opens at stage N2 (Sorgeloos *et al.* 2001). Figueirdo *et al*. (2009) found that mortality of *Artemia* nauplii

increased with enrichment time, in addition, their size increased during the enrichment process, another disadvantage to using *Atermia* as first food.

1.4 Use of copepods as live feed

Copepod nauplii are the natural food source of fish larvae in the wild and constitute a major part of their diet (McKinnon *et al*. 2003, Chen *et al*. 2006, Castonguay 2008). Thus cultivation of copepods as alternative or supplement live feed has received attention from a number of researchers (see Lavens and Sorgeloos 1996, Rippingale and Payne 2001, Evjemo *et al.* 2003, McKinnon *et al.* 2003, Lee and O'Bryen. 2005, Chen *et al*. 2006, Castonguay *et al*. 2008, Camus and Zeng 2009, Ohs *et al*. 2010a, Alajmi and Zeng 2013).

The small body size of copepods and variable life stages (from nuaplii to copepodites) make them important prey for fish larvae and other stages of fish development. The mouth size or gape of the larvae limit what it can feed on, making copepods a better alternative to rotifers and brine shrimp which may be too large to capture. (Lavens and Sorgeloos 1996, Chesney 2005, Fleeger 2005, Marcus 2005). Also, the desired copepod life-cycle stages can be manipulated to meet the demands of the cultured larvae (Kleppel *et al.* 2005). They can be manipulated by prolonging the desired life stage of the copepod or by feeding certain species of microalgae that will produce the desired life stage (Marcus 2005, Puello-Cruz *et al.* 2009). Frozen *T. suecica* fed to *P. euryhalinus* produced mostly nauplii and copepodites and low adult numbers (Puello-Cruz *et al.* 2009). By producing more nauplii, they can be fed to first feeding larvae thereby meeting the nutritional and size requirements that are needed.

In terms of meeting the nutritional requirements of fish larvae, copepods have a more balanced nutritional profile compared to rotifers and *Artemia* and generally have a higher protein content of 44 – 52 % (Lavens and Sorgeloos 1996, McKinnon *et al.* 2003, Fleeger 2005). Their fatty acid profiles vary among species and also depend on their diet during culture; some species such as harpacticoids are able to synthesize longer-chain highly unsaturated fatty acids (HUFA) which are essential to developing fish larvae (Lavens and Sorgeloos 1996, Nanton and Castell 1998, McKinnon *et al.* 2003, Fleeger 2005, Ho 2005, Rhodes and Boyd 2005). Their inclusion has been shown to increase larval growth rate when provided to larval fish (Fleeger 2005,). For example, when *Pseudodiaptomus peligus* nauplii were fed to *Trachinotus carolinus* (Linnaeus 1766) larvae, it resulted in better survival (Cassiano *et al.* 2011), similar to results found when the copepod *Centropages typicus*(Krøyer 1849) was fed to *Amphiprion clarkia* (Bennett 1830), resulting in better survival and growth for the fish larvae (Olivotto *et al.* 2009).

Some species of copepods have an ability to suppress their development at different stages. The dormant eggs can be stored for later use and still retain high nutrient content, depending on the number of days and conditions of storage. This trait is convenient for aquaculturists in the preparation and of feeding to fish larvae (Lavens and Sorgeloos 1996, Marcus 2005). The swimming behaviour of copepods also makes them superior to rotifers and brine shrimp. Since they are much smaller than the larvae, their swimming speed also tends to be slower, especially in the nauplii stages, making it easier for them to be captured by the larvae (Fleeger 2005).

The difficulties with copepods are that some species are difficult to culture in high densities, whereas rotifers and *Artemia* can be cultured at significantly high densities of 2000 individuals/ml and 50 nauplii/ml respectively (Stickeny 2005, Figueiredo *et al.* 2009). Some species of copepods such as *Acartia tonsa* (Dana 1849) have been cultured at densities as low as 600 individuals/L, 500 individuals/L and 1000 individuals/L respectively while maintaining good egg hatching success and good survival rates (Medina and Barata 2004, Jepsen *et al.* 2007). *A. sinjiensis* showed egg hatching success and survival at densities of 2000 individuals/L and *Parvocalanus crassirostris* (Dahl 1894) could be stocked at densities of 5000 individuals/L (Camus and Zeng 2009, Alajmi and Zeng 2014).

1.5 Effect of dietary microalgae on the fecundity and population growth of cultured copepods

Selecting the appropriate microalgal diet to support copepod production is one of the most crucial steps when culturing copepods (Kleppel *et al.* 2005, Puello-Cruz *et al*. 2009, Camus *et al*. 2009). Different species and densities of microalgae have a profound effect on the growth and productivity of copepods due to their nutritional qualities (Tang and Taal 2005, Ohs *et al*. 2010a). Productivity of copepods either decreases or increases depending on the density and species of microalgae fed (Ohs *et al*. 2010). Microalgal diets for certain species of copepods are species specific, where one copepod species may thrive when fed a certain type of microalgae while another may be unsuccessful (Camus *et al.* 2009, Puello-Cruz *et al.* 2009). For example, *P. euryhalinus* was cultured successfully with *C. muelleri* as a mono-algal diet, while the copepod *Bestiolina similis* (Sewell 1949) (showed poor growth when cultured with *C. muelleri*. Successful culture of *B. similis* was with a tri-algal diet not including *C. muelleri* (Camus *et al*. 2009, Puello-Cruz *et al*. 2009).

There are many factors that contribute to defining food quality. These include cell size, cell morphology, and the mineral composition or biochemical components such as amino acids,

polyunsaturated fatty acids and vitamins (Shin *et al*. 2003). In copepods, egg production is linked to the quality of the diet and it is influenced by how much the nutritional needs of the copepod are met, in particular the essential fatty acids DHA and EPA (Kleppel and Burkart 1995, Ianora 2005, Milione and Zeng 2007). Population increase depends on egg production and egg hatching, both of which are affected by the copepod diet (Tang and Taal 2005). Maternal nutrition has a profound influence on the fecundity of copepods and on the reproduction in the next generation (Ohs *et al.* 2010a).

A diverse diet offers the possibility of a balanced ratio of nutrients and a better balanced diet (Kleppel and Burkart 1995, Camus *et al*. 2009). Diversity in the diet increases egg production as seen in *A. sinjiensis* fed *T. chuii* and T-*I. galbana* (Kleppel and Burkart 1995, Milione and Zeng 2007). Similarly, Ohs *et al.* 2010a, observed better growth and productivity in *P. pelagicus* when using mixed algal diets compared to mono-algal diets.

Productivity of copepods is not only dependent on egg production but also on egg hatching and successful recruitment of nauplii and copepodites as sexual mature individuals (Kleppel *et al*. 1998, Milione and Zeng 2007). Reproduction and development involves a number of life stages and biological processes, where at each stage of development, different microalgal diets may be required (Camus *et al*. 2009). For example, microalage such as *T. chuii* may not be ingested by nauplii, but may be good monoalgal diets for adult copepods because they have big cells. Using *Pavlova 50* or *I. galbana* as a mono-algal diet to maintain viable stock densities of *B. similis* during non-production seasons in hatcheries has been suggested because they produce relatively high yields of *B. similis* (Camus *et al*. 2009). Therefore, providing a diverse microalgal diet may result in a better balanced diet for all stages of development (Camus *et al*. 2009).

1.6 *Pseudodiaptomus hessei* as a livefood candidate for marine finfish

Copepods make up a major part of the diet of fish larvae in the natural environment and they possess the essential nutrients, HUFA, that are required by larvae for growth and survival (Evjemo *et al*. 2003).

Of the ten orders of copepods, calanoida has over 1000 described species worldwide and the majority are pelagic, occurring within estuaries and the marine environment (Keppel 1993, Marcus 2005). Calanoid copepods from the family Pseudodiaptomidae are distributed worldwide and occur in freshwater, brackish water and in the marine environment (Grindley 1963). They dominate the zooplankton biomass in the parts of the world they inhabit (Grindley 1963, Jerling and Wooldridge 1991, Montoya-Maya and Strydom 2009).

Table 1.2: Three species of *Pseudodiaptomus* along the coast of South Africa.

Species name	Size range (mm)	Salinity range (ppt)	Location
P. hessei	1.35-1.88	1-74	Estuaries and lagoons west and south coast of South Africa to the mouth of Congo River
P. chateri	1.25-1.55	10-38	Estuaries of St Lucia and Richards Bay
P. nudus	1.15-1.40	35	Cape of Agulhas

ppt- parts per thousand.

Along the coast of southern Africa three members of pseudodiaptomidae are found: *Pseudodiaptomus hessei* (Mrázek 1894), *P. chateri* sp.n. and *P. nudus* (Tanaka 1894) (Grindley 1963).

P. hessei and *P. chareti* are mainly estuarine species, inhabiting areas of varying salinity; *P. nudus* is rarely sampled with these species as it inhabits the marine environment (Grindley 1963). In southern African estuaries, *P. hessei* is distributed throughout the estuary; it is tolerant to a wide range of salinities (Jerling and Wooldridge 1991, Isla and Perissinoto 2004). In culture *P. hessei* showed higher fecundity at salinities of 15 and 25 parts per thousands (Mbandzi *et al*. 2014).

The majority of research on this family along the southern African coastline has been on its the ecology and biology of *P. hessei* (Froneman 2000, Kouassi *et al.* 2001, Pagano *et al*. 2003, Isla 2004, Montoya-Maya and Strydom 2009, Noyon and Froneman 2013). *P. hessei* is known to dominate the zooplankton biomass in southern African estuaries (Grindley 1963, Jerling and Wooldridge 1991, Kouassi *et al.* 2001, Isla and Perissinoto 2004, Montoya-Maya 2009). It is a very robust copepod, typically being the first to recolonize the water after floods (Jerling and Wooldridge 1991).

Members from the family pseudodiaptomidae have been cultured worldwide for use as live food in aquaculture and aspects of their cultivability have also been investigated (Chen *et al*. 2006, Puello-Cruz *et al*. 2009, Rhyne *et al*. 2009, Beyrend-Dur *et al*. 2011, Ohs *et al.* 2010a, Ohs *et al*. 2010b). Chen *et al.* (2006) found that the calanoid copepod *Pseudodiaptomus annandelei* (Sewell 1919) was the dominant species in a mixture of copepods when feeding the seahorses, *Hippocampus kuda* (Bleeker 1852) and *Hippocampus trimaculatus* (Leach 1814),

which is further evidence of the cultivability of *Pseudodiaptomus*. Even though calanoid copepods, unlike harpacticoids, cannot elongate and desaturate 18:3ω-3 to form ω-3 HUFA, they are able to assimilate and incorporate the ω-3 HUFA from their diets (Nanton and Castell 1998), making them optimal live food for fish larvae.

As *P. hessei* is one of the most common copepods in South African estuaries', it is a major part of the diet of estuarine fish such as *Gilchristella aestuaria* (Gilchrist 1913), *Atherina brevicepds* (Valenciennes 1835) and *Rhobdosargus holubi* (Steindachner 1881) (Montoya-Maya and Strydom 2009). In the Kariega Estuary, the diet of *P. hessei* varies throughout the year, with some months having higher levels of PUFAs than others (Noyon and Froneman 2014).

At certain months, the female fatty acid compositions are characterized by fatty acids which are fatty acid trophic markers (FATM) of dinoflagellates, chlorophytes and prymnesiophytes while others are characterized by the diatom FATM and increased saturated fatty acids (Noyon and Froneman 2014). *P. hessei* showed selectivity to their food items and fed on specific phytoplankton and microzooplankton or fed on sediments (Noyon and Froneman 2014).

Local estuarine and marine surveys have collected specimens of *P. hessei* and similar species to those used elsewhere around the world in aquaculture (Isla and Perissinoto 2004, Montoya-Maya and Strydom 2009), suggesting that much of the research techniques and technology could be easily transferred for use under South African conditions. In conclusion there should be extensive research into *P. hessei* as a suitable live food candidate for use in marine larviculture in South Africa.

1.7 Research aim

To investigate the effect of different dietary microalgae on the fatty acid profile, fecundity and population growth and development of the calanoid copepod *Pseudodiaptomus hessei*.

1.8 Research objectives

- Collect, identify and isolate an estuarine copepod species that is a suitable candidate in the Eastern Cape;
- Develop baseline information on maintaining viable stock cultures of *P. hessei* using various microalgae (or combination thereof) as a food source;

- Determine the fatty acid profile of wild *Pseudodiaptomus hessei* as well as those cultured on mono and binary microalgal diets;
- Determine the difference in fecundity based on mono and binary microalgal diets;
- Determine the differences in population growth and development based on the mono and binary microalgal diets.

The objectives are achieved in the chapters as follows; Chapter 2 addresses the general materials and methods used in the experiments, collection, identification and isolation of *P. hessei*. Chapter 3 investigates the fatty acid profile of wild *P. hessei*, as well those cultured on mono and binary microalgal diets. Chapter 4 addresses the difference in population growth and fecundity based on the mono and binary microalgal diets. The last chapter is the conclusion, summary and recommendations made based on the results obtained.

1.9 Research hypothesis

The choice of microalgal diets have no effect on the fatty acid profile, fecundity and population growth of the calanoid copepod, *Pseudodiaptomus hessei*, when maintained under culture conditions.

