Master's thesis

NTNU
Norwegian University of Science and Technology
Faculty of Natural Sciences
Department of Biology

Laura García Calvo

Effects of different live feeding regimes on the development and lipid composition of ballan wrasse (*Labrus bergylta*) larvae

Master's thesis in Ocean Resources
Supervisor: Elin Kjørsvik
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Abstract

Salmon louse (*Lepeophtheirus salmonis*) continues to be one of the greatest challenges hampering the expansion of Norwegian salmon aquaculture, negatively affecting production, fish welfare, and wild salmon populations. The efficiency of chemical and non-medicinal delousing methods has decreased due to the ability of salmon louse to adapt and develop resistances. Cleaner fish, which can feed on this ectoparasite, have been proposed as a suitable alternative to traditional delousing. Among cleaner fish, ballan wrasse (*Labrus bergylta*) is the most robust and efficient salmon louse grazer. However, it is less farmed than other cleaner fish species, such as lumpfish (*Cyclopterus lumpus*). This incongruity can be explained by the problems associated with the rearing of ballan wrasse.

One of the main challenges associated with ballan wrasse farming is feed intake and nutrition during early larval stages, with lipids being one of the most important nutrients. Like other marine pelagic fish species, ballan wrasse larvae are little developed and small after hatch, and they rely on live feeds. The most extended feeding regimes for larval ballan wrasse consist of rotifers followed by brine shrimp (*Artemia* sp.), both enriched in lipids. However, these diets are not optimal, as demonstrated by the high mortalities, incidence of deformities, and poor growth and development observed in larvae fed on rotifers and *Artemia*. A novel live feed available in the market is copepods, which are part of the natural diet of larval ballan wrasse, and their nutritional composition is thus considered appropriate. The use of copepods in marine fish larvae improves survival, development, stress tolerance, and growth. Cirripeds are also part of the natural diet of ballan wrasse and are now commercially available as cryopreserved cirriped nauplii. In the present work, copepods and cirripeds were evaluated as substitutes for rotifers and *Artemia* in ballan wrasse. Four different diets were supplied from the onset of the exogenous feeding phase (4 days post hatch, DPH) until weaning to formulated dry feed, consisting of either rotifers or rotifer-replacers (copepods or experimental small cirripeds) from 4 to 18 DPH, followed by either *Artemia* or *Artemia*-replacers (cryopreserved large cirripeds) from 18 to 32 DPH. Survival, growth, and lipid composition were evaluated.

The results of the present study show that the first feeding diet affects the survival, growth, and lipid composition of ballan wrasse early-stage larvae. The results confirm *A. tonsa* as a suitable first feed for ballan wrasse leading to higher survival and better growth and development than rotifers. We found that cirripeds are a favorable alternative to *Artemia* and that the lipid composition of the diet impacts that of the larvae. The findings in the present work also indicate that DHA levels in the diet are only directly correlated with DHA content in ballan wrasse larvae for some types of diets (not *Artemia*) and that higher DHA/EPA ratios in the feed are correlated with higher standard growth rate.

Sammendrag

Lakselus (*Lepeophtheirus salmonis*) utgjør en av de største utfordringene for utvidelsen av norsk lakseoppdrett, og påvirker produksjonen, fiskevelferden og villaksbestandene negativt. Effektiviteten til både kjemiske og ikke-medisinske avlusingsmetoder har blitt redusert på grunn av lakselusens evne til å tilpasse seg og utvikle resistens. Rensefisk har blitt foreslått som et egnet alternativ til tradisjonell avlusing. Blant rensefisk er berggylt (*Labrus berggylta*) den mest robuste og effektive. Imidlertid er berggylt mindre oppdrettet enn andre rensefiskarter, som rognkjeks (*Cyclopterus lumpus*), noe som kan forklares med utfordringene knyttet til oppdrett av berggylt.

En av hovedutfordringene ved berggylt-oppdrett er fôropptak og ernæring i tidlige larvestadier, hvor lipider er et av de viktigste næringsstoffene. Som andre marine pelagiske fiskearter er berggyltlarver små og lite utviklede etter klekking, og de er avhengige av levende fôr. De mest utbredte fôringsregimene for berggyltlarver inkluderer rotatorier etterfulgt av *Artemia* sp., begge anriket med lipider. Imidlertid er disse diettene ikke optimale, noe som resulterer i høy dødelighet, deformiteter og dårlig vekst og utvikling hos larver som blir matet med rotatorier og *Artemia*. Et nytt levende fôr som nå er tilgjengelig på markedet, er copepoder. Copepoder er en naturlig del av berggyltlarvenes kosthold, og deres ernæringsmessige sammensetning anses som passende. Bruk av copepoder i marine fiskelarver forbedrer overlevelse, utvikling, stresstoleranse og vekst. Cirripedia (rurnauplier) er også en del av berggylts naturlige kosthold og er nå kommersielt tilgjengelige som kryokonserverte rurnauplier. I dette arbeidet ble copepoder og rurnauplier evaluert som erstatninger for rotatorier og *Artemia* i berggylt-oppdrett. Studien omfattet fire ulike dietter gitt til berggyltlarver fra begynnelsen av den eksogene fôringsfasen (4 dager etter klekking, DPH) til avvenning med formulert tørrfôr. De ulike diettene besto av rotatorier eller rotatorier-erstattere (copepoder eller eksperimentelle små rurnauplier) fra 4 til 18 DPH, etterfulgt av *Artemia* eller *Artemia*-erstattere (kryopreserverte store rurnauplier) fra 18 til 32 DPH.

Resultatene viser at den første fôringen har stor innvirkning på overlevelse, vekst og lipidsammensetning hos berggyltlarver. *A. tonsa* (copepod) ble bekreftet som et passende førstefôr for berggylt, da det førte til høyere overlevelse og bedre vekst sammenlignet med rotatorier. Vi fant også at rurnauplier er et gunstig alternativ til *Artemia*, og at lipidsammensetningen i dietten påvirker larvenes egen sammentsetning. Funnene indikerer også at DHA-nivåer i fôret kun har en direkte korrelasjon med DHA-innholdet i berggyltlarver for visse typer fôr (ikke *Artemia*). Videre er et høyere DHA/EPA-forhold i fôret assosiert med bedre standardvekst hos larvene. Dette arbeidet gir verdifull innsikt i fôringspraksis for berggyltlarver og kan bidra til å optimalisere oppdrettet av denne arten.

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3 List of Abbreviations (or Symbols)

ALA	α-linolenic acid
ARA	Arachidonic acid
CE	Colesteryl ester
DHA	Docosahexaenoic acid
DO	Dissolved oxygen
DPA	Docosapentaenoic acid
DPH	Days post hatch
DW	Dry weight
DWI	Daily weight increase
EPA	Eicosapentaenoic acid
ESI	Electrospray ionization

FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organization of the United Nations
FFA	Free fatty acid
FID	Flame ionization detection
GC	Gas chromatography
HUFA	Highly unsaturated fatty acid
LA	Linoleic acid
LC	Liquid chromatography
LOD	Limit of detection
lysoPC	Lysophosphatidylcholine
lysoPE	Lysophosphatidylethanolamine
MAG	Monoacylglycerols
MRM	Multiple reaction monitoring
MS	Mass spectrometry
NL	Neutral lipids
PC	Phosphatidylcholines
PCA	Principal component analysis
PE	Phosphatidylethanolamines
PI	Phosphoinositides
PL	Phospholipids
PS	Phosphoserine
PUFA	Polyunsaturated fatty acid
RAS	Recirculating aquaculture systems
RT	Retention time
SD	Standard deviation
SDG	Sustainable Development Goals
SGR	Specific growth rate
SL	Standard length
sPLS-DA	Sparse partial least squares regression - discriminant analysis
TAG	Triacylglyceride, tryacylglycerols
UN	United Nations
WE	Wax ester

1 Introduction

1.1 Norwegian salmon aquaculture and its challenges

Aquaculture, which can be defined as the farming of aquatic organisms, including fish, mollusks, crustaceans, and aquatic plants, is the world's fastest growing food sector (Afewerki et al., 2022; Bostock et al., 2010; FAO, 2022; Tidwell & Allan, 2001; Troell et al., 2014; Verdegem et al., 2023). Production in the European region is led by Norway, which is also the second world's largest exporter of aquatic animal products and a key player in mariculture of finfish species in sea cages, especially Atlantic salmon (*Salmo salar*) (FAO, 2022; Naylor et al., 2021).

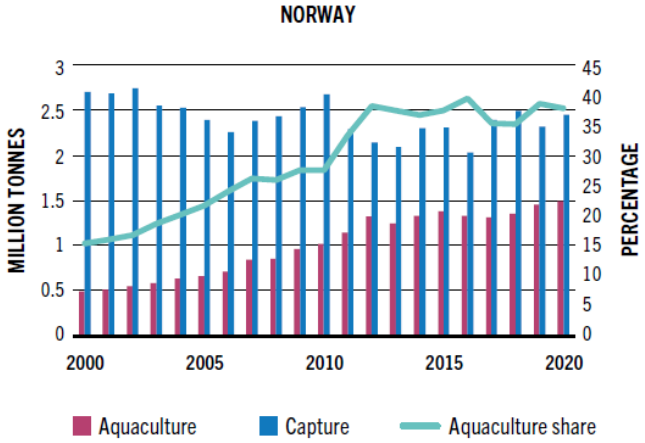


Figure 1.1. Contribution of aquaculture to total fisheries and aquaculture production (excluding algae) by region, 2000–2020. Panel focusing on Norway. Modified from (FAO, 2022).

The history of Norwegian aquaculture began in the 1950s. The introduction of sea pens in the 1970s and further developments in genetics, fish health, juvenile production, feed and primary fish processing have catapulted salmon farming in Norway, making this sector one of the most important and representative of the Norwegian economy (Afewerki et al., 2022; Bjørndal & Tusvik, 2019; Johansen et al., 2019; Moe Føre et al., 2022; Øvrebø et al., 2022). Atlantic salmon aquaculture has been one of the fastest growing economic sectors in Norway in the past decades, with a value increase of more than 50 NOK billion from 1998 to 2018 (Statistisk sentralbyrå (SSB), 2019a, 2019b). The production volume of Norwegian salmon exceeded 1.5 million tons in 2022, which represents a 1.5-fold increase from 2002 (Fiskeridirektoratet, 2022; Olaussen, 2018). The expected future trends for Norwegian aquaculture indicate that further increase is possible, and the industry

intends to grow by a factor of five by 2050 (Olafsen et al., 2012; Tveterås et al., 2019). However, despite the goals of the industry, the expansion of salmon farming in Norway has stagnated in the past years due to important **challenges**, such as environmental pollution, escapes of salmon, and the parasite salmon louse (Naylor et al., 2005; Taranger et al., 2015). Salmon louse and its well-documented increasing resistance to chemotherapeutants constitutes the most persistent problem challenging global salmonid aquaculture (Fjørtoft et al., 2020; Aaen et al., 2015)

1.2 The fight against salmon lice

One of the main challenges hampering the growth of Norwegian aquaculture is the stenohaline caligid copepod salmon louse (*Lepeophtheirus salmonis*), an ectoparasite first described as early as the XVIII century. Its life cycle comprises several stages, including non-feeding planktonic larvae, infective planktonic copepodites, immature chalimi embedded on the host skin, and mobile pre-adults and adults that can change position freely over the host (Pike & Wadsworth, 1999; Torrissen et al., 2013).

Salmon louse affects salmonids (genera *Salmo*, *Oncorhynchus*, and *Salvelinus*) in seawater and occurs naturally with a circumpolar distribution in the Northern Hemisphere, also in the absence of intensive salmon aquaculture (Torrissen et al., 2013). However, the high densities of suitable host fish in sea cages favor the growth and transmission of this parasite (Jackson et al., 2018; Olaussen, 2018; Pike & Wadsworth, 1999; Torrissen et al., 2013). Salmon louse feeds on blood, mucus, skin, and tissue, causing physical and biochemical damage in the form of wounds and risk of secondary infections, osmoregulatory imbalance, increased stress, and immunosuppression (Brooker et al., 2018). The damage caused by this parasite comes with important associated economic costs, biomass losses, fish welfare concerns, as well as conservational worries because of the effects of increased densities of this parasite on wild salmonids (Abolofia et al., 2017; Erkinharju et al., 2020; Groner et al., 2016; Krkosek et al., 2007; Torrissen et al., 2013). In Norway, regulations have been put in place to control infestations, legally mandate treatment, and govern the possibilities of expansion of salmon aquaculture in areas heavily affected by salmon lice (Bailey & Eggereide, 2020; Geitung et al., 2020; Heuch et al., 2005). Although remarkable progress has been achieved in diagnostics, treatment, and management of affected areas, sea lice continue to be a recalcitrant problem (Stentiford et al., 2017).

Different treatments have been developed to try to control salmon lice, known as **delousing methods**. Delousing methods range from chemical to non-medicinal. Some chemotherapeutics that have been in use include pyrethrin/pyrethroid compounds, organophosphates, avermectines, benzoylphenyl ureas, and one oxidizing agent (hydrogen

peroxide) (Roth, 2000). On the other hand, the non-medicinal methods include mechanical treatments (brushes or water jets, such as hydrolicer), thermal treatments (e.g. thermolicer), structural innovations in the sea cages, use of freshwater, feed additives, selective breeding, light modification, vaccines, and passive lice control using laser technology, among others (Afewerki et al., 2022; Barrett et al., 2020; Bergheim, 2012; Brooker et al., 2018; Dempster et al., 2009; Erkinharju et al., 2020; Hevrøy et al., 2003; Jackson et al., 2018; Torrissen et al., 2013). However, although there is a variety of delousing methods, several of them have experienced **decreased efficacy** due to the great capacity of sea louse to develop resistances, adapt, and evolve towards increased early fecundity and earlier reproduction (Mennerat et al., 2010; Torrissen et al., 2013). Some of the medicinal treatments can bioaccumulate, threatening the environment and affecting wild fauna that was not the intended target, for instance, wild crustaceans that are close to salmon net-pens (Hamre, Nordgreen, et al., 2013). Furthermore, many of the non-medicinal treatments pose a threat to the health and welfare of the fish, leading to increased stress and mortality (Overton et al., 2019). Because of this, alternative methods were desperately needed. In this regard, the use of cleaner fish has been introduced as a biological salmon louse control method.

Cleaner fish are fish species that can use specialist feeding strategies to directly capture and eat salmon louse from salmon while cohabitating with them in sea cages. Cleaner fish have increasingly become one of the most important alternative delousing methods in Norwegian salmon aquaculture, especially since 2010 and during the last decade (Brooker et al., 2018; Fiskeridirektoratet, 2023). Five species of cleaner fish were utilized in Norway in 2022: lumpfish (*Cyclopterus lumpus* Linnaeus, 1758) and four different species within the wrasse family (order Labriformes, family Labridae), mainly ballan wrasse (*Labrus bergylta* Ascanius, 1767), followed by goldsinny wrasse (*Ctenolabrus rupestris* Linnaeus 1758), corkwing wrasse (*Symphodus melops* Linnaeus, 1758) and rock cook (*Centrolabrus exoletus* Linnaeus, 1758) (Fiskeridirektoratet, 2023; Skiftesvik et al., 2013). Of these four species, only corkwing and ballan wrasse grow large enough to make them suitable cleaner fish for salmon during their second year in the pens. Ballan wrasse is not only the largest of the two, but also the most robust, fastest growing, and more efficient grazer than other wrasses or lumpfish, which makes it the most valuable wrasse species for the aquaculture industry (Kvenseth et al., 2003; Leclercq et al., 2014; Skiftesvik et al., 2013). Although ballan wrasse is the most effective grazer of sea lice, the most prevalent cleaner fish species in Norway in 2022 was in fact lumpfish. This apparent incongruity is largely due to the difficulties associated with farming of ballan wrasse, especially when it comes to broodstock management and rearing of early larval stages (Brooker et al., 2018), as well as other factors like the ability of lumpfish to continue feeding at lower temperatures (Powell et al., 2017). New research is needed to implement practices that can improve the

quality, growth, and survival of farmed ballan wrasse, and early-stage larval nutrition is a field with considerable room for improvement.

The biology, physiology, and ecology of ballan wrasse, as well as its particularities and challenges as a farmed species, are explored in the following section.

1.3 Biological delousing: ballan wrasse

1.3.1 Ecological, biological, and physiological aspects of *L. bergylta*

The *Labridae* family comprises species that inhabit marine rocky reefs and kelp beds in coastlines of the Pacific, Atlantic, and Indian oceans (Dipper et al., 1977; Villegas-Ríos et al., 2013). Ballan wrasse in particular can be found in the north-east Atlantic Ocean, ranging from the south of Norway to Morocco (Quignard & Pras, 1986). The etymology of the name of this family of fishes derives from the Latin term *labrum*, meaning "lip", due to the aspect of their pharyngeal region. The different wrasse species present distinct morphologies and color patterns and also share some common traits (Quignard & Pras, 1986; Sayer & Treasurer, 1996). Wrasses can feed on prey with hard shells, such as crustaceans, thanks to their strong teeth, protractile mouths, and protruding thick lips (Erkinharju et al., 2020).

L. bergylta is a diurnal and territorial fish (Villegas-Ríos et al., 2013). As a protogynous hermaphrodite, ballan wrasse develops as an immature female, subsequently maturing as a female and then, in some cases, changing sex to a mature male (Godwin, 2019). The natural spawning window for ballan wrasse ranges from April to June, although it can be further extended artificially in captivity (Grant et al., 2016). Puberty occurs at approximately 6 years of age for females and 12 for males, and some individuals surpass longevities of 20-25 years (Dipper et al., 1977; Espeland et al., 2010; Halvorsen et al., 2020). Adults are relatively stationary and display high site fidelity (Espeland et al., 2010; Villegas-Ríos et al., 2013). *L. bergylta* is a predominantly carnivorous fish, and its diet as an adult includes primarily invertebrates such as decapods and other crustaceans, bivalve mollusks, as well as echinoderms, algae, and gastropods, the two latter in lower quantities (Deady & Fives, 2009).

The first report of parasite cleaning activity in wrasses was published in the early 1970s (Potts, 1973), a finding that was subsequently confirmed in field observations (Hilldén, 1981). The first laboratory trials with wrasses as a cleaner fish took place by the end of the 1980s. Later, field experiments in sea cages commenced (Bjordal, 1988; Espeland et al., 2010; Skiftesvik et al., 2013; Torrissen et al., 2013). In 2009, Marine Harvest Labrus (Naturgassparken, Øygarden, Norway) kickstarted large-scale production of *L. bergylta* in

Norway (Hansen et al., 2013). From, 2010, the use of cleaner fish as a biological non-medicinal method for delousing has expanded rapidly (Faust et al., 2018).

1.3.2 Ballan wrasse as a farmed cleaner fish species

Up until around 2011, all wrasses deployed in salmon pens as cleaner fish were wild-caught (Brooker et al., 2018). Out of the wrasse species that are most suitable to be used as cleaner fish, *L. bergylta* is the least abundant in nature (Skiftesvik et al., 2013; Skiftesvik et al., 2015). The increased demand for cleaner fish and the necessity of the industry to become self-sufficient and independent from wild stocks has fueled the incorporation of ballan wrasse into the repertoire of farmed cleaner fish (Brooker et al., 2018; Kousoulaki et al., 2015; Kousoulaki et al., 2021). Ballan wrasse and lumpfish are currently the only cleaner fish species farmed in Norway (Fiskeridirektoratet, 2023; Halvorsen et al., 2020). Ballan wrasse, despite being the most robust and efficient salmon louse grazer, continues to be less farmed, and therefore less used, than lumpfish (Fiskeridirektoratet, 2023). This can be explained by the challenges associated with the rearing of ballan wrasse (Kousoulaki et al., 2015). A study has shown that farmed ballan wrasse can be equally effective as a cleaner fish as its wild counterpart, even with no prior contact with salmon or salmon lice (Skiftesvik et al., 2013), but improvements are needed in order to make ballan wrasse farming sustainable and more efficient.

Broodstock management is challenging in the case of ballan wrasse due to their special physiology and life cycle, which includes a long generation time, a highly skewed sex ratio, and complex hierarchy and mating systems. These aspects determine that, currently, hatchery production is still based on wild-caught broodstock (Brooker et al., 2018).

The deployment size is reached after approximately 18 months, when fish is typically at least 40-50 g, to prevent escapes from the salmon sea cages (Helland et al., 2014). Temperature is an important parameter that determines the deployment window. Ballan wrasse prefers temperate waters, has reduced activity below 10 °C and enters torpor below 6 °C (Geitung et al., 2020; Kottmann et al., 2023; Morel et al., 2013; Yuen et al., 2019). Lower temperatures also negatively impact welfare and lead to increased stress (Kottmann et al., 2023). This is in contrast with lumpfish, which has different habits and behavior and favors lower temperatures. A combined deployment strategy where both lumpfish and ballan wrasse are used in the pens may therefore be more beneficial than only employing one of these species (Brooker et al., 2018).

The low salmon lice densities that are desirable and required in a salmon sea cage are not enough to sustain a population of cleaner fish, and supplying them with additional feed is critical to ensure the health and welfare of ballan wrasse (Leclercq et al., 2015; Skiftesvik et al., 2013). Ballan wrasse deployed in sea cages is not only exposed to nutritional

challenges but also bacterial, fungal, parasitic, and viral infectious diseases (Brooker et al., 2018). There are also increasing welfare concerns around cleaner fish used in salmon net-pens (Brooker et al., 2018; Kottmann et al., 2023; Powell et al., 2017) and a series of operational and laboratory-based welfare indicators have recently been proposed for ballan wrasse (Noble et al., 2019).

One of the main technical and biological challenges in ballan wrasse aquaculture is poor performance during the first larval stages, where high mortality, reduced growth rate, and deformities are common. Poor nutrition and feed intake are important causes of low performance (Evjemo et al., 2003; Hansen et al., 2013; Kousoulaki et al., 2015; Lall & Lewis-McCrea, 2007). The first stages of development are a very vulnerable time for many marine pelagic fish larvae, which are small and little developed. For them to survive and develop correctly, fish larvae require specific biotic and abiotic conditions (Brooker et al., 2018; Hamre, Yúfera, et al., 2013). Moreover, the nutritional quality of the feeds during larval stages follows the fish along its life and determines their growth and development, tolerance to stress, and survival (Kjørsvik et al., 2014). Although the field of larval nutrition for ballan wrasse is progressing rapidly, there are still major knowledge gaps when it comes to the environmental, nutritional, and biological requirements of this species (Brooker et al., 2018). The state of the art of larval nutrition in ballan wrasse aquaculture, with a special focus on lipidic nutrients, is summarized in the following section.

1.4 Larval rearing of ballan wrasse and the importance of the diet

During ontogenesis, marine fish larvae undergo intense changes in morphology and physiology, such as metamorphosis, requiring specific nutritional requirements that may differ qualitatively and quantitatively from their juvenile and adult counterparts. For instance, the requirements of certain essential fatty acids are higher in the early stages (Izquierdo, 1996; Sargent et al., 1997). The diet must also support the dramatic growth rates observed in many species during larval stages (Hamre, Yúfera, et al., 2013).

The digestive system of ballan wrasse is an undifferentiated tube without a stomach and pyloric caeca, and the fish remains agastric as an adult. Furthermore, during larval stages, the production of digestive enzymes is low (Gagnat, 2012; Hansen et al., 2013). These physiological particularities determine that the start feeding diet must include free amino acids, short-chain peptides, and an appropriate lipid profile (Hansen et al., 2013).

The current standard start feeding diet in ballan wrasse hatcheries consists of a two-month live feed period, starting with n-3 highly unsaturated fatty acids (HUFA)-enriched rotifers from the onset of the exogenous feeding phase until 25-30 days post hatch (DPH), and continuing with n-3 HUFA-enriched nauplii of brine shrimp (*Artemia* sp.) (Brooker et al.,

2018; Hamre et al., 2008; Sargent et al., 2003). At around 70-90 DPH, after the initial live feed period, larvae are weaned to formulated feeds (Brooker et al., 2018). Rotifers and *Artemia* are easy to cultivate in a larval rearing facility and can be relatively affordable. However, their nutritional composition differs from that of the natural diet of ballan wrasse larvae in the wild (Sargent et al., 2003; Øie et al., 2015). This may be one of the causes behind the high mortality, deficient growth, and presence of deformities that are commonly observed during intensive farming of ballan wrasse larvae (Gagnat et al., 2016; Kjørsvik et al., 2014). A major part of the diet of marine fish larvae in their natural habitat is zooplankton, such as copepods, which are considered an optimal live first feed for marine fish larvae (Støttrup, 2000; van der Meeren et al., 2014). In this regard, previous studies have explored copepods as live start feed for marine fish larval stages, finding that this improved the survivability, growth, development, and pigmentation of the larvae (Evjemo et al., 2003; Gagnat, 2012; Gagnat et al., 2016; Karlsen et al., 2015; Liu & Xu, 2009; Romundstad, 2015; Rønnestad et al., 1998; Stavrakaki, 2013; van der Meeren et al., 2014; Øie et al., 2015). Positive long-term effects of using copepods as a first feeding live feed have been demonstrated even when they were provided for short periods (Øie et al., 2015). Copepods present high amounts of protein, n-3 fatty acids in the polar lipid fraction, microminerals, and certain vitamins, although their composition may vary with factors such as farming technique and environment, species, feed, and geographical area of harvest or cultivation, among others (Hamre, Yúfera, et al., 2013).

Nutritional analyses have indicated differences between rotifers, which can be considered the industry standard, and zooplankton when it comes to amino acids such as taurine, peptides, vitamins, and minerals like Se, as well as fatty acid composition. Copepods are naturally rich in essential n-3 and n-6 HUFAs, mainly integrated in their phospholipids, which are crucial for correct development, growth, fish welfare, and quality (Bell et al., 2003; Li et al., 2015; Rønnestad et al., 1998; Rønnestad et al., 2013; van der Meeren et al., 2008). These differences might explain the improved overall performance when copepods are used instead of rotifers (Mæhre et al., 2013).

Copepods produced in intensive aquaculture regimes have become available in the market in the past years (CFEED, 2023). Regarding the safety of the use of industrially farmed copepods as live start feed for marine fish larvae, studies have shown that cultures of the copepod *Acartia tonsa* have good microbial water quality, low abundance of bacteria, and no indication of pathogens (Rong et al., 2022). Furthermore, other zooplankton species could be explored and added to the live feed portfolio. On this matter, cirripeds (barnacles) also constitute part of the diet of larval stages of ballan wrasse in the wild and could be therefore an interesting alternative that has not been thoroughly evaluated yet (Dipper et al., 1977). Cryopreserved planktonic crustaceans in the subclass Cirripedia also have

become commercially available in the past years (Planktonic, 2023), but have not been thoroughly evaluated as a first feeding diet for larval stages of ballan wrasse to this date.

Although zooplankton is a promising live feed alternative for early larval stages of ballan wrasse, more in-depth studies are needed. Feeding regimes must be further explored so that they can be optimized for intensive larval rearing, and the implications of the nutritional composition of these novel live feeds must be better understood (Dhont et al., 2013).

Concerning the characterization of novel live feed sources for marine fish larvae, one of the most critical nutrient groups is lipids. Lipids are required by fast-growing larvae as structural components and as an energy source for growth and development (Hansen et al., 2013; Sargent et al., 1997; Watanabe et al., 1983).

1.4.1 Lipid and fatty acid requirements in fish larval first feeding diets

1.4.1.1 Lipids

Lipids can be classified into eight categories: fatty acyls, sterol lipids, prenol lipids, saccharolipids, sphingolipids, polyketides, glycerolipids, and glycerophospholipids (Fahy et al., 2005). Furthermore, the chemical characteristics and polarity of lipids allow us to classify them into two fractions: neutral lipids and polar lipids. The neutral lipid class consists predominantly of storage lipids that function as a main source of energy, with examples such as triacylglycerides (TAGs) (glycerolipids), colesteryl esters (CEs), wax esters (Wes; fatty acyls) and terpenes (prenol lipids). The polar fraction, on the other hand, includes glycerophospholipids (often referred to as phospholipids (PL)) and sphingolipids, among others (Fahy et al., 2005; Sargent et al., 2003).

PL are important nutrients for fish during their whole life, especially at larval stages, where they carry out a plethora of functions. Being amphipathic molecules, PL have a structural function as central components of the cell membrane bilayers. PL are important for lipid digestion, absorption, and transport from the intestine to other parts of the body. On this matter, they enable the transport of hydrophobic lipids, such as TAGs, in aqueous environments, and they play a role in lipid digestion, creating intra-luminal mixed micelles together with dietary lipids and bile salts (Cahu et al., 2009; Izquierdo & Koven, 2011; Izquierdo et al., 2000; Olsen & Ringø, 1997; Rønnestad et al., 2013; Tocher, 1995; Tocher et al., 2008). Apart from their structural function in biomembranes and tissues such as the eye and brain, PL also play a role in the post-transcriptional regulation of proteins, as a messenger molecule and as a precursor for biologically active mediators (Rønnestad et al., 2013; Sargent et al., 2003; Tocher, 1995; Tocher et al., 2008). Although TAGs are the primary lipid class for energy storage and provision, PL can also serve as an energy source, and this is particularly important in embryonic and early larval development in fish species

without a large oil globule in their eggs (PL-rich eggs), such as ballan wrasse (Salze et al., 2005; Tocher et al., 2008; Wiegand, 1996). Most marine fish larvae have limited biosynthetic capacity to synthesize PL *de novo*, which makes them essential nutrients that need to be supplemented with the diet (Cahu et al., 2009; Coutteau et al., 1997; Izquierdo & Koven, 2011).

1.4.1.2 Fatty acids

Fatty acids are carboxylic acids with saturated or unsaturated aliphatic chains. They can be classified into four categories depending on the length of the chain: short-chain fatty acids (<C6), medium-chain fatty acids (C6–C12), long-chain fatty acids (C13–C21), and very-long-chain fatty acids (≥C22) (Chiu & Kuo, 2020). Fish larvae require fatty acids for adequate growth and development. Among the most important fatty acids are the essential long-chain polyunsaturated fatty acids (PUFAs) eicosapentaenoic acid (EPA, 20:5n-3), docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6). Long-chain PUFAs have crucial functions in processes such as regulation of membrane fluidity, synthesis of physiologically active substances, development of neural and sensorial tissue, behavior, pigmentation (in flatfish), as well as modulation of genes, stress, and the immune system. DHA plays an especially important role as a component of PL in biomembranes, facilitating intermembrane processes and reactions. ARA is one of the main precursors for the synthesis of eicosanoids, which are local hormones that regulate a variety of pathways and cascades in the cells. EPA is also a competitive precursor with ARA for the synthesis of eicosanoids (Bell et al., 1995; Bell et al., 1986; Benítez-Santana et al., 2007; Carvalho et al., 2019; Etayo et al., 2021; Gagnat et al., 2016; Ganga et al., 2011; Izquierdo & Koven, 2011; Mourente & Tocher, 1992; Sargent, McEvoy, et al., 1999). These three long-chain PUFAs are similar chemically, which leads to competitive physiological and biochemical interactions between them, their precursors, and their products. Hence, both the quantity, the relative proportion of each fatty acid and their distribution between neutral and polar lipid classes should be considered during the study of fish larval nutrition (Coutteau et al., 1997; Izquierdo et al., 2000; Jin et al., 2017; Olsen et al., 1991; Sargent, Bell, et al., 1999; Sargent, McEvoy, et al., 1999). Dietary DHA/EPA ratios of 2:1, and dietary EPA/ARA ratios of 3.5 to 5:1 (generally, closer to 4:1) have been proposed as adequate for larval nutrition since they are the proportions commonly found in many marine species (Hamre, Yúfera, et al., 2013; Izquierdo & Koven, 2011; Sargent et al., 1997).

The nutritional requirements and relative importance of each fatty acid for marine fish larvae differ between species (Coutteau et al., 1997; Dantagnan et al., 2010). The lipid composition of the diet must not only be adapted to the species of interest but also to the age of the larvae and the degree of development of their digestive system (Izquierdo &

Koven, 2011; Izquierdo et al., 2000; Akio Kanazawa, 1993; Rønnestad et al., 2013). Furthermore, abiotic and biotic factors such as temperature, salinity, and interactions with other nutrients such as vitamins or antioxidants, can also impact the requirements for essential fatty acids (Hamre, Yúfera, et al., 2013).

As mentioned above, when evaluating the fatty acid composition of a feed, it is important to also consider in which lipid class the fatty acids are found, since this affects their digestion and incorporation. Several studies have reported better survival, growth, and organ development when feeding diets containing long-chain PUFAs in the PL class, versus the NL class (Gagnat, 2012; Gisbert et al., 2005; Hamre, Nordgreen, et al., 2013; Izquierdo et al., 2000; Kjørsvik et al., 2009; Wold et al., 2009). The natural prey that is preferred by marine fish larvae, such as copepods, have a much higher content of n-3 HUFA in the PL fraction than the two main live feeds used for marine fish larval farming: enriched rotifers and *Artemia* (Evjemo et al., 2003; Li et al., 2015; Sargent, McEvoy, et al., 1999; van der Meeren et al., 2008). Rotifers and *Artemia* have instead high amounts of α-linolenic acid (ALA, 18:3n−3) and, in less amounts, linoleic acid (LA, 18:2n−6), and must therefore be enriched with n-3 HUFAs to make them suitable for first feeding diets (Sargent, McEvoy, et al., 1999; Sargent et al., 2003). The fatty acid composition has been shown to differ between wild and cultured ballan wrasse, as well as among farmed fish reared on different feeds, which points to the diet as an important factor influencing fatty acid distribution in individuals of this species (Hamre, Nordgreen, et al., 2013).

1.5 Aims and hypotheses

The use of ballan wrasse as a cleaner fish is expanding and has experienced a growing interest in the last decade. The increase in the demand for ballan wrasse has determined that aquaculture practices needed to be developed to increase the availability of individuals to be deployed in sea cages, as well as to become independent from the natural populations of this species. Although remarkable progress has been achieved in ballan wrasse farming, some important bottlenecks need to be addressed. On this matter, rearing of early larval stages continues to be one of the most challenging production stages, where high mortality, reduced growth rate, and deformities are common. Among the main fields to explore and improve in larval farming of ballan wrasse is nutrition.

This master thesis **aimed** to study how different types of novel live feed diets supplied at the beginning of the exogenous nutritional phase affect the **survival**, **development**, and nutritional **lipid composition** of ballan wrasse larvae. Said effects were studied by applying analytical MS-based methods to explore the lipid and fatty acid composition of both the feeds and larvae, as well as monitoring growth and survival along a start feeding experiment.

Based on previous studies pointing to the benefits of using copepods as a live start feed for marine fish larvae (Evjemo et al., 2003; Gagnat, 2012; Gagnat et al., 2016; Karlsen et al., 2015; Liu & Xu, 2009; Romundstad, 2015; Rønnestad et al., 1998; Stavrakaki, 2013; Øie et al., 2015), as well as considering the natural diet of ballan wrasse larvae in the wild (Dhont et al., 2013; Dipper et al., 1977), four live start feeding diets were included in the present work: i) a control treatment or "industry standard regime", corresponding to an initial phase feeding enriched rotifers, followed by *Artemia*; ii) a copepod regime, consisting on copepods of two different sizes during the first feeding period, followed by *Artemia*; iii) experimental cirriped diet, with two sizes of cirripeds and iv) copepod/Cirripedia regime, using two sizes of copepods, followed by cirripeds.

The master thesis work was centered on exploring the following **hypotheses**:

- Hypothesis 1 (H_1): Larvae fed on copepods and Cirripedia will have better growth and development than those fed the industrial standard live feed (rotifers and *Artemia*).
- Hypothesis 2 (H_2): Larvae fed on copepods and Cirripedia will have a higher omega-3 fatty acid content than those fed on rotifers and Artemia.
- Hypothesis 3 (H_3): The lipid composition of the start feeding diets will influence and be reflected in the lipid composition of ballan wrasse larvae.

The results of this work are **important** in the context of sustainable and productive ballan wrasse aquaculture, and they are expected to positively impact the current early larval stage farming of this marine fish species. Increasing the knowledge around how the lipid composition of fish larval feed impacts the survival, development, and composition will contribute to optimizing the aquaculture efforts towards cleaner fish of higher sustainability, welfare, quality, and performance as a control mechanism against salmon lice.

The present master thesis was carried out as part of the FHF #901561 project "Startrens - Optimalisert produksjon av Rensefisk", work package 2, focused on start feeding of ballan wrasse (FHF– Fiskeri- og havbruksnæringens forskningsfinansiering, 2022; Hagemann et al., 2022). As part of the Startrens project, other master theses have studied complementary aspects of the impact of live start feeding diets on ballan wrasse larvae, namely gut and liver histology (Aase, 2022) and microbiota (Rosvoll, 2022).

2 Materials and methods

The experimental work presented in this study was carried out at the Norwegian University of Science and Technology (NTNU) Centre for Fisheries and Aquaculture (SeaLab), CodTech fish larval rearing laboratory (National Animal Experimental Facility No. 154), in Trondheim (Norway). The laboratory work was conducted according to the EU Directive 2010/63/EU for animal experiments and the Animal Welfare Act of 20th December 1974, amended 19th June 2009. The experiment was approved by the Norwegian Food Safety Authority, with project approval FOTS ID 23022.

2.1 Origin of the larvae, reception, and rearing conditions

Newly hatched larvae were provided via air transport by Mowi Rensefisk in Stord (Norway), obtained from photoperiod-manipulated wild-caught broodstock, after incubation of the eggs at 11 °C. Ballan wrasse larvae (N= approx. 736 000) were received on the 21st of February 2020, at 19:00. Larvae corresponded to two hatches: i) the night from 19th February to 20th February 2020 (four bags), and ii) the night from 20th to 21st February 2020 (six bags). Larvae were hence 0 and 1 days post hatch (DPH) at reception. Each bag contained 20 L and the number of larvae per bag was approximately similar (4.6 larvae mL^{-1}). The transport was carried out in oxygen-supersaturated plastic bags containing approximately 34 ppm seawater, stored in styrofoam boxes to minimize temperature changes. The water parameters in the bags were measured at reception with a ProDSS Multiparameter Digital Water Quality Meter (YSI, USA).

The larvae were acclimated to the new water conditions throughout 2 h, by placing the closed bags in two water tanks and progressively transferring water into the bags. The tanks contained filtered seawater thermostatically controlled at 12 °C. Acclimatized larvae were stocked in one water tank per hatch date and allowed to rest for 30 min so that dead larvae would sink to the bottom. Dead larvae were collected by siphoning them out from the bottom of the tanks and subsequently counted. Survival during transport was estimated based on the dead larval counts and live larvae counts from 5 sub-samples of 150 mL from each tank. Survival during transport was calculated to be 36% for the 1 DPH larvae and 40% for the 0 DPH, respectively. The total estimated surviving larvae before distributing between the tanks were 281 650.

The surviving larvae were distributed from the two acclimation tanks to a total of twelve 200 L tanks (Kunststoff-Spranger GMBH, Germany), transferring equal proportions of each of the two hatches to each tank. The tanks included an air hose fixed to the bottom

of a central pipe supplying mild aeration. A central outlet pipe allowed the removal of small debris and organic matter without letting the fish exit the tanks, thanks to a mesh sieve. The mesh sieve sizes were adapted to ensure a pore diameter smaller than the smaller dimension of the larvae, from 64 to 700 µm (Figure 2.1, Table 2.1). The stocking density was approximately 19 716 larvae per tank (corresponding to ~ 100 larvae L^{-1}). The transfer of the larvae to their respective tanks was completed at 00:15. Larvae were kept in the dark and without food supply from the reception day until the beginning of the start feeding experiment at 4 DPH. Water parameters were also measured during these days and tanks were cleaned, minimizing disturbing the larvae as much as possible. Dead larvae were removed for survival calculations.

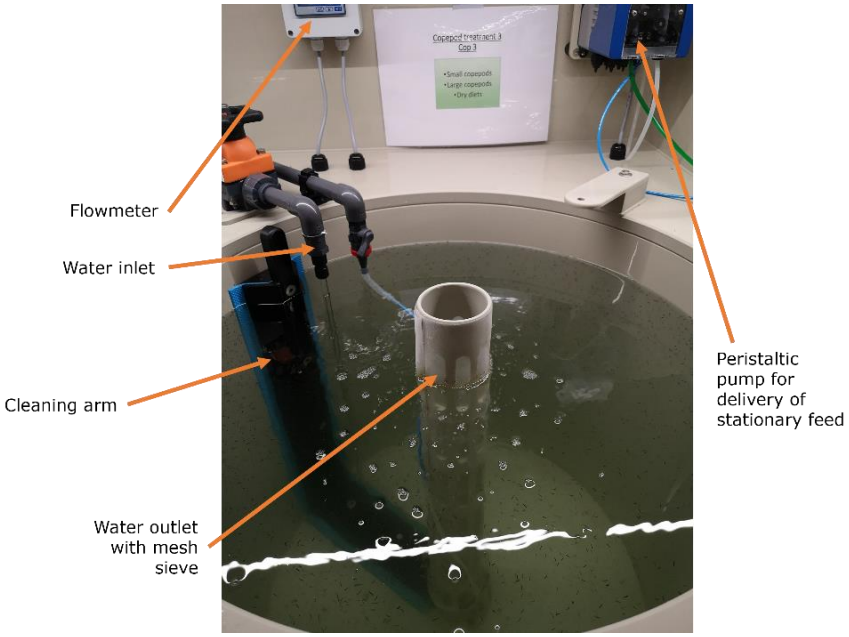


Figure 2.1. Detail of the different elements in one of the rearing tanks.

From day 4 DPH and until the end of the experiment, the setup was constantly illuminated (photoperiod of 24:0) with dimmed light to facilitate feeding behavior, using LED light tubes (FlexTube SC 4000 K of 75 W, Vanpee AS, Norway). Light intensity was increased from 100 to 300 lumen from 4 DPH for 10 days and kept at 300 lumen from 14 DPH until the end of the experiment.

The conditions in the tanks were as follows: 0.2 L min^{-1} water flow, gradually increasing until 1 L min^{-1} at day 32, when weaning to formulated diets started; temperature 12 °C, increasing progressively until reaching 16 °C t day 23, then maintained at 16 ° until the end of the experiment; water exchange from 2 to 8 tank volumes per day over the first 33 days, then maintained constant; gentle aeration using bubbling from the bottom of the tank (Table 2.1). Aeration was adjusted so it was not so high that it disturbed the small and fragile larvae, but that it ensured that the live feed stayed in suspension available for

the larvae, as well as enough access to oxygen in the tank. Tanks were filled with approximately 200 L of seawater collected from 40 m deep in the Trondheim fjord. This water was continuously degassed to avoid nitrogen supersaturation, filtered through a sand filter and 1 µm filter, and thermostatically controlled at 12 °C before its use. Water parameters (Temperature (°C), dissolved oxygen (DO; % saturation), salinity (ppt), pH, turbidity (formazin nephelometric unit, FNU)) were recorded daily in all the tanks using a ProDSS Multiparameter Digital Water Quality Meter (YSI, USA).

Accumulated organic matter, dead feed organisms, and larvae, as well as feces, were removed every morning using cleaning arms integrated into the systems, which were allowed to rotate for 20 mins at speed 2, which resulted in several full rotations. Subsequently, the accumulated debris was siphoned from the bottom of the tank. Dead larvae recovered from the tanks after cleaning were recovered and counted for survival calculations at given time points.

2.2 Experimental procedures for the start feeding experiment

The effects of four live feed regimes on ballan wrasse larvae were studied by means of a start feeding experiment performed between February and April 2020. The start feeding experiment was carried out for 48 days, from the onset of the exogenous feeding phase until completion of weaning to formulated diets.

Four different start feeding regimes were tested, labeled in this thesis as "Rotifer-*Artemia*", "Copepod-*Artemia*", "Experimental cirriped" and "Copepod-cirriped". Diets were evaluated in triplicates (3 tanks per diet, amounting to a total of 12 tanks). The treatments were randomly assigned to the tanks using the random number function of MS Excel (Figure 2.2).

Figure 2.2. Schematic representation of the randomized distribution of the tanks, and overview of the CodTech fish larval rearing laboratory. The color-coded tanks indicate the corresponding diet: Rotifer-Artemia (Industry standard), Copepod-*Artemia* (Copepods), Experimental cirriped (Experimental cirripeds) and Copepod-cirripeds (Mix).

Table 2.1. Summary and overview of the start feeding experiment, including the feeding periods, description of the feeds supplied during the four studied feeding regimes, as well as sampling times for biometry, survival, and lipid analyses. Diets: Rotifer-*Artemia* (Rot-*Art*, industry standard, used as a control); Copepod-*Artemia* (Cop-*Art*); Experimental cirriped diet (ExpCir) and Copepod-cirriped (Cop-cir).

		1st live feed period														Transition					2nd live feed period									Weaning					Formulated dry feed												
		4-17 DPH														18-22 DPH					23-31 DPH									32-36 DPH					37-48 DPH												
	Days post hatch (DPH)	0	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
	Day degrees	12	48	60	73	86	99	112	125	138	152	166	180	195	210	225	240	255	270	285	300	316	332	348	364	380	396	412	428	444	460	476	492	508	524	540	556	572	588	604	620	636	652	668	684	700	716
	Water outlet sieve mesh size (µm)	64												350																	700																
	Temperature (°C)	12			13							14		15								16																									
	Water exchange (%/day)	200															400					600									800																	
Start feed diet plan	**Rot-*Art***	-	Enriched rotifers																												Formulated dry feed																	
																	Enriched *Artemia*																																
	Cop-*Art*	-	Copepods, n1-n3								Copepods, n4-n6																																							
																	Enriched *Artemia*																																
	ExpCir	-	Experimental cirripeds, small																												Formulated dry feed																	
																	Cirripeds, large																																
	Cop-cir	-	Copepods, n1-n3								Copepods, n4-n6																																							
																	Cirripeds, large																																

	Days post hatch (DPH)	0	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
Sampling plan	Dry weight		X								X						X					X									X					X										X
	Standard length		X								X						X					X									X					X										X
	Survival	X	X																																												X
	Lipid composition		X								X						X					X									X					X										X
	Live feed: lipid composition,dry weight[1]		Rotifers, small copepods, exp. small cirripeds								Large copepods						Large cirripeds, *Artemia*															Formulated dry feed																	

All diets included three defined periods adapted to the developmental stage of the animals and their mouth size. The first period corresponded to smaller live feed prey (4-18 DPH), the second period to larger live feed prey (18-32 DPH) and the last period consisted of commercial formulated dry feed in all four diets (32-48 DPH). The introduction of new food items was done progressively to ensure a smooth transition, by mixing the old feed with the new feed and maintaining an overlap period for several days, as shown in Table 2.1. The composition of each of the four studied diets is described in detail below:

1. **Rotifer-*Artemia*** regime. This diet can be considered the industry standard and was used as a control. The regime included a first period where enriched rotifers (*Brachionus plicatilis*) were administered at an increasing density of 3-12 organisms mL^{-1} day^{-1}, distributed in three doses day^{-1}. The second period consisted of enriched *Artemia salina* nauplii, given at 3-9 individuals mL^{-1} day^{-1} densities, also in three doses day^{-1}. For the third period, larvae were weaned to commercial formulated dry feed, namely Gemma Micro 150 (150 µm particle size; Skretting, Norway), Gemma Micro 300 (300 µm particle size; Skretting, Norway), and AgloNorse Extra 2 (200-300 µm particle size; Tromsø Fiskeindustri AS, Norway), supplied *ad libitum* with an automated feeder. Larvae received 12 doses of 0.4 g day^{-1}. The three commercial diets were distributed as follows: 50:50 Gemma Micro 150 and AgloNorse Extra 2 (32-37 DPH); 25:25:50 Gemma Micro 150, Gemma Micro 300 and AgloNorse Extra 2 (37-42 DPH); 50:50 Gemma Micro 300 and AgloNorse Extra 2 (42-48 DPH).
2. **Copepod-*Artemia*** regime. The live feed during the first period was nauplii (n1-n6) of the calanoid copepod species *Acartia tonsa*, followed by *Artemia*. The supplied densities were 10-20 individuals of *A. tonsa* mL^{-1} day^{-1}, divided into three doses day^{-1}, and similar densities of *Artemia* as in the Rotifer-*Artemia* regime. The third period was as indicated for the Rotifer-*Artemia* diet.
3. **Copepod-cirriped** regime. In this diet, the first period consisted of nauplii (n1-n6) of the calanoid copepod *A. tonsa*, followed by a second period with the cirriped species *Semibalanus balanoides* (naupliar stages n1-n2; CryoPlanktonLarge, Planktonic AS, Norway). The densities of *A. tonsa* were those indicated in the Copepod-*Artemia* diet. CryoPlanktonLarge for the second period was supplied at increasing densities of 6-12 organisms mL^{-1} day^{-1}. The third period was as indicated for the Rotifer-*Artemia* diet.
4. **Experimental cirriped** regime. A combination of cirripeds of different sizes were fed in this diet. In the first feeding period, cryopreserved experimental small cirripeds (approximately 1:1 naupliar stages n1-n2 of *Balanus crenatus* and *Balanus balanus*) were fed, at a density of 20 individuals mL^{-1} day^{-1}. *B. crenatus* nauplii were 267 ± 18.8 µm long and 154 ± 26 µm wide, while those of *B. balanus* were 339 ± 21.8 µm long and 194 ± 22.9 µm wide (average length ± standard deviation after

70 measurements). During the second period, CryoPlanktonLarge, large cryopreserved cirripeds (naupliar stages n1-n2 of *S. balanoides*) were supplied at a similar density as indicated for the Copepod-cirriped diet. The third period was as indicated for the Rotifer-*Artemia* diet.

2.2.1 Cultivation and preparation of live feeds

2.2.1.1 Green water and algae cultures

The phytoplankton species utilized for the green water technique (Estévez et al., 2019; Neori, 2011; Palmer et al., 2007; Papandroulakis et al., 2001) were *Nannochloropsis* sp. (Eustigmatophyceae: Monodopsidaceae) Frozen Paste (BlueBioTech Gmbh, Germany), added to the tanks for a daily concentration of 6 mg C L^{-1} day^{-1}, and *Rhodomonas baltica* (clone NIVA 5/91: Cryptophyceae: Pyrenomonadales), at a concentration of 30 000 cells mL^{-1} (corresponding to 1 mg C L^{-1} day^{-1}). Both algal species were administered in 3 doses day^{-1}.

R. baltica was cultivated semi-continuously in the experimental facilities, according to protocols developed by SINTEF Plankton Centre (Malzahn et al., 2022). Cultivation was performed in 100 L acrylic cylinders, employing filtered water from the Trondheim fjord disinfected by chlorination, and neutralized with sodium thiosulfate. The water was fertilized with Conwy medium (Walne, 1970), continuously illuminated by fluorescent light tubes (photoperiod of 24:0, light intensity 150 µmol m^{-2} s^{-1} at cylinder surface), and supplied with 1-2% CO_2 enriched air. Density and cell growth of *R. baltica* were measured using a Multisizer 4e Coulter Counter (Beckmann Coulter, USA).

2.2.1.2 First period live feeds (small feeds)

Rotifers (*Brachionus plicatilis*) were grown semi-continuously at the SINTEF Plankton Centre in 100-L conical tanks with heavily aerated brackish water maintained at 20-22 °C. Rotifers were fed with a 3:1 mix of fresh bakers' yeast (*Saccharomyces cerevisiae*. REMA 1000, Norway) and rotifer diet (Instant algae; Reed Mariculture, USA). Before supplying the rotifers to the larvae, they were lipid enriched with 0.15 g LARVIVA Multigain dry formula enrichment diet (BioMar, Norway) per million rotifers, for 2 h at 24 °C. After the enrichment, rotifers were washed and either directly fed to the larvae or stored at 4 °C for a maximum of 16 h for later feedings on the same day.

Copepod eggs and naupliar stages of the calanoid copepod species *A. tonsa* were provided by CFEED AS (Norway). From 4 to 10 DPH, ballan wrasse larvae received nauplii n1-n3, produced at SINTEF Plankton Centre by hatching eggs in 100-L conical tanks containing heavily aerated seawater at 22 °C for 24 h. Newly hatched copepods were fed with 10 L of *R. baltica* (approx. 1.5 million cells mL^{-1}). Aeration was stopped before harvesting to allow

debris particles, feces, and exoskeletons to settle, after which the first 5 L were drained and discarded. From 10 to 22 DPH, ballan wrasse larvae were fed with nauplii n4-n6, supplied directly by CFEED in 1000-L IBC containers.

The pre-commercial experimental small cirriped diet (Planktonic AS, Norway) consisted of a 1:1 mixture of *B. crenatus* and *B. balanus* nauplii n1-n2. The diet came as cryopreserved pellets which were thawed onsite in cold seawater for several minutes upon arrival, followed by a 5-min rinsing step in a sieve with running sea water. Cirripeds were placed in aerated sea water at 4 °C for 6 h to allow them to revitalize. Cirripeds were supplied to the tanks from heavily aerated 5-L containers, using peristaltic pumps (Kronos 50; Seko, USA), over 4 h, followed by a 4-h pause, three times a day.

2.2.1.3 Second period live feeds (large feeds)

Artemia metal ion-coated cysts (*A. salina*; EG SepArt 225; INVE, Belgium) were prepared and handled according to the protocols from the supplier. In short, cysts were allowed to hatch for 24 h in 60-L conical tanks with heavily aerated sea water at 25-28 °C. Hatched nauplii were separated from the cyst shells using a magnetic separator (INVE, Belgium), rinsed in seawater, and transferred to a new 60-L enrichment tank with similar conditions to a density of 500 individuals mL^{-1}. For lipid enrichment, 1 g LARVIVA Multigain (BioMar) was supplied per million of *Artemia* nauplii. Another dose of the dry formula enrichment diet was added after 18 h. 6 h later, enriched *Artemia* nauplii were rinsed and concentrated for direct use, or they were chilled and stored at 4 °C for a maximum of 16 h for later feedings on the same day.

Large cirripeds (CryoPlanktonLarge; Planktonic AS) were evaluated as a replacer for *Artemia* in the Experimental cirriped and the Copepod-cirriped diets. The diet was thawed, rinsed, revitalized, and supplied similarly to what was previously described for the experimental small cirriped diet.

2.3 Sampling of larvae and feed for analyses

Larvae were randomly harvested for dry weight, standard length, and lipid analyses at eight sampling points corresponding to the start and end of the experiment, as well as every time a new type of feed was introduced in the diet (days 4, 12, 18, 23, 37, 42 and 48 DPH), as illustrated in Table 2.1. Samples were always retrieved in the morning before the new type of feed was introduced in the first feeding of the day. The number of larvae sampled for each analysis is summarized in Table 2.2.

Table 2.2. Number (N) of larvae sampled per tank for each type of analysis and time point. The actual number of larvae sampled was lower in some cases, due to larval mortality at late time points in some feeding regimes. The actual numbers of larvae sampled are indicated in the Results section of the corresponding analyses and in Appendices 1 and 3.

	Days post hatch (DPH)							
Type of analysis	**4**	**12**	**18**	**23**	**32**	**37**	**42**	**48**
Dry weight	10	10	10	10	10	10	10	10
Standard length	10	10	10	10	10	10	10	10
Fatty acids	50	50	25-40	15-30	15-30	15-30	15-30	15-30
Lipidomics	50	50	25-40	15-30	15-30	15-30	15-30	15-30

The different live feed and commercial formulated dry feed were also sampled for lipid analyses once per day for five consecutive days.

2.3.1 Growth parameters: dry weight and standard length

For growth studies, larvae were randomly collected (N=10 per sampling point and tank), by carefully scooping out the fish in a plastic beaker and immediately anesthetizing and euthanizing in 500 mg L-1 tricaine methanesulfonate (MS-222 Finquel®; Agent Chemical Laboratories Inc., USA). Euthanized larvae were rinsed with fresh deionized water and quickly preserved at -20 °C until analysis.

A calibrated research stereomicroscope system was used to image thawed frozen larvae (Olympus SZX10, Japan), and standard length was measured using the software package Infinity Analyze (Teledyne Lumenera, Canada). The standard length values were obtained by measuring from the tip of the larval upper lip to the end of the notochord in younger larvae (pre-flexion), or to the caudal peduncle (the root of the caudal fin) in older larvae (post-flexion) (Figure 2.3).

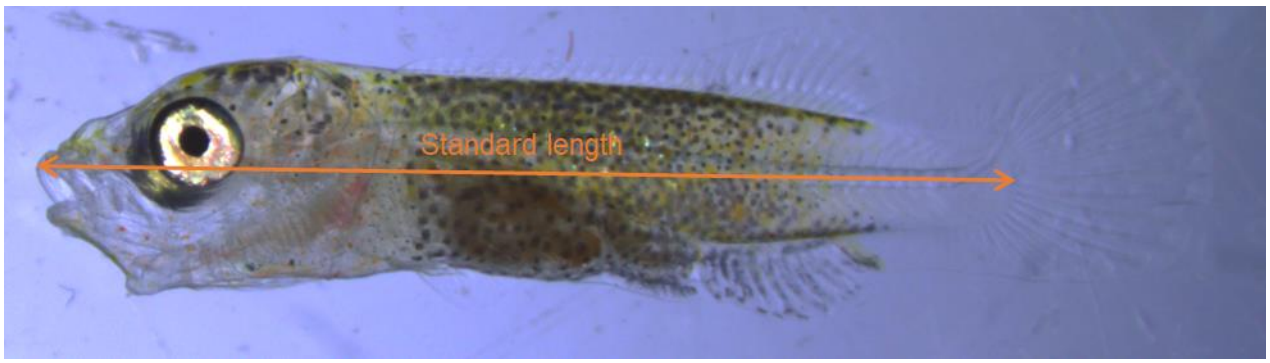


Figure 2.3. Standard length measurement in ballan wrasse larvae at 32 DPH. Standard length was measured from the tip of the upper lip until the end of the caudal peduncle.

Larvae imaged and measured for standard length were rinsed in distilled water and placed in pre-weighed tin capsules for dry weight (DW) analysis. Larvae were dried at 60 °C for 48 h. Dry weight was calculated gravimetrically using a UMX2 ultra-microbalance (Mettler-

Toledo, USA). Daily weight increase (%DWI) and specific growth rate (SGR) were calculated for the distinct time points and feeding regimes using equations 2.1 and 2.2, respectively (Ricker, 1958):

$$SGR = \frac{lnW_2 - lnW_1}{t_2 - t_1} \qquad [2.1]$$

where W_1 and W_2 are dry weight at time t_1 and t_2, respectively.

$$\%DWI = (e^{SGR} - 1) * 100 \qquad [2.2]$$

2.3.2 Mortality

An approximate total of 500-900 larvae were removed from each tank during the experiment, a number that corresponds to 2.5-5% of the larvae that were stocked at 0 DPH. The larvae collected for analyses were alive at the moment of sampling and must therefore be accounted for when estimating mortality. The mortality estimations were corrected for sampled larvae according to Kotani and collaborators (Kotani et al., 2011). In short, this approach assumes that the mortality is constant during the experimental duration and applies this correction to both sampled and non-sampled larvae, according to equation 2.3.

$$N_t = e^{-mt}\left(N_0 - \sum N_{Sn}\, e^{mdn}\right) \qquad [2.3]$$

where N_t is the number of larvae at time t, t is the rearing period in days, N_0 is the initial stocking density (number of larvae at t 0), m is the mortality coefficient, N_{Sn} the number of larvae that were sampled at the n^{th} sampling and dn the period until removal of n^{th} sample. The equation is thus based on an exponential decline of the population and also includes a term that considers the theoretical mortality of the larvae that were sampled (N_{Sn}). The provisional mortality coefficient is calculated from initial and final larval stocking numbers; then, the values for the natural mortality coefficient are substituted into the formula with successive approximation. The coefficient most closely approximating the actual survival is determined as the best fit natural mortality coefficient (Kotani et al., 2011).

2.3.3 Lipid analyses

2.3.3.1 Sampling of larvae and live feed for lipid analyses

For lipid analysis, larvae were randomly collected from the tanks (n >50 for lipidomics and n > 50 for fatty acid analyses, per tank, at the first sampling point. The number of collected larvae was reduced with subsequent sampling points, to ensure similar biomass, as

summarized in Table 2.2). Sampled larvae were anesthetized and euthanized with 500 mg L^{-1} MS222 as indicated for growth parameters. Subsequently, larvae were thoroughly rinsed with water, followed by deionized water, before transferring them to cryotubes. Feed and larval samples were immediately flushed with N_2, snap frozen in liquid N_2, and stored at -80°C until analysis, to protect against degradation by enzymatic lipase activity (release of free fatty acids from acyl lipids) and oxidation of carbon-carbon double bonds (Christie, 1984; Couturier et al., 2020; Rudy et al., 2016).

2.3.3.2 Fatty acid (FA) analyses

Sampling of larvae and feed was performed as indicated in section 2.3.2.1 and processing and analysis were carried out as described elsewhere (Malzahn et al., 2022). FA were studied through transmethylation to fatty acid methyl esters (FAMEs), rendering them suitable for analysis with gas chromatography coupled with mass spectrometry with flame ionization detection (GC-FID) (Chiu & Kuo, 2020). Before use, all glass material used in the lipid extraction was soaked overnight in a 5% deconex 15PF bath, washed, rinsed with deionized H_2O, and heated at 400 °C for 2 h.

Samples were freeze-dried and weighed in a UMX2 ultra-microbalance (Mettler-Toledo, USA). The Folch protocol (Folch et al., 1957) is considered the "golden standard" for lipid extraction from solid samples and was used in the present work, according to previously published methods, with modifications (Iverson et al., 2001; Pati et al., 2016). In short, 10 mL 2:1 chloroform:methanol were added to freeze-dried larvae/feed, which were subsequently homogenized using a blender with a stainless steel tip (IKA T-10 basic ULTRA-TURRAX). Crude extracts were washed with 2.5 mL of a solution containing 0.88% KCl. Phase separation was performed with the help of centrifugation for 3 min at 4000 rpm and 4 °C. The lower phase (organic phase) was transferred with a glass pipette to a pre-weighed clean Kimax 100x150 mm tube and dried under a gentle N_2 stream. The total lipid content was determined gravimetrically by weighing the dried lipid extract (second weighting (mg)- pre-weight (mg) = total lipids in the sample (mg)). Total lipids were resolved in 2:1 chloroform:methanol to a final stock concentration of 10 mg/mL. Samples were spiked with an internal standard: tricosanoic acid (23:0) from NuChekPrep (Chiron, Germany), a FA naturally present in mushrooms, and thus not interfering with FA analyses in marine larvae and live feed. Next, the samples were methylated by the addition of 1 mL chloroform and 2 mL 1% H_2SO_4:MeOH and incubation for 16-18 h at 50°C. Two parallels (technical replicates) were prepared of each sample. Lipophobic phase separation was carried out by adding 6 mL isooctane and 5 mL saturated NaCl, followed by centrifugation at 4000 rpm for 3 min at 4 °C. The upper phase (organic phase) was recovered and dried under a gentle N_2 stream. Finally, samples were reconstituted in 150 µL isooctane and stored at -80°C until analysis.

The analysis of FAMEs was performed according to the protocol described by Daukšas et al. (Daukšas et al., 2005) with the following modifications. An Agilent Technologies 7890B GC–FID equipped with a 7693A autosampler was used. The detector temperature was held at 280 °C, and the flame was maintained with 30 mL/min H_2 gas and 400 mL/min filtered air. The chromatographic separation was performed using a CP-wax 52 CB, 25 m, 250 µm with i.d. 0.2 µm column (Agilent Technologies). Helium was used as the carrier gas at a flow rate of 3 mL/min. GC inlets were held at 250 °C. The initial oven temperature was maintained at 50 °C for 2 min and increased to 150 °C at 30 °C/min, after which the temperature was further risen to 230 °C at 2.5 °C/min, followed by an increase to 240 °C at 10 °C /min, and a 23 min final hold. Free FAMEs were identified through comparing their RTs with those of a commercial standard mix submitted to identical chromatography conditions. Quantification was based on a commercial standard mix (68D mix from NuChekPrep, Chiron), using tricosanoic acid (C23:0, NuChekPrep, Chiron) as internal standard.

2.3.3.3 Lipidomics analyses

Sampling of larvae and feed was performed as indicated in section 2.3.2.1. The first step required before lipid analysis was lipid extraction from the sample (Pati et al., 2016; Saini et al., 2021). Lipid extraction was performed using a high throughput method using methyl-tert-butyl ether (MTBE), chloroform, and ceramic beads, according to Abbott et al. (Abbott et al., 2013) with modifications. Samples were spiked with an internal standard (SPLASH® II LIPIDOMIX® Mass Spec Standard, Avanti, Sigma) before extraction. Lipid extraction was carried out for 1 h at room temperature, followed by the addition of 0.15 M ammonium acetate (MTBE:methanol:0.15 M ammonium acetate 20:6:5). Then, samples were incubated for 10 min and centrifuged at 4 000 rcf and 4 °C for 10 min. The upper phase (organic phase) was transferred to a clean tube, dried under N_2, resuspended in 500 µL $CHCl_3$, and stored at -80 °C until analysis.

Samples in $CHCl_3$ were dried under N_2 and re-dissolved in 100 µL acetonitrile:H_2O (40:60) containing 0.1% formic acid and 10 mM ammonium formate. The lipidomics analyses were performed by Antonio Sarno at SINTEF Ocean analytical laboratory as described elsewhere (Malzahn et al., 2022). In summary, samples were chromatographically separated by injecting 5 µL per run into an Agilent 1260 UPLC instrument with a Waters Acquity CSH C18 HPLC column (2.1 x 100 mm, 1.7 µm particle size) at 45°C, with a flow rate of 0.25 mL/min. The mobile phase consisted of a 60-min gradient of (A) acetonitrile:water (40:60) and (B) isopropanol:acetonitrile (90:10), both with 10 mM ammonium formate and 0.1% formic acid. Three injections were performed per sample to analyze three different sets of lipids, respectively: i) phospholipids; ii) glycerides and free fatty acids, and iii) sphingolipids. The UPLC instrument was coupled to a 4670 triple quadrupole mass

spectrometer with an electrospray ion source (ESI). The data was acquired in multiple reaction monitoring (MRM) mode with mass transitions obtained from the literature (Jouhet et al., 2017; Takeda et al., 2018; Tsugawa et al., 2015; Woodfield et al., 2018; Xuan et al., 2018). All MRMs were scanned on a sample of pooled lipid extract from different sources to obtain retention times (RT), based on the observation that acyl chain length increases and desaturation decreases with increased RT on a reverse phase column (Bromke et al., 2015; Giavalisco et al., 2011). The Agilent MassHunter Quantitative Analysis software package was used to analyze the data from LC-MS/MS runs. All peaks were controlled for peak symmetry, minimum intensity, and RT drift. The response for each mass transition was calculated using a surrogate standard curve elaborated using different injection volumes of an exogenous standard comprising a small aliquot from each sample. A regression curve was generated for each transition, using a R^2 cut-off of 0.7 as an exclusion criterion. Furthermore, peak intensities were normalized to heavy isotope-labelled standards of the same lipid class in each sample, to control for both extraction efficiency and instrument response drift. Finally, signals were normalized to total sample weight.

2.4 Statistical treatment and visualization of the data

Descriptive statistical parameters, including mean and standard deviation, as well as other parameters such as SGR, %DWI, and mortality coefficients, were calculated in Microsoft Office 365, Excel software (v. 2309, build 16.0.16827.20278) and GraphPad Prism 10.1.2. Data visualization and statistical analyses were performed in GraphPad Prism 10.1.2. Python with the libraries Matplotlib v3.7.1 (Hunter, 2007), Seaborn v.0.13.0 (Waskom, 2021), Pandas v.2.0.1, NumPy v.1.24.2 (Harris et al., 2020) and SciPy v.1.10.1 (Virtanen et al., 2020) were used for calculations, data visualization and statistics for the lipid correlations.

Before applying statistical analyses to the data sets, normality (Saphiro-Wilk's test) and homogeneity of the variance (Brown-Forsythe test) were assessed. Normality was also assessed by evaluation of Q-Q plots representing the residuals after fitting a given model, for instance, analysis of variance (ANOVA). Percentual data was arcsine square root transformed when the values were only positive. A significance level of α = 0.05 was employed to evaluate differences in group means and correlations. When the data had normal distribution and homoscedastic variance, one-way ANOVA was applied, with subsequent post hoc Tukey's multiple comparisons test when significant differences were detected by the ANOVA. When data was normally distributed but had heteroscedastic variance, Welch's ANOVA and post hoc Dunnett's T3 test were used. Kruskal-Wallis test (one-way ANOVA on ranks) followed by Dunn's multiple comparisons test was used when the data was not normally distributed. When comparing the means of only two groups with

normally distributed data, unpaired t-test was used. For data that were not normally distributed, two means were compared by the non-parametric unpaired Mann-Whitney test. For the comparison of two means from parametric heteroscedastic distributions, the unpaired Welch's test was used.

Water environmental parameters along the duration of the experiment were statistically assessed utilizing two-way ANOVA followed by Tukey's multiple comparisons tests.

For evaluation of dry weight-length, fatty acid and lipidomics correlations, given that at least one of the data sets in each analysis was not normally distributed, Spearman's correlations were run. Non-linear regression was performed for significant dry weight-length correlations ($p<0.05$). Non-linear models were compared, and the best fit was selected through extra sum of squares F test, selecting, when possible, the simpler model. Differences in slopes of simple linear regression were evaluated using analysis of covariance (ANCOVA).

The differences in average mortality coefficients between the different groups were evaluated by ANOVA followed by Tukey's multiple comparisons test.

Total lipids, fatty acids, and fatty acid groups in the live feeds were compared considering the phase in which they were supplied to the fish (First phase: Rotifers, exp. cirripeds and copepods; second phase: *Artemia* and cirripeds). As for the larvae, total lipids, fatty acids, and fatty acid groups were compared considering groupings based on the last feed that was supplied. MetaboAnalyst 6.0 (Pang et al., 2021) was used for unsupervised principal component analysis (PCA) and hierarchical clustering of the lipid analysis, visualized in heatmaps. Before PCA and hierarchical clustering, data was autoscaled (mean-centered and divided by the square root of the standard deviation of each variable). Missing values were replaced by 1/5 of minimum positive values of their corresponding variables (limit of detection, LOD). Outliers (outside of the 95% confidence interval for each group) were filtered out. In the case of lipidomics data, due to the big volume of data, further filtering was performed as follows: features with a constant or single value across samples were deleted; 10% of the features with lower variance based on interquartile range (IQR) were removed (a total of 31 features) (Hackstadt & Hess, 2009). Sparse partial least squares regression - discriminant analysis (sPLS-DA) was performed for lipidomics (Lê Cao et al., 2011; Pang et al., 2021). For hierarchical clustering, the similarity measure was Euclidean distance (defines which clusters are combined as the dendograms are being generated) and the clustering algorithm was Ward's linkage. Hierarchical clustering was visualized in heatmaps. All features were kept for heatmaps displaying fatty acid composition, while only the top 25 features with the highest differences according to a t-test or ANOVA (carried out in MetaboAnalyst 6.0) were displayed for lipidomics data.

3 Results

3.1 Stability and development of environmental conditions along the experiment

The water parameters in the bags containing the newly hatched larvae were measured at reception and daily during the 48 days of the start feeding experiment. No significant differences were observed in the temperature, pH, and salinity measurements of the bags received from the 20th February hatch or 21st February hatch (Figure 3.1). The DO values were significantly different between the two hatches, with marked supersaturation in the bags from 20th February (DO > 250%) (paired Student's t-test; $p<0.05$). More dead larvae could be seen in the bags from the 21st hatch compared to the 20th.

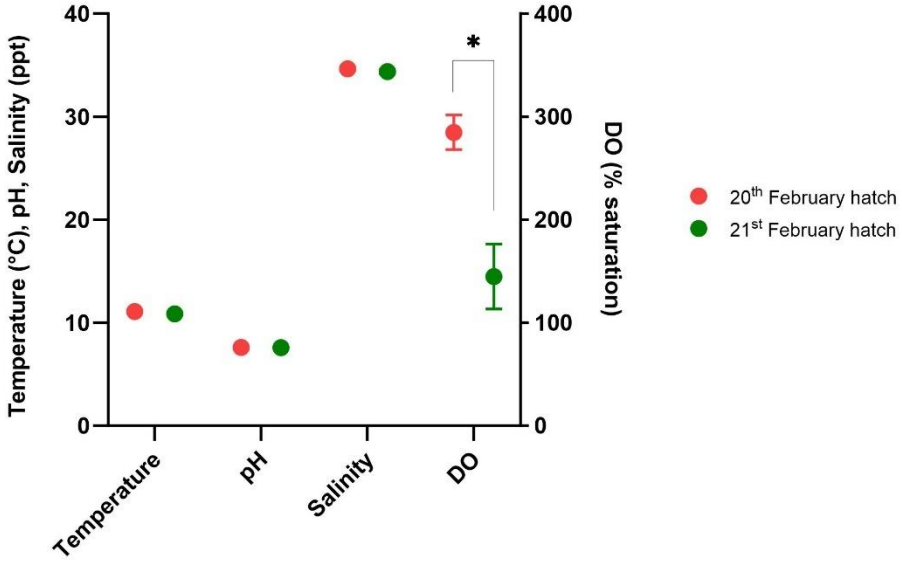


Figure 3.1. Water parameters in the bags containing ballan wrasse larvae at reception. Temperature (°C), pH (unitless), salinity (ppt) and DO (% saturation) are represented. The color of the points indicates the hatch date. Significant differences (paired Student's t-test; $p < 0.05$) between hatches are denoted by asterisks.

The environmental conditions were similar between all tanks along the experiment, except two days with significantly higher temperatures in two tanks of the Rotifer-*Artemia* diet (tank 1 at 10 DPH, and 3 at 7 DPH), and one day with lower DO saturation in tank 3 of the Rot-Art diet, at 42 DPH (Figure 3.2) (two-way ANOVA followed by Tukey's multiple comparisons test; $p<0.0001$). Temperature and DO values returned to levels close to the other tanks on the next measured point.

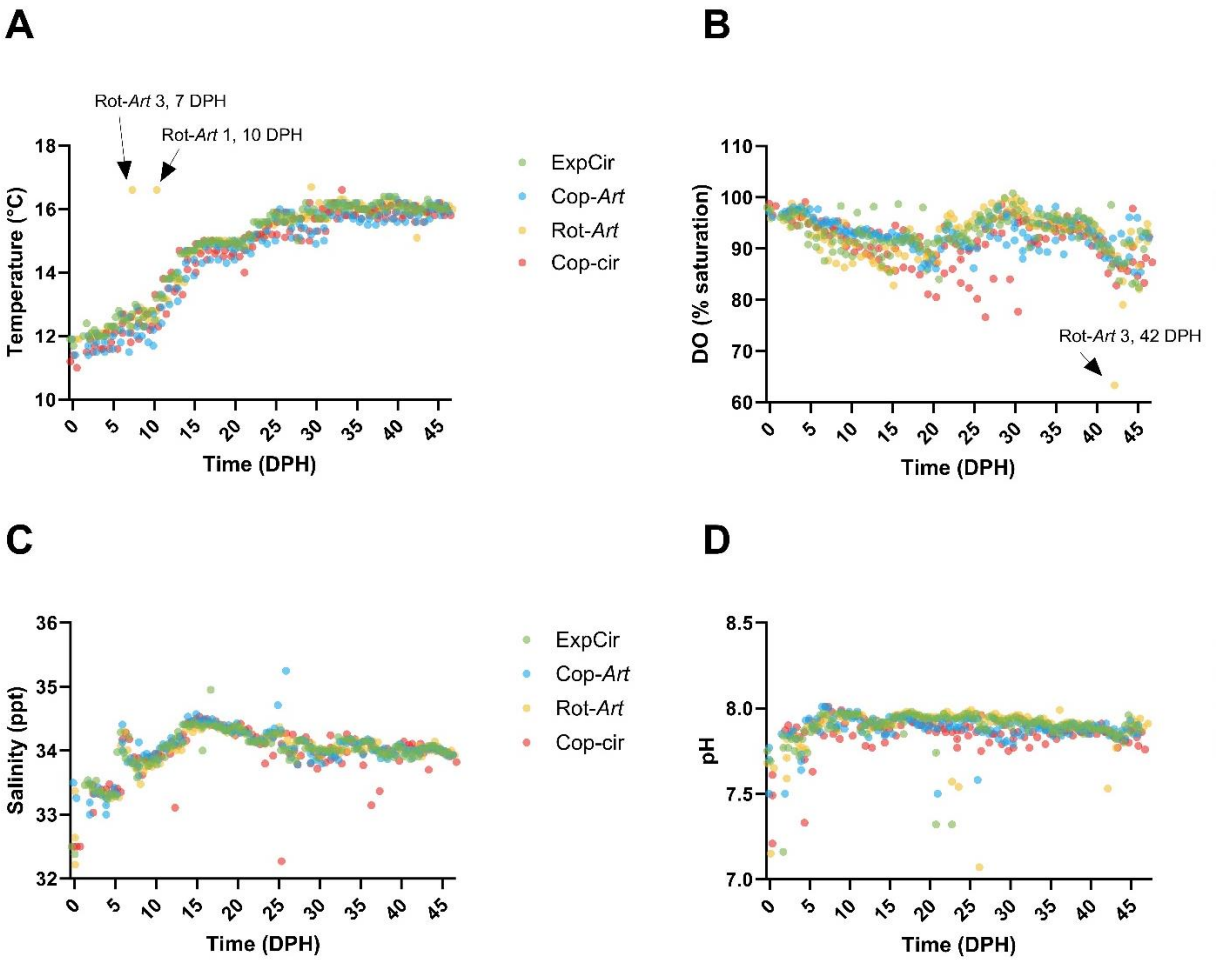


Figure 3.2. Water parameters in the tanks during the 48 days of the first feeding experiment. Each point corresponds to one daily measurement per tank of temperature (panel A; °C), DO (panel B; % saturation), salinity (panel C; ppt) ad pH (panel D; unitless). The color of the points indicates the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirriped (Cop-cir)). Three points with statistically significantly different values (two-way ANOVA followed by Tukey's multiple comparisons test; $p < 0.0001$) are highlighted with arrows and text in panels A and B.

3.2 Larval morphology, growth, and survival

3.2.1 Description of the external larval morphology

At **reception** (1-2 DPH), the external appearance of the larvae was markedly different from that of juveniles or adult fish. Larvae were mostly transparent, elongated, and with a relatively large head-to-body ratio. The yolk sac was ovoid and unsegmented, situated ventrally in the abdomen (yolk sac larvae, *lecithotrophic period*). The finfold was continuous and undifferentiated. The larvae were mostly floating around the surface and relatively stationary, with sporadic sudden forward movements caused by tail contractions. The eyes were relatively little developed and free of pigment. The mouth was undeveloped.

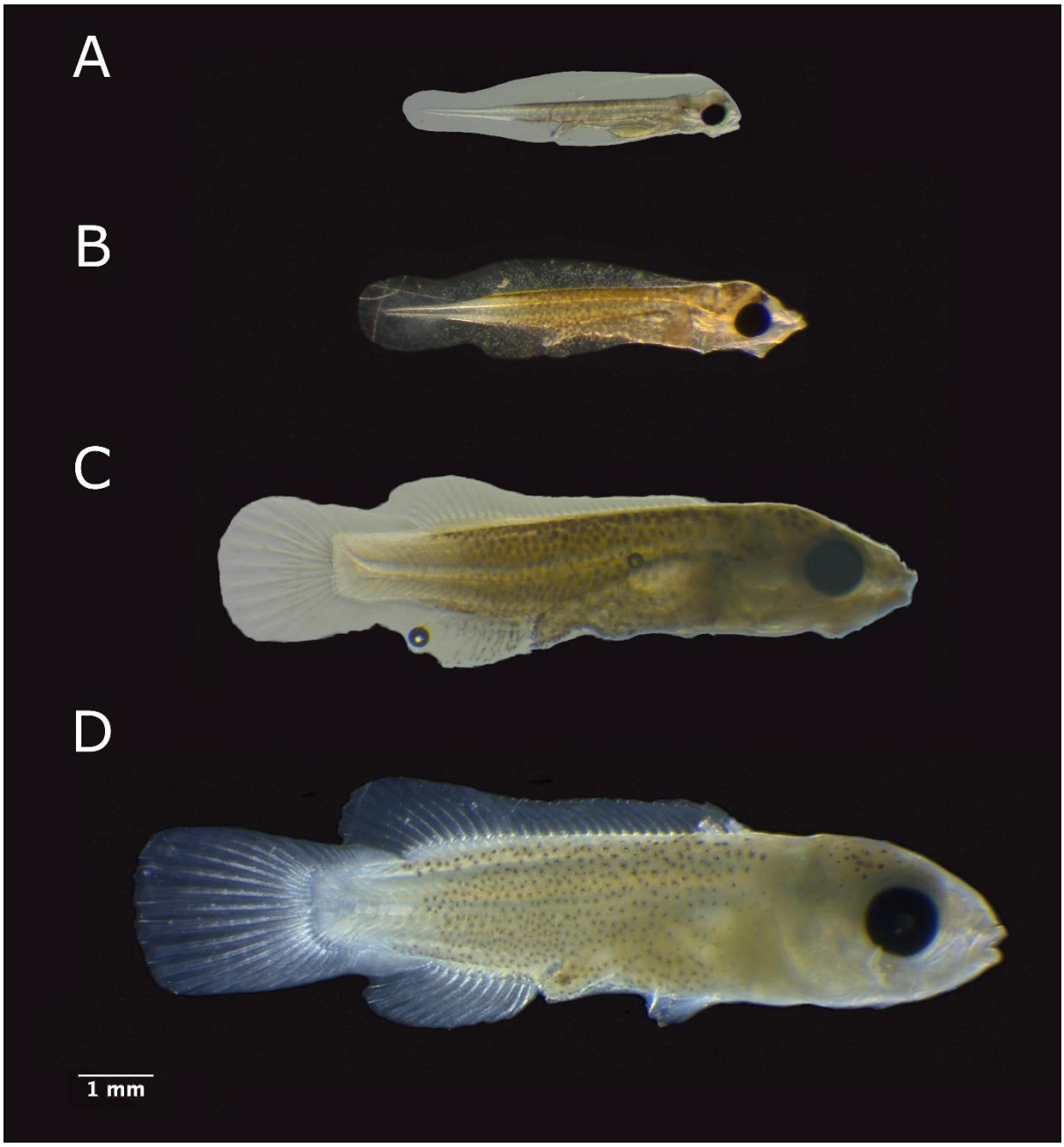


Figure 3.3. Morphology and development of ballan wrasse larvae. The scale bar (1 mm) applies to all the depicted fish. A) 4 DPH, ExpCir treatment; B) 18 DPH Cop-*Art* treatment; C) 32 DPH Cop-*Art* treatment; D) 48 DPH Cop-*Art* treatment. Feeding regimes: Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirriped (Cop-cir). The images are modified from originals taken by Anna Sigrid Norberg Aase.