Chapter 2: Materials and methods

2.1 Field sampling

Pseudodiaptomus hessei was collected at dusk during May 2013 from the Kariega River (33^0 41' S, 26^0 44' E) in the Eastern Cape (Figure 2.1), using a 200 µm WP2 plankton net horizontally deployed at a random site from a boat. The net was towed for 5 to 10 minutes at a speed of 1–2 knots. The zooplankton were sieved through a 1000 µm screen with the larger plankton being discarded. The remaining zooplankton were stored in a 25 L container filled with estuarine water and a mixture of cultured microalgae; they were sorted and identified six hours later in the laboratory. Adult *P. hessei* were isolated from the rest of the zooplankton. The copepods were fed a mixture of microalgae, namely *T. suesica, I. galbana* and *R. salina;* the increasing population was scaled up from flasks to carboys, indoor and outdoor tanks at Pure Ocean Aquaculture in East London.

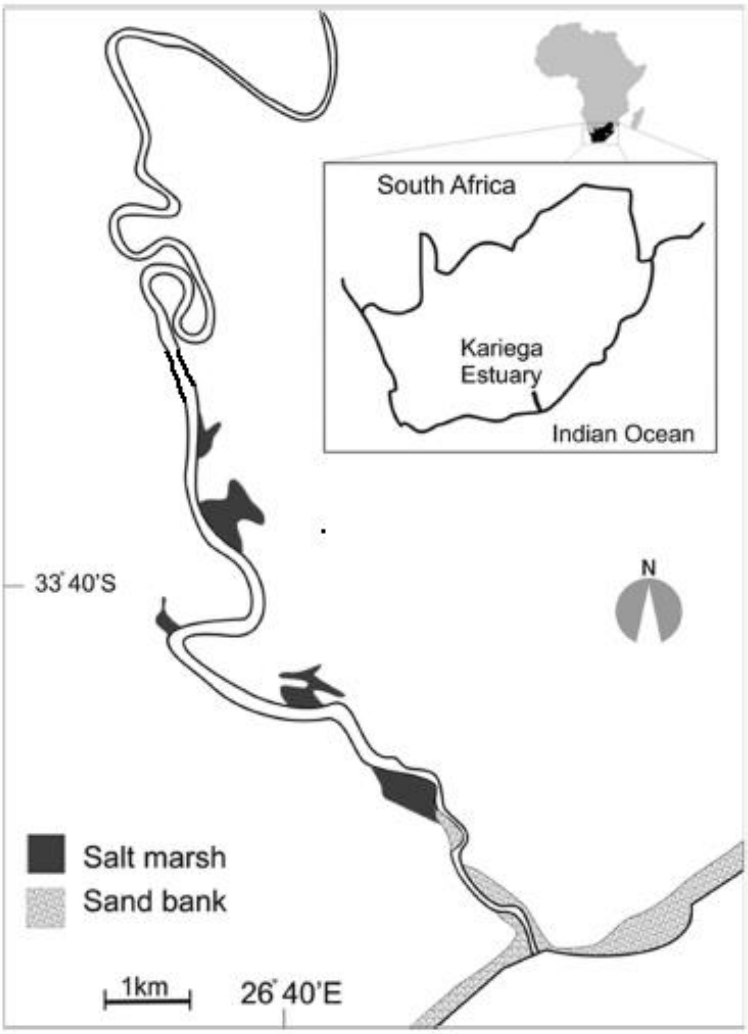

Figure 2.1: Map of the Kariega Estuary, Eastern Cape, South Africa

2.2 Laboratory processing

2.2.1 Algal cultures

The stock cultures of algae: *I. galbana* (Tahitian strain) and *R. salina* were received from the Department of Agriculture, Forestry and Fisheries in Cape Town and from Commonwealth Scientific and Industrial Research Organisation (CSIRO) in Australia, respectively. They were maintained at Pure Ocean Aquaculture in East London at a temperature of 22 °C. The microalgal cultures were inoculated into neutralized, filtered seawater at 28 ppt salinity and gently aerated. All microalgae were fertilized using f/2 (Varicon aqua solutions, Cell-Hi F2P) medium. Every two days, the culture vessels were cleaned and the algae was thinned out. The photoperiod was set at 24 h light with fluorescent light. In the experiments, the copepods required a daily algal feed volume. Cell concentration of microalgal cultures was calculated using a haemocytometer under a microscope, and then converted to carbon concentration (µg C L^{-1}) from cell volume (µm^3) according to Strathamann (1967). Carbon equivalents used were 7 pg C/cell for *I. galbana* and 40.7 pg C/cell for *R. salina*. The carbon concentration used was 2000 µg C L^{-1}, a carbon concentration above that which is known to saturate copepod feeding (Kiørbe *et al*.1985). The copepods were kept in indoor and outdoor tanks, they were fed a mixture of *T. suesica*, *I. galbana* and *R. salina* before the experiment was started.

2.3 Experimental setup and design

The experiment was run for 21 days. Nine 20 litre containers with aeration were set up at a temperature of 26 ± 0.56 °C (Figure 2.2). Three treatments: *I. galbana*, *R. salina* and a 50:50 mixture of *R. salina* and *I. galbana*, with three replicates per treatment were used. The water and microalgae were exchanged every two days depending on the density of the algae. During the experiments, the copepods required a daily algal feed volume. Cell concentration of microalgal cultures was calculated using a haemocytometer under a microscope, and then converted to carbon concentration (µg C L^{-1}) from cell volume (µm^3) according to Strathamann (1967). Carbon equivalents used were 7 pg C/cell for *I. galbana* and 40.7 pg C/cell for *R. salina*. Algae were fed to copepods based on a carbon concentration of approximately 2000 µC L^{-1} for the mono-algal treatments and binary treatments, a carbon concentration above that which is known to saturate copepod feeding (Kiørbe 1985). This equates to 1.65×10^5 cells/ml for *R. salina* and 6.27×10^5 cells/ml for *I. galbana*. Twenty two ovigorous female copepods were inoculated into each 20 litre bucket at the beginning of the experiment to maintain stability of the algal cultures. The photoperiod was set at 24 L:0 D.

Figure 2.2: Experimental set up for investigating the fatty acid profile and population development of *Psuedodiaptomus hessei*.

2.4 Water quality tests

Water quality was tested every two days to measure temperature and dissolved oxygen using an OxyGuard Handy Polaris probe. pH was measured with a Red Sea pH-Alkalinity Test Kit and A7815 Nutrafin pH Wide Range Test (4.5–9.0). Salinity was measured with an Atago Salinity hand refractometer. Water quality tests data were recorded on data sheets every two days.

2.5 Developing baseline information and maintenance

In the outdoor and indoor tanks, copepods had to be separated from rotifers and *Artemia* that contaminated the copepod cultures. The pH that was determined to be most suitable for the culture of *P. hessei* was 7.5–8.0; pH below 7.5 resulted in high mortality. Copepods in outdoor tanks were kept at ambient temperature and those in indoor tanks were kept at 22 ºC in an air conditioned room. Fatty acid analysis experiment, fecundity experiment and population growth data were recorded at the end of the experiment.

Chapter 3: The effect of different dietary microalgae on the fatty acid profile of the calanoid copepod *Pseudodiaptomus hessei*

3.1 Introduction

Zooplankton is the main link of flow of nutrients between phytoplankton (for example primary producers such as microalgae) with higher trophic levels such as fish larvae (Zhang *et al.* 2013). Microalgae with high levels of polyunsaturated fatty acids are necessary to improve the fatty acid profile of zooplankton that are consumed by marine fish larvae (Pratoomyot *et al.* 2005).

Dietary lipids are the main nutrients in energy production, and they play a role in cell membrane functioning and a number of other important roles in living organisms (Budge *et al.* 2006, Guschina and Harwood 2009). Fatty acids are comprised of lipids found in all organisms and are the densest form of energy available for fish (Budge *et al.* 2006, Tocher 2010). Some fatty acids are considered essential fatty acids (EFA) because they cannot be synthesized *de novo* by organisms (Budge *et al.* 2006).

Aquatic food webs are the main source of polyunsaturated fatty acids (Tocher 2010). In aquatic environments, phytoplankton are the primary producers of polyunsaturated fatty acids as they produce large amounts of ω-3 highly unsaturated fatty acids (Sargent *et al.* 1995). In aquaculture hatcheries, mass-cultured microalgae are the main source of nutrition for zooplankton reared as live food for fish larvae (Brown *et al.* 1997, Pratoomyot *et al*. 2005).

Highly unsaturated fatty acids, specifically DHA and EPA, are important for the normal growth of fish (Watanabe 1993). The essential fatty acid requirements of fish larvae differ from species to species (Watanabe 1982). Despite species differences, fish naturally have high levels of DHA and EPA in their tissues and have a high need for these fatty acids (Sargent *et al.* 1997). At first feeding, fish larvae require food with high concentrations of ω-3 polyunsaturated fatty acids or essential fatty acids such as DHA, EPA and ARA in order to obtain better growth, pigmentation and survival (Watanabe 1982, Morais *et al.* 2004). Deficiencies in dietary essential fatty acids lead to poor growth, increased mortality and appetite loss in some species (Watanabe 1993, Tocher 2010). Development of certain areas of the brain in marine finfish larvae are also affected by deficiency of essential fatty acids (Harel *et al.* 2002). The inability to provide these essential fatty acids in the right ratio or quantity is the main cause of fatality and poor growth in cultured marine fish larvae (Watanabe 1993, Rainuzzo *et al.* 1997, Nanton and Castell 1998). Each of these HUFA has a specific and unique role in controlling and

regulating cellular metabolisms and physiology leading to growth of larvae (Watanabe 1993, Tocher 2010).

A high DHA:EPA ratio in marine fish larvae is important because it promotes optimal growth and survival (Bell *et al*. 1985). Unbalanced ratios of DHA and EPA may lead to mortality in some species (Watanabe 1993) and failure to provide the right balance of DHA:EPA may be the cause of unsuccessful rearing of marine fish larvae (Rodríguez *et al.* 1998). Different species of marine fish larvae and juveniles have different DHA:EPA ratio requirements (Watanabe 1993, Rodríguez *et al*. 1998).

The essential fatty acid profile of copepods can be influenced by the dietary microalgae that they are fed. Copepod species such as *Cyclopina kasignete* sp.n. and *Calanus finmarchicus* (Gunnerus 1770) fed different dietary microalgae altered their fatty acid profiles depending on the microalgae that they were fed (Graeve *et al*. 1994, Rasdi *et al.* 2015).

3.2 Materials and methods

3.2.1 Field sampling

Field sampling of *P. hessei* was conducted as per section 2.1

3.2.2 Laboratory processing

The adults were fed a mixture of microalgae: *T. suesica, I. galbana* and *R. salina* for 2 years. The increasing population was scaled up from flasks to carboys, indoor and outdoor tanks at Pure Ocean Aquaculture in East London. Twenty seven random samples of 50 adults were rinsed in clean water and stored at -80 ºC for fatty acid analysis.

3.2.3 Algal cultures

Algal cultures were maintained as per section 2.2.1.

3.2.4 Experimental setup and design

The experimental setup and design is as per section 2.3.

3.2.5 Fatty acid analysis

At the end of the experiment, 50 adult copepods per treatment were collected in a 550 µm sieve and rinsed with clean seawater to remove any algae. Along with the wild collected samples, copepods were deposited onto a pre-ashed glass fibre filter and placed into a micro tube and stored in dry ice before being transferred into a -80 °C freezer for fatty acid analysis at Rhodes University.

The samples were freeze dried for fifteen hours before they were processed using the one-step method from Indarti *et al.* (2005), modified according to Richoux and Ndhlovu (2014)**.** The samples were placed in a test tube with chloroform and 0.01 % butylated hydroxytoluene. A 2 mL solution of sulphuric acid and methanol was added in the ratio 0.3:1.7. The test tube was then flushed with nitrogen (N_2) and sealed with Teflon tape. The test tubes were placed on a heating block at 100 °C for 30 minutes. After cooling at room temperature MilliQ filtered water was added. The test tubes were vortexed for 10 seconds and centrifuged at 3000 rpm for 3 minutes. The top aqueous layer was discarded while the lower fatty acid methyl ester (FAME) containing layer was dried through a cotton wool plugged pipette topped with Na_2SO_4 into a second test tube. The solvent was then evaporated using a gentle N_2 stream. When ~1 mL of the solvent remained in the test tube, the extract was transferred to a 2 mL vial. The solvent was evaporated to dryness using a gentle N_2 stream. Approximately 0.5 mL of hexane was added. The vial was flushed with N_2 and sealed with Teflon tape. The vials were stored in the freezer at -20 °C until analysis. Aliquots (1µl) of the FAME suspensions were injected at 260 °C into an Agilent 7890A/7000 gas chromatograph/mass spectrometer (GC/MS) equipped with a Zebron ZB-WAX *plus* capillary column with helium as a carrier gas. FAME peaks were created using a flame ionization or MS detector and visualized using Chemstation (B04.02) or Masshunter (B.05.00) software, and peak identities were confirmed using a NIST 08 MS library. Fatty acids were reported as the percentage total fatty acids (% TFA, mean ± standard deviation) and grouped into saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA) and essential fatty acids (EFA).

3.2.6 Data analysis

Non-metric multidimensional scaling (NMDS) of the untransformed percentage total fatty acid (%TFA) profiles was used to examine differences amongst the copepods fed different dietary microalgae, wild copepods and microalgae (mean % total fatty acid (TFA) ± SD). Analyses of similarities (ANOSIM) were also performed to determine similarities in the fatty acids of copepods fed different dietary microalgae, wild copepods and microalgae. Differences are considered significant at $p < 0.05$. Similarity percentage (SIMPER) and principal component analysis (PCA) were used to evaluate which fatty acids were most influential on any differences between the copepods fed different dietary microalgae, wild copepods and the microalgae. Analyses were conducted using PAST 3.00 (Hammer *et al.* 2001).