At **4 DPH**, the volume of the yolk sac was considerably reduced but was still identifiable. The anus was observable, opening ventrally in the abdomen around 50% of the total length of the larvae (midbody), between the pre- and postanal finfold. The gut could be observed as a continuous tube. The otic capsule was visible dorsally, and the mouth appeared open in some individuals, allowing for exogenous feeding *((lecitho)exotrophic period*), which was expected to start around 7 DPH. The eyes were round, with black pigmentation, and were

one of the most prominent macroscopic features of the larvae. Both the body and notochord were still straight (Figure 3.3).

All the yolk was resorbed by **18 DPH**. The notochord was surrounded by muscle and continued to be straight, without any visible upward flexion, indicating that the larvae were still at the preflexion stage. The gut was visible and appeared as a straight tube with a larger anterior portion. External pigmentation was more apparent, with colors varying based on the diet and individual and including ochres, yellows (typically in larvae fed on copepods), and reds. No pigmentation was found in the lower jaw, snout, and caudal region. Some of the photographed fish had a swim bladder filled with air (Figure 3.3).

At **32 DPH**, the external pigmentation had increased and covered all the body, making difficult the direct observation of the internal organs on the macroscope. Coloration patterns were clearer and more defined (punctate, contracted melanophores). The tip of the notochord and the posterior part of the urostyle were bent upwards. All fins (anal, pelvic, pectoral, dorsal, and caudal) were developed, and caudal fin rays could be seen separately from the dorsal fin rays in the margin of the finfold, indicating post-flexion stage. The head-to-body ratio changed towards a less large head relative to the body (Figure 3.3).

At **48 DPH**, operculae were visibly formed. Fin rays were very clearly differentiated, and the head and body were better developed. The external pigmentation was present in all the body except for the urostyle region. Pigmentation made difficult the evaluation of the aspect of the internal organs, although the gut appeared to have a coiled appearance (Figure 3.3).

Some differences in development and appearance were observed between the different regimes, with variation in larval size (sections 3.2.2 and 3.2.4) and morphology (data not shown) between individuals of the same tank.

3.2.2 Dry weight (DW)

The DWs recorded for the first sampling point (4 DPH), before the start of the feeding phase, did not show statistically significant differences between groups ($p < 0.05$). The mean value calculated from all the tanks at 4 DPH was 34.0 ± 2.0 µg larva^{-1} (N= 40 larvae) (Figure 3.4 and Appendix 1, Table A1a).

At 12 DPH, a decrease in the mean DW compared to 4 DPH was observed for three of the four feeding regimes (Experimental cirriped, Copepod-*Artemia*, and Rotifer-*Artemia*), which was compatible with the resorption of the yolk sac during the endogenous (*lecithotrophic*) phase. Conversely, the DW at 12 DPH was significantly ($p < 0.05$) higher for the Copepod-cirriped diet, remaining at values close to those observed at 4 DPH (Appendix 1, Table A1a). DW mean values increased progressively from 12 to 37 DPH in

all feeding regimes, although this increment was not equivalent for all the groups and significant differences were present at each of the time points (Figure 3.4).

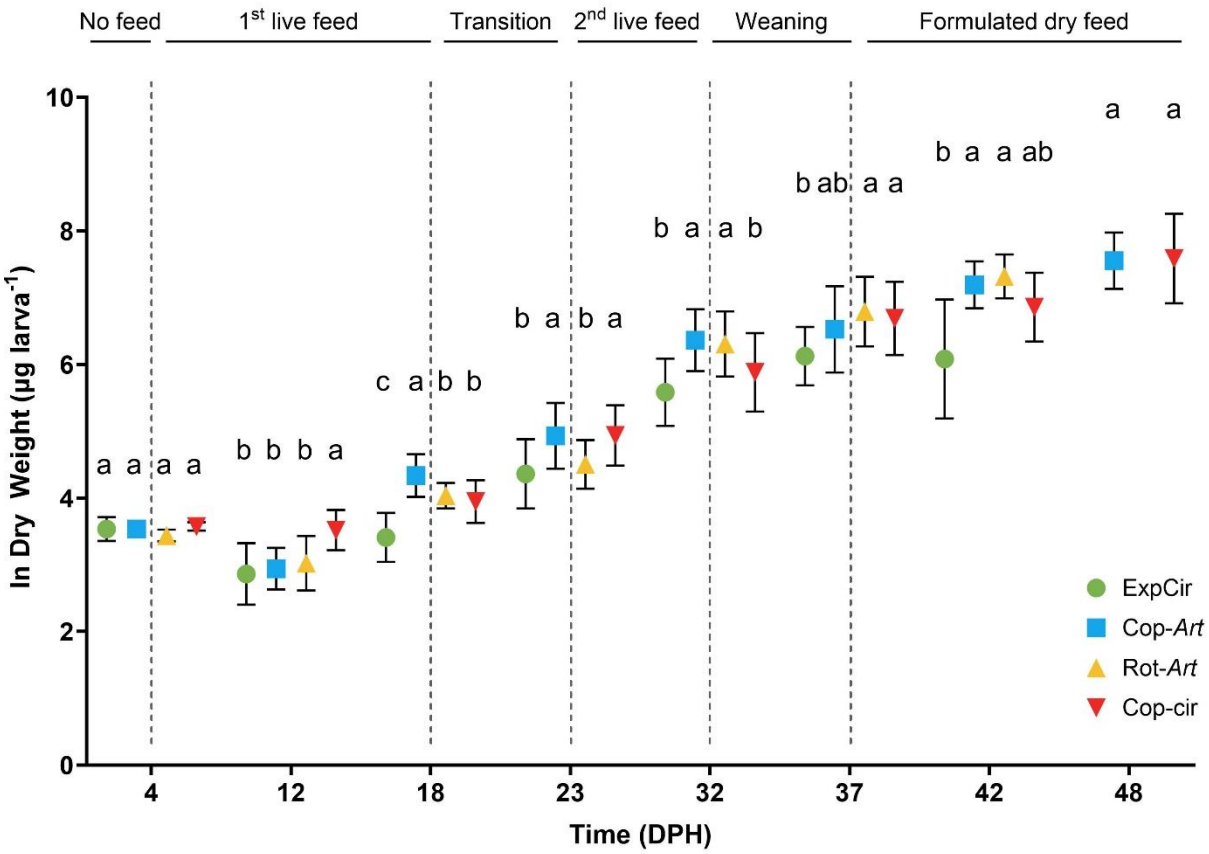


Figure 3.4. Distribution and development of the dry weight of the larvae over time. Mean values and SD are shown. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirripeds (Cop-cir)). Dotted vertical lines in the graph indicate the corresponding feeding period. The horizontal axis represents sampling times as discrete variables, equally spaced independently of the number of days in between points. Statistically significant differences ($p<0.05$) between feeding regimes are depicted by unequal letters over each dot. No values are represented for the Rot-*Art* and ExpCir treatments at 48 DPH due to lack of sampling because of high mortality. Values, statistical tests applied, and violin plots representing the distribution of each group can be consulted in Appendix 1.

Significant differences were found between the Copepod-*Artemia* diet (blue color in Figure 3.4) and the Copepod-cirriped diet (red in Figure 3.4) at 12 DPH, which corresponds to the first live feed period. ANCOVA was applied to evaluate the differences between slopes of a linear regression fit describing the development of DW over time, finding no significant differences between the two groups. On the other hand, Rotifer-Artemia and Experimental cirriped were significantly ($p < 0.05$) different from the two diets that were started with copepods.

The Experimental cirriped diet (green color in Figure 3.4) showed the lowest mean DW at all time points from 12 DPH, and could not be sampled at 48 DPH due to high mortality.

No significant increase in DW could be seen between 37 and 42 DPH. The last studied time-point for this diet (42 DPH) had a very heterogeneous population, with a mean DW of 641.7 ± 729.8 µg larva^{-1}.

Besides the Experimental cirriped diet, no other significant differences in DW were seen after weaning to formulated dry feed (37, 42, and 48 DPH) (Figure 3.4).

The DW mean values at 42 DPH were 641.7 ± 729.8 µg larva^{-1} (Experimental cirriped), 1406.6 ± 494.7 µg larva^{-1} (Copepod-*Artemia*), 1582.4 ± 630.2 µg larva^{-1} (Rotifer-*Artemia*) and 1078.6 ± 545.8 µg larva^{-1} (Copepod-cirriped). At 48 DPH, only the groups that had received copepods at the first live feed period could be sampled due to high mortality in the other tanks. The two groups that could be sampled at 48 DPH showed a mean DW of 2086.1 ± 1032.7 µg larva^{-1} (Cop-Art) and 2357.0 ± 1282.9 µg larva^{-1} (Cop-cir).

3.2.3 Daily weight increase (DWI)

The daily weight increase (DWI) was calculated for each of the feeding periods (4-18 DPH: first live feed; 18-32: second live feed; 32-42: formulated dry feed; 4-42: all periods together) (Figure 3.5).

The largest differences were found during the first live feed period, where the Experimental cirriped diet was the only group with negative DWI. As indicated before for DW, there were statistical differences between both groups being fed on copepods during this period (Copepod-*Artemia* and Copepod-cirriped). The first live feed period had the lowest DWI values out of the whole experiment for all feeding regimes.

The largest DWI were observed during the second live feed period for all the diets, subsequently decreasing at the formulated dry feed phase. The DWI calculated for all the experiment was similar to the values obtained during this last period. No significant differences in DWI were observed between the four diets during the second live feed period and the formulated dry feed period.

The two groups that received *Artemia* during the second live feed phase (Copepod-*Artemia* and Rotifer-*Artemia*) had the highest DWI values, which were significantly ($p < 0.05$) larger than the next group (Copepod-cirriped). Copepod-cirriped showed, in turn, significantly ($p < 0.05$) larger DWI than the Experimental cirripeds (Figure 3.5).

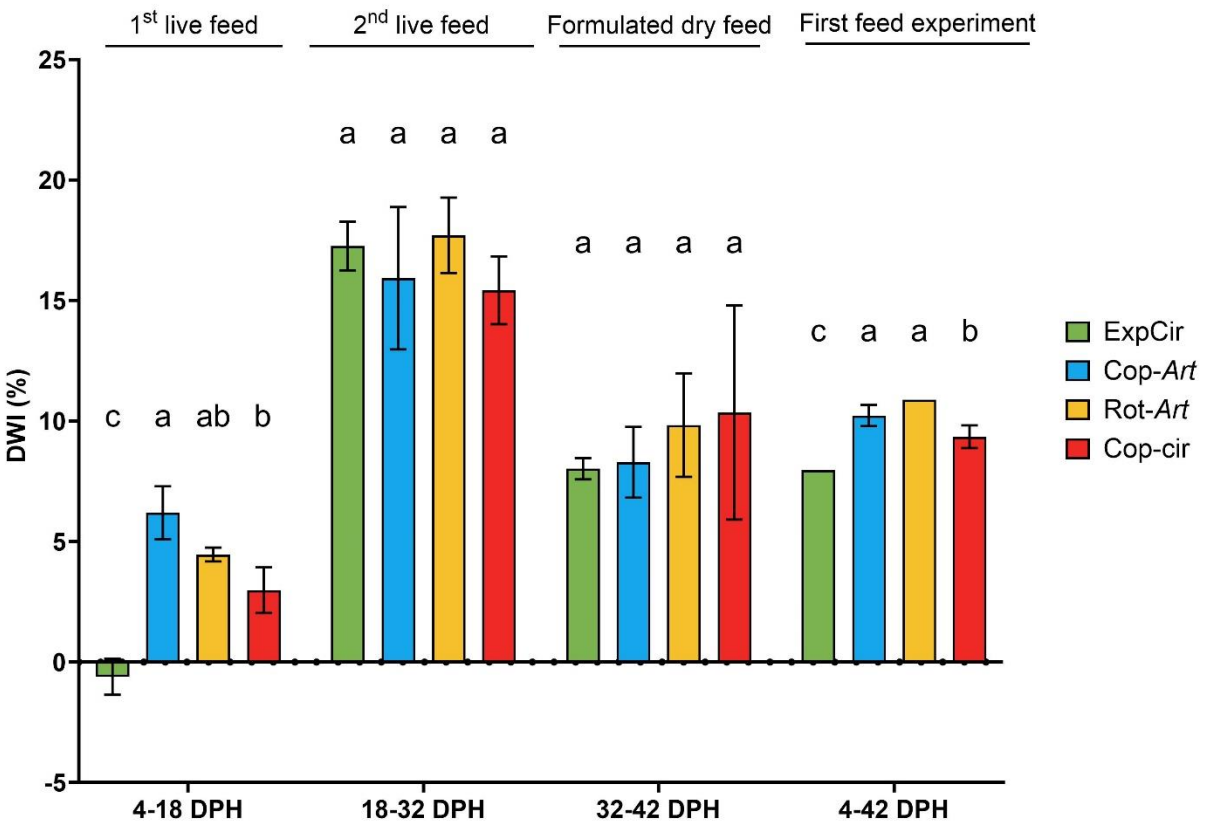


Figure 3.5. Daily weight increase (DWI) during the experiment. DWI (%) is represented for the first live feed period (4-18 DPH), second live feed period (18-32 DPH), formulated dry feed period (32-42 DPH) and for all the mentioned periods altogether. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$) between feeding regimes are depicted by unequal letters over each bar plot. No values are represented from 48 DPH due to lack of sampling because of high mortality in two of the groups (Rot-*Art* and ExpCir treatments). Values can be consulted in Appendix 2.

3.2.4 Standard length (SL)

In line with what was observed for DW, no significant differences were found in the mean standard length (SL) of the larvae at 4 DPH, before the introduction of the live feeds (Figure 3.6, Appendix 4). The mean SL for all the groups at 4 DPH was 4.33 ± 0.31 mm larva^{-1}.

In general, larger differences in SL within the same group were observed as time progressed, with more heterogeneity in the population of each group and larger relative SD values, as shown in Figure 3.6. A progressive increase in mean SL was seen with time, although said increment was not equivalent for all feeding regimes, and statistically significant differences ($p < 0.05$) were present between at least some of the groups at each of the time points (Figure 3.6).

The Copepod-Artemia treatment had significantly ($p < 0.05$) larger mean SL values during the first live feed period (12 and 18 DPH) than the other feeding regimes, including Copepod-cirriped, which had been receiving the same type of diet. This was in line with

the differences observed in DW and DWI between these groups at early sampling points (Figure 3.4 and Figure 3.5).

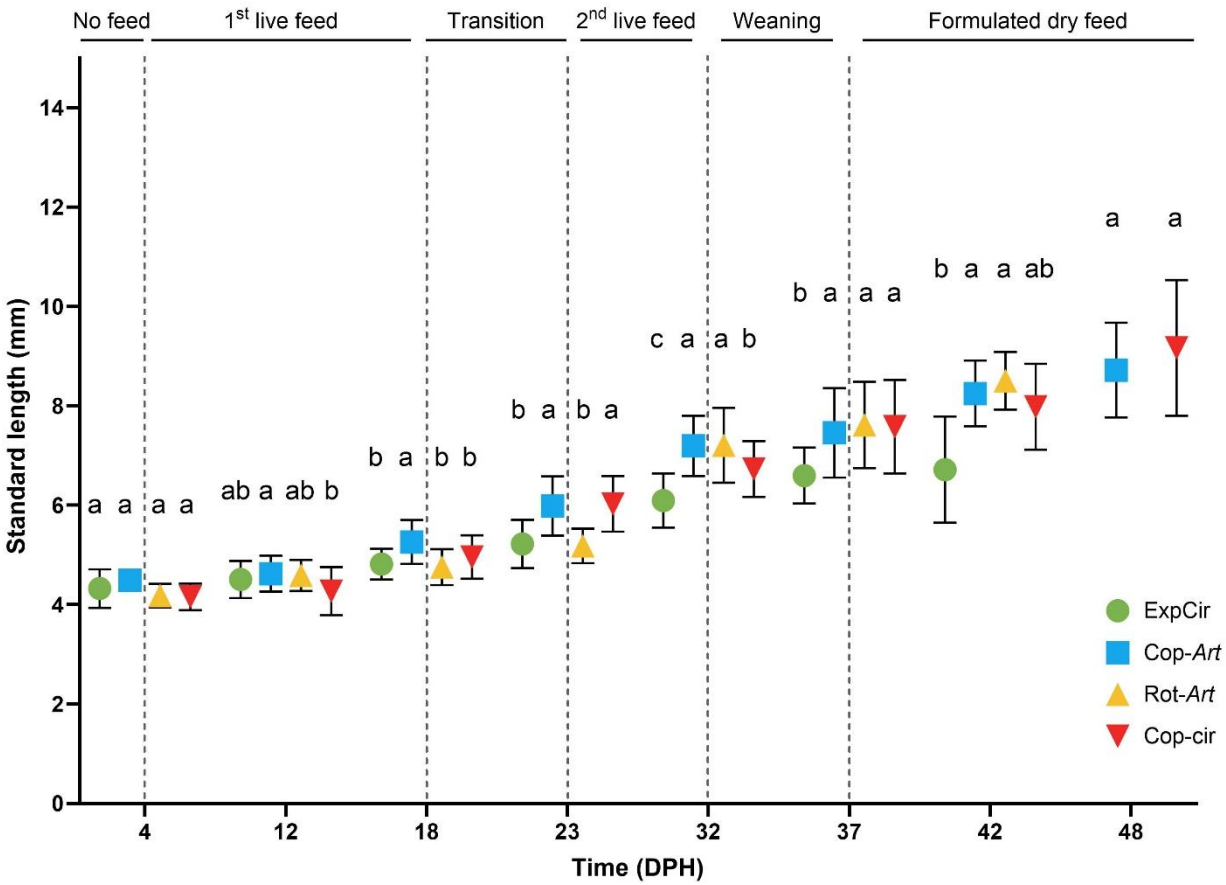


Figure 3.6. Distribution and development of the standard length of the larvae over time. Mean values and SD are shown. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirripeds (Cop-cir)). Dotted vertical lines in the graph indicate the corresponding feeding period. The horizontal axis represents sampling times as discrete variables, equally spaced independently of the number of days in between points. Statistically significant differences ($p<0.05$) between feeding regimes are depicted by unequal letters over each dot. No values are represented for the Rot-*Art* and ExpCir treatments at 48 DPH due to lack of sampling because of high mortality. Values, statistical tests applied, and violin plots representing the distribution of each group can be consulted in Appendix 3.

The development of mean SL versus time for the Experimental cirriped diet showed a significantly different slope compared to the other feeding regimes (ANCOVA; $p < 0.05$). Experimental cirriped had the lowest mean SL from 32 DPH and onwards, corresponding to the weaning and formulated dry feed phases, and did not significantly ($p < 0.05$) increase from 37 to 42 DPH.

The mean SL values at 42 DPH (the last sampling point where all groups could be sampled) were 6.72 ± 1.07 mm larva^{-1} (Experimental cirriped), 8.25 ± 0.66 mm larva^{-1} (Copepod-*Artemia*); 8.50 ± 0.58 mm larva^{-1} (Rotifer-*Artemia*) and 9.16 ± 1.37 mm larva^{-1} (Copepod-cirriped). Copepod-*Artemia* and Copepod-cirriped were also sampled at 48 DPH, displaying mean SL values of 8.72 ± 0.95 mm larva^{-1} and 9.16 ± 1.37 mm larva^{-1}, respectively. Even

though Copepod-cirriped had a greater mean value, it also showed larger heterogeneity within the group, and it was not significantly ($p < 0.05$) different from Copepod-*Artemia*. As indicated for DW, the Experimental cirriped and Rotifer-*Artemia* groups could not be sampled at 48 DPH due to high mortalities.

3.2.5 Relationship between DW and SL

Spearman's correlations were run for each of the different feeding regimes to determine the significance of the relationship between DW and SL, based on values from 4 DPH until 48 DPH (Figure 3.7). It was found that there were statistically significant ($p < 0.0001$) strong, positive, monotonic correlations between the two parameters in all groups (Spearman's correlation coefficients: 0.898, 0.979, 0.901 and 0.980 for the Experimental cirriped, Copepod-*Artemia*, Rotifer-*Artemia*, and Copepod-cirriped diets, respectively). Until approximately 6 mm SL, the larvae grew in length, with a moderate increase in DW. After 6 mm SL, the DW values started to increase more rapidly in all the diets.

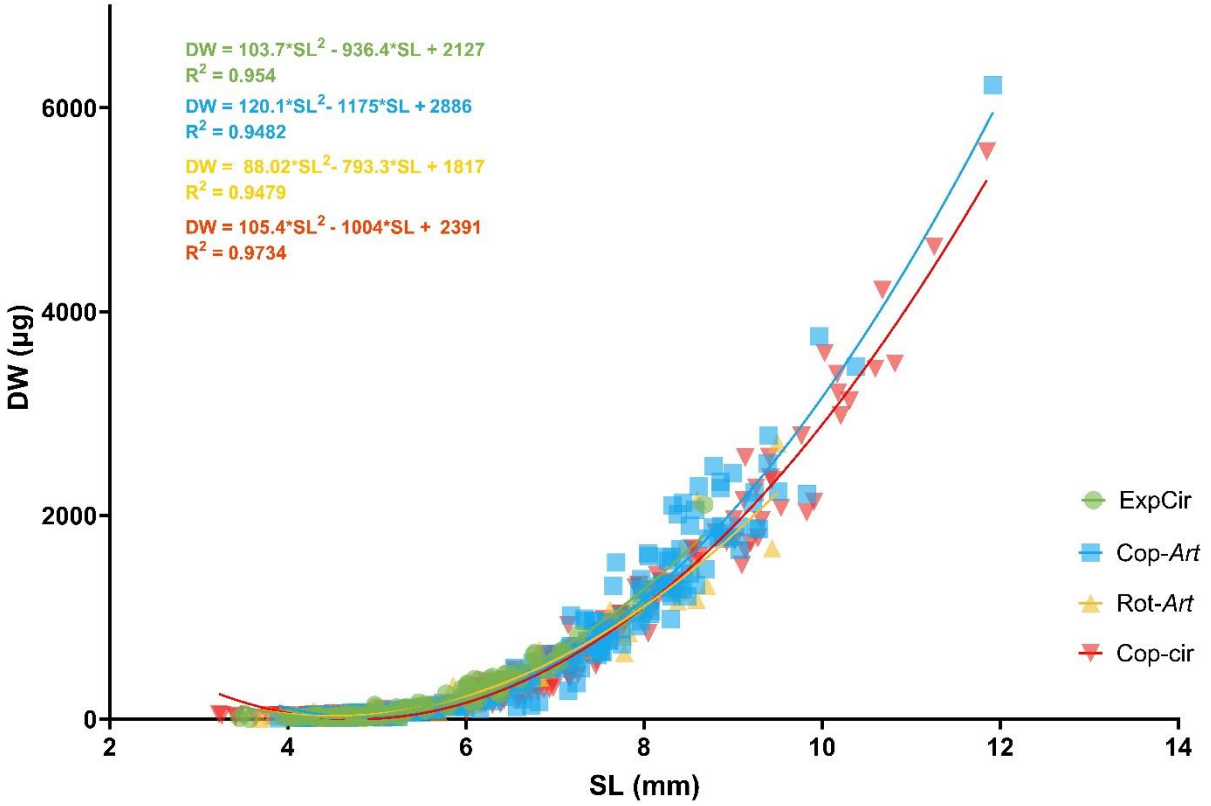


Figure 3.7. Relationship between standard length (SL, mm) and dry weight (DW, µg) in ballan wrasse larvae fed on four different live feed diets. Values from 4 to 48 DPH were included in the study of the correlation. Colors indicate feeding regime: Experimental cirriped (ExpCir; N= 137), Copepod-*Artemia* (Cop-*Art*; N= 212), Rotifer-*Artemia* (Rot-*Art*; N= 140) and Copepod-cirripeds (Cop-cir; N= 214). A statistically significant ($p < 0.05$, Spearman's correlation) strong, positive monotonic correlation was found for all the diets. An extra sum of squares F test revealed that the correlations were significantly ($p < 0.05$) better described by different equations per feeding regime than with a singular shared equation. In all cases, a second order polynomial (squared) model was the best fit.

Different models were tested to describe the regression, namely exponential growth, first order polynomial (straight line), second order polynomial (squared), and third order polynomial (cubic). The fit with the highest R^2 values was the third order polynomial model, followed closely by the second order polynomial (Table 3.1). Given that the graphical exploration of both models yielded very similar results (Appendix 4) and a simpler model is preferable, second order polynomial equations were selected in the present work (Figure 3.7).

Table 3.1. R^2 values for each of the models tested to describe the relationship between DW and SL. Fits of each model were explored for each feeding regime separately. Feeding regimes: Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*), Copepod-cirriped.

	ExpCir	**Cop-*Art***	**Rot-*Art***	**Cop-cir**
Exponential growth	0.964	0.893	0.919	0.942
First order polynomial (straight line)	0.853	0.791	0.843	0.779
Second order polynomial (squared)	0.954	0.948	0.948	0.973
Third order polynomial (cubic)	0.966	0.949	0.948	0.975

A comparison of second order polynomial fits was used to evaluate whether one single equation could adequately describe the relationship between DW and SL for all diets. The results indicated that having a different curve for at least one of the diets was the preferred model (Extra sum-of-squares F test. Null hypothesis: one curve for all datasets; alternative hypothesis: different curve for at least one dataset; $p < 0.0001$). Regressions could be described by equation 3.1 (Experimental cirriped), equation 3.2 (Copepod-*Artemia*), equation 3.3 (Rotifer-*Artemia*), and equation 3.4 (Copepod-cirriped), where DW is dry weight (µg) and SL is standard length (mm).

$$DW = 103.7 * SL^2 - 936.4 * SL + 2127 \quad [3.1]$$

$$DW = 120.1 * SL^2 - 1175 * SL + 2886 \quad [3.2]$$

$$DW = 88.02 * SL^2 - 793.3 * SL + 1817 \quad [3.3]$$

$$DW = 105.4 * SL^2 - 1004 * SL + 2391 \quad [3.4]$$

3.2.6 Mortality

The groups that were fed on copepods as a first live feed prey (Copepod-*Artemia* and Copepod-cirriped) had significantly ($p < 0.05$) lower mortalities than those that received rotifers or experimental cirripeds (Rotifer-*Artemia* and Experimental cirriped) (Table 3.2, Figure 3.8).

Table 3.2. Mortality and survival of the larvae at the end of the experiment. The table shows the mean mortality coefficients *m*, and mean modelled number of surviving larvae at 48 DPH ± SD for each diet (N=3), considering the initial stocking density and number of larvae that were sampled during the experiment. Feeding regimes: Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*), Copepod-cirriped.

	ExpCir	**Cop-*Art***	**Rot-*Art***	**Cop-cir**
Mortality coefficient *m*	0.11 ± 0.02	0.05± 0.00	0.11 ± 0.02	0.06 ± 0.01
Remaining larvae tank^{-1}	142 ± 126	1594 ± 323	141 ± 89	1303 ± 791
Survival at 48 DPH (%)	0.7	8.1	0.7	6.6

The mean surviving larvae at the end of the experiment (48 DPH) were calculated based on a model considering the initial stocking density (19 716 larvae tank-^{1}), and the number of larvae that were removed from the tanks for sampling, which amounted to approximately 2.5-5% of the initial stock (Table 3.2). Two moments of peak mortality were identified, one around 14 DPH, compatible with the complete resorption of the yolk sac, and another one during weaning from live prey to formulated dry feed, in both cases more marked in the feeding regimes that did not receive copepods as the first live feed prey (Experimental cirriped and Rotifer-*Artemia*).

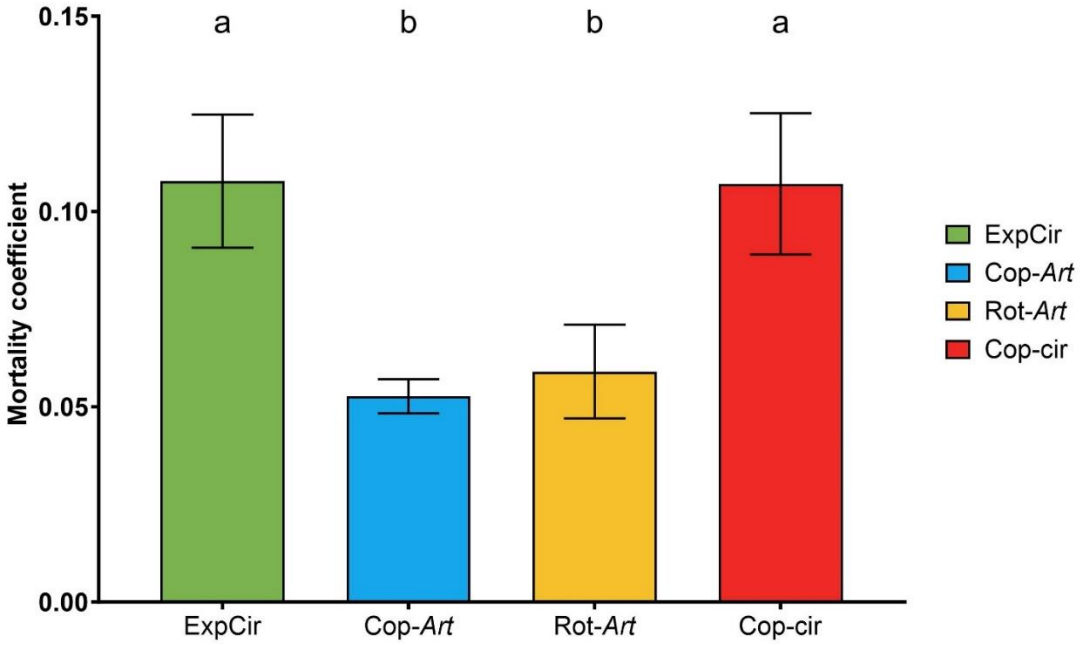


Figure 3.8. Mortality coefficients after 48 days of start feeding experiment. Mean mortality coefficients *m* ±SD are represented for each diet (N=3). The bars indicate SD. Statistically significant differences ($p<0.05$) between feeding regimes are depicted by unequal letters. Feeding regimes: Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*), Copepod-cirriped.

3.3 Lipid analyses

3.3.1 Comparative study of the evolution of fatty acid profiles and total lipids in feed and in ballan wrasse larvae

A total of 30 fatty acids, as well as total lipids, were quantified in the different feeds and in the larvae along the experiment. Values for each fatty acid (% of total fatty acids), sum of fatty acids, and total lipids (% w/w dry weight) are summarized in Appendix 5. Due to time constraints derived from the COVID-19 shutdown, not all sampled time points could be analyzed. Priority was given to those representative for each type of feed and/or feeding period: 4, 12, 18, 32, and 48 DPH.

An initial exploration of the fatty acid composition of the larvae and feed was conducted through hierarchical clustering, visualized in heatmaps. The heatmap containing the whole dataset for all larvae and feed types showed clustering of the larvae together with the last feed item they were receiving in the case of *Artemia* and dry feed (Figure 3.9, groups red and yellow, respectively). On the other hand, no clear clustering was found between the feed and larvae for the other treatment groups.

In general, clear clusters were seen between larvae samples based on the last live feed they were receiving, as well as between replicates of each type of feed. Small and large copepods had a similar composition and were not differentiated as separate groups in the hierarchical clustering analysis. This pattern was also observed in larvae that had small or large copepods as last live feed, which also clustered together independently of copepod stage (Figure 3.9, pink group for small copepods and light blue for large copepods).

Some fatty acids showed different proportions compared to total fatty acids in larvae. Before introduction of the feeds, larvae had high C16:1n5 and C16:1n9, and moderate n-3 fatty acids (purple group in Figure 3.9). The n-3 fatty acids displayed similar levels to those found in larvae fed on copepods and in the cirriped feed. Both *Artemia* (the feed) and larvae that had *Artemia* as their last diet had a lower proportion of n-3 fatty acids, higher C20:4n6 (ARA), C18:1n9, C18:1n7 and C16:1n5 (red group in Figure 3.9). Larvae receiving dry feed (and the dry feed itself) had higher n-6 fatty acids and lower n-3 fatty acids (yellow group in Figure 3.9). Rotifers were characterized by higher C22:5n6 (docosapentaenoic acid, DPA) and C17:1n7, lower n-3 fatty acids, and higher C14:1n5, C14:0, C22:1n9; C20:1n9 and C16:1n7 (orange group in Figure 3.9). Larvae receiving rotifers showed lower n-3 fatty acids. Copepods had lower saturated fatty acids, especially C16:0 and C18:0, and higher unsaturated fatty acids (especially n-3 fatty acids) (pink and light blue groups in Figure 3.9). Finally, cirripeds were characterized by low saturated fatty acids (C16:0 and C18:0), high unsaturated fatty acids (especially n-3) high EPA/ARA proportion, and high EPA (Figure 3.9) (green and dark blue groups in Figure 3.9).

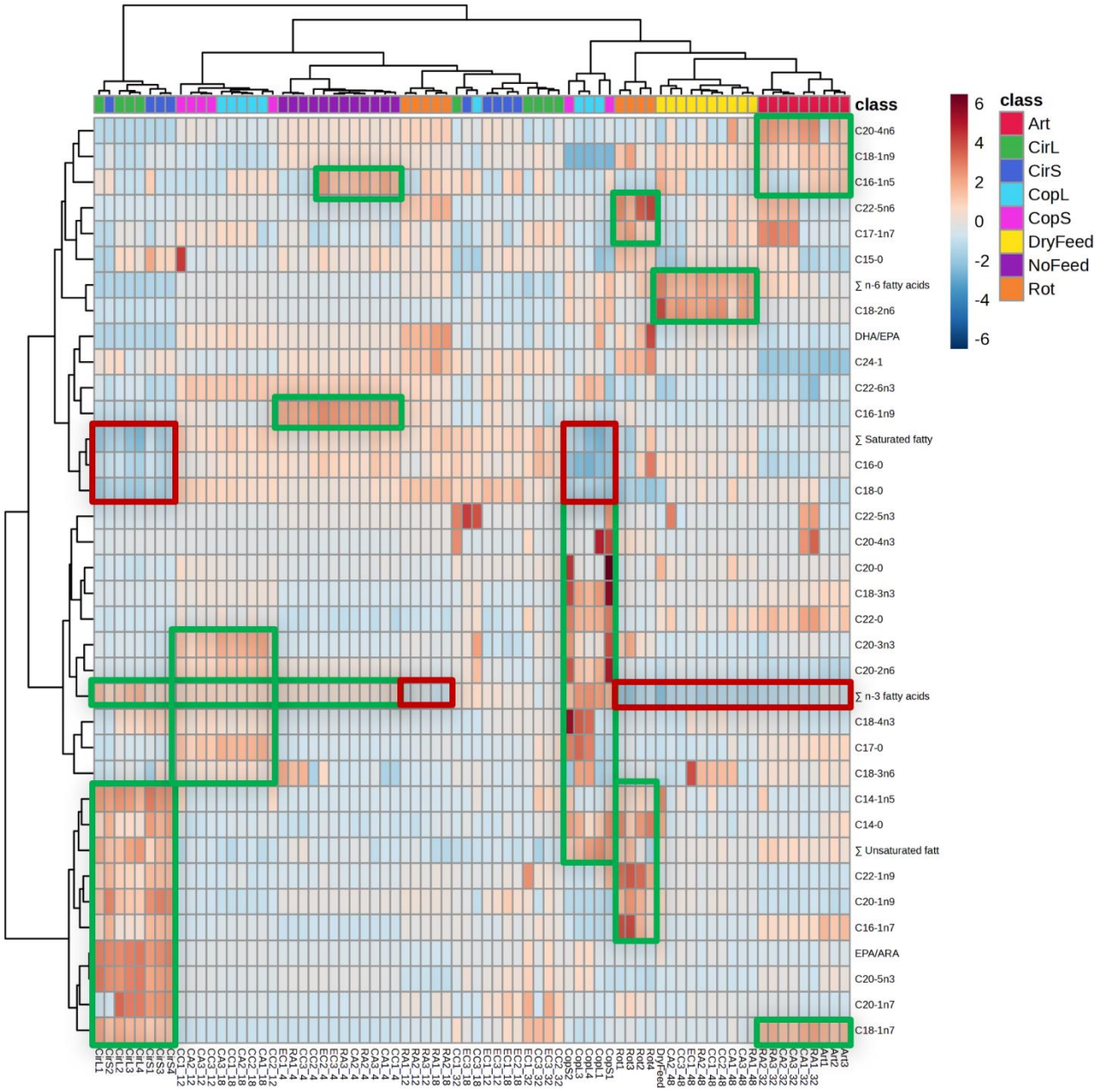


Figure 3.9. Heatmap illustrating hierarchical clustering of larvae and feed based on fatty acid composition. The hierarchical clustering was performed in Metaboanalyst 6.0 based on fatty acid composition expressed as % of total fatty acids. Euclidean distance was used as similarity measure and Ward's linkage as clustering algorithm. The heatmap was generated using normalized data (autoscaling: mean-centered and divided by the square root of the standard deviation of each variable; Appendix 6 Figure A6a and Appendix 7 Figure A7a). Colors from red to blue indicate Z score (standard deviation from the mean), with deeper red reflecting higher abundance of a given fatty acid species, and deeper blue lower abundance. Rows correspond to metabolites and columns to samples, the latter grouped by type of feed (feed samples) and last feed received (larval groups). **Feed samples:** Art (*Artemia*); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers), number indicates replicate. **Larval groups**, based on the diet they received: EC (experimental cirriped), CA (copepod-*Artemia*), RA (rotifer-*Artemia*), CC (Copepod-cirriped), first number indicates replicate; number after underscore indicates time (DPH). Features that are consistently higher (green) or lower (burgundy) per groups are highlighted with surroundings rectangles.

The fatty acid composition in feed and larvae was further studied by unsupervised principal component analysis (PCA) (Figure 3.10 for the feeds, and Figure 3.11 for the larvae).

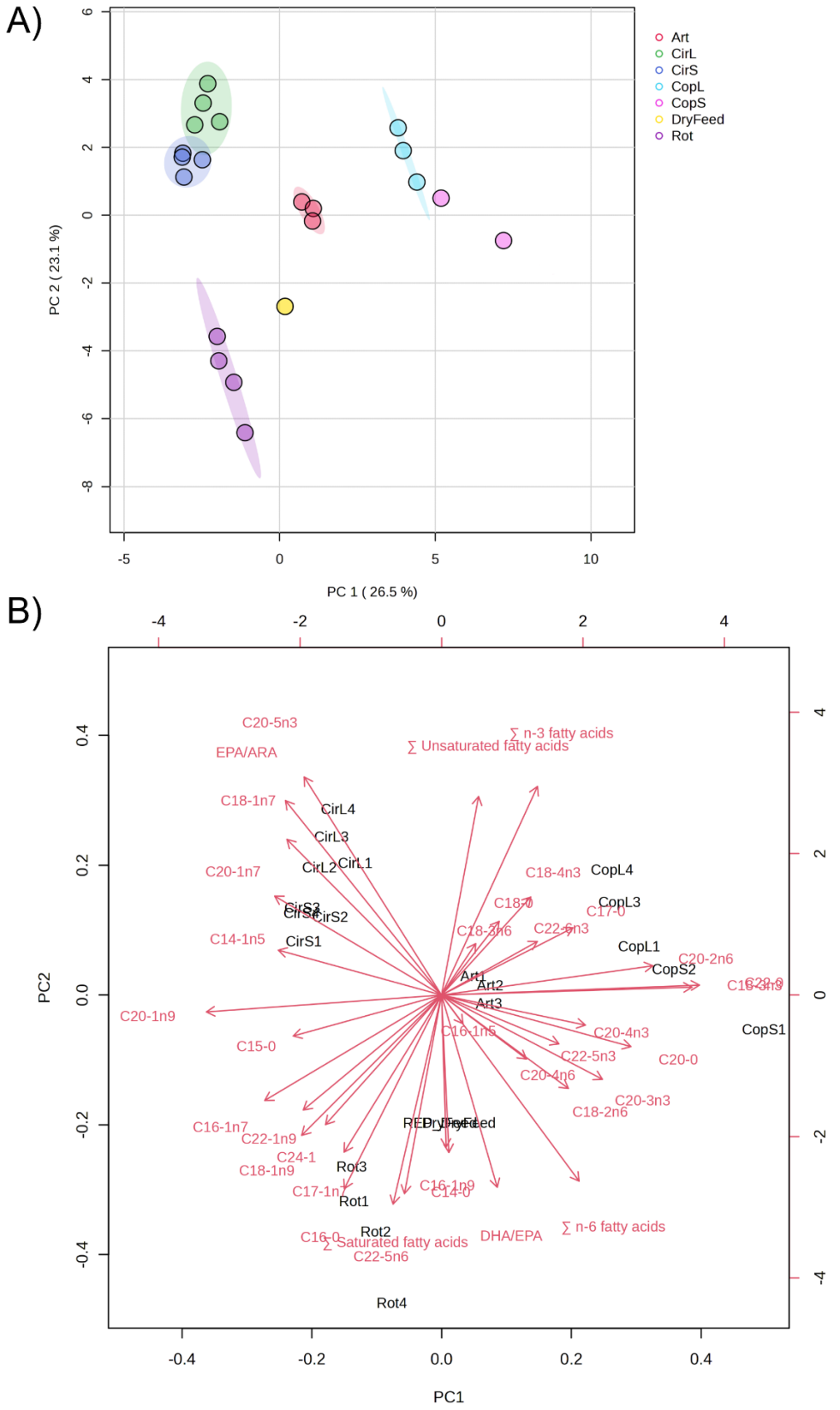


Figure 3.10. PCA analysis of fatty acid composition in feeds. 2d-scores plot **(A)** and biplot **(B)** are shown. Vectors in the biplot indicate increasing concentration. Figures were made using MetaboAnalyst 6.0 (Pang et al., 2021). Groups in A) are indicated by color, and the shading corresponds to the 95% confidence interval for each group, calculated using bootstrap resampling. The explained variances for each principal component (PC) are shown in brackets (A). **Diets:** Art (*Artemia*); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers). Panel A) with sample names can be found in Appendix 6, Figure A6b.

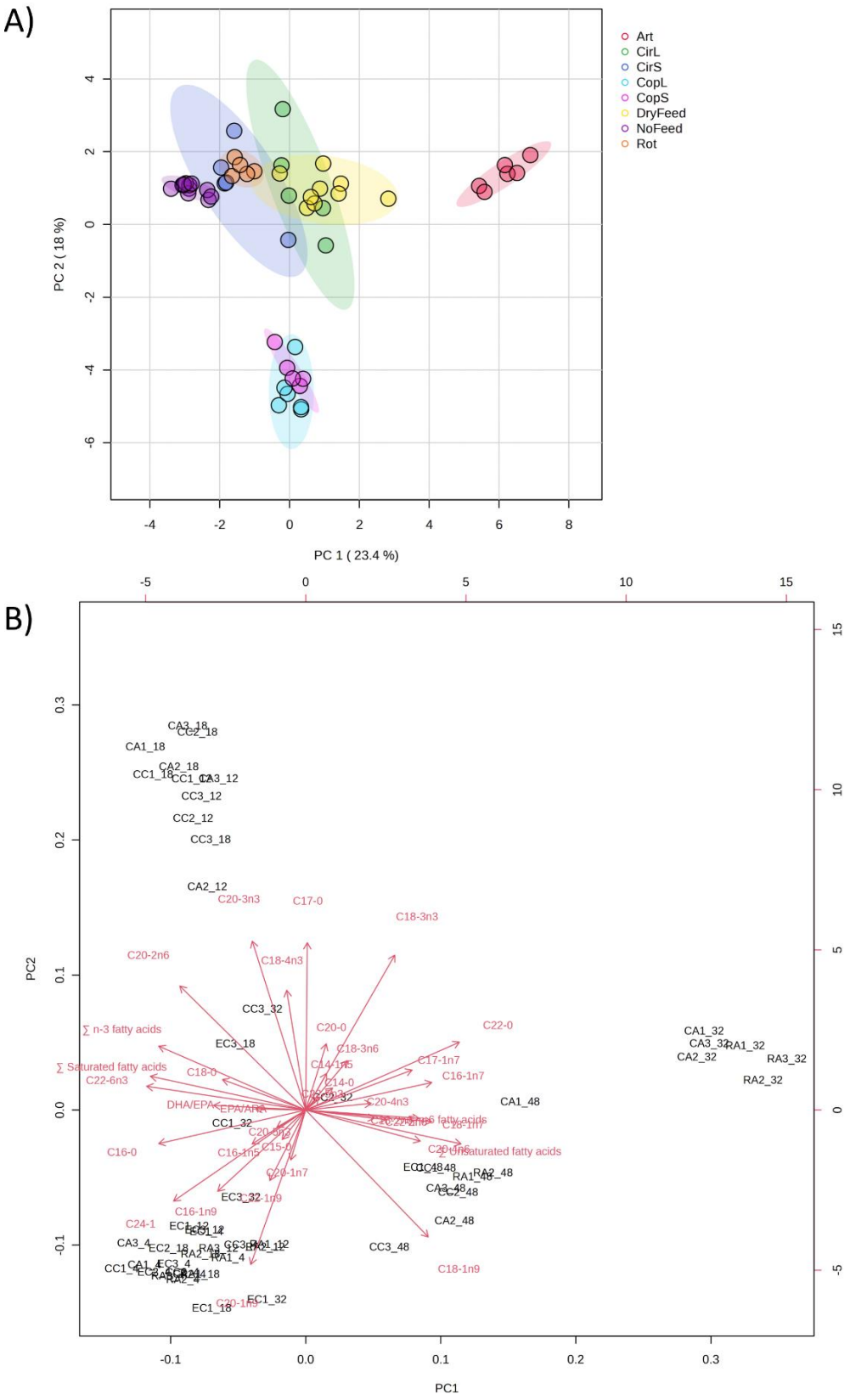


Figure 3.11. PCA analysis of fatty acid composition in larvae. 2d-score plot **(A)** and biplot **(B)** from MetaboAnalyst 6.0 (Pang et al., 2021). Vectors in the biplot indicate increasing concentration. Groups in A) indicate last feed received, and the shading corresponds to the 95% confidence interval for each group, calculated using bootstrap resampling. The explained variances for each principal component (PC) are shown in brackets (A). EC (Experimental cirriped), CA (Copepod-*Artemia*), RA (Rotifer-*Artemia*), CC (Copepod-cirriped), first number indicates replicate; number after underscore indicates time (DPH). Panel A) with sample names can be found in Appendix 7, Figure A7b.

PCA of fatty acid composition in feeds **(Figure 3.10)**

The principal components PC1 and PC2 together explained 49.6% of the variance in the fatty acid composition of the feeds (Figure 3.10). The metabolites contributing the most to PC1 were the proportion of C22:0, C18:3n3 (α-linolenic acid, ALA), C16:1n7, C20:2n6, and C20:0. PC2 was mostly determined by the proportion of C20:5n3 (EPA), the sum of n-3 fatty acids, C22:5n6 (DPA), the sum of unsaturated fatty acids, the sum of saturated fatty acids, EPA/ARA, C16:0, and DHA/EPA ratio.

Separated clusters were observed for each of the feed types, whose replicates grouped. The two cirriped feed types (green and dark blue in Figure 3.10) were close to each other, something which was also observed for the two copepod feed types (pink and light blue groups in Figure 3.10), indicating similar fatty acid compositions between different naupliar stages of each feed. The clustering in the copepod groups was motivated by higher concentrations of unsaturated and n-3 fatty acids, as well as C22:6n3 (DHA). As for the cirripeds, their clustering was mostly driven by higher proportions of C20:5n3 (EPA), EPA/ARA ratio, and other unsaturated fatty acids such as C18:1n7, C20:1n7, and C14:1n5. Among other features, rotifers had higher proportions of saturated fatty acids, such as C16:0.

PCA of fatty acid composition in larvae **(Figure 3.11)**

The principal components PC1 and PC2 together explained 41.4% of the variance in the fatty acid composition of the larvae (Figure 3.11). The metabolites with the highest scores on PC1 were C22:6n3 (DHA), the sum of saturated fatty acids, the sum of unsaturated fatty acids, C22:0, C16:0, and the sum of n-3 fatty acids. As for PC2, it was mostly driven by C20:3n3, C17:0, C18:3n3 (α-linolenic acid, ALA), C20:1n9 and C18:1n9.

Larvae grouped in clusters according to the last feed that they received (Figure 3.11). The separation was clearer in the case of two groups. Firstly, a clear cluster was formed by larvae that had received *Artemia* as last feed item, mostly driven by higher percentage of unsaturated fatty acids (including C18:1n9, C20:4n6, C22:5n6, C17:1n7, C18:3n3, C18:1n7 and C16:1n7), high proportion of C22:0, lower DHA/EPA ratios and lower proportion of saturated fatty acids (Appendix 7, Figure A7c). Secondly, larvae that had received copepods of all naupliar stages grouped together, without distinction between having received "small copepods" (n1-3) or "large copepods" (n4-6) (pink and light blue groups in Figure 3.11). In this case, the grouping of larvae that received copepod as the last feed item was influenced by a high percentage of n-3 fatty acids, including C22:6n3 (DHA), C18:4n3, C20:3n3, C18:3n3 (ALA), as well as C20:2n6.

After the general exploration of the datasets using non-supervised PCA and hierarchical clustering, a deeper study of the fatty acid composition of feed and larvae was performed, as indicated in the following sections.

3.3.1.1 Total lipids and fatty acids of the feed

The total lipid concentrations (% w/w of dry weight) of each of the feeds were quantified. Clear differences were found between the different diets, with enriched *Artemia* showing the highest total lipid concentrations, followed by rotifers and dry feed. Small experimental cirripeds and small copepods had significantly lower total lipid content, and, finally, the lowest concentrations were seen in large copepods and large cirripeds (Figure 3.12).

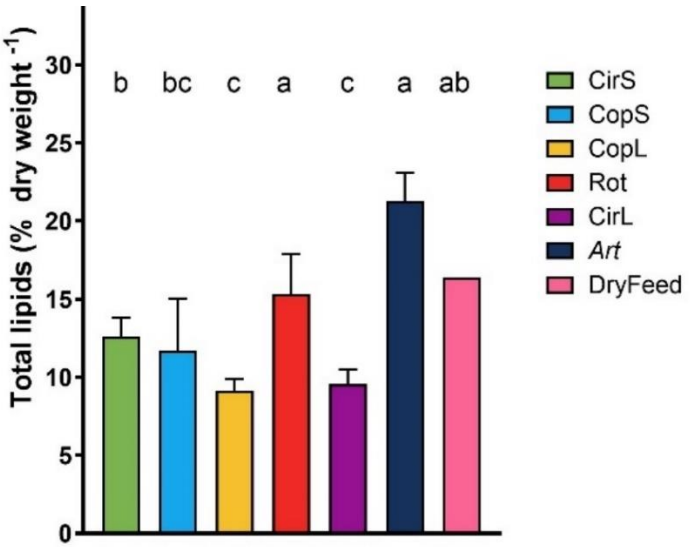


Figure 3.12. Total lipid content of each of the live feeds used in the start feeding experiment. Means and SD are shown (N>3). Colors refer to the type of feed (*Art* (*Artemia*); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers), Dry Feed (formulated dry feed). Statistically significant differences ($p<0.05$, one-way ANOVA followed by Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar. Percentual values were arcsine square root transformed before applying statistics.

The composition of selected fatty acids (% of each fatty acid with respect to the total) was compared for each of the feeds, grouping them according to the phase where they were supplied to the larvae: either the first or second live feed period (Table 2.1).

The **first live feed period** consisted of rotifers or rotifer-replacers, namely small copepods followed by large copepods, or small experimental cirripeds (Figure 3.13 A). Significant differences were found between the feeds for most of the fatty acid groups that were explored, except for ARA, DHA/EPA ratio, the sum of saturated fatty acids, and the sum of unsaturated fatty acids, which were similar in all cases. Small copepods showed higher proportions of LA and ALA, whereas large copepods had the highest proportions of DHA and highest sum of n-3 fatty acids. Experimental cirripeds were rich in EPA and had the

highest EPA/DHA ratio. The sum of n-3 fatty acid was lower in experimental cirripeds than in large copepods, but still significantly above the values observed for rotifers. In turn, experimental cirripeds had the lowest values for the sum of n-6 fatty acids. Finally, rotifers had significantly different fatty acid percentages than the other feeds in several cases, including the lowest levels of EPA, the highest proportion of DPA, and the lowest sum of n-3 fatty acids. Although significant differences were found in total lipids (% w/w of dry weight) between large copepods and rotifers, there was not a feed that had significantly higher or lower total lipids than all the other groups.

During the **second live feed period**, either enriched *Artemia* or *Artemia*-replacers were supplied to the larvae (Figure 3.13 B, which also depicts the values for dry feed, which was supplied during the last phase of the experiment). No significant differences were found in the concentrations of ARA, DPA, DHA, and DHA/EPA ratio between feeds. Dry feed had significantly higher LA content than large cirripeds and *Artemia*, as well as a lower proportion of n-3 fatty acids and higher sum of n-6 fatty acids. In turn, large cirripeds had the highest EPA and EPA/ARA ratio among the feeds in the group, as well as significantly higher values for the sum of unsaturated fatty acids and the sum of n-3 fatty acids, whereas the sum of n-6 fatty acids was the lowest of the three feeds, similarly to what was observed for the experimental cirripeds (Figure 3.13 A). Large cirripeds also had the lowest sum of saturated fatty acids. The total sum of unsaturated and sum of saturated fatty acids was very similar between dry feed and *Artemia*. Finally, *Artemia* showed the highest total lipids proportion in relation to dry weight compared to large cirripeds, as previously stated.

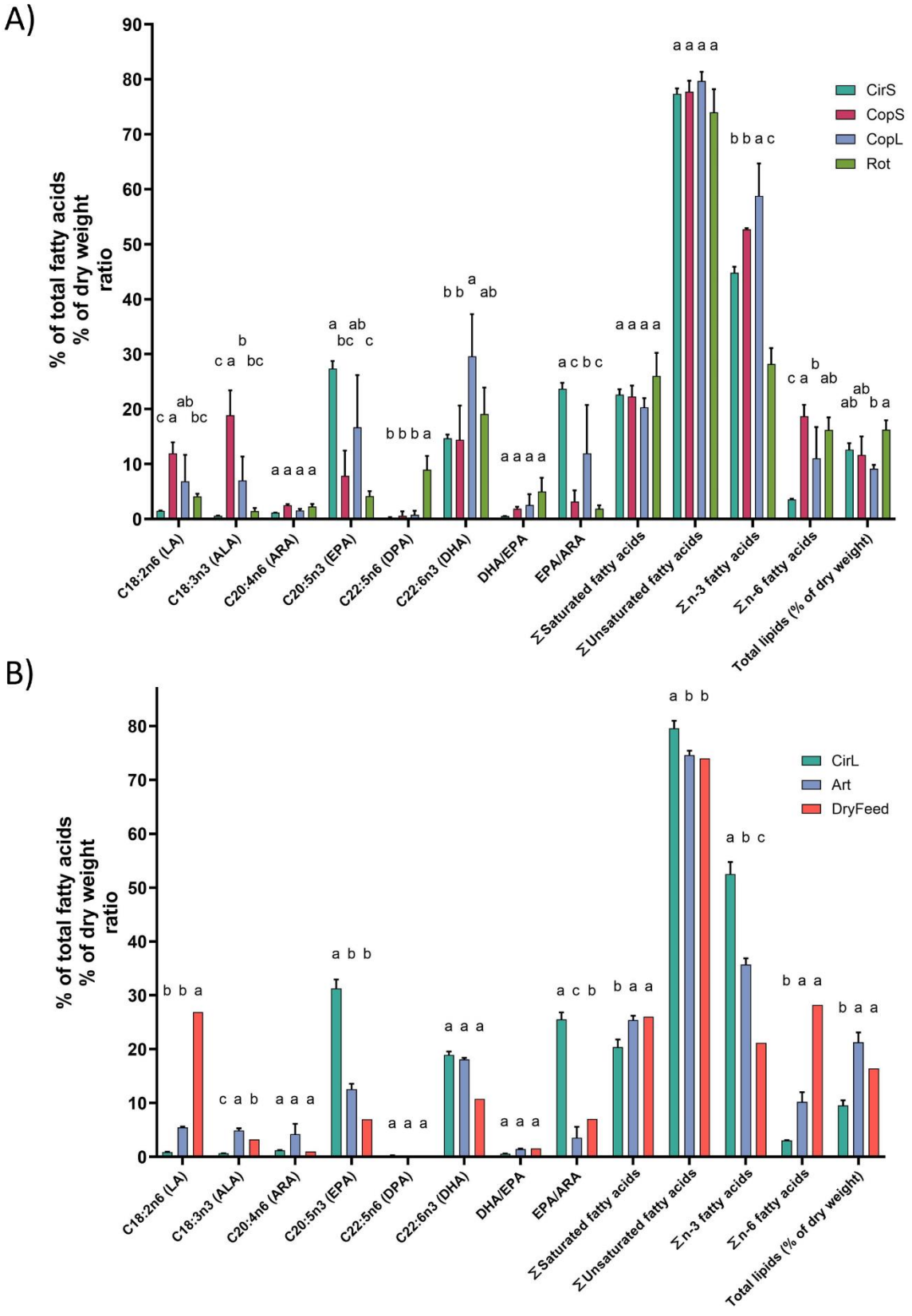


Figure 3.13. Selected fatty acids or groups of fatty acids and total lipids of feed, grouped by feeding phase. Means and SD are shown (N>3). **A)** Feeds supplied during the first live feed phase (rotifers and rotifers replacers: experimental small cirripeds, CirS; small copepods n1-3, CopS; large copepods n4-6; CopL, rotifers, Rot); **B)** Feeds supplied during the second live feed phase and during the last formulated dry feed phase (*Artemia* and replacers: *Artemia*, Art; large cirripeds CirL. As well as dry feed). Statistically significant differences ($p<0.05$, one-way ANOVA and Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar.

3.3.1.2 Total lipids and fatty acids of the larvae

The evolution of the total lipid concentrations (% w/w of dry weight) in the larvae during the experiment showed a general decrease from 4 to 12-18 DPH, compatible with the resorption of the yolk sac. The only significant differences were observed at 32 DPH (significantly higher for larvae that were receiving *Artemia* during this period, namely Cop-*Art* and Rot-*Art*), and at the last time point (48 DPH), between larvae that received the Experimental cirriped diet and those that were fed Copepod-*Artemia* (Figure 3.14).

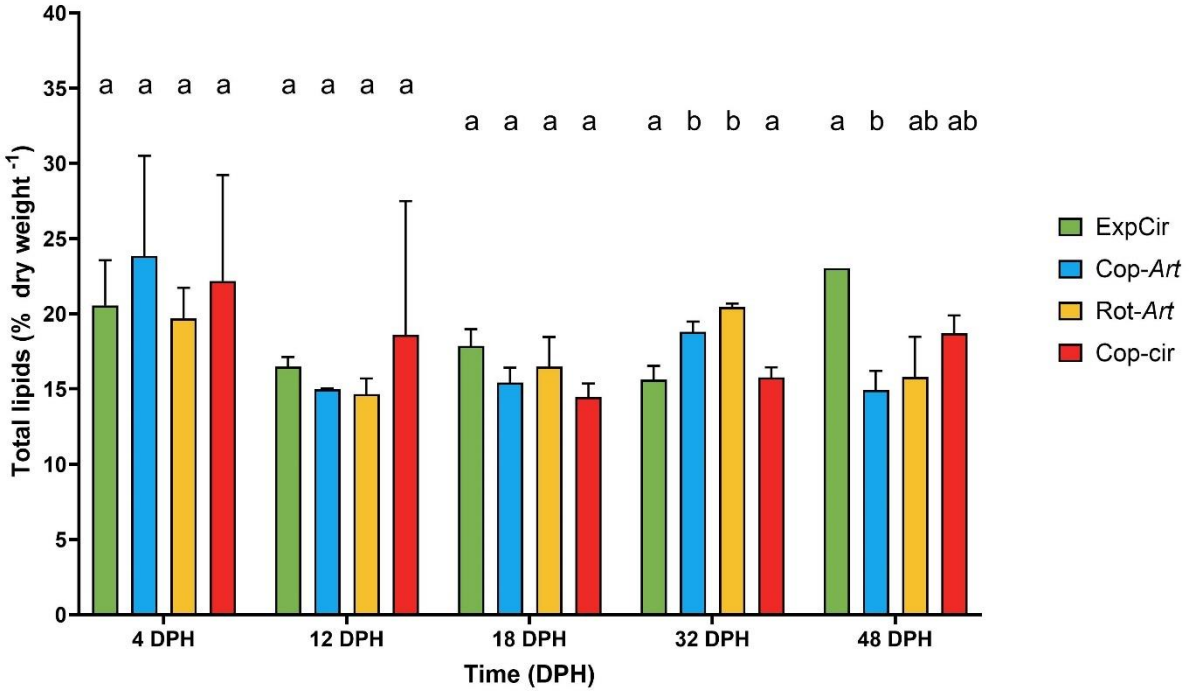


Figure 3.14. Evolution of the total lipid content of the larvae receiving each of the four different first feeding regimes during the experiment. Means and SD for each 3 tanks are shown. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$, one-way ANOVA followed by Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar. Percentual values were arcsine square root transformed before applying statistics.

The fatty acid composition was also studied in the larvae receiving each of the four different feeding regimes along the experiment. A comparison was made by means of one-way ANOVA followed by Tukey's multiple comparison test to explore differences in concentrations of selected fatty acids between the different groups, at each time-point.

No significant differences were found in fatty acid composition for the selected metabolites **at 4 DPH**, before the introduction of the feeds (Appendix 8, Figure A8).

At 12 DPH, significant differences could already be seen in some of the fatty acids or fatty acid groups, while no differences were detected between larvae being fed either of the four diets when it came to DHA/EPA and EPA/ARA ratios, sum of saturated fatty acids, sum of unsaturated fatty acids, and total lipids (Figure 3.15). Both groups receiving copepods as

a first live feed (Cop-*Art* and Cop-cir) showed similar values for all the selected fatty acids. Moreover, significant differences were found between these two feeding regimes and the larvae receiving experimental cirripeds and rotifers, concerning concentrations of LA, ALA, EPA, DPA, sum of n-3 fatty acids, and sum of n-6 fatty acids. Larvae receiving rotifers (Rot-*Art* diet) had significantly higher ARA and DPA proportions compared to the other three feeding regimes, as well as a lower sum of n-3 fatty acids and higher sum of n-6 fatty acids. Finally, lower concentrations of LA and higher EPA were found in larvae fed experimental cirripeds (ExpCir diet).

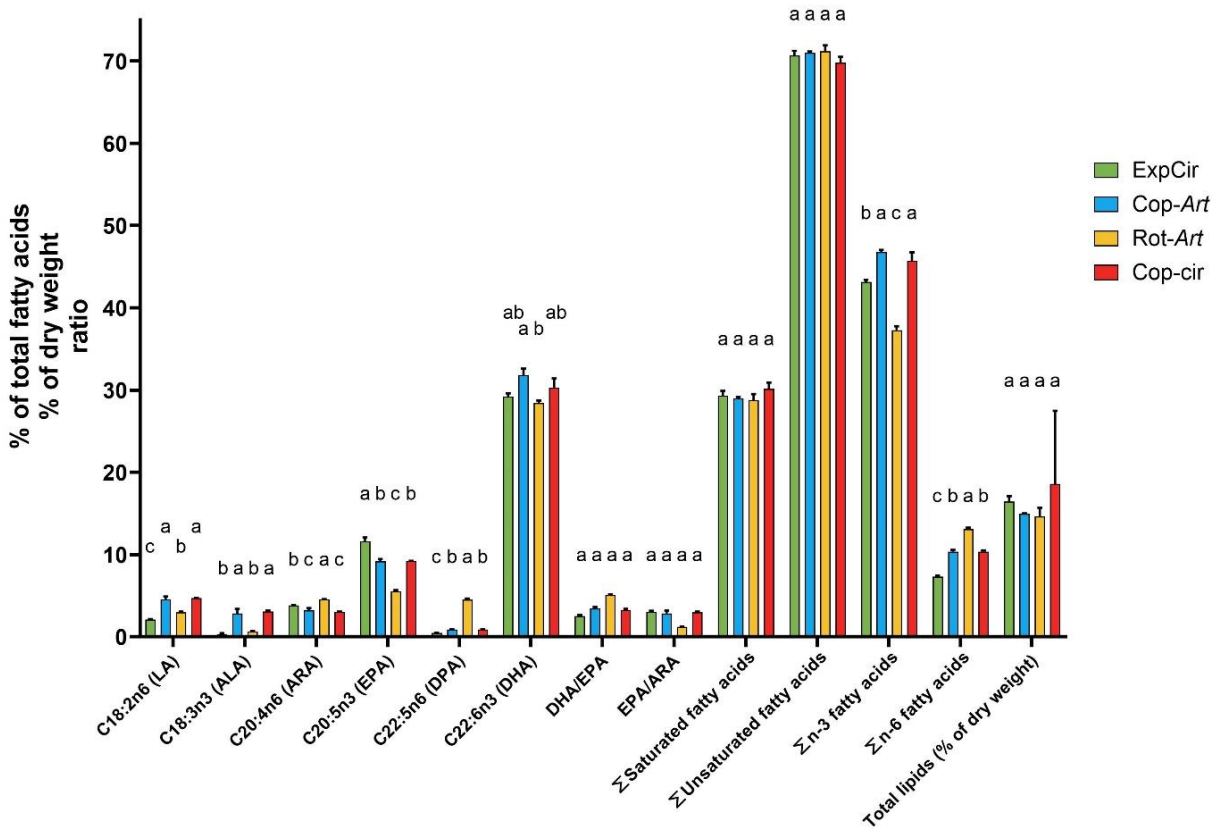


Figure 3.15. Selected fatty acids or groups of fatty acids and total lipids of the larvae at 12 DPH. Means and SD for each 3 tanks are shown. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-Artemia (Cop-Art), Rotifer-Artemia (Rot-Art) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$, one-way ANOVA followed by Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar. Percentual values were arcsine square root transformed before applying statistics.

The feeds supplied to each of the larval groups **at 18 DPH** were still similar to those for 12 DPH. No significant differences between the four feeding regimes were seen in concentrations of ALA, DHA, EPA/ARA ratio, sum of saturated fatty acids, sum of unsaturated fatty acids, and total lipids (Figure 3.16). As also observed at 12 DPH, the larvae receiving a similar feed type (Cop-*Art* and Cop-cir diets, both fed on copepods at this stage) showed no significant differences in any of the selected fatty acids and groups.

Larvae fed on experimental cirripeds (ExpCir) and rotifers (Rot-*Art*) had lower LA proportions, higher ARA, and lower sum of n-3 fatty acids, the latter especially marked in the Rot-*Art* treatment. Rot-*Art* also showed the highest DPA concentrations, lowest EPA, and highest DHA/EPA ratio, as well as the highest sum of n-6 fatty acids. Finally, larvae fed on experimental cirripeds had the highest EPA content and the lowest sum of n-6 fatty acids of all four groups.

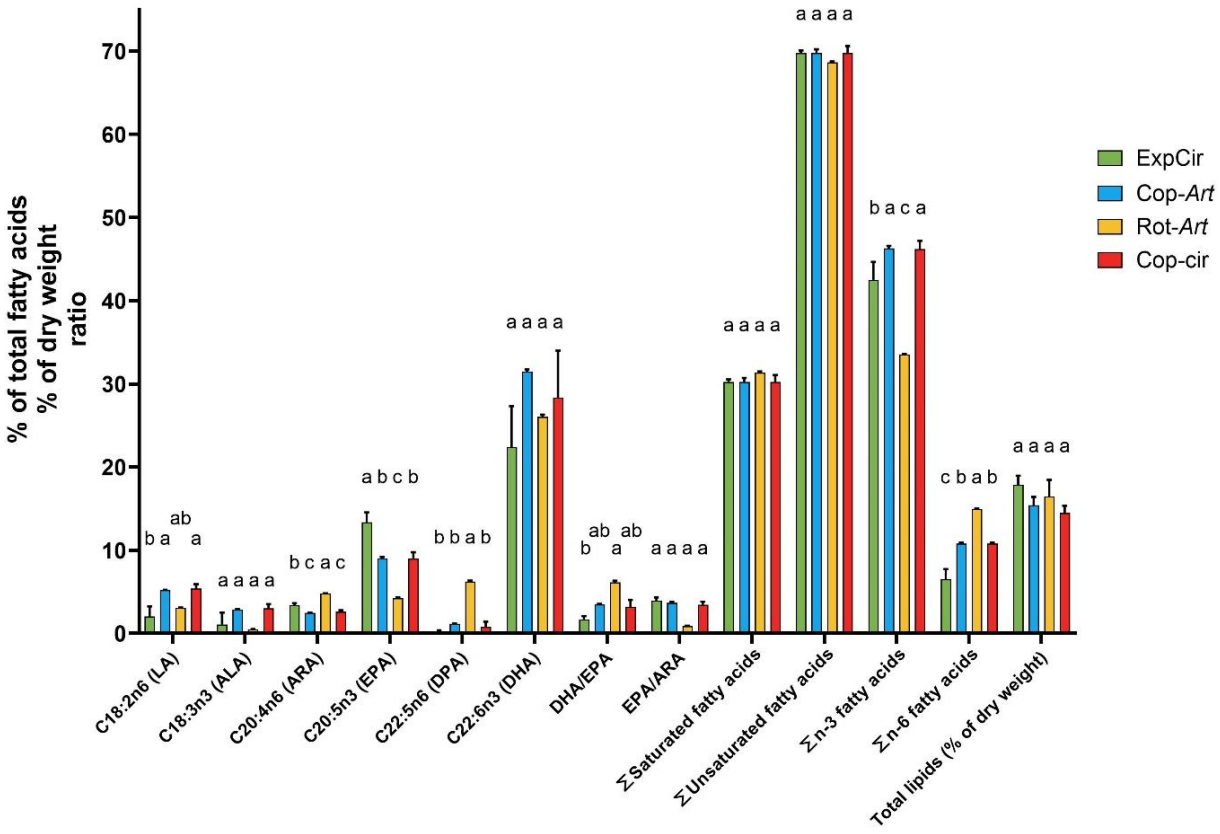


Figure 3.16. Selected fatty acids or groups of fatty acids and total lipids of the larvae at 18 DPH. Means and SD for each 3 tanks are shown. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-Artemia (Cop-Art), Rotifer-Artemia (Rot-Art) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$, one-way ANOVA followed by Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar. Percentual values were arcsine square root transformed before applying statistics.

At **32 DPH**, the larvae had been receiving the second live feed (either *Artemia* or large cirripeds) for 14 days. No significant differences were seen between larvae from the four feeding regimes in concentrations of EPA, DPA, and DHA/EPA ratio (Figure 3.17). Larvae fed on *Artemia* during this period (Cop-*Art* and Rot-*Art* diets) did not show significant differences in the concentration of any of the selected fatty acids, fatty acid groups or total lipid content. Larvae fed on large cirripeds during the second live feed phase (ExpCir and Cop-cir diets) had similar values for all the categories, except for concentrations of LA and ALA (significantly higher in Cop-cir), and the EPA/ARA ratio, which was significantly higher

in ExpCir. The larvae fed on *Artemia* during this phase (Cop-*Art* and Rot-*Art*) and those supplied with large cirripeds (ExpCir and Cop-cir) displayed significant differences between each other in concentrations of LA, ALA, ARA, the sum of saturated fatty acids, the sum of unsaturated fatty acids, the sum of n-3 fatty acids, the sum of n-6 fatty acids and the total lipid content (Figure 3.17). Some of the patterns in larvae fed on *Artemia* (higher proportion of the sum of n-6 fatty acids; lower EPA, DHA, and sum of n-3 fatty acids) were similar to those observed for rotifers at 18 DPH.

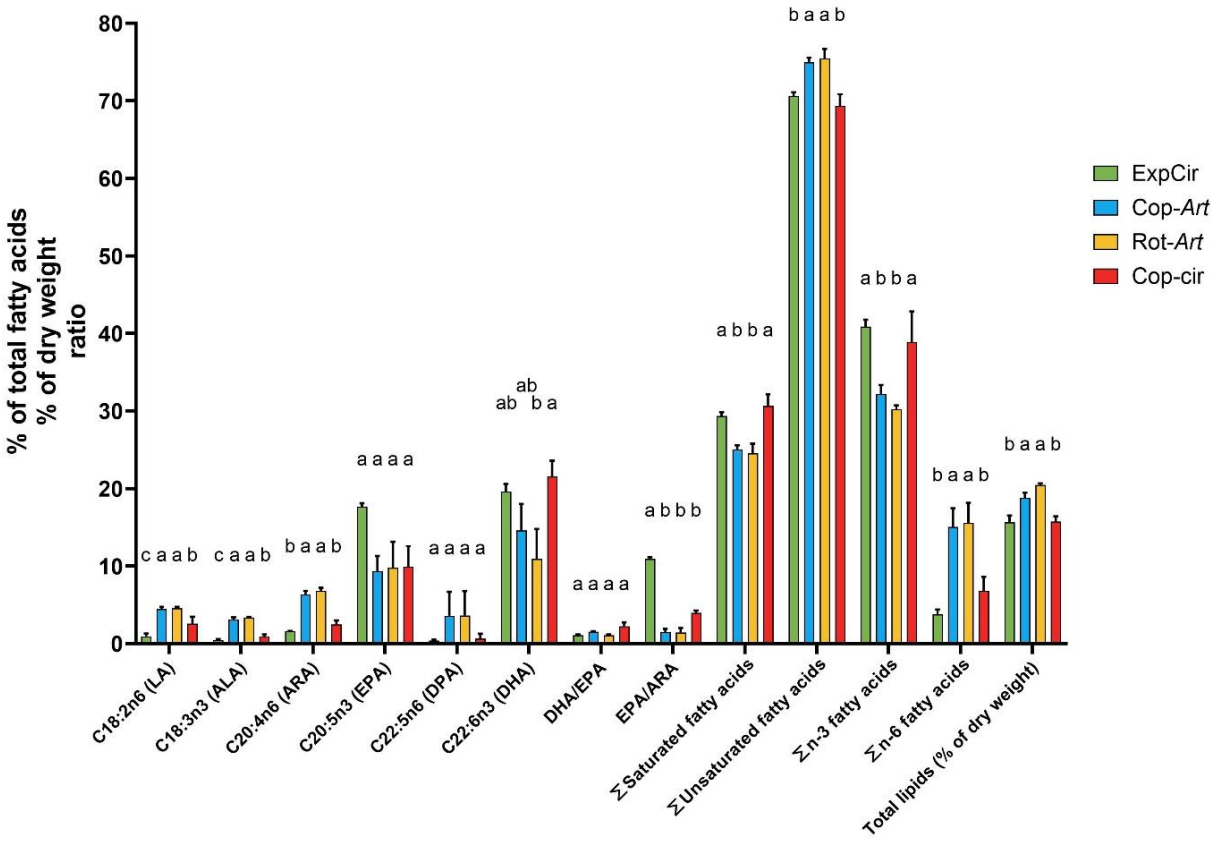


Figure 3.17. Selected fatty acids or groups of fatty acids and total lipids of the larvae at 32 DPH. Means and SD for each 3 tanks are shown. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-Artemia (Cop-Art), Rotifer-Artemia (Rot-Art) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$, one-way ANOVA followed by Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar. Percentual values were arcsine square root transformed before applying statistics.

Finally, less significant differences were seen at the last time point (**48 DPH**) between larvae subject to the different four feeding regimes compared to the other three time points with exogenous feeding. No significant differences were seen in concentrations of LA, ALA, EPA, DPA, DHA, DHA/EPA, EPA/ARA, sum of saturated fatty acids, sum of unsaturated fatty acids, and sum of n-6 fatty acids (Figure 3.18). ARA proportions were higher in larvae from the Cop-*Art* and Rot-*Art* feeding regimes. Rot-*Art* larvae showed a slightly lower sum of n-3 fatty acids compared to the other three diets.

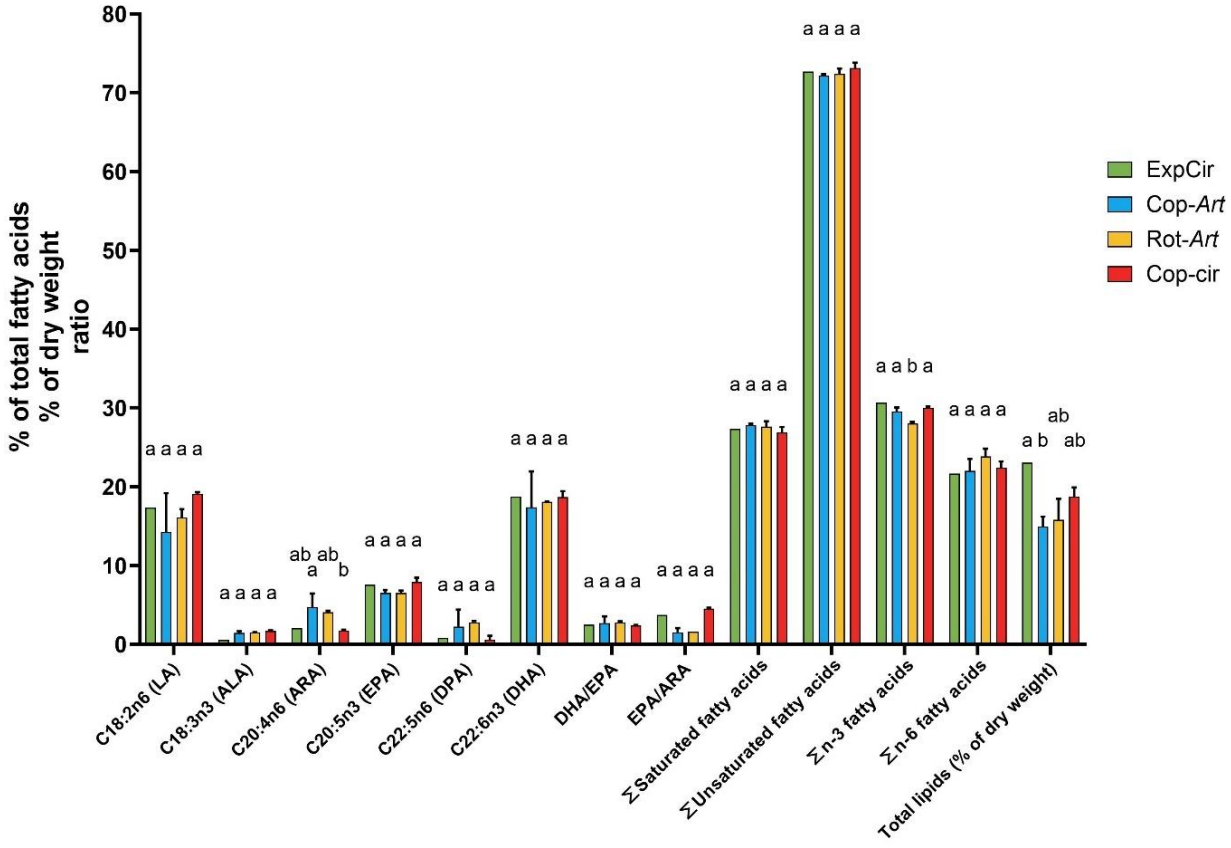


Figure 3.18. Selected fatty acids or groups of fatty acids and total lipids of the larvae at 48 DPH. Means and SD for each 3 tanks are shown. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-Artemia (Cop-Art), Rotifer-Artemia (Rot-Art) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$, one-way ANOVA followed by Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar. Percentual values were arcsine square root transformed before applying statistics.

Considering specific essential fatty acids for marine fish larvae, DHA proportions in the larvae decreased along the experiment for all diets, from approximately 30% before the start of exogenous feeding, to around 18% at 42 DPH. The decline was more evident in larvae receiving the Rotifer-*Artemia* diet during the second live feed phase (32 DPH). EPA was higher in the Experimental cirriped diet for all the time points prior to the second live feed phase, after which no significant differences were found among the surviving larvae for all diets. Larvae receiving the Rotifer-*Artemia* diet showed the lowest EPA proportions of all the diets during the first live feed phase (12 and 18 DPH). Finally, ARA proportions in the larvae changed depending on the supplied diet. The Experimental cirriped diet led to similar ARA concentrations as observed before the start of the exogenous feeding phase. ARA proportions increased in larvae fed on rotifers and decreased in those receiving copepods. With the introduction of the second live feed, the ARA proportions increased significantly in larvae receiving *Artemia*, which displayed higher ARA concentrations until the end.

3.3.2 Lipidomics analyses of the feed and ballan wrasse larvae

A total of 304 metabolites were included in the lipidomics analyses, belonging to nine different lipid classes (FFA: free fatty acids; lysoPC: lysophosphatidylcholines; lysoPE: lysophosphatidylethanolamines; MAG: monoacylglycerols; TAG: triacylglycerols; PC: phosphatidylcholines; PE: phosphatidylethanolamines; PI: phosphoinositides; and PS: phosphoserine). Similar to what was done for the fatty acid composition, lipidomics analyses were carried out in both the larvae and the feed. Data was normalized to an internal standard and to total sample weight, as well as autoscaled in MetaboAnalyst 6.0 before analysis (Appendix 9).

The whole lipidomics dataset (larvae for all time points, as well as feed samples) was firstly explored using sparse partial least squares regression - discriminant analysis (sPLS-DA), considering the top 50 lipids explaining the variance per principal component. Larval samples were categorized depending on the last feed they were receiving (Figure 3.19).