3.3 Results

A total of 31 fatty acids were detected in the calanoid copepod *P. hessei*, 18 were detected in the copepods fed the different dietary microalgae and 25 in the wild copepods. The fatty acid composition of the copepods fed the two mono-algal diets and the 50:50 binary diet are presented in Table 3.1. There were notable differences between the fatty acids in the copepods and the microalgae (Figure 3.1). There were also differences between DHA and EPA production of *P. hessei* and the microalgae (Figure 3.2). Non-metric multidimensional scaling of the fatty acids of copepods fed the two mono-algal diets and the 50:50 binary diet showed differences attributed to the three diets, and SIMPER and PCA showed which fatty acids influenced the differences between the diets. The 50:50 binary diet occupied an intermediate position between the two mono-algal diets. DHA was most influential in separating *I. galbana*, while the fatty acids 16:0 and 17:0 were influential in separating *R. salina* from the two diets (Figure 3.3). When observing the copepods fed *I. galbana*, *R. salina* and the 50:50 binary diet, 12 SFA were detected. The major SFA were 14:0, 16:0, 17:0 – anti-isomer, 17:0 and 18:0.

3.3.1 Fatty acid composition of copepods fed different dietary microalgae

The copepods fed the three different dietary treatments were compared to observe any differences between their fatty acid profiles. The amount of 16:0 was different in copepods fed *R. salina* (5.22 ± 1.97**)**, *I. galbana* (2.83 ± 3.25) and the 50:50 binary diet (4.69 ± 6.78) (ANOSIM: $R = 0.255$, $p = 0.0021$). Futher, the levels of 17:0 were different in copepods fed *R. salina* (1.05 ± 5.97), *I. galbana* (0.00 ± 0.00) and the 50:50 binary diet (0.70 ± 7.31**)** (ANOSIM: $R = 0.604$, $p = 0.0001$). The copepods had similar 17:0-anti-isomer (ANOSIM: $R = 0.020$, $p = 0.334$) and 18:0 content (ANOSIM: $R = 0.023$, $p = 0.150$). Two types of monounsaturated fatty acids were detected in the copepods fed *I. galbana*, *R. salina* and the 50:50 binary mixture: 18:1ω9 and 18:1ω7 (Table 3.1). The 18:1ω7 level was the same in all the copepods, while 18:1ω9 was detected only in those fed *R. salina*. The fatty acid 20:2ω6 was detected only in the copepods fed *R. salina*, while 20:4ω6 (ARA) was not detected in any of the copepods. EPA content was similar in all the copepods, 22:5ω3 ($p = 0.0009$) and DHA ($p = 0.0015$) levels were similar between *I. galbana* and the 50:50 binary diet only.

3.3.2 Fatty acid composition of cultured copepods compared to wild copepods

The wild copepods were compared to the cultured copepods to observe any differences between the wild and the cultured copepods. The polyunsaturated fatty acids; 22:6ω3, 20:5ω3, 18:4ω3, and 22:5ω3 were the major PUFAs in the copepods (Table 3.1) Proportions of 18:4ω3 were similar in the copepods fed the dietary microalgae and were significantly different in the wild copepods (ANOSIM: $R = 0.28$, $p < 0.05$). There was a similarity in EPA levels of wild copepods and those fed the 50:50 binary diet, but those fed *R. salina* (ANOSIM: $R = 0.382$, $p = 0.013$) and *I. galbana* (ANOSIM: $p = 0.003$) had significantly different levels from the wild copepods. The DHA:EPA ratio of the copepods fed the 50:50 binary diet was similar to that of copepods fed *I. galbana*, however there were significant differences between the rarios of copepods fed *R. salina* and *I. galbana* (ANOSIM: $R = 0.340$, $p = 0.0012$) and those fed *I. galbana* and wild to that of the copepods ($p = 0.0036$).

Non-metric multidimensional scaling of the fatty acids of copepods fed the two mono-algal diets, the 50:50 binary diet and wild copepods showed differences between those fed the three diets and the wild copepods. SIMPER and PCA were used to show which fatty acids influenced the differences between the diets. The fatty acids DHA, EPA and 18:4ω3 were most influential in isolating differences between the wild copepods and the cultured copepods (Figure 3.4).

Table 3.1: Fatty acid composition of *Pseudodiaptomus hessei* fed different dietary microalgae and wild copepods and different dietary microalgae fed to copepods. All values are presented as mean % total fatty acid ± standard deviation. (50 copepods per sample).

	Dietary microalgae			**Copepods**			
Fatty acids	*Rhodomonas salina*	*Isochrysis galbana*	50:50 binary diet (calculated)	*Rhodomonas salina*	*Isochrysis galbana*	50:50 binary diet	Wild
Saturated							
14:0	3.13 ± 4.55	5.07 ± 8.18	9.47 ± 9.06	2.07 ± 1.80	1.88 ± 2.71	2.25 ± 7.43	0.94 ± 0.10
15:0 anti-isomer	0.57 ± 0.74	0.41 ± 0.81	1.08 ± 0.16	0.12 ± 0.41	0.23 ± 1.62	0.29 ± 0.68	nd
15:0	nd	nd	nd	nd	nd	nd	0.13 ± 0.003
16:0 isomer	nd	nd	nd	0.21 ± 0.67	0.31 ± 1.23	0.41 ± 0.28	nd
16:0	5.81 ± 8.83	6.54 ± 5.51	13.99±6.70	5.22 ± 1.97	2.83 ± 3.25	4.69 ± 6.78	nd
17:0 anti-isomer	nd	nd	nd	0.59 ± 2.07	0.35 ± 0.74	0.55 ± 1.54	nd
17:0	2.19 ± 9.65	nd	2.19 ± 6.20	1.05 ± 5.97	nd	0.70 ± 7.31	0.15 ± 0.03
18:0	3.36 ± 14.65	0.78 ± 0.97	4.33 ± 6.75	1.47 ± 1.71	1.59 ± 5.39	2.15 ± 8.08	2.57 ± 0.96
20:0	nd	nd	nd	0.10 ± 0.22	0.15 ± 0.36	0.15 ± 0.69	nd
21:0	nd	nd	nd	nd	0.11 ± 0.54	0.22 ± 0.84	nd
22:0	nd	0.24 ± 0.63	0.31 ± 0.86	0.08 ± 0.27	0.12 ± 0.35	0.26 ± 1.10	0.04 ± 0.01
23:0	nd	nd	nd	0.05 ± 0.47	0.10 ± 0.84	0.20 ± 0.89	nd

24:0	nd	0.82 ± 2.62	1.02 ± 2.89	0.04 ± 0.11	0.09 ± 0.63	nd	nd
Monounsaturated							
14:1ω5	0.03 ± 0.87	0.31 ± 1.10	0.67 ± 0.27	nd	nd	nd	0.09 ± 0.03
16:1ω9	0.66 ± 1.52	0.54 ± 3.19	1.34 ± 0.03	nd	nd	nd	0.59 ± 0.20
16:1ω7	0.66 ± 1.41	2.35 ± 5.81	3.60 ± 6.43	nd	nd	nd	0.08 ± 0.02
16:1ω5	nd	nd	nd	nd	nd	nd	0.09 ± 0.02
18:1ω9	0.09 ± 0.10	nd	0.09 ± 0.24	0.21 ± 1.44	nd	nd	0.80 ± 0.66
18:1ω7	0.09 ± 0.10	nd	0.09 ± 0.24	0.11 ± 1.10	0.14 ± 1.46	0.50 ± 2.74	0.47 ± 0.09
20:1ω9	nd	nd	nd	nd	nd	nd	0.21 ± 0.65
24:1ω9	nd	nd	nd	nd	nd	nd	0.14 ± 0.02
Polyunsaturated							
16:2ω4	nd	nd	nd	nd	nd	nd	0.14 ± 0.05
16:3ω4	nd	nd	nd	nd	nd	nd	0.15 ± 0.13
18:2ω6	0.73 ± 1.82	0.15 ± 0.41	0.92 ± 1.55	nd	nd	nd	0.56 ± 0.26
18:3ω3	0.42 ± 0.91	0.41 ± 0.18	0.93 ± 0.25	nd	nd	nd	0.92 ± 0.23
18:4ω3	0.20 ± 0.59	0.13 ± 0.52	0.36 ± 0.09	nd	nd	nd	1.42 ± 0.35
20:2ω6	nd	nd	nd	0.16 ± 0.59	nd	nd	0.10 ± 0.12
20:4ω6 (ARA)	nd	nd	nd	nd	nd	nd	0.12 ± 0.12

20:5ω3 (EPA)	0.64 ± 2.87	0.22 ± 0.31	0.91 ± 1.04	0.31 ± 0.67	0.19 ± 0.60	0.74 ± 4.71	3.09 ± 0.57
22:5ω3	0.22 ± 0.30	0.53 ± 0.69	0.88 ± 1.24	nd	0.31 ± 1.25	0.35 ± 1.66	0.19 ± 0.07
22:5ω6	nd	nd	nd	nd	nd	nd	0.07 ± 0.01
22:6ω3 (DHA)	0.67 ± 1.50	1.08 ± 3.56	2.02 ± 1.92	0.57 ± 0.99	0.69 ± 1.57	0.84 ± 2.84	4.85 ± 1.41
Sum PUFA	81.12	62.75	32.83	180.15	186.57	156.00	183.58
Sum MUFA	59.37	90.77	32.99	20.18	16.78	24.28	40.70
Sum SFA	205.92	346.48	120.78	599.67	896.65	519.73	175.72
Sum EFA	20.92	32.41	11.71	55.93	106.51	77.38	129.05
ω3/ω6	121 ± 4.79	3.63 ± 6.63	5.75 ± 9.42	0.96 ± 4.19	nd	nd	3.10 ± 1.55
DHA: EPA	0.65 ± 2.25	0.97 ± 2.71	1.87 ± 1.61	0.24 ± 0.34	0.34 ± 0.96	0.32 ± 1.82	0.39 ± 0.10

nd indicates the fatty acid was not detected

3.3.1 Fatty acid composition of the dietary microalgae

SIMPER analysis revealed that the fatty acids which were most influential in the differences observed in the two dietary microalgae were the saturated fatty acids 18:0 (24 %), 14:0 (22 %), 16:0 (16 %), 17:0 (13 %) and 24:0 (2 %). For the monounsaturated fatty acids, 16:1ω7 made up 11 %, followed by 18:1ω9 (7 %) while the polyunsaturated fatty acid 22:ω3 made up 2 %. The DHA: EPA ratios between *I. galbana* (0.97 ± 2.71) and *R. salina* (0.65 ± 2.25) were similar. The DHA and EPA contents of the microalgae were also similar (Table 3.1).

3.3.2 Fatty acid composition in dietary microalgae and copepods

When using SIMPER to compare the fatty acid profile of the copepods and the different dietary microalgae, the most differences were observed in the saturated fatty acids (Figure 3.1).

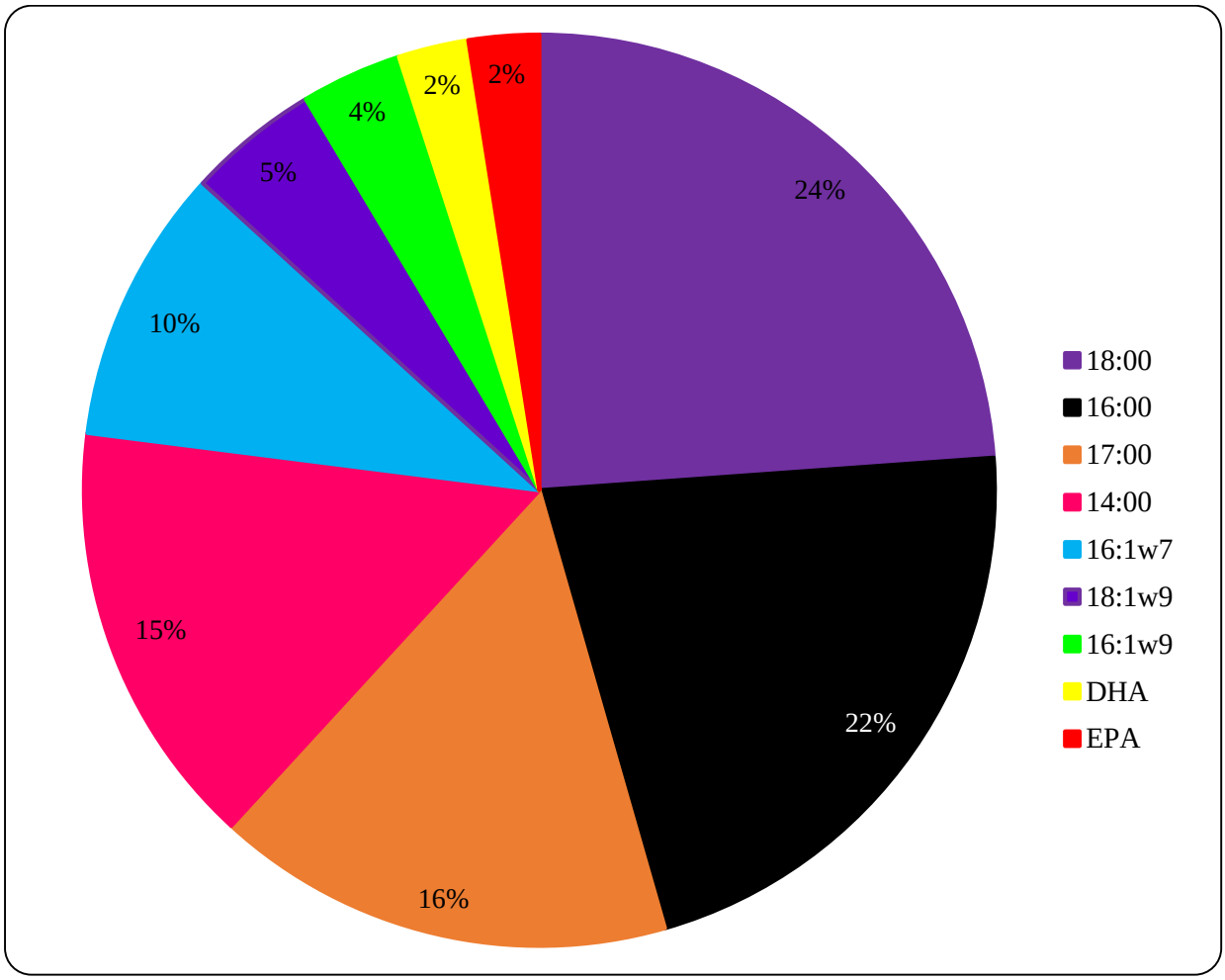

Figure 3.1: Percentage of major fatty acids contributing to differences between copepods and dietary microalgae (SIMPER output).