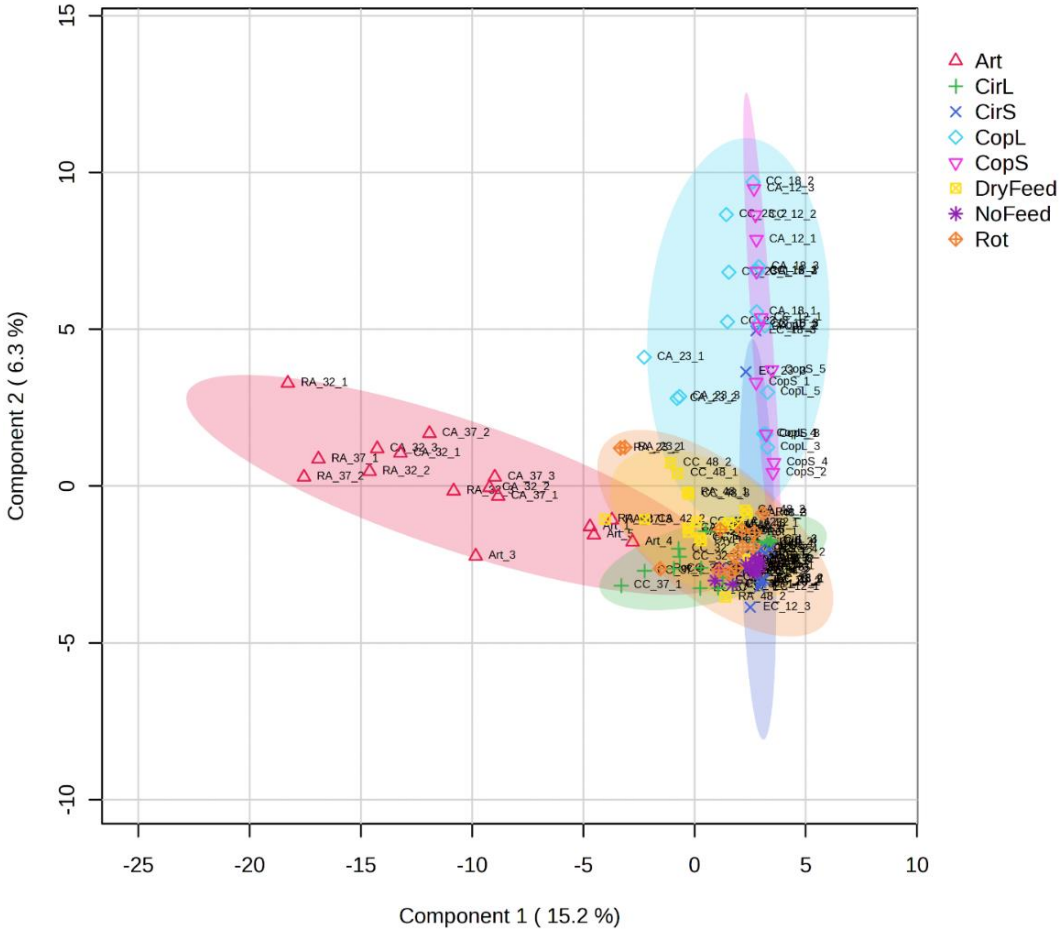


Figure 3.19. sPLS-DA analysis of the lipidomics data in larvae and feeds, together. 2d-scores plot from MetaboAnalyst 6.0 is shown (Pang et al., 2021). Groups are indicated by color, and the shading corresponds to the 95% confidence interval for each group, calculated using bootstrap resampling. The explained variances for each principal component (PC) are shown in brackets. **Abbreviation of feeds**: Art (*Artemia*); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers). **Larvae, based on the diet they received**: EC (Experimental cirriped), CA (Copepod-*Artemia*), RA (Rotifer-*Artemia*), CC (Copepod-cirriped), first number indicates replicate; number after underscore indicates time (DPH).

The principal components PC1 and PC2 explained together 21.5% of the variance in the lipidomics data for larvae and feed (Figure 3.19). The five lipid species with the highest loading scores in PC1 were triacylglycerols, namely TAG49:1-FA17:0, TAG49:1-FA16:0, TAG49:2-FA16:1, TAG49:2-FA18:1 and TAG49:1-FA18-1. As for PC2, it was mostly driven by the phosphatidylcholines PC16:1-16:3, PC18:0-19:1 (or 19:0-18:1), PC20:5-18:3, PC18:1-19:1 (or 18:2-19:0), and phosphatidylethanolamine PE40:8.

A clustering of the data points was observed in larvae receiving *Artemia* and the *Artemia* feed itself. A clustering was also observed in larvae fed on either small copepods (CopS, 12 DPH) or large copepods (CopL, 18 DPH) and the copepods as feed items, in this case especially in PC1. Other clusters were also observed for the rest of the groups but with marked overlap (Figure 3.19).

To further explore the differences between feeds and the impact of the diets on the lipidomics profiles of the larvae, each dataset was studied separately utilizing unsupervised PCA (feed) and sPLS-DA (larvae), as well as hierarchical clustering.

In the case of the **feeds**, PC1 and PC2 together explained 37.5% of the variance (Figure 3.20 A). PC1 was mostly driven by four triacylglycerols (TAG48:2-FA14:0, TAG16:1-16:1-16:2, TAG16:3-16:1-16:0, and TAG48:1-FA16:0) and the phosphatidylcholine PC18:1-20:0. As for PC2, the lipids with highest loading scores in this component were five phosphatidylethanolamines, namely PE36:3, PE18:1-18:2, PE36:1, PE16:0-20:1 (or 18:0-18:1) and PE36:3. Replicates of the same type of live feed clustered closely together in all diets except for rotifers, which displayed less compact grouping. As observed previously for the fatty acid analyses, the samples corresponding to small and large copepods grouped together independently from the naupliar stage. The groupings for large and small cirripeds were close to each other and to the copepods group, with no separation from copepods on PC2. *Artemia*, rotifers, and dry feed were more distant from the cirriped and copepod clusters (Figure 3.20 A). Hierarchical clustering heatmaps showed higher relative content of several TAGs in the small cirripeds, whereas other TAGs and selected PIs, lysoPCs, and PCs were high in *Artemia*. LysoPC18:2 was high in large copepods (Appendix 10, Figure A10a).

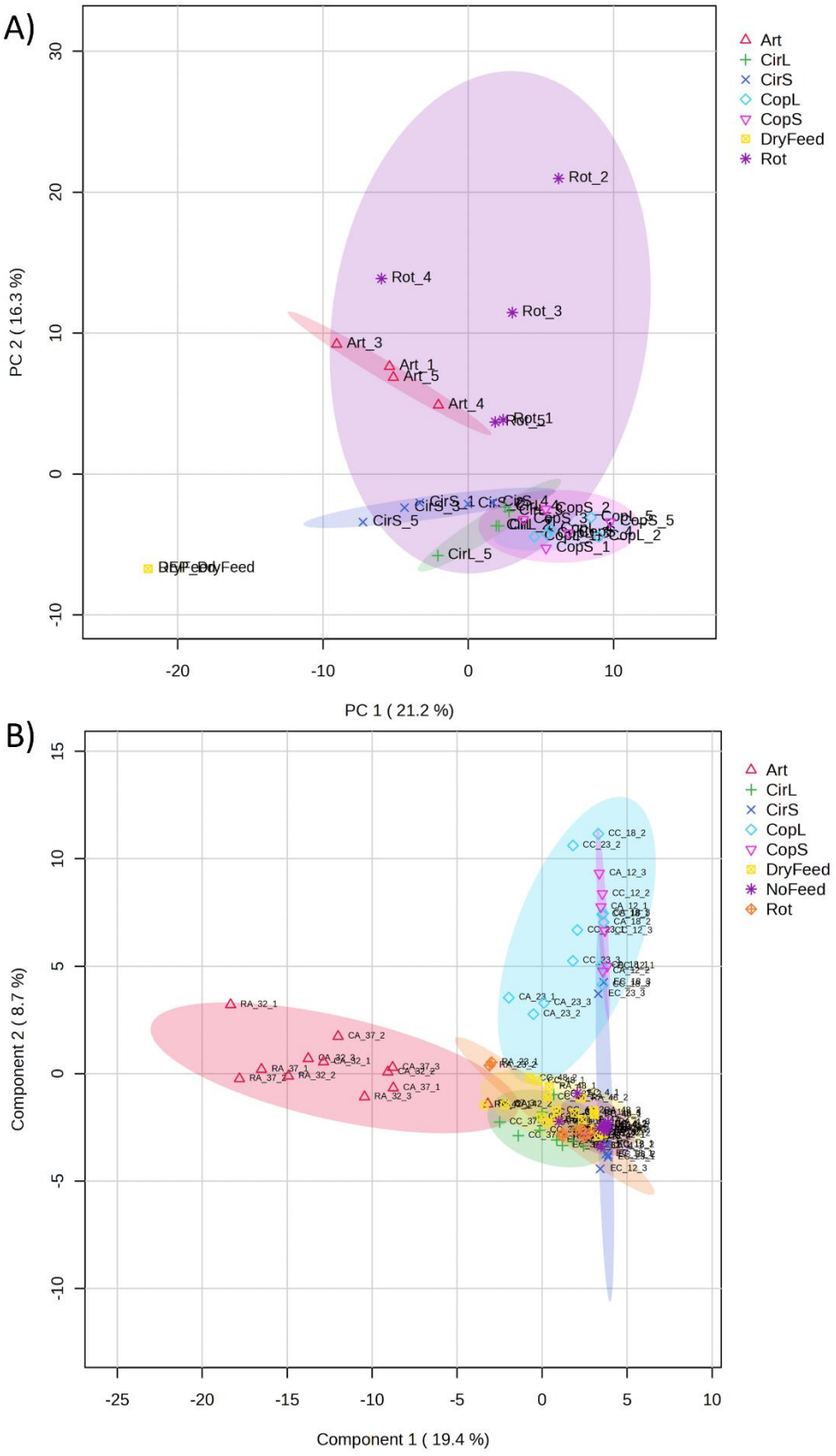


Figure 3.20. Analysis of lipidomics profiles in feeds and in larvae, separately. A) 2d-scores plot from PCA analysis of feeds. **Feeds:** Art (*Artemia*); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers). **B)** 2d-scores plot from sPLS-DA analysis of larvae. Groups in are indicated by color. Shading corresponds to the 95% confidence interval for each group. The explained variances for each principal component (PC) are shown in brackets. **Larvae**: EC (Experimental cirriped), CA (Copepod-*Artemia*), RA (Rotifer-*Artemia*), CC (Copepod-cirriped), first number indicates replicate; number after underscore indicates time (DPH).

Concerning the lipidomics profiles of the **larvae**, the principal components PC1 and PC2 could explain together 25.2% of the variance (Figure 3.20 B). PC1 was driven by the triacylglycerols TAG49:2-FA16:1, TAG49:1-FA18:1, TAG49:1-FA17:0 and TAG49:2-FA18:1, as well as the phosphatidylcholine PC18:0-22:5. PC2, in turn, was mostly influenced by five phosphatidylcholines: PC14:0-20:4 (or 16:1-18:3), PC17:1-22:6, PC18:0-19:1 (or 19:0-18:1), PC16:1-16:3 and PC20:5-18:3. Clear clustering was observed based on sPLS-DA for larvae that were receiving *Artemia* as last feed item (red group in Figure 3.20 B). As observed in the general sPLS-DA analysis for all the data, larvae that were receiving copepods also clustered together, independently of the naupliar stage of the copepods supplied (small n1-3, or large n4-6). Hierarchical clustering heatmaps showed a high content of several MAGs, lysoPCs, PCs and TAGs in larvae fed on *Artemia* as the last feed. TAG46:1-FA16:0 was high in large cirripeds, and several PCs were high in small and large copepods (Appendix 10, Figure A10b).

The lipid class composition of the larvae during the experiment was studied by comparing the total abundances of each group between the different feeding regimes (Figure 3.21 and Figure 3.22). Larvae displayed significant differences in **saturated FFAs** according to their diets from 23 DPH (2nd live feed period) until the end of the experiment (48 DPH). In general, larvae supplied with the Experimental cirriped diet had higher saturated FFA from 23 DPH on, and values were also higher for those fed on Rotifer-*Artemia* at 48 DPH. **Unsaturated FFAs** were found to be especially higher for the Rotifer-*Artemia* diet at 23 and 48 DPH and for the Copepod-*Artemia* diet at 32 DPH. **MAGs** were high for the Experimental cirriped diet at 12 and 23 DPH. In the case of **TAGs**, significantly higher values were found for the Rotifer-*Artemia* diet at 18 and 32 DPH, and for the Copepod-cirriped diet at 48 DPH (Figure 3.21). **LysoPCs** were more represented in the Copepod-*Artemia* diet from 23 to 42 DPH, and high for the Copepod-cirriped diet at 23 DPH. Significant differences in **PCs** were only observed between 23 and 37 DPH, and the values were lower for larvae that received the Experimental cirriped diet. **PEs** were not significantly different between groups at any time point. **PIs** differed solely at 23 and 32 DPH when the larvae supplied with the Experimental cirriped diet had lower values. Finally, **PS** abundances were lower than for the rest of the lipid classes in general and decreased around 12-28 DPH for all groups, compatible with the resorption of the yolk sac.

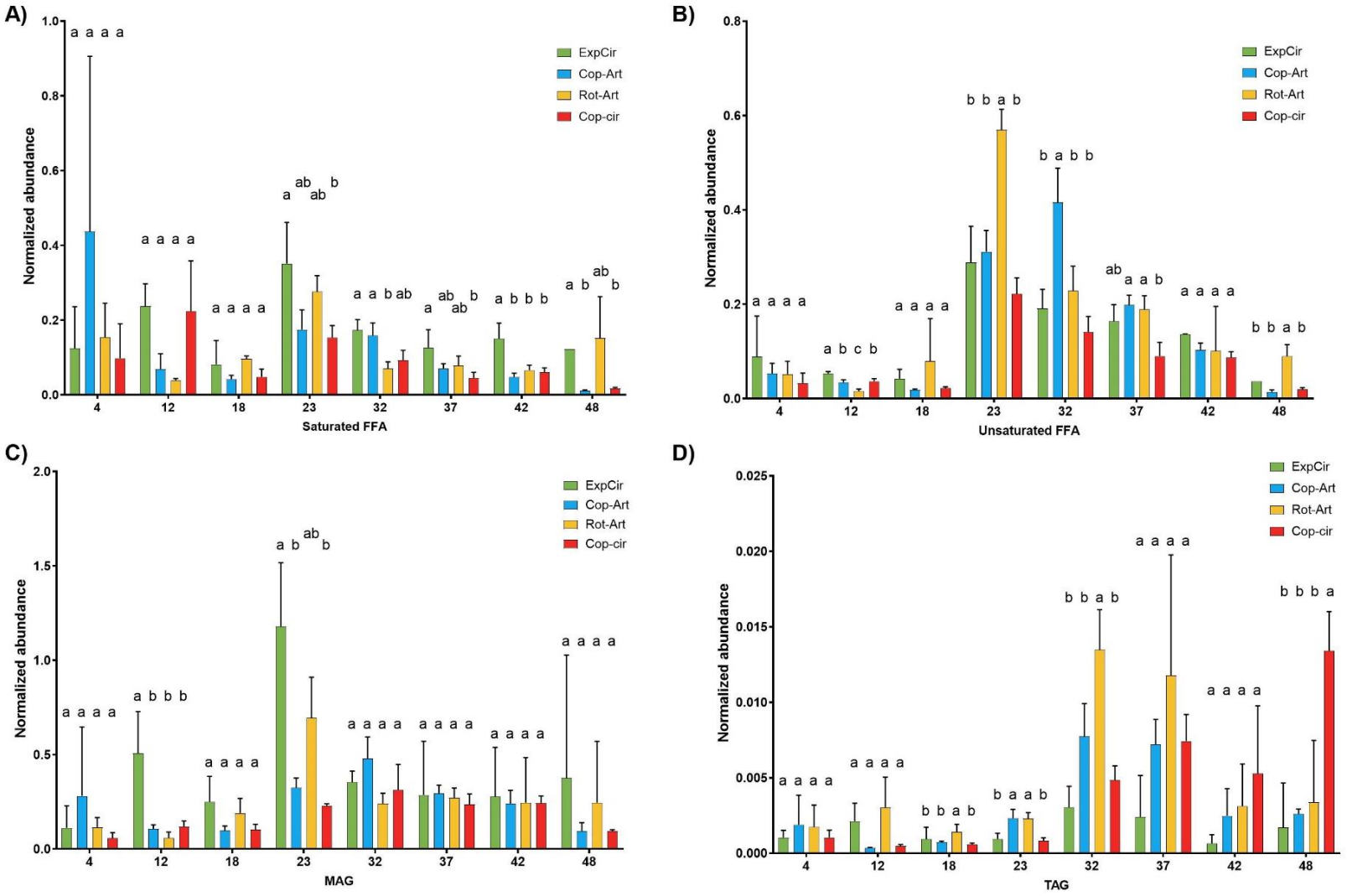


Figure 3.21. Evolution of selected lipid classes in the larvae along the first feeding experiment. Means and SD for 3 tanks are shown (A: saturated FFA, B) unsaturated FFA, C) MAGs, D) TAGs). Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$, one-way ANOVA and Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar. Values correspond to abundances normalized to an internal standard and sample weight.

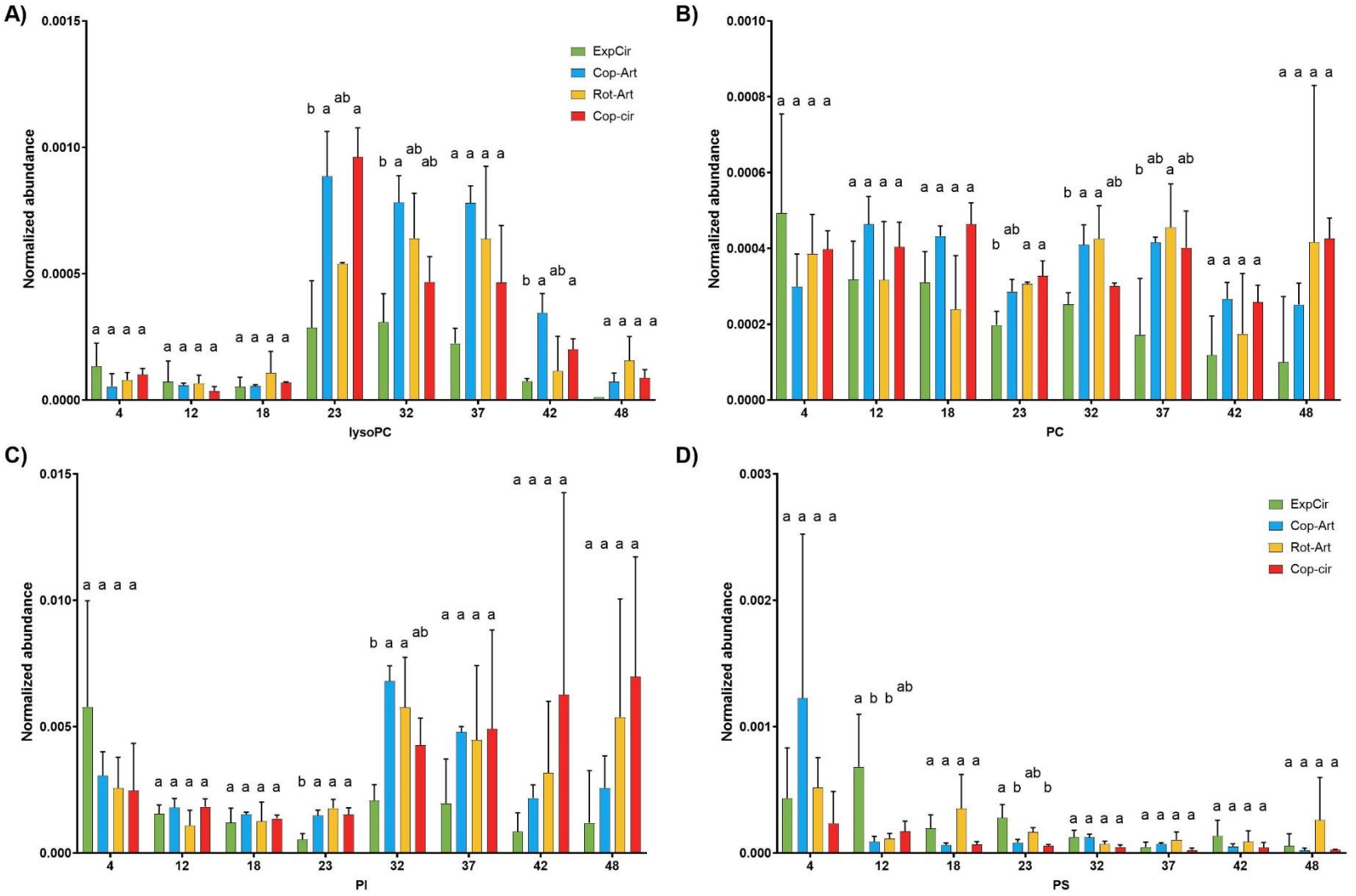


Figure 3.22. Evolution of selected lipid classes in the larvae along the first feeding experiment. Means and SD for 3 tanks are shown (A: lysoPCs, B) PCs, C) PIs, D) PSs). Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$, one-way ANOVA and Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar. Values correspond to abundances normalized to an internal standard and total sample weight.

3.3.3 Study of the relationship between selected lipid species and indicators for growth and survival in ballan wrasse

The relationship between the fatty acid composition in the larvae and in the last feed they were receiving, as well as between the feed fatty acid composition and performance indicators (mortality coefficient, SL, and SGR) were studied. Moreover, the lipidomics dataset was surveyed by studying correlations between lipid species in the feed and performance indicators in the larvae. The objective was to find meaningful correlations that could identify important lipid species for early larval growth and development in ballan wrasse.

3.3.3.1 Fatty acids

FAs in the feed vs FAs in the larvae

Significant correlations were found between 22 of the 37 average values for fatty acids or fatty acid sums/ratios in the larvae, and the average values of the same metabolite in the last feed they received (Spearman's correlations, $p < 0.05$) (Figure 3.23). Out of those, 11 were identified as very strong positive correlations (rho coefficient over 0.8). Said 11 fatty acids were C17:0, C24:1, C18:1n9, LA, ALA, C20:1n9, C20:2n6, C20:3n3, EPA, the sum of n-3 fatty acids and the DHA/EPA ratio. The scatter plots for the significant very strong correlations, as well as p values and Spearman's rho coefficients, can be seen in Appendix 11. DHA was positively correlated between feed and larvae, but the correlation was not significant due to differences in the case of *Artemia* (Appendix 11). When evaluating the correlation for DHA without including the *Artemia* samples, a significant moderate positive correlation (rho = 0.55; $p = 0.04$) was found.

FAs in the feed vs performance indicators

The study of the relationship between individual fatty acids, groups of fatty acids, or fatty acid ratios in the feeds, with larval mortality coefficients, showed significant very strong positive correlations between **mortality** and concentrations of C20:1n7, C15:0, C20:1n9, C16:1n9 (Figure 3.24). Furthermore, very strong significant negative correlations between mortality and C18:3n3 (ALA), C18:2n6 (LA), and C22:0 were found. **SGR** showed a significant very strong positive correlation with the proportion of DHA and a significant very strong negative correlation with C22:5n3 concentrations. Finally, larval **SL** had a significant very strong negative correlation with the concentrations of C22:5n3 and C14:0. Scatter plots for the significant very strong correlations, p values, and Spearman's rho coefficients are shown in Appendix 12.

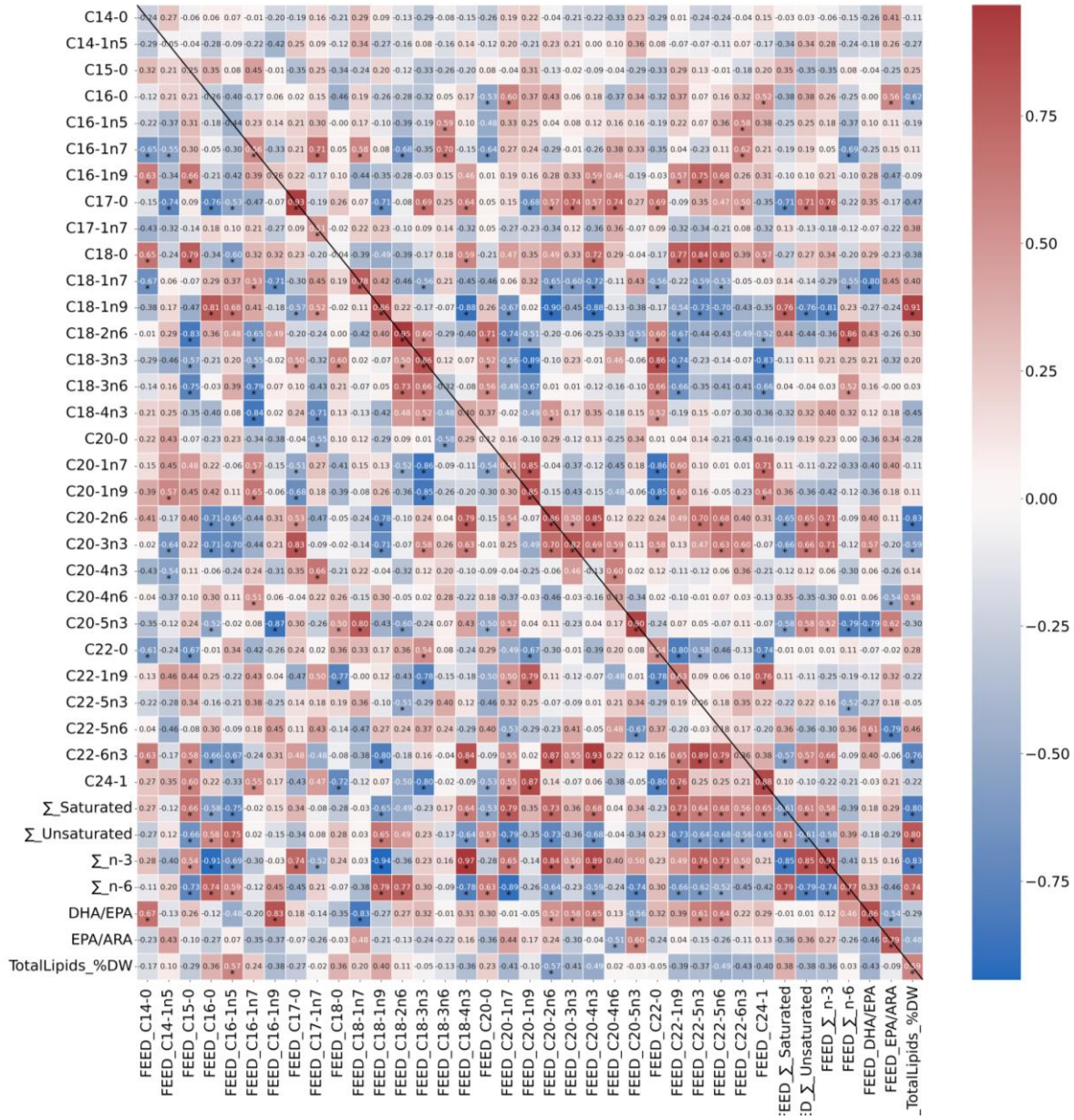


Figure 3.23. Correlations between the proportion of fatty acids (or sums/ratios) in the larvae and in the last feed they consumed. The heatmap represents Spearman's rho coefficients for the correlations between fatty acids in the larvae (rows) and the feed (columns). The central diagonal line indicates pairs corresponding to the same fatty acid in the larvae and feed. Correlations are color-coded according to their Spearman's rho coefficient, with deeper red cells indicating proximity to 1 and deeper blue to -1. Significant correlations ($p < 0.05$) are marked with an asterisk.

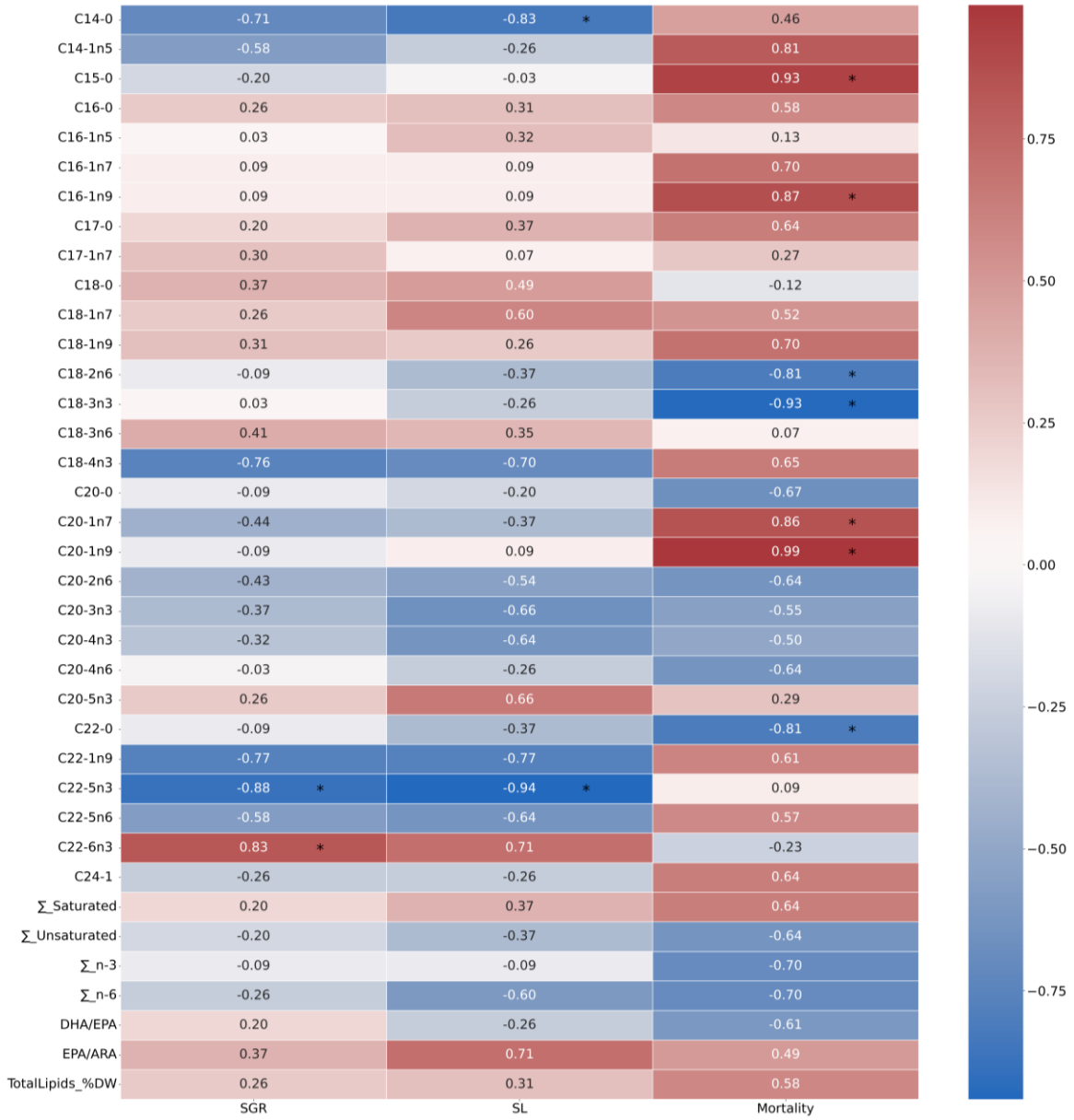


Figure 3.24. Correlations between fatty acid, fatty acid sums or ratios of fatty acids in the feed, and performance indicators in the larvae. The heatmap represents Spearman's rho coefficients for the correlations between fatty acids in the feed (rows) and SGR, SL and mortality coefficients (columns). Correlations are color-coded according to their Spearman's rho coefficient, with deeper red cells indicating proximity to 1 and deeper blue to -1. Significant correlations ($p < 0.05$) are marked with an asterisk.

3.3.3.2 Lipidomics

Spearman's correlations were run between performance indicators of the larvae (SL, SGR, mortality coefficient) and lipid species found in the different live feeds (Figure 3.25). Significant very strong positive correlations were found between SGR and lysoPC17:1, MAG18:1 and MAG22:6, and negative for MAG22:1, MAG22:5, PC18:1-20:1, and TAG14:0-16:1-16:1. SL showed a significant very strong positive correlation with FFA20:5, lysoPC15:0, lysoPC20:5, MAG18:1 and the sum of lysoPC, while there was a very strong

negative correlation with MAG22:1, PC18:1-20:1, PC18:0-20:3 (or 18:1-20:2), P32:0 (or 16:0-16:0), PS20:0-18:2, PS20:0-22:6, and the sum of PS. Finally, mortality had a significant very strong positive correlation with a total of 26 lipid species, including FFA20:1, lysoPC20:1, lysoPE:20, PC14:0-16:1, several PE, PS18:1-20:1 and 17 TAGs, and it also displayed a very strong negative correlation with 16 lipid species (Figure 3.25).

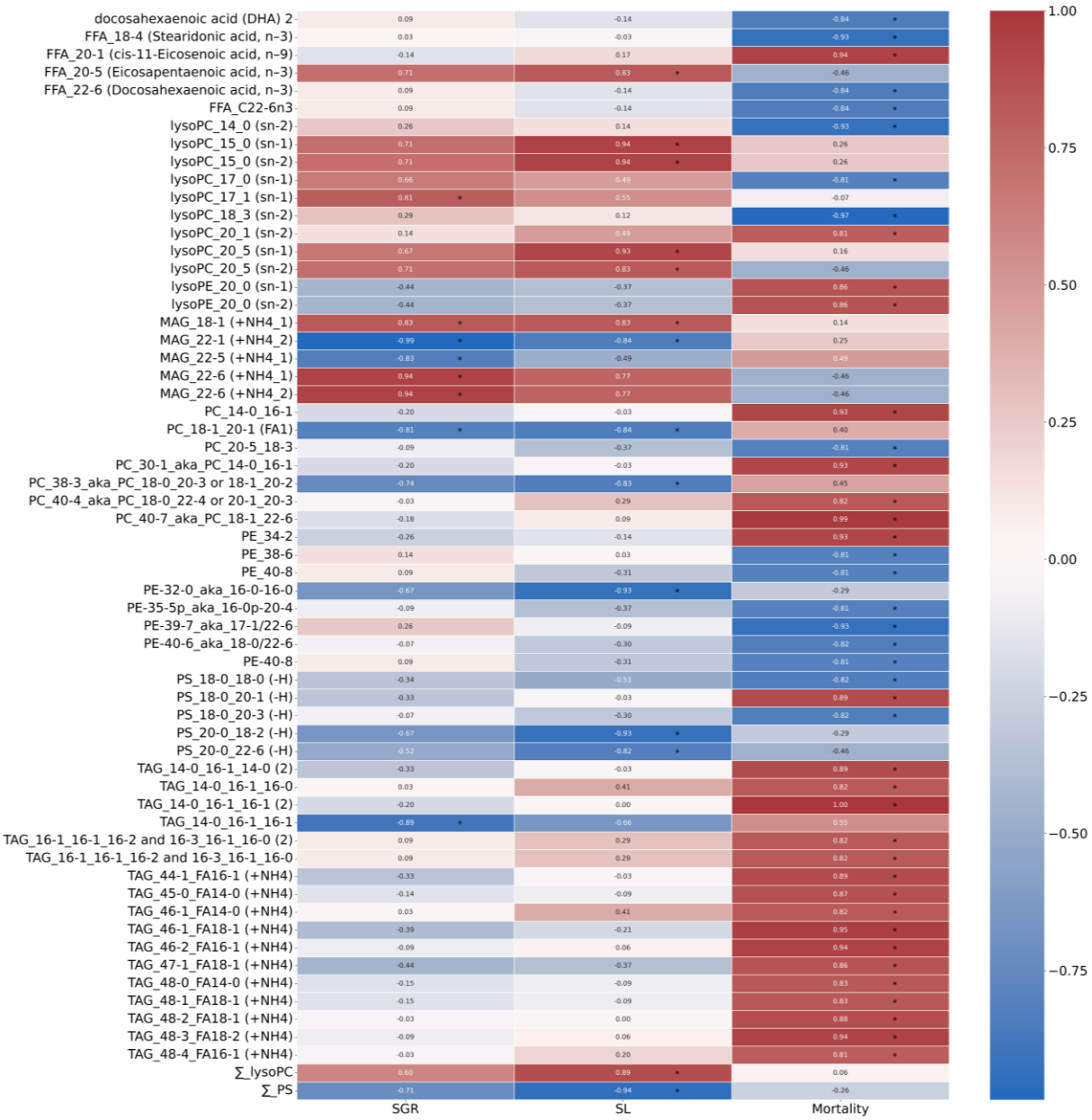


Figure 3.25. Correlations between lipidomics data from the feed and performance indicators in the larvae. The heatmap represents Spearman's rho coefficients for the correlations between lipid species or lipid classes in the feed (rows) and SGR, SL and mortality (columns). Correlations are color-coded according to their Spearman's rho coefficient, with deeper red cells indicating proximity to 1 and deeper blue to -1. Significant correlations ($p < 0.05$) are marked with an asterisk. Only lipid species with rho values corresponding to very strong negative (rho < -0.8) or positive (rho > 0.8) correlations are shown.

4 Discussion

4.1 Effects of the diet on morphology, growth, and survival

The appearance of the larvae during the experiment followed, in general, the expected development compared to other works on ballan wrasse (Gagnat, 2012; Gagnat et al., 2016; Ottesen et al., 2012) and other wrasse species (Kožul et al., 2011). A large portion of the yolk sac still remained at 4 DPH, similar to what Gagnat *et al.* reported (Gagnat et al., 2016). Differences in appearance were observed in the four diets studied in the present work, with some tanks showing considerable variation in larval size and development. The morphology, ossification, and histological development of the fish supplied with different diets was not the focus of study of the present master thesis but has partially been explored in other works, as discussed further below (Malzahn et al., 2022; Aase, 2022).

The importance of the very first live feed was evident in the present work, where the two diets that had copepods as the first prey item (Copepod-*Artemia* and Copepod-cirriped diets) displayed significantly lower mortality coefficients than those receiving experimental cirripeds or enriched rotifers (Experimental cirriped and Rotifer-*Artemia* diets, respectively). At the end of the experiment (48 DPH), only the groups that had received copepods during the first live feed period had enough remaining larvae to be sampled. These results are in line with what has been observed in previous works, where copepods resulted in lower mortality, more favorable development and pigmentation than other live feeds that are common in the industry, such as rotifers, in larvae of ballan wrasse (Gagnat et al., 2016; Romundstad, 2015; Øie et al., 2015) and other marine fishes (Betancor et al., 2019; Busch et al., 2010; Cassiano et al., 2011; Conceição et al., 2010; Evjemo et al., 2003; Imsland et al., 2006; Koedijk et al., 2010; Næss et al., 1995; Payne et al., 2001; Rajkumar & Kumaraguru vasagam, 2006; Rocha et al., 2017; Shields et al., 1999; Støttrup, 2003; Øie et al., 2015). Zooplanktonic naupliar stages of copepods are part of the natural diet of larval ballan wrasse, and their nutritional composition is believed to be adequate for marine fish larvae (Støttrup, 2003). The improved growth, survival, and normal pigmentation observed when supplying copepods as a first live feed has been especially attributed to the ratios of essential PUFAs and a large proportion of polar lipids (Evjemo et al., 2003; Fraser et al., 1989; Sargent et al., 1997). The effects of the lipid composition of the feed on the growth, survival, and lipid profile of the larvae are further discussed in section 4.2. Apart from their beneficial lipid composition, copepods also have high levels of antioxidants, vitamin A and are a good source of exogenous digestive enzymes that can improve fish larval digestion, with rotifers and *Artemia* showing much

lower values (Hansen et al., 2013; Munilla-Moran et al., 1990; Rønnestad et al., 1998; Sargent et al., 1997). Moreover, the erratic movements of the copepods in the water column are believed to stimulate feeding behavior in the small marine fish larvae (Buskey, 2005; Chesney, 2005; Conceição et al., 2010; van der Meeren, 1991).

In contrast with what was found for the Copepod-*Artemia* and Copepod-cirriped diets, the groups that received experimental cirripeds and enriched rotifers as the first feed item (Experimental cirriped and Rotifer-*Artemia* diet, respectively) displayed lower survival and did not seem to be able to adapt to the transition towards dry feed, showing stagnation in growth (DW and SL) and a mortality peak during the weaning phase. Said mortality peak could be explained by an immature state of the larvae at the time of introduction of the formulated feed, triggered by initiating the weaning phase at a pre-set age. Considering that the growth and development of larvae belonging to each group differed, more favorable results could have been obtained by performing a dynamic transition to dry feed, adapting the start of the weaning phase to developmental status (Estévez et al., 2019).

Since both copepods and cirripeds constitute part of the natural diet of larval stages of ballan wrasse (Dhont et al., 2013; Dipper et al., 1977; Martinez-Silva et al., 2018), and should therefore be nutritionally and ethologically appropriate, experimental cirripeds were expected to result in high growth and survival. In contrast, poor performance was observed when supplying experimental cirripeds as a first prey. One hypothesis that can explain this finding is the presence of underfeeding because some of the prey size exceeded that of the larval gape size. The experimental cirriped diet contained approximately 50% nauplii of *B. balanus*, which are larger than those of *B. crenatus* (on average, 339 x 194 µm for the larger nauplii and 267 x 154 µm for the smaller, according to measurements done in the present work). In contrast, the supplied naupliar stages of the copepod *A. tonsa* were significantly smaller (around 150 µm in body length at n1-n3 stages and 230 µm for the n4-n6 stages) and may have been a more appropriate prey. It is therefore believed that only one third of the biomass that was supplied to the Experimental cirriped group during the first live feed period was in an appropriate size range for the larvae, which can explain the poorer performance and survival of this group. The size of the live prey is a crucial factor determining the ability of the larvae to capture and swallow the prey (Bengtson, 2003; Bremigan & Stein, 1994; Chesney, 2005; Devries et al., 1998; Fernández-Díaz et al., 1994; Pepin, 2022). Given that cirripeds constituted a good second live feed item, as shown by the good performance in the Copepod-cirriped group, further studies should evaluate cirripeds of a more appropriate size as a first prey.

The higher mortalities and lower performance of the larvae fed on enriched rotifers as opposed to those fed on copepods can be explained by a less favorable nutritional composition of the rotifers (Conceição et al., 2010; Sargent et al., 2003; Øie et al., 2015).

Although rotifers were lipid-enriched in the present work, it is difficult to control the levels of n-3 HUFAs, as these vary with time, developmental stage, and nutritional composition of the green water algae (Sargent et al., 2003). In contrast, copepods are naturally rich in nutrients that are important for correct growth and development, such as essential n-3 and n-6 HUFAs, a greater amount of free amino acids and proteins (Bell et al., 2003; Evjemo et al., 2003; Li et al., 2015; Mæhre et al., 2013; Rønnestad et al., 1998; Rønnestad et al., 2013; Tocher et al., 2008; van der Meeren et al., 2008). Better tolerance to stress has been reported in ballan wrasse larvae that were fed on copepods instead of rotifers (Øie et al., 2015), which may have led to reduced mortality during stressful feed transitions.

The four diets affected DW differently at the studied times. At the first sampling point (12 DPH), the diet did not have a marked effect, since no significant differences were found between most of the groups. Since the period between 4 and 12 DPH included both an endogenous (yolk sac) and exogenous feeding phase, possible early effects of the live feed diets on DW could be masked by the progressive DW loss caused by the resorption of the yolk sac. Although there were significant differences in mean DW for the four feeding regimes in the midway sampling points, no differences were found between the Copepod-*Artemia*, Copepod-cirriped, and Rotifer-*Artemia* diets at the last time points (37-48 DPH). This finding points to an equalizing effect of the formulated feed on the DW of the larvae that had survived and were fit to transition to a dry diet. In turn, the Experimental cirriped group had consistently significantly lower mean DW, indicating an effect of the early live feed diet on weaning success.

The development of the SL in the Rotifer-*Artemia* diet in the present experiment was similar to the values reported for an enriched rotifers-*Artemia* diet in Ottesen *et al*. (Ottesen et al., 2012), except for 32 DPH, where we observed significantly higher mean SL, and 48 DPH, where our SL values were lower. The experimental setup reported by Ottesen *et al.* was not directly comparable to the conditions in the present work, with longer live feed periods, differing day-degrees, and diets, so the conclusions that can be obtained from such a comparison are limited. The mean SL reported in (Øie et al., 2015) were similar to our values until 28 DPH, and higher for their studies compared to the present work from 28 DPH and onwards. They also showed higher survival rates than we observed in the present work. These could be due to the differences between our study and the one reported by Øie, where the introduction of formulated dry feed occurred later (40 DPH, instead of the 32 DPH used in the present study), and with a longer weaning period (5 days of co-feeding, versus our 5 days). A shorter transition and earlier introduction of the dry feed in our work may have been detrimental to the survival and growth of the larvae that were least developed and still relied on live feed. The hypothesis of unsuccessful or too early weaning in our work could be further supported by the results of another study

on ballan wrasse larvae, where a steep increase in SL was observed after 40 DPH (not found in the present work), and interpreted as successful weaning (Hansen et al., 2013). Moreover, the last reported SL value was significantly higher for Ottesen et al., which did not use dry feed, than in our work, further indicating problems in our weaning phase (Ottesen et al., 2012). Høyland observed higher DW values at around 45 days than in our work at 48, possibly attributed to the different diets used in the former, where only live feed (either copepods of different sizes, or rotifers-*Artemia*) were used (Høyland, 2015). Piccinetti *et al*. reported higher growth than what was observed in the present work, also possible due to differences in rearing conditions, such as higher temperatures, which can positively affect growth (Cavrois-Rogacki et al., 2019; Kottmann et al., 2023; Piccinetti et al., 2017).

The differences found in DW, DWI, and SL between the two groups that received copepods during the first live feed period (Copepod-*Artemia* and Copepod-cirriped) at early sampling points (12 and 18 DPH) were unexpected since the diet had been equivalent. It was not possible to identify the cause of this variability. DW and SL values were not significantly different between groups before the beginning of the first live feed phase, indicating a homogenous starting point. Moreover, the measured environmental factors were in the ranges recommended for ballan wrasse (Noble et al., 2019) and not significantly different between the tanks supplied with copepods. Finally, the live feeds originated from the same feed tanks. Unexpected variability between equivalent groups is not uncommon in first feeding experiments and has been previously observed in other works on ballan wrasse using similar live feeds (Gagnat, 2012). In the absence of predation, the two main causes for differences in growth and low survival in fish larvae can be nutrition and microbiota (Cushing D, 1973; Lasker, 1981; Vadstein, Attramadal, Bakke, Forberg, et al., 2018; Vadstein et al., 2013). Live feeds for larval rearing may have high organic loads, which can support the growth of a variety of microorganisms, including bacteria, viruses, and fungi, as has been reported for rotifers and *Artemia* (Haché & Plante, 2011). The water and larval microbiota were not studied in the present work and could be presumed to vary between the tanks, since they were not interconnected and received water through a flow-through system, which tends to select for opportunistic microbes or r-strategists and favors perturbations (Vadstein, Attramadal, Bakke, & Olsen, 2018). Other works part of the Startrens project evaluated the diversity of the microbial community of the larvae, feed, and water, finding significant differences between the treatments and sampling times (Malzahn et al., 2022; Rosvoll, 2022). Differences in microbiota could be a plausible cause for the unexpected differences we observed between similar diets, but further investigation is needed.

The DWI during the first live feed period seemed to impact the DWI for the whole experiment, whose values mirrored those observed during the first phase. In turn, the DWI

during the second live feed and formulated dry feed periods was not significantly different between the four tested diets. No significant differences were detected in DWI at early stages between larvae that received rotifers or copepods, in contrast to what Øie *et al.* found (Øie et al., 2015).

A non-significant reduction in DWI was observed when transitioning from copepods to *Artemia*, in line with what Øie *et al*. and Gagnat reported (Gagnat, 2012; Øie et al., 2015). This could be explained by a more stressful adaptation of the larvae from a "higher nutritional quality feed" to a "lower nutritional quality" feed. Other works have reported negative effects on growth when a short transition period was carried out from a higher to a lower quality diet, and the opposite effect with a change from lower to higher quality (Koedijk et al., 2010). Based on the DWI results, both *Artemia* and large cirripeds can be an appropriate second feed item, but it may be beneficial to adapt the transition phases or prolong the weaning stage depending on the supplied diet and fish development (Dutton, 1992; Rosenlund et al., 1997). Parallel works in the STARTRENS project focusing on other aspects have indicated that cirripeds may lead to a more favorable liver and gut development than *Artemia*, with improved energy storage and digestive capacity. Moreover, no major rearrangement in gene expression seemed to be necessary when transitioning to cirripeds as a second feed item, pointing at cirripeds as a favorable second feed item (Malzahn et al., 2022; Aase, 2022).

Concerning the relationship between SL and DW, a steep increase in DW was found once the larvae reached approximately 6 mm in SL, coinciding with the transition from preflexion to postflexion stage (also coinciding with the start of the metamorphosis and change from positive allometric to isometric growth) (Gagnat et al., 2016). Other works on ballan wrasse larvae fed on copepods or rotifers have reported similar findings (Gagnat et al., 2016; Norland et al., 2022; Ottesen et al., 2012). We found that the relationship between SL and DW was better described by dedicated equations for each feeding regime, instead of one single equation for all the groups. This is in contrast with what was reported in another study based on the same experiment (Malzahn et al., 2022). The differences may be explained by the fact that Malzahn *et al.* correlated SL to larval volume instead of DW, which is the parameter used in the present work.

4.2 Fatty acid and lipidomics profiles of the larvae during the first feeding experiment and its interplay with the diet

4.2.1 Fatty acid profiles and total lipids

The lipid composition of the larvae that received the four different diets, as well as the feeds themselves, were studied in the present work through fatty acid analysis, total lipid quantification, and LC-MS based lipidomics.

A clear clustering of the replicates of the same **feed** type was observed in both the fatty acid and lipidomics data, indicating the existence of common profiles in their lipid composition. In general, marked differences in fatty acid profiles were found between the different feed types, except for the two copepod feeds (small and large copepods: n1-3, and n4-6, respectively). The small and large **copepods** clustered together irrespective of their naupliar stage, which shows a consistent lipid composition in this live feed during different stages of its life cycle. Copepods showed a total lipid content of approximately 10% of dry weight (w/w), which is in line with the values of 6.9-22.5% reported by van der Meeren *et al*. for other calanoid copepod species (van der Meeren et al., 2008). Moreover, the three most abundant fatty acids in the present work were the same as reported by van der Meeren, and in the same range (22% DHA of total fatty acids, 13% palmitic acid C16:0, and 12.3% EPA, followed by 12% ALA and 9.4% LA) (van der Meeren et al., 2008). Our ARA concentrations in the copepods (approx. 2% of total fatty acids) were in the range reported by van der Meeren *et al.* (0-2.6% of total fatty acids), as were the EPA/ARA and DHA/EPA ratios. The fatty acid composition reported in our work for copepods is also comparable to that indicated by Øie *et al*. for *A. tonsa* (Øie et al., 2015), and in Rocha *et al.* for wild zooplankton samples containing *Temora* sp., *Oithona* sp. and *Pseudocalanus* sp. (Rocha et al., 2017). These findings point to a quite consistent fatty acid composition of copepods irrespective of species, in agreement with the results shown by Martinez-Silva *et al*., where two species of copepods (*Eurytemora* spp. and *Acartia* sp.) displayed similar fatty acid compositions (Martinez-Silva et al., 2018), as well as with the findings in Karlsen *et al.*, were comparable fatty acid distributions were found in naupliar stages versus adult copepods (Karlsen et al., 2015). A consistent, predictable, and nutritionally adequate lipid composition is required for a good live feed, which further points to copepods as a favorable diet for marine fish larvae.

To the best of our knowledge, the present work contains the first in-depth study of the fatty acid composition of **cirripeds** used as live feed for early larval stages of marine fish. The most abundant fatty acids in cirripeds were EPA (27.4% of total fatty acids in small copepods and 31.3% in large copepods, respectively), DHA (14.7% and 18.9%), and palmitic acid (14.9% and 14.3%). The proportions of DHA and palmitic acid in cirripeds

were close to the values we observed for small copepods. However, cirripeds had a higher EPA content than the studied copepods. Cirripeds had a lower DHA/EPA ratio than the other live feeds used in the present work. A lower content of DHA in relation to EPA has led to decreased growth and survival in other marine fish larvae (Rodríguez et al., 1997; Watanabe, 1993), and could be one of the causes behind the lower growth and higher mortality rates we observed in the larvae that received the Experimental cirriped diet. This hypothesis can be supported by the significant very strong positive correlations we found between the DHA/EPA ratio and EPA levels in the feeds and the larvae that received them, as well as between higher levels of DHA in the feed and higher larval SGR. DHA levels and DHA/EPA ratios have correlated positively with survival in previous works on marine fish larvae, such as Atlantic cod (Cutts et al., 2006). Moreover, DHA has been regarded as having a higher value as an essential fatty acid than EPA in marine fish (Ibeas et al., 1994; Kalogeropoulos et al., 1992; Tocher, 2010; Watanabe, 1993).

The total lipid content of small cirripeds was similar to that of small copepods (12.6 and 11.7%, respectively), while the total lipids in large cirripeds were close to large copepods (9.6% and 9.2%, respectively). This similarity in the lipid composition was reflected in the gene expression patterns studied in other works on larvae from our same experiment, where larvae that were fed on cirripeds or copepods showed fewer differences between each other than with those that received rotifers (Malzahn et al., 2022). A more similar composition between copepods and cirripeds may be beneficial, as less rearrangement in gene expression may lead to reduced stress and more availability of energy to be redirected into growth.

Martinez-Silva *et al*. and Li *et al*. reported lower EPA and DHA proportions in enriched **rotifers** compared to copepods (Li et al., 2015; Martinez-Silva et al., 2018). In the present work, we also found lower EPA in rotifers, but the DHA levels were comparable to copepods. Given that the lipid composition of the diets was tested on morning samples (after enrichment), and the same feed was supplied for up to 16 h, it could have been interesting to sample the live feeds at the end of the day, to evaluate the capacity of enriched rotifers and *Artemia* to retain the added essential PUFAs. Karlsen *et al.* and Øie *et al*. both found similar DHA levels in enriched rotifers and copepods, while their EPA values were higher for copepods than for rotifers, in agreement with what we observed (Karlsen et al., 2015; Øie et al., 2015). Choi *et al*. reported a relationship between DHA/EPA ratios above 4 and increased growth rate, survival, and resistance to stress in Pacific cod (*Gadus macrocephalus*) (Choi et al., 2021), while Sargent *et al.*, proposed 2 as the optimal ratio for seabass and turbot (Sargent, Bell, et al., 1999). In the present work, only enriched rotifers surpassed a DHA/EPA ratio of 4 (5.0 ± 2.5) and copepods were close to a DHA/EPA ratio of 2 (2.2 ± 0.3). Kousoulaki *et al.* reported a positive correlation between fish larvae final body weight and DHA/EPA ratio in the diet, but the fish in their experiment were older

(from 40 DPH) than those used in the present work (Kousoulaki et al., 2015). To the best of our knowledge, no studies have been published indicating optimal DHA/EPA ratios specifically for early larval stages of ballan wrasse, but a DHA/EPA ratio of 2 may be appropriate, considering the improved survival and growth observed in the present work for larvae fed on copepods. A DHA/EPA ratio of 2 as optimal for ballan wrasse would be in agreement with what has been found for other marine fish larvae such as (Hamre, Yúfera, et al., 2013; Izquierdo & Koven, 2011; Sargent, Bell, et al., 1999). As for EPA/ARA ratio in larval first feed, Rocha *et al*. proposed 3.8 and 4.7 as optimal values for Atlantic cod (*G. morhua*), similar to the optimal EPA/ARA ratios of 3.5-5.1 reported for other marine fish species (Hamre, Yúfera, et al., 2013; Izquierdo & Koven, 2011; Rocha et al., 2017; Sargent, Bell, et al., 1999). The EPA/ARA ratios for the feed studied in the present work were approximately 23.7, 3.2, 12.0, 1.9, 25.5, 3.6 and 7.0 for small cirripeds, small copepods, large copepods, rotifers, large cirripeds, *Artemia* and dry feed, respectively, which is similar to the ranges indicated in the literature, where EPA/ARA ratios of up to 20 have been described for copepods (Conceição et al., 2010). No optimal EPA/ARA ratio has been published to this date for early larval diets in ballan wrasse. Optimal EPA/ARA ratios can help the larvae cope better with stress, which may explain the better adaptation to weaning and survival in larvae that were fed copepods as a first live feed (Cop-*Art* and Cop-cir) (McEvoy & Sargent, 1998; Støttrup, 2003). EPA, DHA, and ARA are essential PUFAs for marine fish larvae, where they play crucial roles in physiological processes, including regulation of the fluidity of membranes, gene modulation, pigmentation, behavior, development of sensorial and neural tissue, and biosynthesis of regulatory local hormones (Bell et al., 1995; Bell et al., 1986; Benítez-Santana et al., 2007; Cahu et al., 2003; Carvalho et al., 2019; Etayo et al., 2021; Gagnat et al., 2016; Ganga et al., 2011; Izquierdo & Koven, 2011; Mourente & Tocher, 1992; Reitan et al., 1994; Sargent, McEvoy, et al., 1999).

Enriched ***Artemia*** had higher total lipids, LA, and ALA than the other feeds, which was consistent with the literature (Conceição et al., 2010; Sargent, McEvoy, et al., 1999; Sargent et al., 2003; Øie et al., 2015). The higher percentage of total lipids in this feed was reflected in the total lipid percentages of the larvae that received *Artemia*, which also displayed higher total lipid values after weaning to formulated dry feed. LA and ALA are precursors of EPA, DHA, and ARA, but the specific enzymes involved in their biosynthesis (desaturase and elongase) are limited in marine fish larvae (Estévez et al., 2019; Mejri et al., 2021; Tocher, 2010). *Artemia* did not show a lower DHA/EPA ratio than the other live feeds. A low DHA/EPA ratio has been described as a common problem in *Artemia*, due to the interconversion of DHA to EPA in this species (Navarro et al., 1999; Sargent, McEvoy, et al., 1999). This did not seem to be the case in the present work, where the lipid enrichment successfully led to higher essential PUFA concentrations. However, as

discussed further below, it is not only important to quantify PUFAs, but also to know in which lipid fraction (PL or NL) they are (Kjørsvik et al., 2009). Our results illustrate this, considering that a correlation between DHA in larvae and feed was found for the other feeds, but not for *Artemia*. Rotifers and *Artemia* have higher concentrations of essential PUFAs in neutral lipids, as opposed to other feeds such as copepods, which are naturally rich in PL-PUFAs (Conceição et al., 2010; Dhont et al., 2013; van der Meeren et al., 2008). Specific distributions of fatty acids per lipid class were not studied in the present work, and this could be an interesting analysis to further explore the links between lipid composition and increased mortality in ballan wrasse larvae fed on Rotifer-*Artemia*.

Fatty acid composition data from the larvae only clustered together with the feed's composition in the case of *Artemia* and dry feed. This clustering was not evident for the other diets that were studied, suggesting that the composition of ballan wrasse larvae only directly reflects the composition of their diets in some cases. In other words, ballan wrasse larvae only directly "are what they eat" for selected fatty acids and in some feeds such as enriched *Artemia*. In the case of dry feed, the clustering was motivated by high proportions of n-6 fatty acids. However, given that the mortality at the last time point was high in two of the studied diets (Rotifer-*Artemia* and Experimental cirriped), this higher n-6 content may be motivated by survivor bias, and it cannot be inferred if this finding reflects higher n-6 fatty acids in the dry feed or a higher n-6 content in survivors in general. The differences in the lipid composition of larvae and their feed for the rest of the diets could be explained by a selective incorporation of some of the fatty acids during growth (Mejri et al., 2021).

Before the introduction of the feeds, **larvae** clustered together in PCA and hierarchical clustering analyses, indicating a homogeneous start point for their fatty acid composition. Once the diets were introduced, larvae also clustered together based on the last feed item they were receiving, pointing to an effect of the last diet on the lipid composition of the larvae themselves. In this regard, 22 out of the 37 analyzed fatty acids (or fatty acid groups/ratios) showed a significant correlation between content in the feed and in the larvae that received them. The fatty acid composition of fish can be influenced by the diets and has been used as a tool to explore habitat use and feeding history (Hielscher et al., 2015; Perga et al., 2006). Long-time effects of the diet on the fatty acid composition were less apparent in the present work, as larvae receiving a different first feed item still clustered together when receiving the same second live feed. In this regard, no significant differences in fatty acid composition were found at 32 DPH between the two groups receiving *Artemia* (Rotifer-*Artemia* and Copepod-*Artemia* diets), independently from their first live feed item. This suggests an equalizing effect of the last supplied diet on the lipid composition of the larvae. A similar effect was observed at 48 DPH, after the introduction of formulated dry feed. As observed for DWI, the larvae that had survived up to this point

showed in general less marked differences in fatty acid composition, despite their feeding history. The higher ARA concentrations at 48 DPH for the larvae that received *Artemia* as a second live feed reflect the higher values observed for these groups at 32 DPH, which may indicate a diluted but present effect of the nutritional fingerprint of *Artemia* in the larval lipid composition.

A lower n-3 fatty acid content was found in both rotifers and *Artemia* as feeds, as well as the larvae that received them. Given that both rotifers and *Artemia* were lipid-enriched, this finding suggests a poor capacity of these live feeds to retain n-3 fatty acids. A deficiency in n-3 fatty acids has been associated with higher mortalities, delayed growth, reduced resistance to stress, and the presence of anatomical alterations in marine fish larvae (Izquierdo, 1996), which could explain the lower survival found in larvae fed on the Rot-*Art* diet. Not only the amount of n-3 fatty acids but also the relative proportions between essential PUFAs are important, since the enzymes involved in lipid metabolism have different affinities for each fatty acid, leading to competitive interactions (Sargent, McEvoy, et al., 1999). The proportion between 20 (ARA, EPA) and 22 carbon (DHA) essential PUFAs must be balanced (Izquierdo et al., 2000), given that EPA and DHA compete for phospholipid biosynthesis and ARA and EPA for the eicosanoid response (Martinez-Silva et al., 2018; Sargent, McEvoy, et al., 1999). The balance between the n-3 and the n-6 fatty acid series also plays a role in immune response, development, and resistance to disease in early larval stages (Bou et al., 2017; Bou et al., 2020; Izquierdo et al., 2000).

The similarities between the lipid profiles of small and large copepods were also reflected in the lipid composition of the larvae that received them, which clustered together irrespective of the copepod naupliar stage they received. Said clustering indicates a consistent lipid profile in copepods that positively impacts larval growth and development of ballan wrasse, based on the DWI and survival. This confirms previous findings indicating that cultivated copepods are a favorable live feed item for ballan wrasse and other marine fish larvae (Betancor et al., 2019; Busch et al., 2010; Cassiano et al., 2011; Conceição et al., 2010; Evjemo et al., 2003; Gagnat et al., 2016; Imsland et al., 2006; Koedijk et al., 2010; Næss et al., 1995; Payne et al., 2001; Rajkumar & Kumaraguru vasagam, 2006; Rocha et al., 2017; Romundstad, 2015; Shields et al., 1999; Støttrup, 2003; Øie et al., 2015). Considering previous studies, it is likely that the essential fatty acids present in the copepods were incorporated mostly as PLs instead of NLs (which is typical for enriched rotifers and *Artemia*), which facilitates its bioavailability and utilization for growth and development (Conceição et al., 2010; Evjemo & Olsen, 1997; Gisbert et al., 2005; Izquierdo et al., 2000; Li et al., 2015; van der Meeren et al., 2008; Wold et al., 2009).

The fatty acid and lipid requirements of fish larvae should not only be studied in the light of survival and growth but also taking into account the development of biological functions and processes such as muscle-skeleton, pigmentation, neural system development, and digestive organs development (Hamre, Yúfera, et al., 2013). Some of these aspects have been evaluated in other master theses carried out as part of the Startrens project (Rosvoll, 2022; Aase, 2022). Aase carried out histological analysis of the gut and liver and found that the nutritional condition of larval ballan wrasse fed on copepods was more favorable than those seen for the other tested diets, especially when followed up with feeding with cirripeds instead of *Artemia* (Aase, 2022). A correct development of the gut can have an impact on the absorption and transport of lipids (Izquierdo et al., 2000).

4.2.2 Lipidomics

The study of the lipid profiles in biological systems is known as **lipidomics**. The field of lipidomics takes advantage of a variety of sample processing and chromatographic methods coupled to mass spectrometry (MS) to study relative and absolute amounts of lipid species (Han, 2016). Lipidomics was applied in the present work to study the relative distributions of lipid classes during the experiment in larvae receiving each of the four different diets, as well as the feeds themselves.

The feed lipidomics data for small and large copepods clustered together and independently from the naupliar stage, as seen for fatty acid composition. A cluster constituted by larvae fed on either small copepods (CopS, 12 DPH) or large copepods (CopL, 18 DPH) and these feed items was also observed, pointing to a similar lipidomics profile in these two cases and a connection with the feed they were receiving.

PLs such as PCs and PEs appeared to be one of the main driving factors behind the clustering of the lipidomics data according to the supplied diet. PLs are naturally high in feeds such as copepods and cirripeds, and have been connected to increased growth, survival, improved digestibility of feed, and enhanced lipid metabolism and transport in marine fish larvae (Cahu et al., 2009; Coutteau et al., 1997; Izquierdo et al., 2000; A Kanazawa, 1993; Overrein, 2010; van der Meeren et al., 2008). The hypothesis of better lipid metabolism in larvae fed diets that are naturally rich in PL (cirripeds and copepods) was supported by the histological findings reported by Aase, where lipid droplets were accumulated in enterocytes from larvae fed Rotifer-*Artemia* (Aase, 2022). The accumulation of fat droplets can be reduced by supplying PC in the diet (Fontagné, 1996; Gisbert et al., 2005; Izquierdo et al., 2000; Salhi et al., 1999). Salhi *et al.* found that feeding larval gilthead seabream with natural live feed containing marine PL instead of marine TG significantly improved growth (Salhi et al., 1999). Several more recent studies have shown that marine fish larvae utilize LC-PUFAs from the feed more efficiently when they are present in the PL fraction instead of the NL, leading to better growth, development

and survival (Gagnat, 2012; Gisbert et al., 2005; Hamre, Nordgreen, et al., 2013; Izquierdo et al., 2000; Kjørsvik et al., 2009; Wold et al., 2009).

A study of the relationship between mortality rates and larval lipidomics composition at end time of the same experiment as the one present in this work has been carried out in another publication (Malzahn et al., 2022). Given that this data already exists, and that the larvae at the end of the experiment were those that survived (and, thus, could have a different lipid composition than those that died), this set of correlations were not included in the present work.

The start feeding diet of fish larvae is not only conformed by lipids but also other nutrients. The protein content and amino acid composition in feed are important for larval development and growth, and so are micronutrients such as vitamins (vitamins A, C, D, E, K) and minerals (Zn, Mn, Se, I) (Hamre et al., 2008; Hamre, Yúfera, et al., 2013; Mazurais et al., 2009; Nguyen et al., 2008; Penglase et al., 2010). For instance, the aminosulphonic acid taurine has been identified as a nutrient that improves growth rates in the larval stages of some marine fish species (Chen et al., 2005; Chen et al., 2004; Conceição et al., 2010; Karlsen et al., 2015; Pinto et al., 2010; Rotman et al., 2017). These nutritional groups were not evaluated in the present work. It could therefore be interesting to study them together with the lipids to further explore the impact and interplay of different nutrients in ballan wrasse larval performance.

4.3 Limitations of the study and recommendations for further work

The shutdown caused by the COVID-19 pandemic affected the experimental phase of the present work. Not all the initially planned replicates and analyses could be run, which may have impacted the results at later time points, where the number of sampled larvae was not equal for all groups. A higher number of replicates could have been beneficial to increase the statistical power of the analyses.

As previously mentioned, the size of the first live feed in the Experimental cirriped diet was not fully appropriate for the larval gape size, which could have led to underfeeding. This starvation may have impacted the survival of the larvae that received this diet. The experimental cirripeds are, nevertheless, promising, considering their nutritional lipid composition and the fact that surviving larvae fed on cirripeds had good growth and development of hepatic and intestinal tissue (Aase, 2022). A recommendation for further studies would be to carry out a first feeding experiment using experimental cirripeds of an appropriate size, adjusted to the developmental stage of the fish.

Unexpected variations were found in some growth indicators between larvae receiving copepods as their first live feed. No significant differences were seen in environmental parameters during the first feeding experiment that could explain this. Maintaining tight control of the water quality is of paramount importance, given that environmental parameters may influence growth, development, and survival (Planas & Cunha, 1999). We observed marked oxygen supersaturation upon reception of the newly hatched larvae. Oxygen supersaturation can have varied effects on fish, but its consequences are not well understood on larval stages specifically (Helvik et al., 2009; Person-Le Ruyet et al., 2002; Stuart et al., 2015; Thorarensen et al., 2017; Weitkamp & Katz, 1980). In the present study, the larvae were pooled after reception and before distribution to experimental tanks, so it is unlikely that differences in oxygen supersaturation in the two hatches that constituted the larval population gave rise to the differences in growth we observed. The study of other possible factors at play, such as the environmental microbiota, may help identify the cause behind unexpected differences between tanks receiving the same diet.

Our lipidomics methodology was not able to distinguish between isobaric species of the same lipid class with different chain configurations, and there was a degree of uncertainty to each lipid species (for instance, a diacylglyceride with 18:0 and 16:1 and one with 18:1 and 16:0 would both be detected as 34:1) (Hansen et al., 2022). High-resolution MS could be implemented in future works to confirm the identification of the mass transitions.

Given the experimental design of the present work, where different live feeds were combined into different diets, it was difficult to evaluate the effects on the larval lipid composition and performance of each live feed independently. Further studies could implement more mortality estimations along the trial, which may improve the capacity to identify the underlying cause behind differences in the studied indicators.

Finally, there is a lack of long-term studies that evaluate the whole feeding history of cleaner fish, from the onset of the exogenous feeding phase until they begin their journey as delousers. Such comprehensive studies may help shed light on the effects of first feeding on the quality of ballan wrasse as a cleaner fish.

5 Conclusions

The results of the present work showed that the first feeding diet impacts the survival, growth, and lipid composition of early stage ballan wrasse larvae. Based on the indicators and parameters evaluated, a recommendation for an optimal diet would be to supply copepods as a first live feed item, followed by either *Artemia* or large cirripeds. The present work contains the first in-depth lipid characterization of cirripeds as a live feed for the early larval stages of ballan wrasse. Given the similarities between the lipid composition of cirripeds and copepods, the fact that they are one of the natural prey items of ballan wrasse, and the improved development of liver and gut found in other studies done on fish from this same experiment, cirripeds can be considered an appropriate live feed to follow up a first phase with copepods. Both copepods and cirripeds can be easy to handle in a first feeding setting and may be a good substitute for the extensively used rotifers and *Artemia*, respectively. Future studies should evaluate small cirripeds of an appropriate size as a first live feed for ballan wrasse, as well as dive deeper into the lipid composition of feeds and larvae and their relationship with performance in the fish.

The lipid composition of the diet impacted growth, survival, and development in ballan wrasse larvae in the present work. Moreover, the distribution of fatty acids in the feed also influenced the lipid composition of the larvae, especially based on the last feed that was supplied. A very strong positive correlation was found between the EPA content, DHA/EPA ratio, and total n-3 fatty acids in feeds and in the larvae that received them. The n-3 fatty acids levels were higher in natural diets, such as copepods and cirripeds, versus enriched live feeds (rotifers and *Artemia*). A higher n-3 content, together with a more favorable fatty acid profile, may be behind the observed improved growth, development, and survival in larvae fed on natural zooplankton diets. In this regard, higher levels of DHA in the diet were found to be very strongly correlated with higher larval SGR.

Despite the changes observed in lipid composition among the different diets, we found an equalizing effect of the introduction of formulated dry feed on the lipid composition of the larvae that had received different feeds. This finding points at the diet affecting growth and survival of ballan wrasse during critical early stages, but not having a clear effect on the larval composition after successful weaning. The results reported in the present work after weaning to dry feed should be considered carefully, since the number of surviving larvae in some of the feeding regimes was very reduced. Further studies should also focus on the long-term effects of first feeding diets on fish performance.

6 References

Abbott, S. K., Jenner, A. M., Mitchell, T. W., Brown, S. H., Halliday, G. M., & Garner, B. (2013). An improved high-throughput lipid extraction method for the analysis of human brain lipids. *Lipids*, *48*(3), 307-318. https://doi.org/10.1007/s11745-013-3760-z

Abolofia, J., Wilen, J. E., & Asche, F. (2017). The cost of lice: Quantifying the impacts of parasitic sea lice on farmed salmon. *Marine Resource Economics*, *32*(3), 329-349. https://doi.org/10.1086/691981

Afewerki, S., Asche, F., Misund, B., Thorvaldsen, T., & Tveteras, R. (2022). Innovation in the Norwegian aquaculture industry. *Reviews in Aquaculture*, *15*(2), 759-771. https://doi.org/10.1111/raq.12755

Bailey, J. L., & Eggereide, S. S. (2020). Indicating sustainable salmon farming: The case of the new Norwegian aquaculture management scheme. *Marine Policy*, *117*, 103925. https://doi.org/https://doi.org/10.1016/j.marpol.2020.103925

Barrett, L. T., Oppedal, F., Robinson, N., & Dempster, T. (2020). Prevention not cure: a review of methods to avoid sea lice infestations in salmon aquaculture. *Reviews in Aquaculture*, *12*(4), 2527-2543. https://doi.org/10.1111/raq.12456

Bell, J. G., McEvoy, L. A., Estevez, A., Shields, R. J., & Sargent, J. R. (2003). Optimising lipid nutrition in first-feeding flatfish larvae. *Aquaculture*, *227*(1), 211-220. https://doi.org/https://doi.org/10.1016/S0044-8486(03)00504-0

Bell, M. V., Batty, R. S., Dick, J. R., Fretwell, K., Navarro, J. C., & Sargent, J. R. (1995). Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). *Lipids*, *30*(5), 443-449. https://doi.org/10.1007/BF02536303

Bell, M. V., Henderson, R. J., & Sargent, J. R. (1986). The role of polyunsaturated fatty acids in fish. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *83*(4), 711-719. https://doi.org/https://doi.org/10.1016/0305-0491(86)90135-5

Bengtson, D. A. (2003). Status of marine aquaculture in relation to live prey: Past, present and future. In *Live Feeds in Marine Aquaculture* (pp. 1-16). https://doi.org/https://doi.org/10.1002/9780470995143.ch1

Benítez-Santana, T., Masuda, R., Juárez Carrillo, E., Ganuza, E., Valencia, A., Hernández-Cruz, C. M., & Izquierdo, M. S. (2007). Dietary n-3 HUFA deficiency induces a reduced visual response in gilthead seabream *Sparus aurata* larvae. *Aquaculture*, *264*(1), 408-417. https://doi.org/https://doi.org/10.1016/j.aquaculture.2006.10.024

Bergheim, A. (2012). Recent growth trends and challenges in the Norwegian aquaculture industry. *Latin American Journal of Aquatic Research*, *40*(3 SPL. ISS.), 800-807. https://doi.org/10.3856/vol40-issue3-fulltext-26

Betancor, M. B., Ortega, A., de la Gándara, F., Varela, J. L., Tocher, D. R., & Mourente, G. (2019). Evaluation of different feeding protocols for larvae of Atlantic bluefin tuna (*Thunnus thynnus* L.). *Aquaculture*, *505*, 523-538. https://doi.org/https://doi.org/10.1016/j.aquaculture.2019.02.063

Bjordal, Å. (1988). *Cleaning symbiosis between wrasses (Labridae) and lice infested salmon (Salmo salar) in mariculture.* International Council for the Exploration of the Sea, Committee on Mariculture. Copenhagen.

Bjørndal, T., & Tusvik, A. (2019). Economic analysis of land based farming of salmon. *Aquaculture Economics & Management*, *23*(4), 449-475. https://doi.org/10.1080/13657305.2019.1654558

Bostock, J., McAndrew, B., Richards, R., Jauncey, K., Telfer, T., Lorenzen, K., Little, D., Ross, L., Handisyde, N., Gatward, I., & Corner, R. (2010). Aquaculture: Global

status and trends. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1554), 2897-2912. https://doi.org/10.1098/rstb.2010.0170
Bou, M., Berge, G. M., Baeverfjord, G., Sigholt, T., Østbye, T. K., & Ruyter, B. (2017). Low levels of very-long-chain n-3 PUFA in Atlantic salmon (*Salmo salar*) diet reduce fish robustness under challenging conditions in sea cages [Article]. *Journal of Nutritional Science*, *6*, Article e32. https://doi.org/10.1017/jns.2017.28
Bou, M., Torgersen, J. S., Ostbye, T. K., Ruyter, B., Wang, X., Skugor, S., Kristiansen, I. O., & Todorcevic, M. (2020). DHA modulates immune response and mitochondrial function of Atlantic salmon adipocytes after LPS treatment. *Int J Mol Sci*, *21*(11), 4101. https://doi.org/10.3390/ijms21114101
Bremigan, M. T., & Stein, R. A. (1994). Gape-dependent larval foraging and zooplankton size: Implications for fish recruitment across systems. *Canadian Journal of Fisheries and Aquatic Sciences*, *51*(4), 913-922. https://doi.org/10.1139/f94-090
Bromke, M. A., Hochmuth, A., Tohge, T., Fernie, A. R., Giavalisco, P., Burgos, A., Willmitzer, L., & Brotman, Y. (2015). Liquid chromatography high-resolution mass spectrometry for fatty acid profiling. *The Plant Journal*, *81*(3), 529-536.
Brooker, A. J., Papadopoulou, A., Gutierrez, C., Rey, S., Davie, A., & Migaud, H. (2018). Sustainable production and use of cleaner fish for the biological control of sea lice: Recent advances and current challenges. *Veterinary Record*, *183*(12), 383-383. https://doi.org/10.1136/vr.104966
Busch, K. E. T., Falk-Petersen, I.-B., Peruzzi, S., Rist, N. A., & Hamre, K. (2010). Natural zooplankton as larval feed in intensive rearing systems for juvenile production of Atlantic cod (*Gadus morhua* L.). *Aquaculture Research*, *41*(12), 1727-1740. https://doi.org/https://doi.org/10.1111/j.1365-2109.2009.02450.x
Buskey, E. J. (2005). Behavioral characteristics of copepods that affect their suitability as food for larval fishes. In *Copepods in Aquaculture* (pp. 91-106). https://doi.org/https://doi.org/10.1002/9780470277522.ch8
Cahu, C., Zambonino Infante, J., & Takeuchi, T. (2003). Nutritional components affecting skeletal development in fish larvae. *Aquaculture*, *227*(1), 245-258. https://doi.org/https://doi.org/10.1016/S0044-8486(03)00507-6
Cahu, C. L., Gisbert, E., Villeneuve, L. A. N., Morais, S., Hamza, N., Wold, P.-A., & Zambonino Infante, J. L. (2009). Influence of dietary phospholipids on early ontogenesis of fish. *Aquaculture Research*, *40*(9), 989-999. https://doi.org/10.1111/j.1365-2109.2009.02190.x
Carvalho, M., Montero, D., Gesto, M., Lencina, A., Lund, I., & Izquierdo, M. (2019). The effect of dietary n-3 LC-PUFA on the responses to acute and prolonged stress of meagre (*Argyrosomus regius*, Asso 1801) juveniles. *Aquaculture*, *506*, 112-118. https://doi.org/https://doi.org/10.1016/j.aquaculture.2019.03.033
Cassiano, E. J., Ohs, C. L., Weirich, C. R., Breen, N. E., & Rhyne, A. L. (2011). Performance of larval florida pompano fed nauplii of the calanoid copepod *Pseudodiaptomus pelagicus*. *North American Journal of Aquaculture*, *73*(2), 114-123. https://doi.org/10.1080/15222055.2011.545598
Cavrois-Rogacki, T., Davie, A., Monroig, O., & Migaud, H. (2019). Elevated temperature promotes growth and feed efficiency of farmed ballan wrasse juveniles (*Labrus bergylta*). *Aquaculture*, *511*, 734237. https://doi.org/https://doi.org/10.1016/j.aquaculture.2019.734237
CFEED. (2023). *CFEED*. Retrieved 10th November 2023 from https://cfeed.no/home
Chen, J. N., Takeuchi, T., Takahashi, T., Tomoda, T., Koisi, M., & Kuwada, H. (2005). Effect of rotifers enriched with taurine on growth in larvae of Japanese flounder *Paralichthys olivaceus* [Article]. *Nippon Suisan Gakkaishi*, *71*(3), 342-347. https://doi.org/10.2331/suisan.71.342
Chen, J. N., Takeuchi, T., Takahashi, T., Tomoda, T., Koiso, M., & Kuwada, H. (2004). Effect of rotifers enriched with taurine on growth and survival activity of red sea bream *Pagrus major* larvae [Article]. *Nippon Suisan Gakkaishi*, *70*(4), 542-547. https://doi.org/10.2331/suisan.70.542

Chesney, E. J. (2005). Copepods as live prey: A Review of factors that Influence the feeding success of marine fish larvae. In *Copepods in Aquaculture* (pp. 133-150). https://doi.org/https://doi.org/10.1002/9780470277522.ch11

Chiu, H.-H., & Kuo, C.-H. (2020). Gas chromatography-mass spectrometry-based analytical strategies for fatty acid analysis in biological samples. *Journal of Food and Drug Analysis*, *28*(1), 60-73. https://doi.org/https://doi.org/10.1016/j.jfda.2019.10.003

Choi, J., Han, G. S., Lee, K. W., Byun, S.-G., Lim, H. J., Lee, C.-H., Lee, D.-Y., & Kim, H. S. (2021). Effects of feeding differentially enriched *Artemia* nauplii on the survival, growth, fatty acid composition, and air exposure stress response of Pacific cod (*Gadus macrocephalus*) larvae. *Aquaculture Reports*, *21*, 100829. https://doi.org/https://doi.org/10.1016/j.aqrep.2021.100829

Christie, W. W. (1984). The chemistry and biochemistry of simple and complex lipids. *Nat Prod Rep*, *1*(5), 499-511. https://doi.org/10.1039/np9840100499

Conceição, L. E. C., Yúfera, M., Makridis, P., Morais, S., & Dinis, M. T. (2010). Live feeds for early stages of fish rearing. *Aquaculture Research*, *41*(5), 613-640. https://doi.org/10.1111/j.1365-2109.2009.02242.x

Coutteau, P., Geurden, I., Camara, M. R., Bergot, P., & Sorgeloos, P. (1997). Review on the dietary effects of phospholipids in fish and crustacean larviculture. *Aquaculture*, *155*(1), 149-164. https://doi.org/https://doi.org/10.1016/S0044-8486(97)00125-7

Couturier, L. I. E., Michel, L. N., Amaro, T., Budge, S. M., da Costa, E., De Troch, M., Di Dato, V., Fink, P., Giraldo, C., Le Grand, F., Loaiza, I., Mathieu-Resuge, M., Nichols, P. D., Parrish, C. C., Sardenne, F., Vagner, M., Pernet, F., & Soudant, P. (2020). State of art and best practices for fatty acid analysis in aquatic sciences. *ICES Journal of Marine Science*, *77*(7-8), 2375-2395. https://doi.org/10.1093/icesjms/fsaa121

Cushing D, H. (1973). The natural regulation of fish populations. *Sea Fisheries Research*, 399-411. https://cir.nii.ac.jp/crid/1574231874681677312

Cutts, C. J., Sawanboonchun, J., Mazorra de Quero, C., & Bell, J. G. (2006). Diet-induced differences in the essential fatty acid (EFA) compositions of larval Atlantic cod (*Gadus morhua* L.) with reference to possible effects of dietary EFAs on larval performance. *ICES Journal of Marine Science*, *63*(2), 302-310. https://doi.org/10.1016/j.icesjms.2005.11.002

Dantagnan, P., Bórquez, A., Hernández, A., & Izquierdo, M. (2010). Effect of EPA/DHA ratios on the growth and survival of *Galaxias maculatus* (Jenyns, 1842) larvae reared under different salinity regimes. *Aquaculture Research*, *41*(9), e239-e244. https://doi.org/10.1111/j.1365-2109.2010.02512.x

Daukšas, E., Falch, E., Šližytė, R., & Rustad, T. (2005). Composition of fatty acids and lipid classes in bulk products generated during enzymic hydrolysis of cod (*Gadus morhua*) by-products. *Process Biochemistry*, *40*(8), 2659-2670.