There was no significant difference between the EPA levels of the algae and the copepods fed the dietary microalgae, however copepods fed *R. salina* had significantly different levels from *R. salina* algae (ANOSIM: *R* = 0.589, p = 0.037) and *I. galbana* algae (ANOSIM: *R*= 0.492, p = 0.045). The copepods fed the 50:50 binary diet had similar levels of DHA to the monoalgal diets *I. galbana* and *R. salina*. *R. salina* fed copepods had a similar DHA level to *R. salina* microalgae, but had significantly different DHA levels to *I. galbana* microalgae. The copepods fed *I. galbana* had a similar DHA level to that of the *I. galbana* microalgae but a significantly different level to the *R. salina* microalgae (ANOSIM: R = 0.535, p=0.006). The DHA:EPA ratio of the copepods fed the 50:50 binary diet and those fed *I. galbana* was similar with the DHA:EPA ratio of both the mono-algal diets. Copepods fed *R. salina* had a similar DHA:EPA ratio to the *R. salina* microalgae, but had significantly different levels to that of *I. galbana* (p = 0.02). Figure 3.5 shows a comparison between dietary microalgae and copepods where *R. salina*, *I. galbana*, and 50:50 the binary diet fed copepods were spatially separated from *R. salina* algae with 14:0, 16:1ω7, 16:1ω9 and 18:1ω9 being most influential in the separation. There was an overlap between the copepods fed the three diets and *I. galbana* algae.

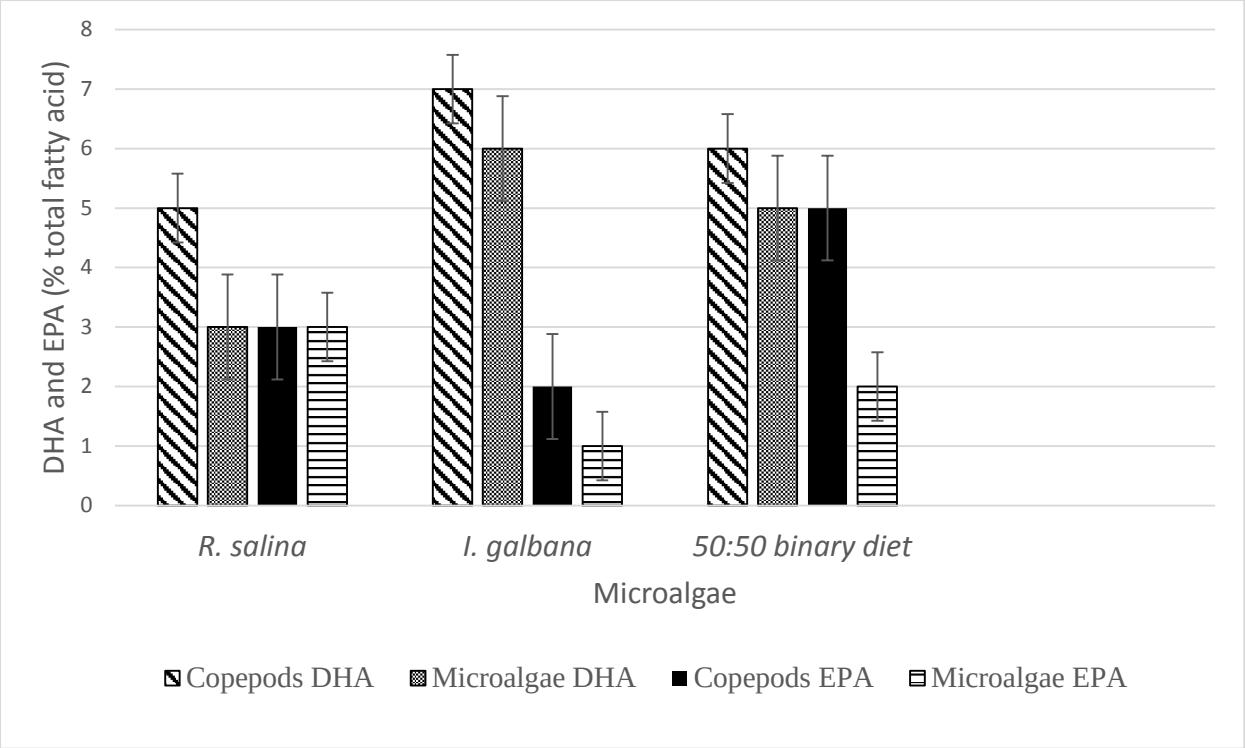

Figure 3.2: Production of DHA and EPA by *Psuedodiaptomus hessei* corresponding to different dietary microalgae. Values are average ± standard error of three replicates.

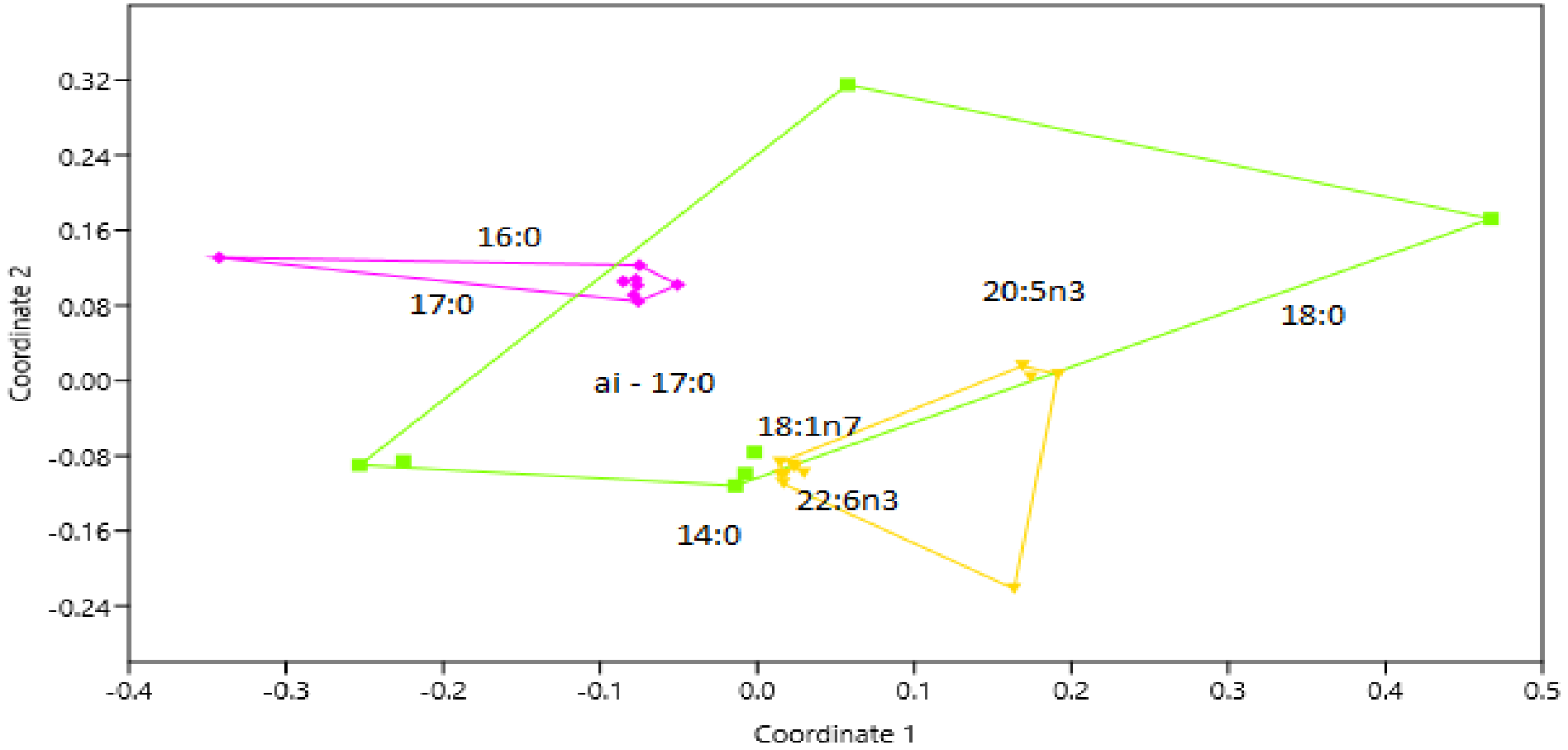

Figure 3.3: Non-metric multidimensional scaling output using fatty acid profiles of *Pseudodiaptomus hessei* fed dietary microalgae; *Isochrysis galbana*, *Rhodomonas salina* and a 50:50 binary diet of the two microalgae. Polygons representing the fatty acid groups of the different dietary microalgae. Fatty acids influential in separating the 3 diets (derived from SIMPER and PCA) are superimposed in the plot. Green squares = 50:50 binary diet, pink diamonds = *R. salina* & yellow triangles = *I. galbana.*

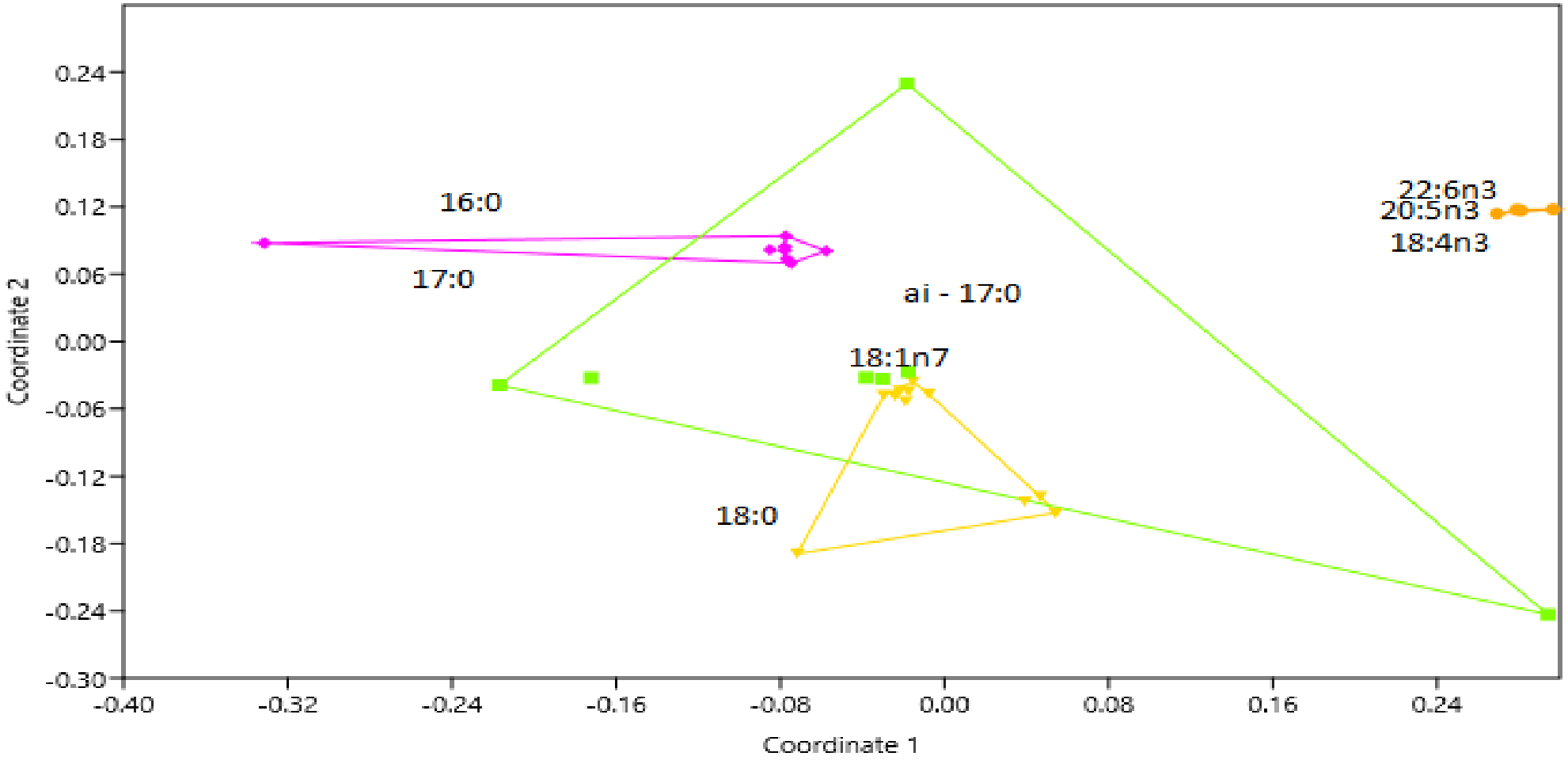

Figure 3.4: Non-metric multidimensional scaling output using fatty acid profiles of *Pseudodiaptomus hessei* fed microalgal diets; *Isochrysis galbana*, *Rhodomonas salina*, a 50:50 binary diet of the two microalgae and wild copepods. Polygons representing the fatty acid groups of the different dietary microalgae and wild copepods. Fatty acids influential in separating the 3 diets and wild copepods (derived from SIMPER and PCA) are superimposed in the plot. Green squares = 50:50 binary diet, pink diamonds = *R. salina* & yellow triangles = *I. galbana*, orange circles are wild copepods.