Deady, S., & Fives, J. M. (2009). Diet of ballan wrasse, *Labrus bergylta*, and some comparisons with the diet of corkwing wrasse, *Crenilabrus melops*. *Journal of the Marine Biological Association of the United Kingdom*, *75*(3), 651-665. https://doi.org/10.1017/s0025315400039072

Dempster, T., Korsøen, Ø., Folkedal, O., Juell, J. E., & Oppedal, F. (2009). Submergence of Atlantic salmon (*Salmo salar* L.) in commercial scale sea-cages: A potential short-term solution to poor surface conditions. *Aquaculture*, *288*(3-4), 254-263. https://doi.org/10.1016/j.aquaculture.2008.12.003

Devries, D. R., Stein, R. A., & Bremigan, M. T. (1998). Prey selection by larval fishes as influenced by available zooplankton and gape limitation. *Transactions of the American Fisheries Society*, *127*(6), 1040-1050. https://doi.org/https://doi.org/10.1577/1548-8659(1998)127<1040:PSBLFA>2.0.CO;2

Dhont, J., Dierckens, K., Støttrup, J., Van Stappen, G., Wille, M., & Sorgeloos, P. (2013). *Rotifers, Artemia and copepods as live feeds for fish larvae in aquaculture*. Woodhead Publishing Limited. https://doi.org/10.1533/9780857097460.1.157

Dipper, F. A., Bridges, C. R., & Menz, A. (1977). Age, growth and feeding in the ballan wrasse *Labrus bergylta* Ascanius 1767. *Journal of Fish Biology*, *11*(2), 105-120. https://doi.org/10.1111/j.1095-8649.1977.tb04103.x

Dutton, P. (1992). Effects of experience on feeding success by larval white seabass, *Atractoscion nobilis*. *Journal of Fish Biology*, *41*(5), 765-773. https://doi.org/https://doi.org/10.1111/j.1095-8649.1992.tb02705.x

Erkinharju, T., Dalmo, R. A., Hansen, M., & Seternes, T. (2020). Cleaner fish in aquaculture: review on diseases and vaccination. *Reviews in Aquaculture*, *13*(1), 189-237. https://doi.org/10.1111/raq.12470

Espeland, S. H., Nedreaas, K., Mortensen, S., Skiftesvik, A. B., Agnalt, A.-L., Caroline Durif, Harkestad, L. S., Karlsbakk, E., Knutsen, H., Thangstad, T., Knut Jørstad, Bjordal, Å., & Gjøsæter, J. (2010). *Kunnskapsstatus leppefisk. Utfordringer i et økende fiskeri*. Havforskningsinstituttet.

Estévez, A., Papandroulakis, N., Wille, M., & Sorgeloos, P. (2019). Early life stages and weaning. In G. Lembo & E. Mente (Eds.), *Organic Aquaculture : Impacts and Future Developments* (pp. 79-102). Springer International Publishing. https://doi.org/10.1007/978-3-030-05603-2_5

Etayo, A., Le, H., Araujo, P., Lie, K. K., & Sæle, Ø. (2021). Dietary lipid modulation of intestinal serotonin in ballan wrasse (*Labrus bergylta*)-In Vitro Analyses. *Front Endocrinol (Lausanne)*, *12*, 560055. https://doi.org/10.3389/fendo.2021.560055

Evjemo, J. O., & Olsen, Y. (1997). Lipid and fatty acid content in cultivated live feed organisms compared to marine copepods. *Hydrobiologia*, *358*(1), 159-162. https://doi.org/10.1023/A:1003196829109

Evjemo, J. O., Reitan, K. I., & Olsen, Y. (2003). Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. *Aquaculture*, *227*(1-4), 191-210. https://doi.org/10.1016/S0044-8486(03)00503-9

Fahy, E., Subramaniam, S., Brown, H. A., Glass, C. K., Merrill, A. H., Murphy, R. C., Raetz, C. R. H., Russell, D. W., Seyama, Y., Shaw, W., Shimizu, T., Spener, F., van Meer, G., VanNieuwenhze, M. S., White, S. H., Witztum, J. L., & Dennis, E. A. (2005). A comprehensive classification system for lipids. *Journal of Lipid Research*, *46*(5), 839-861. https://doi.org/https://doi.org/10.1194/jlr.E400004-JLR200

FAO. (2022). *The State of World Fisheries and Aquaculture 2022. Towards Blue Transformation*. FAO.

Faust, E., Halvorsen, K. T., Andersen, P., Knutsen, H., & Andre, C. (2018). Cleaner fish escape salmon farms and hybridize with local wrasse populations. *R Soc Open Sci*, *5*(3), 171752. https://doi.org/10.1098/rsos.171752

Fernández-Díaz, C., Pascual, E., & Yúfera, M. (1994). Feeding behavior and prey size selection of gilthead seabream, *Sparus aurata*, larvae fed on inert and live food [Article]. *Marine Biology*, *118*(2), 323-328. https://doi.org/10.1007/bf00349800

FHF– Fiskeri- og havbruksnæringens forskningsfinansiering. (2022). *Program rensefisk: Optimalisert startfôring av rensefisk (STARTRENS)*. Retrieved 20th November 2023 from https://www.fhf.no/prosjekter/prosjektbasen/901561/

Fiskeridirektoratet. (2022, June 6th, 2023). *Akvakulturstatistikk: laks, regnbueørret og ørret. Matfiskproduksjon: Salg av laks og regnbueørret, og salg per sysselsatt 1994-2022*. Retrieved June 6th, 2023 from https://www.fiskeridir.no/Akvakultur/Tall-og-analyse/Akvakulturstatistikk-tidsserier/Laks-regnbueoerret-og-oerret/salg-av-laks-og-regnbueorret

Fiskeridirektoratet. (2023). *Total utsett av rensefisk i merd 1998-2022*. Retrieved November 14th, 2023 from https://www.fiskeridir.no/Akvakultur/Tall-og-analyse/Akvakulturstatistikk-tidsserier/Rensefisk

Fjørtoft, H. B., Nilsen, F., Besnier, F., Espedal, P. G., Stene, A., Tveten, A.-K., Bjørn, P. A., Aspehaug, V. T., & Glover, K. A. (2020). Aquaculture-driven evolution: distribution of pyrethroid resistance in the salmon louse throughout the North Atlantic in the years 2000–2017. *ICES Journal of Marine Science*, *77*(5), 1806-1815. https://doi.org/10.1093/icesjms/fsaa062

Folch, J., Lees, M., & Sloane Stanley, G. H. (1957). A simple method for the isolation and purification of total lipides from animal tissues. *Journal of Biological Chemistry*, *226*(1), 497-509.

Fontagné, S. (1996). *Effet des phospholipides alimentaires sur la structure histologique du foie et de l'intestin de larves de carpe. Disertation Thesis.* University Bordeaux].

Fraser, A. J., Sargent, J. R., & Gamble, J. C. (1989). Lipid class and fatty acid composition of *Calanus finmarchicus* (Gunnerus), *Pseudocalanus* sp. and *Temora longicornis* Muller from a nutrient-enriched seawater enclosure. *Journal of Experimental Marine Biology and Ecology*, *130*(1), 81-92. https://doi.org/https://doi.org/10.1016/0022-0981(89)90020-8

Gagnat, M. R. (2012). *The effect of different live feed on the early growth and development of ballan wrasse (Labrus bergylta Ascanius, 1767) larvae and its organs (master thesis)* Norwegian University of Science and Technology (NTNU)]. Trondheim.

Gagnat, M. R., Wold, P. A., Bardal, T., Øie, G., & Kjørsvik, E. (2016). Allometric growth and development of organs in ballan wrasse (*Labrus bergylta* Ascanius, 1767) larvae in relation to different live prey diets and growth rates. *Biology Open*, *5*(9), 1241-1251. https://doi.org/10.1242/bio.017418

Ganga, R., Montero, D., Bell, J. G., Atalah, E., Ganuza, E., Vega-Orellana, O., Tort, L., Acerete, L., Afonso, J. M., Benitez-Sanatana, T., Fernández Vaquero, A., & Izquierdo, M. (2011). Stress response in sea bream (*Sparus aurata*) held under crowded conditions and fed diets containing linseed and/or soybean oil. *Aquaculture*, *311*(1), 215-223. https://doi.org/https://doi.org/10.1016/j.aquaculture.2010.11.050

Geitung, L., Wright, D. W., Oppedal, F., Stien, L. H., Vågseth, T., & Madaro, A. (2020). Cleaner fish growth, welfare and survival in Atlantic salmon sea cages during an autumn-winter production. *Aquaculture*, *528*, 735623. https://doi.org/https://doi.org/10.1016/j.aquaculture.2020.735623

Giavalisco, P., Li, Y., Matthes, A., Eckhardt, A., Hubberten, H. M., Hesse, H., Segu, S., Hummel, J., Köhl, K., & Willmitzer, L. (2011). Elemental formula annotation of polar and lipophilic metabolites using 13C, 15N and 34S isotope labelling, in combination with high-resolution mass spectrometry. *The Plant Journal*, *68*(2), 364-376.

Gisbert, E., Villeneuve, L., Zambonino-Infante, J. L., Quazuguel, P., & Cahu, C. L. (2005). Dietary phospholipids are more efficient than neutral lipids for long-chain polyunsaturated fatty acid supply in European sea bass *Dicentrarchus labrax* larval development. *Lipids*, *40*(6), 609-618. https://doi.org/10.1007/s11745-005-1422-0

Godwin, J. (2019). Sex change in reef fishes: Behavior and physiology. In J. C. Choe (Ed.), *Encyclopedia of Animal Behavior (Second Edition)* (pp. 364-372). Academic Press. https://doi.org/https://doi.org/10.1016/B978-0-12-809633-8.20825-X

Grant, B., Davie, A., Taggart, J. B., Selly, S. L. C., Picchi, N., Bradley, C., Prodohl, P., Leclercq, E., & Migaud, H. (2016). Seasonal changes in broodstock spawning performance and egg quality in ballan wrasse (*Labrus bergylta*). *Aquaculture*, *464*, 505-514. https://doi.org/https://doi.org/10.1016/j.aquaculture.2016.07.027

Groner, M. L., Rogers, L. A., Bateman, A. W., Connors, B. M., Frazer, L. N., Godwin, S. C., Krkosek, M., Lewis, M. A., Peacock, S. J., Rees, E. E., Revie, C. W., & Schlagel, U. E. (2016). Lessons from sea louse and salmon epidemiology. *Philos Trans R Soc Lond B Biol Sci*, *371*(1689). https://doi.org/10.1098/rstb.2015.0203

Haché, R., & Plante, S. (2011). The relationship between enrichment, fatty acid profiles and bacterial load in cultured rotifers (*Brachionus plicatilis* L-strain) and *Artemia* (*Artemia salina* strain *Franciscana*). *Aquaculture*, *311*(1), 201-208. https://doi.org/https://doi.org/10.1016/j.aquaculture.2010.11.034

Hackstadt, A. J., & Hess, A. M. (2009). Filtering for increased power for microarray data analysis. *BMC Bioinformatics*, *10*(1), 11. https://doi.org/10.1186/1471-2105-10-11

Hagemann, A., Malzahn, A., Hansen, B. H., Ribicic, D., Sarno, A., Kjørsvik, E., Vu, T. A., & Jager, T. (2022). *STARTRENS – Optimalisert startfôring av rensefisk. Sluttrapport*

for FHF-prosjekt 901561. SINTEF Ocean AS. https://hdl.handle.net/11250/2837772

Halvorsen, K., Skiftesvik, A. B., Bjelland, R., & Larsen, T. (2020). *Kunnskapstøtte og råd for regulering av fisket etterleppefisk i 2021*.

Hamre, K., Nordgreen, A., Grotan, E., & Breck, O. (2013). A holistic approach to development of diets for Ballan wrasse (Labrus berggylta) - a new species in aquaculture. *PeerJ*, *1*(1), e99. https://doi.org/10.7717/peerj.99

Hamre, K., Srivastava, A., RØNnestad, I., Mangor-Jensen, A., & Stoss, J. (2008). Several micronutrients in the rotifer *Brachionus* sp. may not fulfil the nutritional requirements of marine fish larvae. *Aquaculture Nutrition*, *14*(1), 51-60. https://doi.org/10.1111/j.1365-2095.2007.00504.x

Hamre, K., Yúfera, M., Rønnestad, I., Boglione, C., Conceição, L. E. C., & Izquierdo, M. (2013). Fish larval nutrition and feed formulation: knowledge gaps and bottlenecks for advances in larval rearing. *Reviews in Aquaculture*, *5*(s1), S26-S58. https://doi.org/10.1111/j.1753-5131.2012.01086.x

Han, X. (2016). Lipidomics for studying metabolism. *Nat Rev Endocrinol*, *12*(11), 668-679. https://doi.org/10.1038/nrendo.2016.98

Hansen, B. H., Kjørsvik, E., Malzahn, A. M., Sarno, A., Kulild, O. M., Farkas, J., Nordtug, T., Rye, R., Kvæstad, B., Lein, I., & Hagemann, A. (2022). Ova lipid profiling and egg quality in wild and captive lumpfish, *Cyclopterus lumpus* (Linnaeus, 1758). *Aquaculture*, *560*, 738556. https://doi.org/https://doi.org/10.1016/j.aquaculture.2022.738556

Hansen, T. W., Folkvord, A., Grøtan, E., & Sæle, Ø. (2013). Genetic ontogeny of pancreatic enzymes in *Labrus bergylta* larvae and the effect of feed type on enzyme activities and gene expression. *Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology*, *164*(3), 176-184. https://doi.org/https://doi.org/10.1016/j.cbpb.2012.12.001

Harris, C. R., Millman, K. J., van der Walt, S. J., Gommers, R., Virtanen, P., Cournapeau, D., Wieser, E., Taylor, J., Berg, S., Smith, N. J., Kern, R., Picus, M., Hoyer, S., van Kerkwijk, M. H., Brett, M., Haldane, A., del Río, J. F., Wiebe, M., Peterson, P., . . . Oliphant, T. E. (2020). Array programming with NumPy. *Nature*, *585*(7825), 357-362. https://doi.org/10.1038/s41586-020-2649-2

Helland, S., Dahle, S. V. W., Hough, C., & Børge, J. (2014). *Production of ballan wrasse (Labrus bergylta). Science and Practice*.

Helvik, J. V., Hamre, K., Hordvik, I., Van der Meeren, T., Ressem, H., Tveiten, H., Øie, G., & Schartl, M. (2009). *The fish larva: a transitional life form, the foundation for aquaculture and fisheries. Report from a working group on research on early life stages of fish.* T. R. C. o. Norway.

Heuch, P. A., Bjørn, P. A., Finstad, B., Holst, J. C., Asplin, L., & Nilsen, F. (2005). A review of the Norwegian 'National Action Plan Against Salmon Lice on Salmonids': The effect on wild salmonids. *Aquaculture*, *246*(1), 79-92. https://doi.org/https://doi.org/10.1016/j.aquaculture.2004.12.027

Hevrøy, E. M., Boxaspen, K., Oppedal, F., Taranger, G. L., & Holm, J. C. (2003). The effect of artificial light treatment and depth on the infestation of the sea louse *Lepeophtheirus salmonis* on Atlantic salmon (*Salmo salar* L.) culture. *Aquaculture*, *220*(1-4), 1-14. https://doi.org/10.1016/S0044-8486(02)00189-8

Hielscher, N. N., Malzahn, A. M., Diekmann, R., & Aberle, N. (2015). Trophic niche partitioning of littoral fish species from the rocky intertidal of Helgoland, Germany. *Helgoland marine research*, *69*(4), 385-399. https://doi.org/10.1007/s10152-015-0444-5

Hilldén, N.-O. (1981). Territoriality and reproductive behaviour in the goldsinny, *Ctenolabrus rupestris* L. *Behavioural Processes*, *6*(3), 207-221. https://doi.org/https://doi.org/10.1016/0376-6357(81)90001-2

Hunter, J. D. (2007). Matplotlib: A 2D Graphics Environment. *Computing in Science & Engineering*, *9*(3), 90-95. https://doi.org/10.1109/mcse.2007.55

Høyland, O. S. (2015). *The effect of different live feeds and water qualities on growth, survival, ossification and skeletal anomalies of ballan wrasse (Labrus bergylta)*

larvae (master thesis) Norwegian University of Science and Technology]. Trondheim, Norway.
Ibeas, C., Izquierdo, M. S., & Lorenzo, A. (1994). Effect of different levels of n−3 highly unsaturated fatty acids on growth and fatty acid composition of juvenile gilthead seabream (*Sparus aurata*). *Aquaculture*, *127*(2), 177-188. https://doi.org/https://doi.org/10.1016/0044-8486(94)90424-3
Imsland, A. K., Foss, A., Koedijk, R., Folkvord, A., Stefansson, S. O., & Jonassen, T. M. (2006). Short- and long-term differences in growth, feed conversion efficiency and deformities in juvenile Atlantic cod (*Gadus morhua*) startfed on rotifers or zooplankton. *Aquaculture Research*, *37*(10), 1015-1027. https://doi.org/https://doi.org/10.1111/j.1365-2109.2006.01523.x
Iverson, S. J., Lang, S. L., & Cooper, M. H. (2001). Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids*, *36*(11), 1283-1287. https://doi.org/10.1007/s11745-001-0843-0
Izquierdo, M. S. (1996). Essential fatty acid requirements of cultured marine fish larvae. *Aquaculture Nutrition*, *2*(4), 183-191. https://doi.org/https://doi.org/10.1111/j.1365-2095.1996.tb00058.x
Izquierdo, M. S., & Koven, W. (2011). Lipids. In G. J. Holt (Ed.), *Larval fish nutrition* (pp. 47–84). Wiley-Blackwell, John Wiley and Sons. https://doi.org/https://doi.org/10.1002/9780470959862.ch2
Izquierdo, M. S., Socorro, J., Arantzamendi, L., & Hernández-Cruz, C. M. (2000). Recent advances in lipid nutrition in fish larvae. *Fish Physiology and Biochemistry*, *22*(2), 97-107. https://doi.org/10.1023/A:1007810506259
Jackson, D., Moberg, O., Stenevik Djupevåg, E. M., Kane, F., & Hareide, H. (2018). The drivers of sea lice management policies and how best to integrate them into a risk management strategy: An ecosystem approach to sea lice management. *Journal of Fish Diseases*, *41*(6), 927-933. https://doi.org/10.1111/jfd.12705
Jin, M., Monroig, Ó., Lu, Y., Yuan, Y., Li, Y., Ding, L., Tocher, D. R., & Zhou, Q. (2017). Dietary DHA/EPA ratio affected tissue fatty acid profiles, antioxidant capacity, hematological characteristics and expression of lipid-related genes but not growth in juvenile black seabream (*Acanthopagrus schlegelii*). *PLoS One*, *12*(4), e0176216. https://doi.org/10.1371/journal.pone.0176216
Johansen, U., Bull-Berg, H., Vik, L. H., Stokka, A. M., Richardsen, R., & Winther, U. (2019). The Norwegian seafood industry – Importance for the national economy. *Marine Policy*, *110*(June), 103561-103561. https://doi.org/10.1016/j.marpol.2019.103561
Jouhet, J., Lupette, J., Clerc, O., Magneschi, L., Bedhomme, M., Collin, S., Roy, S., Maréchal, E., & Rebeille, F. (2017). LC-MS/MS versus TLC plus GC methods: consistency of glycerolipid and fatty acid profiles in microalgae and higher plant cells and effect of a nitrogen starvation. *PLoS One*, *12*(8), e0182423.
Kalogeropoulos, N., Alexis, M. N., & Henderson, R. J. (1992). Effects of dietary soybean and cod-liver oil levels on growth and body-composition of gilthead bream (*Sparus aurata*) [Article]. *Aquaculture*, *104*(3-4), 293-308. https://doi.org/Doi 10.1016/0044-8486(92)90211-3
Kanazawa, A. (1993). Essential phospholipids of fish and crustaceans. In S. Kaushik & P. Luquet (Eds.), *Fish nutrition in practice* (pp. 519-530). INRA.
Kanazawa, A. (1993). Nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish. *Journal of the World Aquaculture Society*, *24*(2), 162-166. https://doi.org/10.1111/j.1749-7345.1993.tb00005.x
Karlsen, Ø., van der Meeren, T., Rønnestad, I., Mangor-Jensen, A., Galloway, T. F., Kjørsvik, E., & Hamre, K. (2015). Copepods enhance nutritional status, growth and development in Atlantic cod (*Gadus morhua* L.) larvae — can we identify the underlying factors? *PeerJ*, *3*(5), e902-e902. https://doi.org/10.7717/peerj.902
Kjørsvik, E., Gagnat, M. R., Wold, P.-A., Bardal, T., Attramadal, K., Sørøy, M., Hansen, M. H., Almli, M., Størseth, T., Dahle, S., & Øie, G. (2014). Functional development of ballan wrasse and effects of start feed quality. In S. Helland, S. Wiborg Dahle, C.

Hough, & J. Borthen (Eds.), (pp. 56-59). Fiskeri- og havbruksnæringens forskningsfond (FHF) - The Norwegian Seafood Reserach Fund. www.rensefisk.no
Kjørsvik, E., Olsen, C., Wold, P. A., Hoehne-Reitan, K., Cahu, C. L., Rainuzzo, J., Olsen, A. I., Øie, G., & Olsen, Y. (2009). Comparison of dietary phospholipids and neutral lipids on skeletal development and fatty acid composition in Atlantic cod (*Gadus morhua*). *Aquaculture*, *294*(3-4), 246-255. https://doi.org/10.1016/j.aquaculture.2009.06.012
Koedijk, R. M., Folkvord, A., Foss, A., Pittman, K., Stefansson, S. O., Handeland, S., & Imsland, A. K. (2010). The influence of first-feeding diet on the Atlantic cod *Gadus morhua* phenotype: survival, development and long-term consequences for growth. *Journal of Fish Biology*, *77*(1), 1-19. https://doi.org/10.1111/j.1095-8649.2010.02652.x
Kotani, T., Yokota, M., Fushimi, H., & Watanabe, S. (2011). How to determine the appropriate mortality in experimental larval rearing? *Fisheries Science*, *77*, 255-261.
Kottmann, J. S., Berge, G. M., Kousoulaki, K., Ostbye, T. K., Ytteborg, E., Gjerde, B., & Lein, I. (2023). Welfare and performance of ballan wrasse (*Labrus bergylta*) reared at two different temperatures after a preparatory feeding trial with enhanced dietary eicosapentaenoic acid. *Journal of Fish Biology*, *103*(5), 906-923. https://doi.org/10.1111/jfb.15482
Kousoulaki, K., Bogevik, A. S., Skiftesvik, A. B., Jensen, P. A., & Opstad, I. (2015). Marine raw material choice, quality and weaning performance of Ballan wrasse (*Labrus bergylta*) larvae. *Aquaculture Nutrition*, *21*(5), 644-654. https://doi.org/10.1111/anu.12186
Kousoulaki, K., Grøtan, E., Kortner, T. M., Berge, G. M., Haustveit, G., Krogdahl, Å., Nygaard, H., Sæle, Ø., Chikwati, E. M., & Lein, I. (2021). Technical feed quality influences health, digestion patterns, body mineralization and bone development in farming of the stomachless cleaner fish ballan wrasse (*Labrus bergylta*). *Animal Feed Science and Technology*, *274*, 114830. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2021.114830
Kožul, V., Glavić, N., Tutman, P., Bolotin, J., & Onofri, V. (2011). The spawning, embryonic and early larval development of the green wrasse *Labrus viridis* (Linnaeus, 1758) (*Labridae*) in controlled conditions. *Animal Reproduction Science*, *125*(1), 196-203. https://doi.org/https://doi.org/10.1016/j.anireprosci.2011.01.013
Krkosek, M., Ford, J. S., Morton, A., Lele, S., Myers, R. A., & Lewis, M. A. (2007). Declining wild salmon populations in relation to parasites from farm salmon. *Science*, *318*(5857), 1772-1775. https://doi.org/10.1126/science.1148744
Kvenseth, P. G., Andreassen, J., & Solgaard, J. (2003). Ballan wrasse – strong medicine. In A. N. Hjelme (Ed.), (pp. 18-23). Norsk Fiskeoppdrett A/S.
Lall, S. P., & Lewis-McCrea, L. M. (2007). Role of nutrients in skeletal metabolism and pathology in fish — An overview. *Aquaculture*, *267*(1), 3-19. https://doi.org/https://doi.org/10.1016/j.aquaculture.2007.02.053
Lasker, R. (1981). The role of a stable ocean in larval fish survival and subsequent recruitment. *Marine fish larvae: morphology, ecology and relation to fisheries*, *1*, 80-89.
Lê Cao, K.-A., Boitard, S., & Besse, P. (2011). Sparse PLS discriminant analysis: biologically relevant feature selection and graphical displays for multiclass problems. *BMC Bioinformatics*, *12*(1), 253. https://doi.org/10.1186/1471-2105-12-253
Leclercq, E., Davie, A., & Migaud, H. (2014). Delousing efficiency of farmed ballan wrasse (*Labrus bergylta*) against *Lepeophtheirus salmonis* infecting Atlantic salmon (*Salmo salar*) post-smolts. *Pest Management Science*, *70*(8), 1274-1282. https://doi.org/10.1002/ps.3692
Leclercq, E., Graham, P., & Migaud, H. (2015). Development of a water-stable agar-based diet for the supplementary feeding of cleaner fish ballan wrasse (*Labrus bergylta*) deployed within commercial Atlantic salmon (*Salmon salar*) net-pens. *Animal Feed*

Science and Technology, *208*, 98-106. https://doi.org/https://doi.org/10.1016/j.anifeedsci.2015.06.026
Li, K., Kjørsvik, E., Bergvik, M., & Olsen, Y. (2015). Manipulation of the fatty acid composition of phosphatidylcholine and phosphatidylethanolamine in rotifers *Brachionus* Nevada and *Brachionus* Cayman. *Aquaculture Nutrition*, *21*(1), 85-97. https://doi.org/10.1111/anu.12140
Liu, G., & Xu, D. (2009). Effects of calanoid copepod *Schmackeria poplesia* as a live food on the growth, survival and fatty acid composition of larvae and juveniles of Japanese flounder, Paralichthys olivaceus. *Journal of Ocean University of China*, *8*(4), 359-365. https://doi.org/10.1007/s11802-009-0359-3
Malzahn, A. M., Ribičić, D., Hansen, B. H., Sarno, A., Kjørsvik, E., Aase, A. S. N., Musialak, L. A., García-Calvo, L., & Hagemann, A. (2022). First feed matters: The first diet of larval fish programmes growth, survival, and metabolism of larval ballan wrasse (*Labrus bergylta*). *Aquaculture*, *561*. https://doi.org/10.1016/j.aquaculture.2022.738586
Martinez-Silva, M. A., Audet, C., Winkler, G., & Tremblay, R. (2018). Prey quality impact on the feeding behavior and lipid composition of winter flounder (*Pseudopleuronectes americanus*) larvae. *Aquaculture and Fisheries*, *3*(4), 145-155. https://doi.org/https://doi.org/10.1016/j.aaf.2018.06.003
Mazurais, D., Glynatsi, N., Darias, M. J., Christodoulopoulou, S., Cahu, C. L., Zambonino-Infante, J. L., & Koumoundouros, G. (2009). Optimal levels of dietary vitamin A for reduced deformity incidence during development of European sea bass larvae (*Dicentrarchus labrax*) depend on malformation type [Article]. *Aquaculture*, *294*(3-4), 262-270. https://doi.org/10.1016/j.aquaculture.2009.06.008
McEvoy, L., & Sargent, J. R. (1998). Problems and techniques in live prey enrichment. *Proceedings of the Live Feeds Session, Aquaculture Canada*, *98*, 12-16.
Mejri, S. C., Tremblay, R., Audet, C., Wills, P. S., & Riche, M. (2021). Essential fatty acid requirements in tropical and cold-water marine fish larvae and juveniles. *Frontiers in Marine Science*, *8*. https://doi.org/10.3389/fmars.2021.680003
Mennerat, A., Nilsen, F., Ebert, D., & Skorping, A. (2010). Intensive farming: Evolutionary implications for parasites and pathogens. *Evolutionary Biology*, *37*(2), 59-67. https://doi.org/10.1007/s11692-010-9089-0
Moe Føre, H., Thorvaldsen, T., Osmundsen, T. C., Asche, F., Tveterås, R., Fagertun, J. T., & Bjelland, H. V. (2022). Technological innovations promoting sustainable salmon (*Salmo salar*) aquaculture in Norway. *Aquaculture Reports*, *24*. https://doi.org/10.1016/j.aqrep.2022.101115
Morel, G. M., Shrives, J., Bossy, S. F., & Meyer, C. G. (2013). Residency and behavioural rhythmicity of ballan wrasse (*Labrus bergylta*) and rays (*Raja* spp.) captured in Portelet Bay, Jersey: implications for Marine Protected Area design. *Journal of the Marine Biological Association of the United Kingdom*, *93*(5), 1407-1414. https://doi.org/10.1017/S0025315412001725
Mourente, G., & Tocher, D. R. (1992). Effects of weaning onto a pelleted diet on docosahexaenoic acid (22: 6 n-3) levels in brain of developing turbot (*Scophthalmus maximus* L.). *Aquaculture*, *105*(3), 363-377. https://doi.org/https://doi.org/10.1016/0044-8486(92)90100-Y
Munilla-Moran, R., Stark, J. R., & Barbour, A. (1990). The role of exogenous enzymes in digestion in cultured turbot larvae (*Scophthalmus maximus* L.). *Aquaculture*, *88*(3), 337-350. https://doi.org/https://doi.org/10.1016/0044-8486(90)90159-K
Mæhre, H. K., Hamre, K., & Elvevoll, E. O. (2013). Nutrient evaluation of rotifers and zooplankton: Feed for marine fish larvae. *Aquaculture Nutrition*, *19*(3), 301-311. https://doi.org/10.1111/j.1365-2095.2012.00960.x
Navarro, J. C., Henderson, R. J., McEvoy, L. A., Bell, M. V., & Amat, F. (1999). Lipid conversions during enrichment of *Artemia* [Article]. *Aquaculture*, *174*(1-2), 155-166. https://doi.org/10.1016/s0044-8486(99)00004-6
Naylor, R., Hindar, K., Fleming, I. A., Goldburg, R., Williams, S., Volpe, J., Whoriskey, F., Eagle, J., Kelso, D., & Mangel, M. (2005). Fugitive Salmon: Assessing the Risks of

Escaped Fish from Net-Pen Aquaculture. *Bioscience*, *55*(5), 427-427. https://doi.org/10.1641/0006-3568(2005)055[0427:fsatro]2.0.co;2
Naylor, R. L., Hardy, R. W., Buschmann, A. H., Bush, S. R., Cao, L., Klinger, D. H., Little, D. C., Lubchenco, J., Shumway, S. E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. *Nature*, *591*(7851), 551-563. https://doi.org/10.1038/s41586-021-03308-6
Neori, A. (2011). "Green water" microalgae: the leading sector in world aquaculture. *Journal of Applied Phycology*, *23*(1), 143-149. https://doi.org/10.1007/s10811-010-9531-9
Nguyen, V. T., Satoh, S., Haga, Y., Fushimi, H., & Kotani, T. (2008). Effect of zinc and manganese supplementation in *Artemia* on growth and vertebral deformity in red sea bream (*Pagrus major*) larvae [Article]. *Aquaculture*, *285*(1-4), 184-192. https://doi.org/10.1016/j.aquaculture.2008.08.030
Noble, C., Iversen, M. H., Lein, I., Kolarevic, J., J., –H., L., Burgerhout, E., Puvanendran, V., Kousoulaki, K., Aas, G. H., Stene, A., & Espmark, Å. M. (2019). RENSVEL OWI FACT SHEET SERIES: An introduction to Operational and Laboratory based Welfare Indicators for ballan wrasse (*Labrus bergylta*). In (pp. 1-43).
Norland, S., Saele, Ø., & Rønnestad, I. (2022). Developmental stages of the ballan wrasse from first feeding through metamorphosis: Cranial ossification and the digestive system. *Journal of Anatomy*, *241*(2), 337-357. https://doi.org/10.1111/joa.13686
Næss, T., Germain-Henry, M., & Naas, K. E. (1995). First feeding of Atlantic halibut (*Hippoglossus hippoglossus*) using different combinations of *Artemia* and wild zooplankton. *Aquaculture*, *130*(2), 235-250. https://doi.org/https://doi.org/10.1016/0044-8486(94)00323-G
Olafsen, T., Winther, U., Olsen, Y., & Skjermo, J. (2012). Value created from productive oceans in 2050. 83-83.
Olaussen, J. O. (2018). Environmental problems and regulation in the aquaculture industry. Insights from Norway. *Marine Policy*, *98*(July), 158-163. https://doi.org/10.1016/j.marpol.2018.08.005
Olsen, R. E., Henderson, R. J., & Pedersen, T. (1991). The influence of dietary lipid classes on the fatty acid composition of small cod *Gadus morhua* L. juveniles reared in an enclosure in northern Norway. *Journal of Experimental Marine Biology and Ecology*, *148*(1), 59-76. https://doi.org/https://doi.org/10.1016/0022-0981(91)90147-O
Olsen, R. E., & Ringø, E. (1997). Lipid digestibility in fish: a review. *Recent Research Developments in Lipid Research*, *1*, 199-264.
Ottesen, O. H., Dunaevskaya, E., & D'Arcy, J. (2012). Development of *Labrus Bergylta* (Ascanius 1767) Larvae from Hatching to Metamorphosis. *Journal of Aquaculture Research and Development*, *3*, 1-4.
Overrein, I. (2010). *Copepod lipids in aquaculture (PhD thesis)* Norwegian University of Science and Technology (NTNU)].
Overton, K., Dempster, T., Oppedal, F., Kristiansen, T. S., Gismervik, K., & Stien, L. H. (2019). Salmon lice treatments and salmon mortality in Norwegian aquaculture: a review. *Reviews in Aquaculture*, *11*(4), 1398-1417. https://doi.org/10.1111/raq.12299
Palmer, P. J., Burke, M. J., Palmer, C. J., & Burke, J. B. (2007). Developments in controlled green-water larval culture technologies for estuarine fishes in Queensland, Australia and elsewhere. *Aquaculture*, *272*(1), 1-21. https://doi.org/https://doi.org/10.1016/j.aquaculture.2007.06.018
Pang, Z., Chong, J., Zhou, G., de Lima Morais, D. A., Chang, L., Barrette, M., Gauthier, C., Jacques, P. E., Li, S., & Xia, J. (2021). MetaboAnalyst 5.0: narrowing the gap between raw spectra and functional insights. *Nucleic Acids Research*, *49*(W1), W388-W396. https://doi.org/10.1093/nar/gkab382
Papandroulakis, N., Divanach, P., Anastasiadis, P., & Kentouri, M. (2001). The pseudo-green water technique for intensive rearing of sea bream (*Sparus aurata*) larvae. *Aquaculture International*, *9*(3), 205-216. https://doi.org/10.1023/A:1016813623122

Pati, S., Nie, B., Arnold, R. D., & Cummings, B. S. (2016). Extraction, chromatographic and mass spectrometric methods for lipid analysis. *Biomedical Chromatography*, *30*(5), 695-709. https://doi.org/10.1002/bmc.3683

Payne, M. F., Rippingale, R. J., & Cleary, J. J. (2001). Cultured copepods as food for West Australian dhufish (*Glaucosoma hebraicum*) and pink snapper (*Pagrus auratus*) larvae. *Aquaculture*, *194*(1), 137-150. https://doi.org/https://doi.org/10.1016/S0044-8486(00)00513-5

Penglase, S., Nordgreen, A., van der Meeren, T., Olsvik, P. A., Sæle, O., Sweetman, J. W., Baeverfjord, G., Helland, S., & Hamre, K. (2010). Increasing the level of selenium in rotifers (*Brachionus plicatilis* 'Cayman') enhances the mRNA expression and activity of glutathione peroxidase in cod (*Gadus morhua* L.) larvae [Article]. *Aquaculture*, *306*(1-4), 259-269. https://doi.org/10.1016/j.aquaculture.2010.05.011

Pepin, P. (2022). Feeding by larval fish: how taxonomy, body length, mouth size, and behaviour contribute to differences among individuals and species from a coastal ecosystem. *ICES Journal of Marine Science*, *80*(1), 91-106. https://doi.org/10.1093/icesjms/fsac215

Perga, M. E., Kainz, M., Matthews, B., & Mazumder, A. (2006). Carbon pathways to zooplankton: insights from the combined use of stable isotope and fatty acid biomarkers. *Freshwater Biology*, *51*(11), 2041-2051. https://doi.org/10.1111/j.1365-2427.2006.01634.x

Person-Le Ruyet, J., Pichavant, K., Vacher, C., Le Bayon, N., Sévère, A., & Boeuf, G. (2002). Effects of O_2 supersaturation on metabolism and growth in juvenile turbot (*Scophthalmus maximus* L.). *Aquaculture*, *205*(3), 373-383. https://doi.org/https://doi.org/10.1016/S0044-8486(01)00689-5

Piccinetti, C. C., Grasso, L., Maradonna, F., Radaelli, G., Ballarin, C., Chemello, G., Evjemo, J. O., Carnevali, O., & Olivotto, I. (2017). Growth and stress factors in ballan wrasse (*Labrus bergylta*) larval development. *Aquaculture Research*, *48*(5), 2567-2580. https://doi.org/https://doi.org/10.1111/are.13093

Pike, A. W., & Wadsworth, S. L. (1999). Sealice on salmonids: Their biology and control. In (Vol. 31, pp. 233-337). https://doi.org/10.1016/S0065-308X(08)60233-X

Pinto, W., Figueira, L., Ribeiro, L., Yúfera, M., Dinis, M. T., & Aragão, C. (2010). Dietary taurine supplementation enhances metamorphosis and growth potential of *Solea senegalensis* larvae. *Aquaculture*, *309*(1), 159-164. https://doi.org/https://doi.org/10.1016/j.aquaculture.2010.08.031

Planas, M., & Cunha, I. (1999). Larviculture of marine fish: problems and perspectives. *Aquaculture*, *177*(1), 171-190. https://doi.org/https://doi.org/10.1016/S0044-8486(99)00079-4

Planktonic. (2023). *Planktonic*. Retrieved 2nd November 2023 from https://planktonic.no/

Potts, G. W. (1973). Cleaning symbiosis among British fish with special reference to *Crenilabrus melops* (Labridae). *Journal of the Marine Biological Association of the United Kingdom*, *53*(1), 1-10. https://doi.org/10.1017/S0025315400056587

Powell, A., Treasurer, J. W., Pooley, C. L., Keay, A. J., Lloyd, R., Imsland, A. K., & Garcia de Leaniz, C. (2017). Use of lumpfish for sea-lice control in salmon farming: challenges and opportunities. *Reviews in Aquaculture*, *10*(3), 683-702. https://doi.org/10.1111/raq.12194

Quignard, J. P., & Pras, A. (1986). Labridae. In P. J. P. Whitehead, M. L. Bauchot, J. C. Hureau, J. Nielsen, & E. Tortonese (Eds.), *Fishes of the North-eastern Atlantic and the Mediterranean* (pp. 919-942). UNESCO.