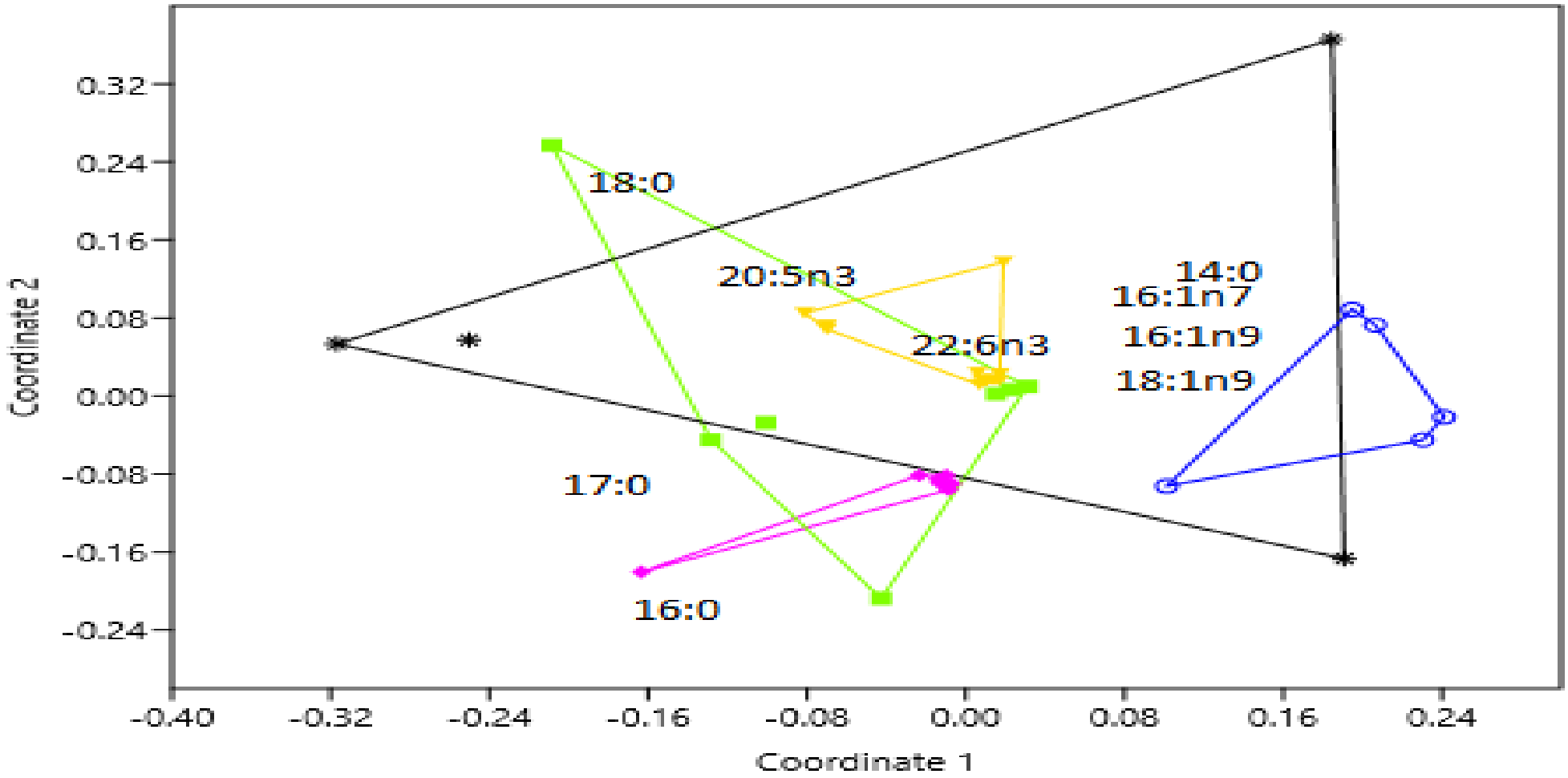

Figure 3.5: Non-metric multidimensional scaling output using fatty acid profiles of *Pseudodiaptomus hessei* fed microalgal diets; *Isochrysis galbana*, *Rhodomonas salina*, a 50:50 binary diet and the microalgae. Fatty acids influential in separating the 3 copepods fed different diets and the microalgae (derived from SIMPER and PCA) are superimposed in the plot. Green squares = 50:50 binary diet, pink diamonds = *R. salina*, yellow triangles = *I. galbana*, black stars = *R. salina* algae & blue circles = *I. galbana* algae.

3.4 Discussion

Microalgae have a crucial role as primary producers in marine aquaculture, where they serve as food for consumers such as copepods which are in turn fed to fish larvae (Pratoomyot *et al*. 2005).

In this study, the dietary microalgae fed to the calanoid copepod *P. hessei* had a significant impact on its fatty acid profile. Some fatty acids were detected only in copepods fed *R. salina* (18:1ω9, 20:2ω6), others in copepods fed *I. galbana* and the 50:50 binary diet (21:0) while others were detected in *R. salina* and the 50:50 binary diet only (24:0). Most importantly, the copepods could incorporate essential fatty acids from their diet. These findings are consistent with studies on other copepod species fed different diets that changed the copepod fatty acid profiles (Graeve *et al.* 1994, Rasdi *et al*. 2015). Rasdi *et al.* (2015) found that the fatty acid profile of *C. kasignete* was altered when it was fed dry *Nannochloropsis oculata* (Droop 1955), dry *Melosira* sp., *Schroederiella apiculata* sp. n., *Scenedesmus pectinatus* sp. n and *Tetraedrom minimum* (Hansg 1888). Graeve *et al*. (1994), also found similar results, in that the copepods *Calanus finmarchicus* (Gunnerus 1770), *Calanus hyperboreus* (Krøyer 1838) and *Calanus glacialis* (Jaschnov 1955) fed *Thalassiosira antarctica* (Fryxell, Doucette, & Hubbard 1981) and *Amphidinium cartera* sp. n. could directly incorporate fatty acids from their diets. The fatty acid profile of the copepods in this study fed the different dietary microalgae were dominated by SFA and MUFA, with lower concentrations of PUFAs, similar to findings by McKinnon *et al.* (2003). McKinnon *et al.* (2003) studied the calanoid copepods, *B. similis* and *P. crassirostris*, to which they fed different dietary algae. They found that the fatty acid profiles of the copepods were dominated by SFA (16:0, 18:0, 14:0) and MUFA (22:1, 20:1) and that the EFA, DHA and EPA were present in lower proportions. The proportions of SFA 16:0, 17:0 and 24:0 in our study varied amongst the copepods fed the different dietary microalgae. Copepods fed the 50:50 binary diet had a fatty acid profile which occupied an intermediate position between copepods fed both the mono-algal diets *I. galbana* and *R. salina* (Figure 3.3). This implies that a combination of different dietary microalgae can be used to provide copepods with a varied and possibly adequate fatty acid profile required by fish larvae. Milione and Zeng (2007) found that, when fed a mixture of dietary microalgae, the copepod *A. sinjiensis* had better growth than copepods fed a mono-algal diet. The better growth could be attributed to the combined fatty acids of the multi-algal diets as single diets can be limiting in EFAs (Camus *et al.* 2009).

Twenty five fatty acids were detected in wild copepods compared to the eighteen found in those fed either the mono or binary diets. The higher levels of fatty acids detected in wild copepods are likely due to the fact that copepods in the wild do not feed exclusively on microalgae but also on heterotrophic protists and dinoflagellates, giving them a more diverse fatty acid profile (Tang and Taal 2005). In this study, the wild zooplankton had a significantly higher proportion of long chain EFA, DHA and EPA, and low levels of ARA were detected, as opposed to copepods fed the different dietary microalgae, which had none. These results are similar to the findings of Nanton and Castell (1999). Nanton and Castell (1999) studied the effect of temperature and dietary fatty acids on the fatty acid composition of the harpacticoid copepods *Tisbe* sp. and *Amonardia* sp. They also found that wild zooplankton contained large amounts of both long chain EFA, EPA and DHA. The DHA:EPA ratio of the wild copepods was similar to the DHA:EPA ratio of the copepods fed *R. salina* (Table 3.1). The DHA:EPA ratio of the wild copepods in this study is similar to the DHA: EPA ratio of *P. hessei* females sampled from Kariega Estuary by Noyon and Froneman (2014), and similarly Nanton and Castell (1999) found that wild copepods had lower DHA:EPA compared to copepods fed microalgae (*I. galbana* and *Dunaliella. tertiolecta*). The low DHA:EPA ratio in the wild copepods found in this study can be attributed to high levels of EPA than DHA, resulting in a lower DHA:EPA ratio (Noyon and Froneman 2014).

When comparing the transfer of fatty acids from the microalgae to the copepods, the copepods had similar DHA and EPA levels to the dietary microalgae (Figure 3.2). This relates to findings by Nanton and Castell (1998) and Rasdi *et al*. (2015) who obseverd that even though the microalgae provided to copepods had low levels of DHA and EPA, the copepods had high levels of DHA and EPA. This suggests that copepods are able to accumulate essential fatty acids from their diet (Graeve *et al*. 1994, Rasdi *et al.* 2015). The EPA content was higher in the 50:50 binary diet than in both *R. salina* and *I. galbana*. Copepods fed the 50:50 binary diet had fatty acid levels similar to both *R. salina* and *I. galbana* (Figure 3.3), suggesting that a mixed diet can provide the nutritional values of both the mono-algal diets combined. ARA is not considered to be as important as DHA and EPA in the diets of marine fish larvae (Castell *et al*. 1998, Koven *et al*. 2001). In this study, ARA was not detected in the copepods or the dietary microalgae, however Nanton and Castell (1998) found low levels of this fatty acid in the microalgae *I. galbana*, *D. tertiolecta* and *Chaetocerus calcitrans* while copepods fed these diets had > 1% ARA levels. Not having detected this fatty acid in both the dietary microalgae and the copepods might suggest that it was present in very small amounts in the algae and in

turn was in trace amounts in the copepods fed the algae to the extent that it was not detected. SIMPER analysis revealed that the major differences between the dietary microalgae and the copepods were mainly due to the fatty acids; 18:0, 16:0, 17:0, 14:0 and 16:1ω7 (Figure 3.1 & 3.4).

The results of this study show that copepod fatty acid profiles can be manipulated through feeding different diets to meet the requirements of the fish larvae (Rasdi *et al.* 2015). Because the fatty acid profile of *P. hessei* can be manipulated through their diet, it shows good potential as a live feed for marine fish larvae. Dietary manipulation can produce a high DHA: EPA ratio and high DHA and EPA levels when *I. galbana* or a 50:50 binary diet is used.

Chapter 4: Fecundity and population growth of *Pseudodiaptomus hessei* fed different dietary microalgae

4.1 Introduction

Different species of microalgal diets have been shown to have varying effects on the growth and population increase of copepod species which suggests that it is important to select the appropriate microalgal diet for each species (Kleppel *et al.* 2005, Puello-Cruz *et al*. 2009, Camus *et al.* 2009). In order to increase copepod productivity, the nutritional quality of the selected microalgae must match the nutritional needs of the cultured copepod (Ohs *et al.* 2010a). Nutritional quality of food depends on compounds such as long chain fatty acids, amino acids, sterols, proteins and vitamins (Shin *et al*. 2003, Arendt *et al*. 2005).

One of the factors affecting population growth is fecundity. It is significantly affected by the extent to which the nutritional requirements of the copepod are met by its diet (Kleppel and Burkart 1995, Milione and Zeng 2007). In order to understand the population dynamics and production rate of copepods, knowledge of fecundity is necessary (Liang and Uye 1997). Productivity of copepods is not only linked to fecundity but also to hatching rate, survival and development of nauplii and copepodites to adults (Milione and Zeng 2007), with a diverse diet generally being associated with increased productivity (Kleppel and Burkart 1995). Mixed diets complement each other if the mixed species lack different nutritional compounds (Koski *et al*. 2006). Other factors that affect fecundity in cultured copepods include stocking density (Camus and Zeng 2009), temperature, salinity (Beyrend-Dur *et al*. 2011, Devreker *et al.* 2012), cannibalism (Camus and Zeng 2009) and lifespan of the female (Devreker *et al*. 2012).

When all other aspects such as stocking density, temperature and salinity that affect fecundity or population are optimum for the cultured copepod, diet is the only determinant of fecundity and population growth (Kleppel *et al.* 1998, Anzueto-SÃnchez *et al*. 2014).

The need and use of nutrients in the diet for copepods may also be different between copepod life stages (Arendt *et al*. 2005, Koski *et al*. 2006). For example, egg production may require certain nutrients different from those required for egg hatching or naupliar and copepodite development (Koski *et al.* 2006). The ability of copepods to manipulate food may also differ between life stages and species (Koski *et al*. 2006).The feeding history of copepods has been found to affect egg production in some species of copepods (Arendt *et al.* 2005), where the availability of nutrients such as amino acids and essential fatty acids (EFAs) have been

positively correlated with fecundity, and life stage development in copepods (Broglio *et al*. 2003, Arendt *et al.* 2005, Jónasdóttir *et al*. 2009).

This chapter compares the effect of three different dietary microalgae; *I. galbana*, *R. salina* and a 50:50 binary diet on the fecundity, population growth and development of different life stages of the calanoid copepod *P. hessei*.

4.2 Materials and Methods

4.2.1 Experimental setup and design

The experimental setup and design is as per section 2.3, with the exception that on the final day of the experiment, 20 ovigerous females from each container were collected and preserved in 10% buffered formalin. The number of females was taken based on sample size and considering that a minimum of 50 copepods was need for the fatty acid analysis experiment. The copepods were placed gently in a petri dish, where the copepod was carefully separated from the egg sac membrane. The egg sac was placed in a 5% solution of sodium hypochlorite and gently agitated to dissolve the egg sac membrane. The total number of eggs in each sac were counted under a dissecting stereo microscope (BestScope BS3040). Apart from those removed for the fatty acids analysis, all the remaining copepods were preserved in 10 % formalin, counted and each stage of development identified according to Jerling and Wooldridge (1989). *P. hessei* nauplii, copepodites and adults were counted from each bucket and the final population estimated as the average of three replicates. Based on Fenchel (1974), the intrinsic rate of population increase r was calculated for each treatment using the formula:

$$r = \ln (N_1/N_0)/ t,$$

where N_0 = population number at the beginning of the experiment, N_1 = population number at the end of the experiment while t (days) is the duration of the experiment.

4.2.2 Data analysis

The fecundity data were confirmed to be normally distributed (Kolmogorov-Smirnov test), the population and development data were confirmed by the Shapiro-Wilk test. The data were analysed using one-way ANOVA. When significant differences ($p < 0.05$) were found, Tukey's multiple comparisons test was used to determine specific differences among treatments ($p < 0.05$). To test whether copepod fatty acids were correlated to fecundity and hatching success, a linear regression analysis was performed. All statistical analyses were conducted using SPSS version 23. Data are presented as mean ± standard error.

4.3 Results

4.3.1 Fecundity

The three different dietary microalgal diets used in this study had an effect on the fecundity of the calanoid copepod *P. hessei* (Table 4.1). There was a significant difference in the fecundity of the copepods between all three groups fed the dietary microalgae and the wild caught copepods (ANOVA: $F_{(3, 3)} = 95.54$, $p < 0.001$). The wild caught copepods had the highest number of eggs (40.70 ± 0.91) followed by the copepods fed the mono-algal diet *R. salina* (34.60 ± 0.77) and those fed the 50:50 binary diet (23.30 ± 0.87). Copepods fed *I. galbana* had the least number of eggs (22.8 ± 5.44). All the groups were significantly different from each other except for the copepods fed *I. galbana* and those fed the 50:50 binary diet (Table 4.1). No significant correlations were found between HUFAs; DHA and EPA measured in the copepods and fecundity of the copepods ($p > 0.05$).

Table 4.1: Mean *P. hessei* egg production of wild caught and copepods fed three different microalgal diets for 21 days.

Treatment	Egg production
Rhodomonas salina	34.60 ± 0.77^{a}
Isochrysis galbana	22.88 ± 0.70^{b}
50:50 Binary diet	23.30 ± 0.87^{b}
Wild caught copepods	40.70 ± 0.91^{c}

Data are presented as mean ± standard error; different superscript letters in the same column indicate significant differences ($p < 0.05$).

4.3.2 Population growth

After 21 days of culture on the three different dietary microalgae, the average final population numbers of *P. hessei* were collated into four categories, namely 'All Stages Included' (including adults, copepodites & nauplii); 'Adults Only' only stage C6); 'Copepodites Only' (C1 – C5); 'Nauplii Only'(N2 – N6) as presented in Table 4.2. The final population of all stages were highest for the *I. galbana* (709 ± 92.23); it was significantly higher than all other diets (ANOVA: $F_{(2, 3)} = 13.918$, $p = 0.009$). The second most productive diet was the 50:50 binary diet (437 ± 40.02), however it was not statistically different from *R. salina* (433 ± 78.08) which produced the lowest population numbers. Nauplii accounted for the highest numbers in the final population including all stages in the groups fed *R. salina* and *I. galbana*. All-Stages-Included r ranged from 0.14 ± 0.005 for the 50:50 binary diet to 0.20 ± 0.06 for *I. galbana*. A breakdown of the *P. hessei* final population composition based on 3 groups of life stages (nauplii, copepodites and adults) is shown in Figure 4.1. *I. galbana* produced the highest number of copepods across all life stages. The only life stages that were similar between the three microalgal diets were the copepodite stages of all the treatments (Table 4.2). No correlations were found between the fatty acids DHA, EPA, 18:0, 23:0 and 22:5ω3, and the hatching success of copepods ($p > 0.05$).

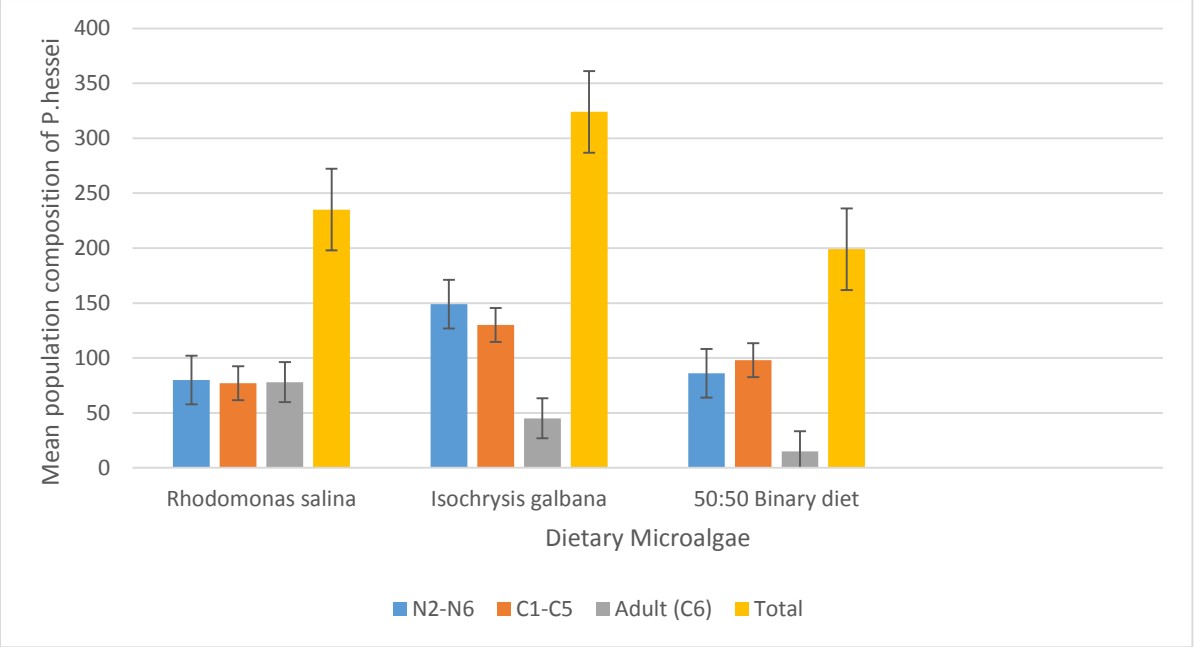

Figure 4.1 Effect of three microalgal diets on the average population composition of *Pseudodiaptomus hessei* over a 21 day culture period. The experiment started with 21 ovigerous females of *P. hessei*. Data are presented as mean ± standard error. N2 – N6 = naupliar stage 2 to 6; C1 – C5 = copepodite stage 1 to 5.

Table 4.2 Final population and intrinsic rate (r) of population increase of *Pseudodiaptomus hessei* cultures over a 21 day period fed on three microalgal diets. Data are presented as mean ± standard error; different superscript letters in the same column indicate significant differences ($p < 0.05$). FNP denotes; Final Population Number.

Microalgal diet	FNP –All Stages Included	r (All Stages Included)	FNP -Adults Only	r (Adults Only)	FNP- Copepodite Only	r (Copepodite Only)	FNP- Nauplii Only	r (Nauplii Only)
Rhodomonas salina	144.3 ± 45.08^{a}	0.05 ± 0.01	65.1 ± 8.84^{a}	$0.03 \pm 1.7\times10^{-17}$	25.9 ± 20.88^{a}	0.02 ± 0.02	26.7 ± 9.54^{a}	0.02 ± 0.004
Isochrysis galbana	236.4 ± 53.25^{b}	0.07 ± 0.03	93.8 ± 18.11^{ba}	0.04 ± 0.003	43.3 ± 12.42^{a}	0.03 ± 0.01	49.7 ± 11.59^{b}	0.03 ± 0.003
50:50 Binary diet	145.8 ± 23.10^{ac}	0.05 ± 0.002	56.1 ± 34.83^{ba}	0.03 ± 0.01	32.6 ± 8.84^{a}	0.02 ± 0.003	28.6 ± 9.68^{ac}	0.02 ± 0.01

4.4 Discussion

Microalgae is the primary diet of zooplankton cultured as prey for marine finfish larvae (Brown *et al*. 1997), and is generally pelagic and in the nannoplankton size range (2 – 20 µm) (Muller-Feuga *et al*. 2003). Selecting the appropriate microalgal diet is therefore crucial for enhancing the productivity of the copepod species under culture (Camus *et al*. 2009). The copepods were cultured for 21 days in order to have a new generation (F2) of copepods fed the dietary microalgae. To ensure food was not a limiting factor, copepods were fed at a carbon concentration (2000 µg C L^{-1}) above that which is known to saturate copepod feeding (Kiørbe *et a1*. 985).

The mono-algal diet *R. salina* produced copepods with the highest fecundity. This is similar to the findings of Shin *et al.* (2003) who obtained high fecundity when *Acartia omorii* (Bradford 1976) was fed the mono-algal diets *Heterocapsa triquetra* (Stein 1883) and *Scrippsiella trochoidea* (Loeblich III 1976) respectively.

Some authors have found that when combinations of microalgae are fed to copepods, they perform better in terms of productivity than mono-algal diets (Camus *et al*. 2009, Ohs *et al*. 2010a, Camus and Zeng 2012). For example, Camus *et al.* (2009) found that when *B. similis* was fed a tri-algal diet, it produced better egg production, hatching success and population increase. Similarly, Ohs *et al.* (2010a) and Camus and Zeng (2012) found that productivity of copepods increased when copepods were fed a mixed diet rather than a mono-algal diet. In this study, when the mono-algal diet *R. salina* was fed to *P. hessei* it performed better than the 50:50 binary diet and mono-algal, *I. galbana* in terms of fecundity. These results are contrary to the findings of Støttrup and Jensen (1990), who found that *A. tonsa* had higher egg production when fed *I. galbana* than *Rhodomonas* sp. Støttrup and Jensen (1990) attributed this to the high DHA: EPA ratio in *I. galbana*. Similar results were not obtained in this study, this may be due to species specific differences between *P. hessei* and *A. tonsa*. Diverse results were also observed by McKinnon *et al*. (2003), who found that egg production rates of the copepods *B. similis, P. crassirostris* and *A. sinjiensis* were affected differently when fed similar algal diets. However in McKinnon's study when fed as a mono-algal diet, *Rhodomonas* sp. maintained the second highest egg production rate after the microalagae *Heterocapsa niei* (Morrill & Loeblich III 1981) of all the cultured copepod species (McKinnon *et al*. 2003).

Many authors have discussed the positive relationship between certain fatty acids and fecundity (Payne and Rippingale 2001, Broglio *et al*. 2003). The availability of the amino acids

glutamate, methionine, arginine and histidine in the diet of copepods had been positively correlated with increased egg production in the copepod *A. tonsa* (Kleppel *et al*. 1998). Noyon and Froneman (2013) found that the fatty acids 16:1ω7, 16PUFAs, EPA, 18:4ω3 and DHA were positively linked to egg production in the copepod *P. hessei*, while Kleppel *et al.* (1998) found the fatty acids 18:0, 22:5ω3 and 23:0 were positively correlated with egg production in the calanoid copepod *A. tonsa*. This is however, contrary to our findings, we found no significant correlation between the fatty acids of copepods fed microalgae and increased egg production ($p = 0.355$).

Broglio *et al*. (2003) found a positive correlation between dietary EPA and egg production when culturing *A. tonsa* with heterotrophic and autotrophic diets. However in this study, no significant correlation ($p = 0.810$) was found between EPA and egg production. The performance of *R. salina* as a superior microalgal diet for fecundity could not be attributed to higher EPA levels in *R. salina* as compared to *I. galbana* (Dunstan *et al*. 1994, Brown *et al.* 1997) or the 50:50 binary diet. Moreover, Arendt *et al*. (2005) also did not find any correlation between EPA and fecundity of the copepod *Temora longicornis* (Müller 1785). From these observations one may conclude that different copepods may have different nutritional requirements other than the essential fatty acid such as EPA when it comes to fecundity.

The 50:50 binary diet and *I. galbana* in this study were the second highest towards the increase of fecundity. In the copepod *B. similis* a multi-algal diet containing *I. galbana* led to high egg production compared to *I. galbana* fed as a mono-algal diet (Camus *et al.* 2009). The poor performance of the binary diet compared to *R. salina*, may be because energy was diverted to capturing preferred food within the mixture instead of reproduction, hence lowering fecundity (Kleppel *et al.* 1998). Consumption rate in copepods is influenced by size, quality and quantity of the food item (Kiøbe *et al*. 1985), therefore it is possible that the two dietary algal species were not being ingested at an equal rate as the capture of the cells was not equal (DeMott 1989). Støttrup and Jensen (1990) found that *A. tonsa* ingested *I. galbana* at a higher rate than *Rhodomonas* sp. This may have been the case in this study as well; copepods may have selected *I. galbana* over *R. salina*, leading to lower fecundity in copepods fed the 50:50 binary diet. The accumulation of metabolites from the algae that was not assimilated quickly in the mixture might have caused sedimentation and settling at the bottom of the culture vessels, making it difficult for the copepods to feed. An alternative hypothesis is that the algae may have also caused super-saturation (Muller-Feuga *et al*. 2003, Jónasdóttir *et al*. 2009), causing the sedimentation observed at the bottom of the culture vessels. Based on the findings of this

study, a 50:50 combination of *I. galbana* and *R. salina* diets may not be as nutritionally adequate as *R. salina* for enhancing the fecundity of *P. hessei*. However, different combinations of *I. galbana* and *R. salina* as well as other microalgal species may possibly improve the fecundity and productivity of *P. hessei*.

When comparing the fecundity of the wild–caught copepods to the copepods fed the different dietary microalgae, the wild–caught copepods had a significantly higher fecundity (40.70±4.96) when compared to any of the others (Table 4.1). Noyon and Froneman (2013) studying the fecundity of wild *P. hessei* from Kariega Estuary found similar results, they found a mean clutch size of 34 ± 5 eggs per sac (26 – 46 eggs). They found that the clutch size of *P. hessei* in Kariega Estuary was positively correlated to DHA and a (ω3): (ω6) ratio. The difference between the fecundity of wild caught copepods and those fed the cultured microalgae may be attributed to the diversity of diet in the wild. The pelagic food web in the wild is complex as, heterotrophic protists and dinoflagellates also form part of the diet of wild copepods, and copepods do not depend entirely on planktonic algae (Kleppel 1993, Tang and Taal 2005). In the Kariega Estuary, the diet of *P. hessei* consists of dinoflagellates, chlorophytes, prymnesiophytes, diatoms and possibly ciliates at varying times of the year (Noyon and Froneman 2014).

In this study the microalgal diets significantly affected the population of the calanoid copepod *P. hessei*. This effect of microalgae on copepod population is similar to the findings of Milione and Zeng (2007), who obsevered that different microalgal diets significantly affected the population growth of the copepod *A. sinjiensis*. They found that a binary diet of *T. chuii* and *I. galbana* was more successful than any mono or binary diet. However in this study, the mono-algal diet of *I. galbana* was more successful than a 50:50 binary diet. The intrinsic rate of increase of a population (r), which is described as the increase of a population which is not affected by density-dependent factors (Birch 1948), was calculated for the different life stages of *P. hessei*. The population of *P. hessei* increased exponentially over time in all the treatments. An exponential increase in a population is expected when a species encounters sufficient quantities of preferred food, in this case, the microalgae. The growth rate increased as the population (N) became larger (Birch 1948).

The mono-algal diet *I. galbana* gave the highest population of copepods as compared to the other two diets (Table 4.2). In contrast with this, fecundity was lower in *I. galbana* (All Stages Included). Koski *et al*. (2006) observed a similar trend with *T. longicornis*, which produced a

high number of eggs followed by lower hatching success, leading to low nauplii numbers. These findings were attributed to the fact that; egg production in *T. longicornis* was positively correlated to nitrogen content in the diet, but it was independent of the presence HUFAs (DHA and EPA). Støttrup and Jensen (1990) also found that egg production of *A. tonsa* was not influenced by the quantity of HUFAs ingested. However, egg hatching success for *T. longicornis* was positively correlated to the concentration of DHA and EPA in the diet (Koski *et al.* 2006). This was also the case in our study, egg production was independent of HUFAs; hatching success was also been found to be independent of HUFA concentration ($p > 0.05$). Results by other authors suggest that egg production, egg viability, hatching and subsequent growth to naupliar and copepodite stages are controlled by different factors in some copepod species. Factors such as carbon and nitrogen contents and HUFA concentration may all play a role (Arendt *et al.* 2005, Koski *et al*. 2006). Although this may be the case in my study, I did not test the effect of carbon and nitrogen on hatching success and egg production. Moreover, Knuckey *et al.* (2005) found *Rhodomonas* sp. to be a superior monoalgal diet than *I. galbana* for the development of the calanoid copepod *A. sinjiensis*. These findings have led us to conclude that there are species-specific and life stage-specific differences in the nutritional requirements of copepods (Koski *et al*. 2006).

In other studies *Rhodomonas* sp. fed to the calanoid copepod *A. sinjiensis* produced a higher number of nauplii compared to *I. galbana* (Milione and Zeng 2009), while *Rhodomonas* sp. also supported higher development rate for *A. sinjiensis* nauplii when compared to the binary diets of *Tetraselmis* sp. and *I. galbana* (Knuckey *et al*. 2005). Conversely, in this study *R. salina* produced the least number of nauplii along with the 50:50 binary diet. This suggests that the suitability of a microalgal diet is species-specific and certain diets may be nutritionally deficient in promoting some life stages of copepods, while being sufficient to promote others (Camus *et al.* 2009). For example, *Pavlova* 50 was not nutritionally sufficient to promote moulting of *B. similis* from the nauplii stage 6 to the first copepodite stage, even though it was nutritionally balanced in terms of DHA and EPA (Camus *et al.* 2009).

Hatching success or failure of eggs has been linked to food limitation, along with the availability of the fatty acids; 18:3ω3, 18:5ω3 and DHA (Koski *et al*. 2006). Some nutrients may be required in higher quantities for hatching and growth than for egg production (Arendt *et al*. 2005, Koski *et al*. 2006). It has also been obseverd that different life stages of copepods respond differently to food in terms of capturing and ingestion, for example nauplii may select a different food size to copepodites or adults (Koski *et al*. 2006). In this study this implies

nauplii may have been selecting a smaller food size than copepodites and adults. This might also explain the population growth observed in copepods fed *I. galbana*. Nauplii may have been selecting food based on size and when fed *R. salina* the size of the food particle may have been above its threshold size, leading to a lower nauplii production when fed *R. salina* (Price *et al.* 1983). Therefore, while *R. salina* supports higher fecundity of *P. hessei*, *I. galbana* appears to better support copepod growth from nauplii to adult life stages.

I conclude, therefore, that the mono-algal diet *R. salina* can be used in the culture of *P. hessei* to promote fecundity while the 50:50 binary diet can be used to maintain viable stock cultures of the copepods. Based on this study the best diet for maintaining high stock density for *P. hessei* is the mono-algal diet *I. galbana*, which despite promoting low fecundity produces the highest population over 21 days.

Chapter 5: Discussion and concluding remarks

Marine fish larvae are generally small and have limited endogenous reserves and therefore require live food soon after hatching (Fleeger 2005). Even though the use of copepods has been recommended as an alternative livefood, rotifers and *Artemia* are still the most commonly used live feed organisms in mariculture hatcheries worldwide (Fleeger 2005). The DHA: EPA ratio and DHA content of a larval fish's diet significantly affects its growth and survival with different species of fish having different DHA: EPA requirements (Bell *et al*. 1985, Watanabe 1993, Nanton and Castell 1998).

When selecting suitable copepod live food in the culture of marine finfish larvae, it is important to understand aspects of the copepod's nutritional value and population dynamics (Strøttrup 2000). The main objective of this study was to determine the effect of different dietary microalgae on the fatty acid profile of the calanoid copepod *P. hessei*, with the future aim of optimising the growth and survival of marine fish larvae through the provision of required fatty acids. The aspects investigated in this study were DHA, EPA, DHA: EPA ratio, fecundity and population development which are summarised in Table 5.1.

Table 5.1: Summary of data on the use of *Rhodomonas salina*, *Isochrysis galbana* and the 50:50 binary diet in the culture of *Pseudodiaptomus hessei*.

Parameter	***R. salina***	***I. galbana***	**50:50 binary diet**	**Optimal diet**
DHA content (µg/copepod)	0.57 ± 0.99^{a}	0.69 ± 1.57^{b}	0.84 ± 2.84^{b}	*I. galbana* and 50:50 binary diet
DHA: EPA ratio	1.88^{m}	3.73^{n}	2.23^{n}	*I. galbana* and 50:50 binary diet
Fecundity (no. of eggs/female)	34.60 ± 0.77^{x}	22.88 ± 0.70^{y}	23.3 ± 0.87^{y}	*R. salina*
Population development (no. of copepods/treatment)*	144.3 ± 45.08^{q}	236 ± 53.25^{p}	145.8 ± 23.10^{p}	*I. galbana*

Different superscripts in each row denote significant difference ($p < 0.05$). *treatment= 20 L

It is evident from the study that *P. hessei* can accumulate fatty acids from its diet, and more importantly, the essential fatty acids; DHA and EPA. In terms of incorporation of DHA, the best diets were the mono-algal diets *I. galbana* and the 50:50 binary diet. In analysisng the

EPA content was similar for all three diets. The sum of essential fatty acids was different between copepods fed *R. salina* and those fed *I. galbana* ($p = 0.003$) respectively, however, those fed the binary diet had similar levels to those fed both mono-algal diets. More importantly, the DHA:EPA ratio of copepods fed *I. galbana* (3.73) was the highest. Although the ratio was not significantly different from the binary diet (2.23), it was different from that of *R. salina* (1.88), therefore we concluded that the DHA:EPA ratio of *P. hessei* is affected by diet. A similar observation was made by Nanton and Castell (1999), when feeding *I. galbana* and *D. tertiolecta* as a mono-algal diets to the harpacticoids *Tisbe* sp. and *Amonardia* sp. They found that the DHA:EPA ratio of copepods were affected by diet, with the ratio significantly higher when fed *D. tertiolecta* than *I. galbana*. ‘

Despite expections that, the best performing diet in this study would be the 50:50 binary diet because it had a balanced profile, it did not improve the essential fatty acid profile or population development of *P. hessei* any more than the mono-algal diet *I. galbana*. The total sum of EFA in the binary diet was lower than both monoalgal diets; this may be one of the reasons it did not perform better than *I. galbana* in terms of increasing DHA and the DHA’: EPA ratio.

The binary diet could possibly be improved in order to meet the nutritional needs of *P. hessei* by altering the feeding ratios of the microalgae. Based on Table 5.1, it is recommended that testing be done on a 75:25 *I. galbana* to *R. salina* treatment. The mono-algal diet *R. salina* had a significant impact on the fecundity while *I. galbana* had the impact on the DHA:EPA ratio and population development, therefore feeding at this ratio may help balance the nutritional profile of *P. hessei*. This ratio should also ensure that a sufficient concentration of *R. salina* is available to enhance fecundity, and that a sufficient concentration of *I. galbana* is available to maintain the DHA: EPA ratio and population development.

Various nutritional studies have been conducted on a range of copepods to determine their suitability for marine larviculture, with species being affected differently by the diets consumed, and the ratio at which the diets are provided (Støttrup and Jensen 1990, Graeve *et al*. 1994, Kleppel and Burkart 1995, Nanton and Castell 1998, Nanton and Castell 1999, McKinnon et al. 2003, Milione and Zeng 2007, Koski *et al*. 2006, Camus *et al*. 2009, Puello-Cruz *et al.* 2009, Camus and Zeng 2012).

Table 5.2: Summary of studies investigating the effect of diet on DHA and EPA levels, DHA: EPA ratio, fecundity and population development on various copepod species.

Copepod species	Diet	Findings	Reference
Acartia sinjiensis	*Isochrysis* sp., *H. niei*, *Rhodomonas* sp., *T. chuii*	A high DHA: EPA ratio was obtained when fed a 1:2:1:1 combination of *Isochrysis*/*Rhodomonas*/*Tetraselmis*/*Heterocapsa*. Fecundity was highest when the copepods were fed a mono-algal diet of *H. niei* (40 eggs/female/day), the second most productive diet was *Rhodomonas* sp. (33eggs/female/day).	McKinnon *et al.* (2003)
Acartia sinjiensis	*Nannochlopsis* sp., *R. maculate*, *Isochrysis* sp., *T. chuii*, *Tetraselmis* sp.	Population development was highest when copepods were fed a binary diet of *T. chuii* and *Isochrysis*, of the mono-algal diets, *Isochrysis* produced the highest population. High egg hatching success was obtained with the binary diets *Nannochloropsis* sp. and *Isochysis*, *Tetraselmis* and *Isochrysis* and the mono-algal diet of *Isochrysis*.	Milione and Zeng (2007)
Acartia tonsa	*I. galbana*, *D. tertiolecta*, *R. baltica*, *T. weissflogii*, *D. brightwellii*	Fecundity was highest when fed mono-algal diets of *I. galbana* and *R. baltica*.	Støttrup and Jensen (1990)
Acartia tonsa	*T. weissfloggi*, *A. carterii*, *I.galbana*, *D. hanseni*, *O. marina*	Egg production was highest when the copepods were fed *T. weissfloggi*. Copepods fed *I. galbana*, *D. hanseni* and *O. marina* had similar egg production rates.	Kleppel and Burkart (1995)
Amonardia sp.	*D. tertiolecta*, *I. galbana*	*Amonardia* sp. is able to synthesize DHA and EPA from EFA poor *D. tertiolecta*. It had a higher EPA content when fed *I. galbana*, DHA: EPA ratio was higher when it was fed *D. tertilecta*.	Nanton and Castell (1999)
Bestiolina similis	*Isochrysis* sp., *H. niei*, *Rhodomonas* sp., *T. chuii*	A high DHA: EPA ratio was obtained when fed a 2:1:1:1 combination of *Isochrysis*/*Rhodomonas*/*Tetraselmis*/*Heterocapsa*. Fecundity was highest when fed a mono-algal diet of *H. niei* (48 eggs/female/day), the second most productive diet was *Rhodomonas* sp. (25 eggs/female/day).	McKinnon *et al.* (2003)

Bestiolina similis	*Isochrysis* sp., *Pavlova* 50, *T. chuii*, *C. muelleri*	The highest egg production rate (44.1± 2.8 eggs/female/day) was produced by a tri-algal diet; *Isochrysis* sp., *T. chuii* and *Pavlova* 50 (1:1:1). The highest population (886.8±139.4) was obtained when the copepods were fed the tri-algal diet; *Isochrysis* sp., *T. chuii* and *Pavlova* 50.	Camus *et al*. (2009)
Calanus finmarchicus	*T. antarctica*	There was unchanged incorporation of dietary fatty acids, the dinoflagellate lipid pattern (18:4ω3 and DHA) of the copepod was replaced by the diatom lipid pattern (16:1ω7 and EPA) of the algae.	Graeve *et al*. (1994)
Calanus hyperboreus	*A. carterae*	The DHA and 18:4ω3 content of the copepod increased over time, replacing the diatom lipid pattern (16:1ω7 and EPA) of the copepod.	Graeve *et al*. (1994)
Cyclopina kasignete	*S. apiculata*, *S. pectinatus*, *T. minimum*, *Mesotaenium* sp., *Desmodesmus* sp., *N. oculata*, *Melosira* sp.	Copepods fed dry *Melosira* sp. had a higher EPA and DHA content than those fed either mixed dry algae or dry *N. oculata*. The DHA and EPA content of copepods fed dry and fresh *N. oculata* were similar. EPA content of copepods were higher than in algal diets when fed mixed dry algae, dry *Melosira* sp. or fresh *T. lutea*. Copepods fed dry *Melosira* sp., mixed dry algae, fresh *T. lutea*, dry *N. oculata* or fresh *N. oculata* had higher DHA content than the diets.	Rasdi *et al*. (2015)
Euterpina acutifrons	*Isochrysis* sp., *P. salina*, *T. chuii*, *C. muelleri*	The binary diet of *C. muelleri* and *T. chuii* supplied in 1:1 ratio supported the highest productivity (19.5±1.7 nauplii/female/day) of *E. acutifrons* naupliar, similar to the mono-algal diet of *C. muelleri* (13.5±1.1 nauplii/female/day).	Camus and Zeng (2012)
Parvocalanus crassirostris	*Isochrysis* sp., *H. niei*, *Rhodomonas* sp., *T. chuii*	When fed a combination of *Isochrysis*/*Rhodomonas*/*Tetraselmis*/*Heterocapsa*, 2:1:1:1, DHA:EPA ratio was 2:1. The highest fecundity was when fed a mono-algal diet of *H. niei* (31 eggs/female/day), the second most productive diet was *Rhodomonas* sp (15 eggs/female/day).	McKinnon *et al*. (2003)
Pseudocalanus elongatus	*Dunaliella* sp., *Amphidinium* sp., *C. polylepis*, *Synechococcus* sp. *Rhodomonas* sp.	A mixture of *Dunaliella* sp. and *Rhodomonas* sp. supplied in 10:1 ratio resulted in the highest egg production, along with a combination of *Dunaliella* sp. and *Synechococcus* sp. supplied in 1:1 ratio.	Koski *et al*. (2006)
Pseudodiaptomus euryhalinus	*C. muelleri*, *N. oculata*, *I. galbana*, *T. suecica*, *Tetrasemis* sp.	The highest number of copepods produced were those fed *C. muelleri* (462.92±152.5 organism/L) as a mono-algal diet, followed by *I. galbana* and a binary diet of *C. muelleri* and *I. galbana* (296±114.74 organism/L) supplied in 1:1 ratio.	Puello-Cruz *et al*. (2009)

Pseudodiaptomus hessei	*I. galbana, R. salina*	The highest DHA: EPA ratio was obtained when the copepods were fed *I. galbana* and a 50:50 binary diet of *I. galbana* and *R. salina*. The highest population was obtained with a diet *of I. galbana* (236.4±53.25 no. of copepods/treatment) and the highest fecundity obtained when fed *R. salina* (34.60±0.77 no. of eggs/female).	This study
Pseudodiaptomus pelagicus	*I. galbana, T. weissfloggi, C. glacilis, R. lens, T. suecica*	*T. weissfloggi* produced the highest population (1250.0±100.7) and the highest fecundity. The binary diet of *I. galbana* and *T. weissfloggi* provided in 1:1 ratio produced the highest population (830.0±283.8), and the mono-algal diet *T. weissfloggi* produced the highest mean fecundity.	Ohs *et al.* (2010a)
Tisbe sp.	*C. calcitrans, D. tertiolecta, I. galbana, S. cerevisiae*	*Tisbe* sp. fed *I. galbana* and *C. calcitrans* both produced copepods with a high DHA content, but were lacking EPA, however copepods fed yeast had the highest DHA and EPA content. The DHA: EPA ratio was similar in all diets.	Nanton and Castell (1998)
Temora longicornis	*T. weissfloggi, P. globosa, Isochrysis* sp., *D. tertiolecta*	The highest egg production rate and hatching success was obtained with *Isochrysis* sp. and *T. weissfloggi*. There was a positive correlation between dietary EPA and egg production rate and hatching success. There was a positive correlation between DHA: EPA ratio and hatching success.	Arendt *et al.* (2005)
Temora longicornis	*Dunaliella* sp., *Amphidinium* sp., *C. polylepis, Synechococcus* sp. *Rhodomonas* sp.	The mixture of *Dunaliella* sp. and *Rhodomonas* sp. provided in 10:1 ratio resulted in the highest egg production, along with a 1:1 mixture of *Dunaliella* sp. and any of the microalgal diets (*Amphidinium* sp., *C. polylepis, Synechococcus* sp.)	Koski *et al.* (2006)
Temora longicornis	*T. weissfloggi, L. danicus, D. tertriolecta, T. suecica, A. carterii*	The mono-algal diet *T. weissfloggi* produced the highest fecundity followed by a binary diet of *T. weissfloggi* and *L. danicus* supplied at a ratio of 1:1.	Jónasdóttir *et al.* (2009)

A. caterii= Amphidinium caterii; C. calcitrans= Chaetocerus calcitran; C. glacilis= Chaetoceros glacilis; C. muelleri= Chaetoceros mueller; C. polylepis= Chrysochromulina polylepis; D. hanseni= Debariomyces hanseni; D. tertiolecta= Dunaliella tertiolecta; H. niei= Heterocapsa niei; I. galbana= Isochrysis galbana; L. danicus= Leptocyclindricus danicus; N. oculata= Nannochloropsis oculata; O. marina= Oxyrrhis marina; P. salina= Pavlova salina; P. globosa= Phaeocystis globosa; R. lens= Rhodomonas lens; R. maculata= Rhodomonas maculate; R. salina= Rhododmonas salina; S. cerevisiae=Saccharomyces cerevisiae; S. pectinatus= Scenedesmus pectinatus; S. apiculata= Schroederiella apiculata; T. antarctica= Thalassiosira antarctica; T. minimum= Tetraedrom minimum; T. chuii= Tetraselmis chuii; T. suecica=Tetraselmis suecica; T. weissfloggi= Thalassiosira weissfloggi.

Table 5.2 summerizes the literature on the effect of diet on copepod productivity, development and nutrition. Based on this, it is evident that species of microalgae such as *T. weissfloggi* and *Tetraselmis* sp. should be tested to investigate whether they can improve the fatty acid profile and productivity of cultured *P. hessei*. These microalgal species have been used to successfully culture various copepods such as; *T. longicornis* and *A. tonsa*, although with *T. weissfloggi* high productivity and good nutritional was obtained (Kleppel and Burkart 1995, Arendt *et al.* 2005, Jónasdóttir *et al*. 2009, Ohs *et al.* 2010a). *Tetraselmis* sp. has been used in the culture of *A. sinjiensis* (Milione and Zeng 2007).

It is important to consider the labour requirements and costs involved in algal culture (Milione and Zeng 2007). From an operational perspective, the use of mono-algal diets would be easier for hatchery management compared to a combination of microalgae. This reduces the cost of labour, space and more effort can be used to produce a higher quality algae under specific conditions (Rivero-Rodriguez *et al*. 2007).

One of the major bottlenecks in the production of copepods as live food for marine fish larvae is their low productivity in mass culture (Milione and Zeng 2007, Camus and Zeng 2009). This trend was observed in this study. The number of eggs produced was not proportional to the number of nauplii available. This may be due to several reasons, first that *P. hessei* adults consume nauplii. Cannibalism has been observed in some calanoid copepods such as *Acartia sinjiensis*, where cannibalism of nauplii by adults and late copepodite stages (C5) increased as stocking density increased (Camus and Zeng 2009), however the densities obtained by Camus and Zeng (2009) were not reproduced in this study. Another reason could be the provision of inappropriate or insufficient food for the developing nauplii, as different stages feed on different food particles (DeMott 1989). Third, abiotic factors may be play a role in the survival of the copepods (Chen *et al.* 2006). The nauplii may have also been killed when cleaning the culture vessels using sieves. Further stuies towards improving the culture of *P. hessei* may involve investigating cannibalism and stocking density. Larger culture vessels may also improve the productivity and reduce cannibalism and delayed hatching we believe due to increased stocking density (Camus and Zeng 2009).

When comparing *P. hessei* with other copepod species such as *C. kasignete* fed different dietary microalgae (Rasdi *et al*. 2015), *P. hessei* was similar in that its fatty acid profile could be altered by its diet. Moreover, copepods such as *A. sinjiensis*, *B. similis* and *P. crassirostris*, were found to meet the required DHA: EPA ratio of 2:1 or more when fed dietary microalgae, *P. hessei*

also met and exceeded the required DHA: EPA when fed *I. galbana* and the binary diet. When fed *R. salina, P. hessei* could improve its fecundity, as seen with *A. sinjiensis*, *B. similis* and *P. crassirostris* when fed *H. niei* and *Rhodomonas* sp. (McKinnon *et al*. 2003). *P. hessei* was however, different to the harpacticoid *Tisbe* sp., which can synthesise the fatty acids DHA and EPA (Nanton and Castell 1998). Harpacticoid copepods, however, are difficult to culture as they adhere to walls and stay at the bottom of culture tanks, unlike calanoid copepods (Nanton and Castell 1998).

The marine fish species, *T. carolinus* fed *P. pelagicus* nauplii, either as a sole diet or mixed with rotifers resulted in significantly better survival of marine fish larvae than copepods fed only rotifers (Cassiano *et al*. 2011), showing that there may be potential in feeding *P. hessei* to marine fish larvae.

The average final population numbers of *P. hessei* are comparable to those reported by other studies. For example, *B. similis* cultured over 12 days with different dietary microalgae had final population numbers of 886 ± 139.4 when fed a tri-algal diet of *Pavlova* 50, *Tetraselmis chuii* and *Isochrysis* sp. and 541 ± 53.6 when fed a binary diet of *T. chuii* and *Isochrysis* sp. (Camus *et al*. 2009). With *P. hessei,* the final population numbers were 709 ± 92.23 over 21 days of culture when fed a mono-algal diet of *I. galbana,* and 433 ± 78.08 when fed *R. salina*

In conclusion, *P. hessei* has a DHA:EPA ratio that met or exceeded the recommended 2:1 DHA:EPA ratio required by marine fish larvae. Moreover, when fed *I. galbana*, high yields of *P. hessei* could be obtained while providing the copepods with a HUFA rich diet and maintaining a high DHA:EPA ratio. Therefore, the calanoid copepod, *P. hessei* is recommended as alive feed diet for marine fish larvae.

Additional research is necessary to better investigate the effect of other diets on the fatty acid and amino acid profiles of *P. hessei*. Further, investigation into the effects of high stocking density on culture productivity, egg hatching rate, survival of nauplii and copepodites under different culture conditions is necessary. The effect of high stocking density on the above mentioned factors may be investigated by conducting experiments at different stocking densities, with at least five replicates per treatment. Daily egg production, hatching success and cannibalism of adults and late copepodites toward nauplii could be monitored daily. Optimal culture conditions of *P. hessei* could then be assessed over a number of days.

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