Rajkumar, M., & Kumaraguru vasagam, K. P. (2006). Suitability of the copepod, *Acartia clausi* as a live feed for Seabass larvae (*Lates calcarifer* Bloch): Compared to traditional live-food organisms with special emphasis on the nutritional value. *Aquaculture*, *261*(2), 649-658. https://doi.org/https://doi.org/10.1016/j.aquaculture.2006.08.043

Reitan, K. I., Rainuzzo, J. R., & Olsen, Y. (1994). Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. *Aquaculture International*, *2*(1), 33-48. https://doi.org/10.1007/BF00118531

Ricker, W. E. (1958). *Handbook of computations for biological statistics of fish populations*. Fisheries Research Board of Canada.
Rocha, G. S., Katan, T., Parrish, C. C., & Kurt Gamperl, A. (2017). Effects of wild zooplankton versus enriched rotifers and *Artemia* on the biochemical composition of Atlantic cod (*Gadus morhua*) larvae. *Aquaculture*, *479*, 100-113. https://doi.org/https://doi.org/10.1016/j.aquaculture.2017.05.025
Rodríguez, C., Pérez, J. A., Díaz, M., Izquierdo, M. S., Fernández-Palacios, H., & Lorenzo, A. (1997). Influence of the EPA/DHA ratio in rotifers on gilthead seabream (*Sparus aurata*) larval development. *Aquaculture*, *150*(1), 77-89. https://doi.org/https://doi.org/10.1016/S0044-8486(96)01472-X
Romundstad, M. (2015). *Effects of different live feed on ballan wrasse (Labrus bergylta) larval hepatocyte and enterocyte development* Norwegian University of Science and Technology]. Trondheim.
Rong, R. L., Gagnat, M. R., Attramadal, Y., & Vadstein, O. (2022). Microbial water quality of the copepod *Acartia tonsa* in cultures for use as live feed. *Aquaculture*, *560*, 738439. https://doi.org/https://doi.org/10.1016/j.aquaculture.2022.738439
Rosenlund, G., Stoss, J., & Talbot, C. (1997). Co-feeding marine fish larvae with inert and live diets. *Aquaculture*, *155*(1), 183-191. https://doi.org/https://doi.org/10.1016/S0044-8486(97)00116-6
Rosvoll, A. I. C. (2022). *Effect of different live feed organisms on microbiota, growth and survival during start-feeding of ballan wrasse (Labrus bergylta)* Norwegian University of Science and Technology]. Trondheim.
Roth, M. (2000). The availability and use of chemotherapeutic sea lice control products. *Contributions to Zoology*, *69*(1/2), 109-118.
Rotman, F., Stuart, K., & Drawbridge, M. (2017). Effects of taurine supplementation in live feeds on larval rearing performance of California yellowtail *Seriola lalandi* and white seabass *Atractoscion nobilis*. *Aquaculture Research*, *48*(3), 1232-1239. https://doi.org/https://doi.org/10.1111/are.12964
Rudy, M. D., Kainz, M. J., Graeve, M., Colombo, S. M., & Arts, M. T. (2016). Handling and storage procedures have variable effects on fatty acid content in fishes with different lipid quantities. *PLoS One*, *11*(8), e0160497. https://doi.org/10.1371/journal.pone.0160497
Rønnestad, I., Helland, S., & Lie, Ø. (1998). Feeding *Artemia* to larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) results in lower larval vitamin A content compared with feeding copepods. *Aquaculture*, *165*(1), 159-164. https://doi.org/https://doi.org/10.1016/S0044-8486(98)00258-0
Rønnestad, I., Yúfera, M., Ueberschär, B., Ribeiro, L., Sæle, Ø., & Boglione, C. (2013). Feeding behaviour and digestive physiology in larval fish: current knowledge, and gaps and bottlenecks in research. *Reviews in Aquaculture*, *5*(s1). https://doi.org/10.1111/raq.12010
Saini, R. K., Prasad, P., Shang, X., & Keum, Y. S. (2021). Advances in lipid extraction methods-A review. *Laboratory Investigation*, *22*(24). https://doi.org/10.3390/ijms222413643
Salhi, M., Hernández-Cruz, C. M., Bessonart, M., Izquierdo, M. S., & Fernández-Palacios, H. (1999). Effect of different dietary polar lipid levels and different n−3 HUFA content in polar lipids on gut and liver histological structure of gilthead seabream (*Sparus aurata*) larvae. *Aquaculture*, *179*(1), 253-263. https://doi.org/https://doi.org/10.1016/S0044-8486(99)00194-5
Salze, G., Tocher, D. R., Roy, W. J., & Robertson, D. A. (2005). Egg quality determinants in cod (*Gadus morhua* L.): egg performance and lipids in eggs from farmed and wild broodstock. *Aquaculture Research*, *36*(15), 1488-1499. https://doi.org/10.1111/j.1365-2109.2005.01367.x
Sargent, J., Bell, G., McEvoy, L., Tocher, D., & Estevez, A. (1999). Recent developments in the essential fatty acid nutrition of fish. *Aquaculture*, *177*(1), 191-199. https://doi.org/https://doi.org/10.1016/S0044-8486(99)00083-6
Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., & Tocher, D. (1999). Lipid nutrition of marine fish during early development: current status and future

directions. *Aquaculture*, *179*(1), 217-229. https://doi.org/https://doi.org/10.1016/S0044-8486(99)00191-X
Sargent, J. R., McEvoy, L. A., & Bell, J. G. (1997). Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. *Aquaculture*, *155*(1), 117-127. https://doi.org/https://doi.org/10.1016/S0044-8486(97)00122-1
Sargent, J. R., Tocher, D. R., & Bell, J. G. (2003). 4 - The Lipids. In J. E. Halver & R. W. Hardy (Eds.), *Fish Nutrition (Third Edition)* (pp. 181-257). Academic Press. https://doi.org/https://doi.org/10.1016/B978-012319652-1/50005-7
Sayer, M. D. J., & Treasurer, J. W. (1996). North European wrasse: Identification, distribution and habitat. In *Wrasse: Biology and Use in Aquaculture* (pp. 3-12).
Shields, R. J., Bell, J. G., Luizi, F. S., Gara, B., Bromage, N. R., & Sargent, J. R. (1999). Natural copepods are superior to enriched *Artemia* nauplii as feed for halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. *The Journal of Nutrition*, *129*(6), 1186-1194. https://doi.org/https://doi.org/10.1093/jn/129.6.1186
Skiftesvik, A. B., Bjelland, R. M., Durif, C. M. F., Johansen, I. S., & Browman, H. I. (2013). Delousing of Atlantic salmon (*Salmo salar*) by cultured vs. wild ballan wrasse (*Labrus bergylta*). *Aquaculture*, *402-403*, 113-118. https://doi.org/10.1016/j.aquaculture.2013.03.032
Skiftesvik, A. B., Durif, C. M. F., Bjelland, R. M., & Browman, H. I. (2015). Distribution and habitat preferences of five species of wrasse (Family Labridae) in a Norwegian fjord. *ICES Journal of Marine Science*, *72*(3), 890-899. https://doi.org/10.1093/icesjms/fsu211
Statistisk sentralbyrå (SSB). (2019a, June 6th, 2023). *07326: Aquaculture. Sales of slaughtered fish for food, by fish species, contents and year*. https://www.ssb.no/en/statbank/table/07326/
Statistisk sentralbyrå (SSB). (2019b, June 6th, 2023). *Aquaculture statistics*. https://www.ssb.no/en/jord-skog-jakt-og-fiskeri/statistikker/fiskeoppdrett
Stavrakaki, M. G. (2013). *Ballan wrasse (Labrus bergylta) larvae and live-feed quality; effects on growth and expression of genes related to mitochondrial functions (master thesis)* Norwegian University of Science and Technology (NTNU)]. Trondheim.
Stentiford, G. D., Sritunyalucksana, K., Flegel, T. W., Williams, B. A. P., Withyachumnarnkul, B., Itsathitphaisarn, O., & Bass, D. (2017). New paradigms to help solve the global aquaculture disease crisis. *PLoS Pathogens*, *13*(2), e1006160. https://doi.org/10.1371/journal.ppat.1006160
Stuart, K., Losordo, M., Olin, P., & Drawbridge, M. (2015). Effects of stocking density and water conditioners on yolk-sac larvae of two marine finfish during simulated air transport. *Aquaculture Research*, *46*(9), 2124-2132. https://doi.org/https://doi.org/10.1111/are.12368
Støttrup, J. G. (2000). The elusive copepods: their production and suitability in marine aquaculture. *Aquaculture Research*, *31*(8-9), 703-711. https://doi.org/10.1046/j.1365-2109.2000.318488.x
Støttrup, J. G. (2003). Production and nutritional value of copepods. In J. G. Støttrup & L. A. McEvoy (Eds.), (pp. 145-205). Blackwell Science Ltd. https://doi.org/10.1002/9780470995143.ch5
Takeda, H., Izumi, Y., Takahashi, M., Paxton, T., Tamura, S., Koike, T., Yu, Y., Kato, N., Nagase, K., & Shiomi, M. (2018). Widely-targeted quantitative lipidomics method by supercritical fluid chromatography triple quadrupole mass spectrometry. *Journal of Lipid Research*, *59*(7), 1283-1293.
Taranger, G. L., Karlsen, Ø., Bannister, R. J., Glover, K. A., Husa, V., Karlsbakk, E., Kvamme, B. O., Boxaspen, K. K., Bjørn, P. A., Finstad, B., Madhun, A. S., Morton, H. C., & Svåsand, T. (2015). Risk assessment of the environmental impact of Norwegian Atlantic salmon farming. *ICES Journal of Marine Science*, *72*(3), 997-1021. https://doi.org/10.1093/icesjms/fsu132
Thorarensen, H., Gústavsson, A., Gunnarsson, S., Árnason, J., Steinarsson, A., Björnsdóttir, R., & Imsland, A. K. D. (2017). The effect of oxygen saturation on the

growth and feed conversion of juvenile Atlantic cod (*Gadus morhua* L.). *Aquaculture*, *475*, 24-28. https://doi.org/https://doi.org/10.1016/j.aquaculture.2017.04.002
Tidwell, J. H., & Allan, G. L. (2001). Fish as food: aquaculture's contribution. Ecological and economic impacts and contributions of fish farming and capture fisheries. *EMBO Rep*, *2*(11), 958-963. https://doi.org/10.1093/embo-reports/kve236
Tocher, D. R. (1995). Chapter 6 - Glycerophospholipid metabolism. In P. W. Hochachka & T. P. Mommsen (Eds.), *Biochemistry and Molecular Biology of Fishes* (Vol. 4, pp. 119-157). Elsevier. https://doi.org/https://doi.org/10.1016/S1873-0140(06)80009-3
Tocher, D. R. (2010). Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquaculture Research*, *41*(5), 717-732. https://doi.org/https://doi.org/10.1111/j.1365-2109.2008.02150.x
Tocher, D. R., Bendiksen, E. Å., Campbell, P. J., & Bell, J. G. (2008). The role of phospholipids in nutrition and metabolism of teleost fish. *Aquaculture*, *280*(1), 21-34. https://doi.org/https://doi.org/10.1016/j.aquaculture.2008.04.034
Torrissen, O., Jones, S., Asche, F., Guttormsen, A., Skilbrei, O. T., Nilsen, F., Horsberg, T. E., & Jackson, D. (2013). Salmon lice - impact on wild salmonids and salmon aquaculture. *Journal of Fish Diseases*, *36*(3), 171-194. https://doi.org/10.1111/jfd.12061
Troell, M., Naylor, R. L., Metian, M., Beveridge, M., Tyedmers, P. H., Folke, C., Arrow, K. J., Barrett, S., Crépin, A. S., Ehrlich, P. R., Gren, Å., Kautsky, N., Levin, S. A., Nyborg, K., Österblom, H., Polasky, S., Scheffer, M., Walker, B. H., Xepapadeas, T., & De Zeeuw, A. (2014). Does aquaculture add resilience to the global food system? *Proceedings of the National Academy of Sciences of the United States of America*, *111*(37), 13257-13263. https://doi.org/10.1073/pnas.1404067111
Tsugawa, H., Ohta, E., Izumi, Y., Ogiwara, A., Yukihira, D., Bamba, T., Fukusaki, E., & Arita, M. (2015). MRM-DIFF: data processing strategy for differential analysis in large scale MRM-based lipidomics studies. *Frontiers in genetics*, *5*, 471.
Tveterås, R., Reve, T., Haus-Reve, S., Misund, B., & Blomgren, A. (2019). En konkurransedyktig og kunnskapsbasert havbruksnæring. 1-140.
Vadstein, O., Attramadal, K. J. K., Bakke, I., Forberg, T., Olsen, Y., Verdegem, M., Giatsis, C., Skjermo, J., Aasen, I. M., Gatesoupe, F. J., Dierckens, K., Sorgeloos, P., & Bossier, P. (2018). Managing the microbial community of marine fish larvae: A holistic perspective for larviculture. *Front Microbiol*, *9*, 1820. https://doi.org/10.3389/fmicb.2018.01820
Vadstein, O., Attramadal, K. J. K., Bakke, I., & Olsen, Y. (2018). K-Selection as microbial community management strategy: A method for improved viability of larvae in aquaculture. *Front Microbiol*, *9*, 2730. https://doi.org/10.3389/fmicb.2018.02730
Vadstein, O., Bergh, Ø., Gatesoupe, F.-J., Galindo-Villegas, J., Mulero, V., Picchietti, S., Scapigliati, G., Makridis, P., Olsen, Y., Dierckens, K., Defoirdt, T., Boon, N., De Schryver, P., & Bossier, P. (2013). Microbiology and immunology of fish larvae. *Reviews in Aquaculture*, *5*(s1), S1-S25. https://doi.org/https://doi.org/10.1111/j.1753-5131.2012.01082.x
van der Meeren, T. (1991). Selective feeding and prediction of food consumption in turbot larvae (*Scophthalmus maximus* L.) reared on the rotifer *Brachionus plicatilis* and natural zooplankton. *Aquaculture*, *93*(1), 35-55. https://doi.org/https://doi.org/10.1016/0044-8486(91)90203-J
van der Meeren, T., Karlsen, Ø., Liebig, P. L., & Mangor-Jensen, A. (2014). Copepod production in a saltwater pond system: A reliable method for achievement of natural prey in start-feeding of marine fish larvae. *Aquacultural Engineering*, *62*, 17-27. https://doi.org/https://doi.org/10.1016/j.aquaeng.2014.07.003
van der Meeren, T., Olsen, R. E., Hamre, K., & Fyhn, H. J. (2008). Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. *Aquaculture*, *274*(2), 375-397. https://doi.org/https://doi.org/10.1016/j.aquaculture.2007.11.041

Verdegem, M., Buschmann, A. H., Latt, U. W., Dalsgaard, A. J. T., & Lovatelli, A. (2023). The contribution of aquaculture systems to global aquaculture production. *Journal of the World Aquaculture Society*, *54*(2), 206-250. https://doi.org/10.1111/jwas.12963

Villegas-Ríos, D., Alós, J., March, D., Palmer, M., Mucientes, G., & Saborido-Rey, F. (2013). Home range and diel behavior of the ballan wrasse, *Labrus bergylta*, determined by acoustic telemetry. *Journal of Sea Research*, *80*, 61-71. https://doi.org/https://doi.org/10.1016/j.seares.2013.02.009

Virtanen, P., Gommers, R., Oliphant, T. E., Haberland, M., Reddy, T., Cournapeau, D., Burovski, E., Peterson, P., Weckesser, W., Bright, J., van der Walt, S. J., Brett, M., Wilson, J., Millman, K. J., Mayorov, N., Nelson, A. R. J., Jones, E., Kern, R., Larson, E., . . . SciPy, C. (2020). SciPy 1.0: fundamental algorithms for scientific computing in Python. *Nature Methods*, *17*(3), 261-272. https://doi.org/10.1038/s41592-019-0686-2

Walne, P. R. (1970). Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera *Ostrea*, *Crassostrea*, *Mercenaria* and *Mytilus*. *Fish. Invest. Ser. 2*, *26*(5).

Waskom, M. (2021). seaborn: statistical data visualization. *Journal of Open Source Software*, *6*(60). https://doi.org/10.21105/joss.03021

Watanabe, T. (1993). Importance of docosahexaenoic acid in marine larval fish. *Journal of the World Aquaculture Society*, *24*(2), 152-161. https://doi.org/https://doi.org/10.1111/j.1749-7345.1993.tb00004.x

Watanabe, T., Kitajima, C., & Fujita, S. (1983). Nutritional values of live organisms used in Japan for mass propagation of fish: A review. *Aquaculture*, *34*(1), 115-143. https://doi.org/https://doi.org/10.1016/0044-8486(83)90296-X

Weitkamp, D. E., & Katz, M. (1980). A review of dissolved gas supersaturation literature. *Transactions of the American Fisheries Society*, *109*(6), 659-702. https://doi.org/https://doi.org/10.1577/1548-8659(1980)109<659:ARODGS>2.0.CO;2

Wiegand, M. D. (1996). Composition, accumulation and utilization of yolk lipids in teleost fish. *Reviews in Fish Biology and Fisheries*, *6*(3), 259-286. https://doi.org/10.1007/BF00122583

Wold, P. A., Hoehne-Reitan, K., Cahu, C. L., Infante, J. Z., Rainuzzo, J., & Kjørsvik, E. (2009). Comparison of dietary phospholipids and neutral lipids: Effects on gut, liver and pancreas histology in Atlantic cod (*Gadus morhua* L.) larvae. *Aquaculture Nutrition*, *15*(1), 73-84. https://doi.org/10.1111/j.1365-2095.2008.00569.x

Woodfield, H. K., Cazenave-Gassiot, A., Haslam, R. P., Guschina, I. A., Wenk, M. R., & Harwood, J. L. (2018). Using lipidomics to reveal details of lipid accumulation in developing seeds from oilseed rape (*Brassica napus* L.). *Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids*, *1863*(3), 339-348.

Xuan, Q., Hu, C., Yu, D., Wang, L., Zhou, Y., Zhao, X., Li, Q., Hou, X., & Xu, G. (2018). Development of a high coverage pseudotargeted lipidomics method based on ultra-high performance liquid chromatography–mass spectrometry. *Analytical Chemistry*, *90*(12), 7608-7616.

Yuen, J. W., Dempster, T., Oppedal, F., & Hvas, M. (2019). Physiological performance of ballan wrasse (*Labrus bergylta*) at different temperatures and its implication for cleaner fish usage in salmon aquaculture. *Biological Control*, *135*, 117-123. https://doi.org/https://doi.org/10.1016/j.biocontrol.2019.05.007

Øie, G., Galloway, T., Sørøy, M., Holmvaag Hansen, M., Norheim, I. A., Halseth, C. K., Almli, M., Berg, M., Gagnat, M. R., Wold, P. A., Attramadal, K., Hagemann, A., Evjemo, J. O., & Kjørsvik, E. (2015). Effect of cultivated copepods (*Acartia tonsa*) in first-feeding of Atlantic cod (*Gadus morhua*) and ballan wrasse (*Labrus bergylta*) larvae. *Aquaculture Nutrition*, *23*(1), 3-17. https://doi.org/10.1111/anu.12352

Øvrebø, T. K., Balseiro, P., Imsland, A. K. D., Stefansson, S. O., Tveterås, R., Sveier, H., & Handeland, S. (2022). Investigation of growth performance of post-smolt Atlantic salmon (*Salmo salar* L.) in semi closed containment system: A big-scale benchmark

study. *Aquaculture Research*, *53*(11), 4178-4189. https://doi.org/10.1111/are.15919

Aaen, S. M., Helgesen, K. O., Bakke, M. J., Kaur, K., & Horsberg, T. E. (2015). Drug resistance in sea lice: a threat to salmonid aquaculture. *Trends in Parasitology*, *31*(2), 72-81. https://doi.org/https://doi.org/10.1016/j.pt.2014.12.006

Aase, A. S. N. (2022). *Nutritional condition of ballan wrasse (Labrus bergylta) larvae reared on different live feed diets – an investigation of gut and liver histology (master thesis)* Norwegian University of Science and Technology]. Trondheim.

7 Appendices

Appendix 1: Dry Weight (DW)

Table A1a. Descriptive statistics of larval DW along the experiment. The table compiles the mean DW values ± standard deviation (SD) (µg larva^{-1}) and number of larvae sampled (N), at times 4, 12, 18, 23, 32, 37, 42 and 48 DPH for each feeding regime. Feeding regimes: Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Industry standard (Rot-*Art*) and Copepod-cirripeds (Cop-cir).

	ExpCir		**Cop-*Art***		**Rot-*Art***		**Cop-cir**	
Time (DPH)	**Mean DW ± SD (µg larva-1)**	**N**	**Mean DW ± SD (µg larva-1)**	**N**	**Mean DW ± SD (µg larva-1)**	**N**	**Mean DW ± SD (µg larva-1)**	**N**
4	34.8 ± 6.9	10	34.4 ± 3.1	10	31.2 ± 2.7	9	35.7 ± 2.2	10
12	18.9 ± 6.0	30	19.8 ± 5.8	30	22.1 ± 8.0	30	34.9 ± 7.9	29
18	32.3 ± 12.6	26	80.0± 25.4	29	57.5 ± 10.5	29	54.3 ± 16.7	30
23	88.5 ± 44.7	20	155.6 ± 80.5	27	96.0 ± 34.8	23	152.9 ± 66.9	29
32	298.0 ± 142.7	24	636.3 ± 264.4	28	609.4 ± 280.6	25	421.8 ± 250.7	27
37	502.1 ± 237.5	21	824.8 ± 505.0	29	1004.2 ± 495.8	19	923.8 ± 491.4	30
42	641.7 ± 729.8	6	1406.6 ± 494.7	30	1582.4 ± 630.2	5	1078.6 ± 545.8	30
48	NA		2086.1 ± 1032.6	29	NA		2357.0 ± 1282.9	29

NA: no availability of samples for that time point and feeding regime.

Table A1b. Statistical tests applied for analysis of each time point and feeding regime. The tests were selected according to the criteria indicated in Section 2.4.

Time (DPH)	**Statistical test**
4	Ordinary One-way ANOVA + Tukey's multiple comparisons test
12	Kruskal-Wallis test (one-way ANOVA on ranks) + Dunn's multiple comparisons test
18	Welch's ANOVA test, corrected by multiple comparisons using statistical hypothesis testing with Dunnet T3
23	Ordinary One-way ANOVA + Tukey's multiple comparisons test
32	Ordinary One-way ANOVA + Tukey's multiple comparisons test
37	Ordinary One-way ANOVA + Tukey's multiple comparisons test
42	Kruskal-Wallis test (one-way ANOVA on ranks) + Dunn's multiple comparisons test
48	Unpaired Welch's t-test

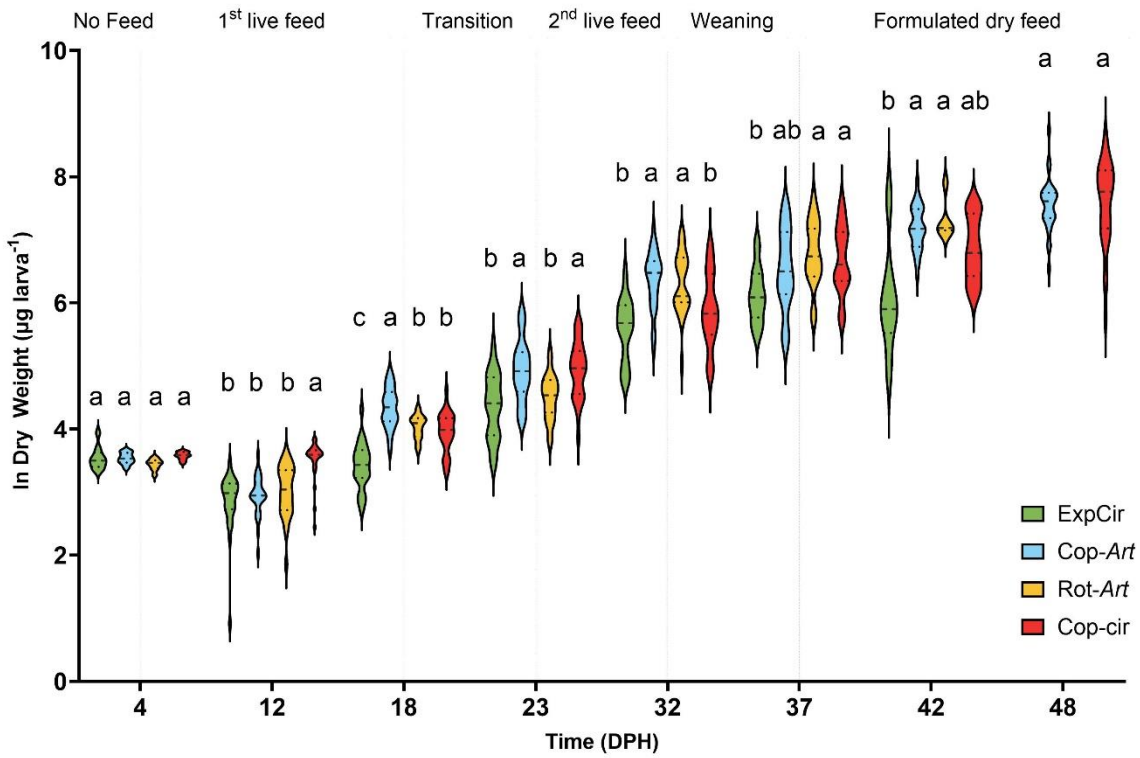


Figure A1. Distribution and development of the dry weight of the larvae over time. Dotted horizontal black lines inside each violin plot indicate the first and third quartiles. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirripeds (Cop-cir)). Dotted vertical lines in the graph indicate the corresponding feeding period. The horizontal axis represents sampling times as discrete variables, equally spaced independently of the number of days in between points. Statistically significant differences ($p<0.05$) between feeding regimes are depicted by unequal letters over each violin plot. No values are represented for the Rot-*Art* and ExpCir treatments at 48 DPH due to lack of sampling because of high mortality. Values and statistical tests applied can be consulted in Appendix 1, Table A1a and Table A1b.

Appendix 2: Daily weight increase (DWI)

Table A2. Daily weight increase (DWI). The table indicates the mean DWI ± standard deviation (SD) (%) for each feeding period and for all the experiment from 4 to 42 DPH. The last sampling point (48 DPH) is omitted from this analysis, since the Experimental cirriped and Rotifer-*Artemia* diets could not be sampled due to high mortalities. Feeding regimes: Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Industry standard (Rot-*Art*) and Copepod-cirripeds (Cop-cir).

Period (DPH)	**Feed type**	**ExpCir** **Mean DWI ± SD (%)**	**Cop-*Art*** **Mean DWI ± SD (%)**	**Rot-*Art*** **Mean DWI ± SD (%)**	**Cop-cir** **Mean DWI ± SD (%)**
4-18	First live feed	-0.6 ± 0.7	6.2 ± 1.1	4.5 ± 0.3	3.0 ± 1.0
18-32	Second live feed	17.3 ± 1.0	16.0 ± 3.0	17.7 ± 1.6	15.4 ± 1.4
32-42	Formulated dry feed	8.0 ± 0.4	8.3 ± 1.5	9.8 ± 2.2	10.4 ± 4.4
4-42	All periods	8.0 ± NA	10.2 ± 0.4	10.9 ± NA	9.4 ± 0.5

NA: Only one tank available to sample at the end of the experiment, no SD calculated.

Appendix 3: Standard length (SL)

Table A3a. Descriptive statistics of larval SL along the experiment. The table compiles the mean SL values ± standard deviation (SD) (mm larva^{-1}) and number of larvae sampled (N), at times 4, 12, 18, 23, 32, 37, 42 and 48 DPH for each feeding regime. Feeding regimes: Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Industry standard (Rot-*Art*) and Copepod-cirripeds (Cop-cir).

	ExpCir		**Cop-*Art***		**Rot-*Art***		**Cop-cir**	
Time (DPH)	**Mean SL ± SD (mm larva^{-1})**	**N**	**Mean SL ± SD (mm larva^{-1})**	**N**	**Mean SL ± SD (mm larva^{-1})**	**N**	**Mean SL ± SD (mm larva^{-1})**	**N**
4	4.32 + 0.39	10	4.49 ± 0.22	10	4.18 ± 0.24	10	4.16 ± 0.27	10
12	4.51 ± 0.37	30	4.62 ± 0.36	30	4.59 ± 0.31	30	4.27 ± 0.48	30
18	4.81 ± 0.31	26	5.26 ± 0.44	30	4.76 ± 0.36	29	4.96 ± 0.44	30
23	5.22 ± 0.49	20	5.98 ± 0.60	27	5.18 ± 0.35	23	6.03 ± 0.56	30
32	6.09 ± 0.54	24	7.19 ± 0.61	28	7.20 ± 0.75	26	6.73 ± 0.56	27
37	6.60 ±0.56	21	7.46 ± 0.90	30	7.61 ± 0.87	19	7.58 ± 0.94	30
42	6.72 ± 1.07	6	8.25 ± 0.66	30	8.50 ± 0.58	5	7.98 ± 0.87	30
48	NA	NA	8.72 ± 0.95	29	NA	NA	9.16 ± 1.37	29

NA: no availability of samples for that time point and feeding regime.

Table A3b. Statistical tests applied for analysis of each time point and feeding regime. The tests were selected according to the criteria indicated in Section 2.4.

Time (DPH)	**Statistical test**
4	Ordinary One-way ANOVA + Tukey's multiple comparisons test
12	Kruskal-Wallis test (one-way ANOVA on ranks) + Dunn's multiple comparisons test
18	Ordinary One-way ANOVA + Tukey's multiple comparisons test
23	Ordinary One-way ANOVA + Tukey's multiple comparisons test
32	Ordinary One-way ANOVA + Tukey's multiple comparisons test
37	Kruskal-Wallis test (one-way ANOVA on ranks) + Dunn's multiple comparisons test
42	Kruskal-Wallis test (one-way ANOVA on ranks) + Dunn's multiple comparisons test
48	Unpaired Mann-Whitney test

Appendix 4: Comparison of equations to describe the relationship between SL and DW

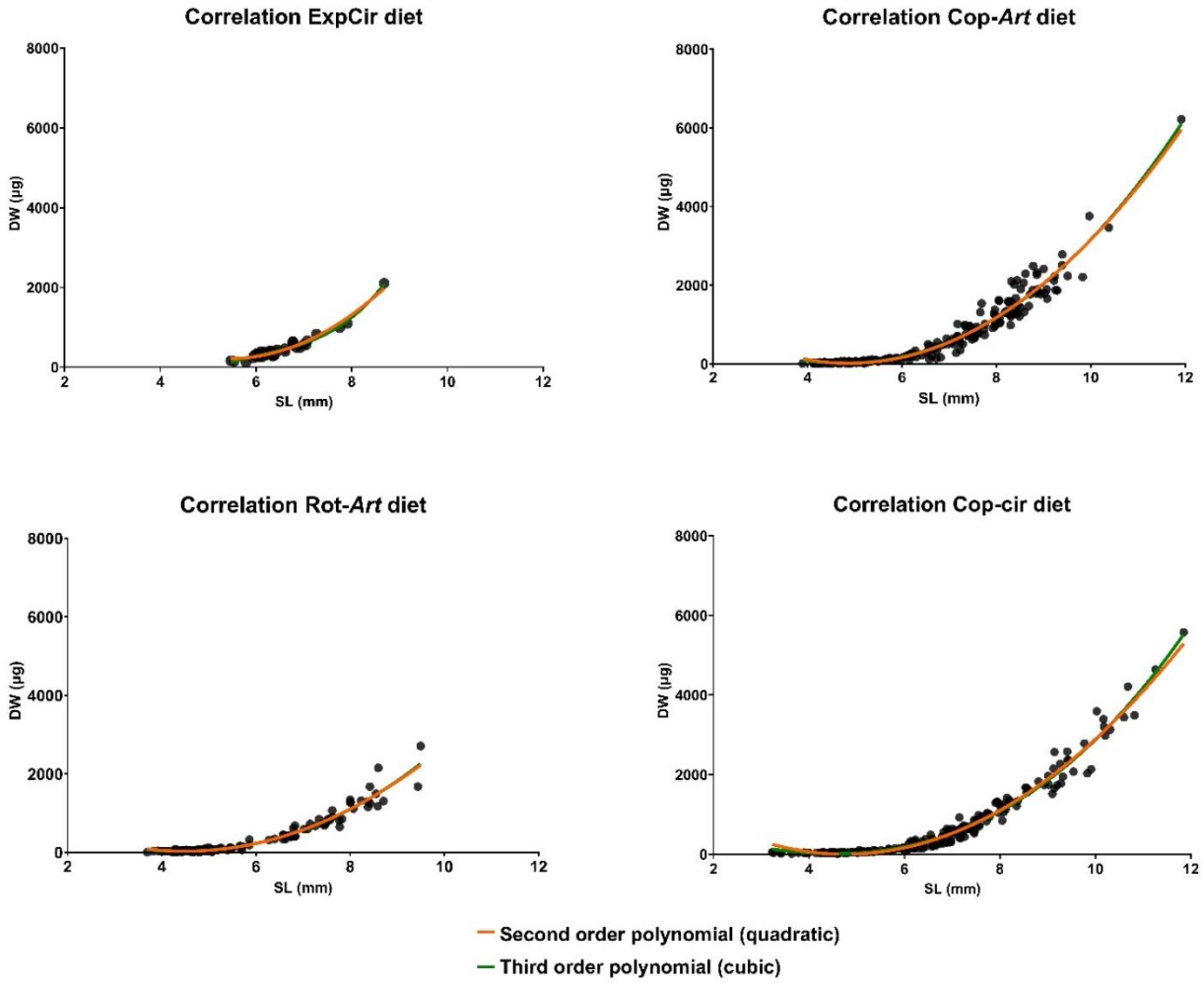


Figure A4. Comparison of fits of different equations to describe the relationship between SL and DW. Fits for a second order polynomial (quadratic) equation (orange color) and a third order polynomial (cubic) equation (green color). Each panel corresponds to one of the four feeding regimes.

Appendix 5: Fatty acid composition of the feed and larvae along the first feeding experiment

Table A5a. Fatty acid composition of the different feeds. Fatty acids are expressed as mean % of total fatty acids (3 biological replicates, corresponding to 3 tanks) ± standard deviation. Total lipids (last row) are given as % (w/w) of dry weight. Feeds: Art (*Artemia*); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers).

Diet	CirS	CopS	CopL	Rot	CirL	Art	DryFeed
C14:0 (Myristic acid)	2.53±0.22	2.67±0.18	1.9±0.39	2.67±0.56	1.55±0.13	1.45±0.22	2.48±0
C14:1n5 (Myristoleic acid)	0.06±0.01	0.03±0.01	0.01±0.03	0.02±0	0.05±0.01	0±0	0.06±0
C15:0 (Pentadecanoic Acid)	0.35±0.18	0.19±0.19	0.18±0.14	0.41±0.03	0.27±0.13	0.12±0.02	0.08±0
C16:0 (Palmitic acid)	14.87±0.33	12.79±0.1	12.23±1.75	18.7±4.52	14.32±0.85	16.95±0.32	18.9±0
C16:1n5 (Hexadecenoic acid)	0.11±0.13	0.09±0.13	0±0	0±0	0.04±0.08	0.26±0.01	0.33±0
C16:1n7 (Palmitoleic acid)	6.15±0.14	1.3±0.19	1.25±1.52	8.01±3.37	3.75±0.31	5.91±0.46	3.34±0
C16:1n9 (Hypogeic acid)	0.26±0.07	0.26±0.12	0.38±0.2	0.57±0.14	0.16±0.11	0.21±0.05	0.36±0
C17:0 (Heptadecanoic acid)	0.48±0.11	1.26±1.42	1.42±1.22	0.52±0.14	0.52±0.15	1.24±0.08	0.33±0
C17:1n7 (Heptadecenoic acid)	0.14±0.16	0.13±0.18	0.15±0.13	0.71±0.2	0.24±0.16	0.2±0.02	0.17±0
C18:0 (Stearic acid)	4.17±0.34	4.04±0.65	4.05±0.43	3.41±0.63	3.52±0.47	5.09±0.32	3.62±0
C18:1n7 (Vaccenic Acid)	7.66±0.22	3.04±0.29	3.9±3.61	3±0.73	8.93±0.52	8.02±0.63	3.38±0
C18:1n9 (Oleic acid)	8.51±0.2	0.83±0.16	2.11±2.6	12.71±4.08	5.95±0.32	13.52±0.46	14.43±0
C18:2n6 (Linoleic acid, LA)	1.52±0.07	11.94±2.03	6.89±4.81	4.14±0.47	0.88±0.07	5.46±0.18	26.85±0
C18:3n3 (Alpha linolenic acid, ALA)	0.62±0.02	18.86±4.56	7.03±4.35	1.48±0.55	0.65±0.04	4.91±0.4	3.24±0
C18:3n6 (Gamma linolenic acid)	0.17±0.14	0.03±0.05	0.35±0.31	0.19±0.02	0.16±0.05	0.33±0.04	0.09±0
C18:4n3 (Stearidonic acid)	1.05±0.7	2.53±3.46	1.97±1.73	0.28±0.02	0.87±0.58	0.02±0.03	0±0
C20:0 (Arachidic acid)	0.14±0.01	0.7±0.14	0.17±0.03	0.17±0.02	0.12±0.01	0.19±0.01	0.35±0
C20:1n7 (Eicosenoic Acid)	1.35±0.9	0.1±0.14	0.69±1.15	0.64±0.21	1.68±1.13	0±0	0±0
C20:1n9 (Gondoic acid)	4.03±0.13	0.16±0.02	0.85±1.34	2.96±0.74	2.66±0.13	0.44±0.09	2.13±0

Diet	CirS	CopS	CopL	Rot	CirL	Art	DryFeed
C20:2n6 (Eicosadienoic acid)	0.54±0.03	3.6±0.61	1.5±0.6	0.6±0.1	0.61±0.03	0.15±0	0.26±0
C20:3n3 (Eicosatrienoic acid)	0.14±0.01	0.84±0.21	0.29±0.09	0.45±0.15	0.19±0.01	0.18±0	0.16±0
C20:4n3 (Eicosatetraenoic acid)	0.35±0.23	4.37±5.42	2.71±4.71	1.56±0.37	0.27±0.18	0±0	0±0
C20:4n6 (Arachidonic acid, ARA)	1.16±0.04	2.53±0.18	1.56±0.3	2.29±0.47	1.22±0.04	4.23±1.93	0.99±0
C20:5n3 (Eicosapentaenoic acid, EPA)	27.37±1.39	7.87±4.61	16.73±9.45	4.17±0.92	31.26±1.67	12.55±1.03	6.97±0
C22:0 (Behenic acid)	0.1±0.01	0.61±0.02	0.37±0.16	0.13±0.01	0.1±0.01	0.36±0.06	0.26±0
C22:1n9 (Erucic acid)	0.23±0.01	0.13±0.14	0.09±0.08	0.35±0.07	0.17±0.01	0.03±0	0.07±0
C22:5n3 (Docosapentaenoic acid)	0.62±0.22	3.83±4.37	0.46±0.33	1.18±0.24	0.35±0.23	0±0	0±0
C22:5n6 (Docosapentaenoic acid, DPA)	0.21±0.14	0.62±0.8	0.78±0.76	8.97±2.53	0.18±0.12	0.03±0.02	0±0
C22:6n3 (Docosahexaenoic acid, DHA)	14.68±0.69	14.39±6.27	29.6±7.69	19.09±4.84	18.95±0.61	18.08±0.31	10.8±0
C24:1 (Nervonic acid)	0.42±0.03	0.24±0.02	0.36±0.04	0.62±0.08	0.37±0.08	0.08±0.01	0.35±0
DHA/EPA	0.54±0.01	1.93±0.33	2.55±2	5±2.53	0.61±0.02	1.45±0.09	1.55±0
EPA/ARA	23.67±1.12	3.19±2.06	11.94±8.84	1.88±0.62	25.54±1.28	3.57±2.04	7.02±0
∑ Saturated fatty acids	22.64±0.98	22.27±2.01	20.32±1.67	26.01±4.21	20.41±1.39	25.4±0.82	26.02±0
∑ Unsaturated fatty acids	77.36±0.98	77.73±2.01	79.68±1.67	73.99±4.21	79.59±1.39	74.6±0.82	73.98±0
∑ n-3 fatty acids	44.83±1.09	52.7±0.21	58.8±5.88	28.2±2.89	52.54±2.22	35.73±1.14	21.17±0
∑ n-6 fatty acids	3.6±0.11	18.73±2.08	11.08±5.67	16.19±2.32	3.05±0.06	10.21±1.82	28.19±0
Total lipids (% of dry weight)	12.61±1.2	11.69±3.35	9.15±0.74	16.25±1.72	9.56±0.95	21.28±1.82	16.41±1

Table A5b. Fatty acid composition of the larvae fed different start feeding diets, at different time points (DPH). Fatty acids are expressed as mean % of total fatty acids (3 biological replicates, corresponding to 3 tanks) ± standard deviation. Total lipids (last row) are given as % (w/w) of dry weight. Feeds: Art (*Artemia*); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers).

Diet	**ExpCir**					**Cop-*Art***					**Rot-*Art***					**Cop-cir**				
Time (DPH)	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**
C14:0 (Myristic acid)	0.73± 0.16	0.58± 0	0.92± 0.18	0.98± 0.17	0.9 8±0	0.62± 0.06	0.51± 0.04	0.78± 0.13	0.77± 0.15	0.73± 0.26	0.56± 0.08	0.58± 0.13	0.84± 0.03	0.75± 0.06	0.75± 0.25	0.55± 0.14	0.81± 0.13	0.76± 0.05	0.96± 0.2	0.91± 0.09
C14:1n5 (Myristoleic acid)	0±0	0±0	0±0	0±0.0 2	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0±0	0.01± 0.02	0±0	0±0	0.02± 0.01	0±0.0 1	0.02± 0.02	0.01± 0.01
C15:0 (Pentadecanoic Acid)	0.34± 0.04	0.29± 0	0.26± 0.15	0.31± 0.02	0.3 1±0	0.35± 0.05	0.21± 0	0.21± 0.01	0.25± 0.14	0.22± 0.13	0.28± 0.01	0.3±0 .04	0.38± 0.01	0.26± 0.15	0.31± 0.02	0.29± 0.03	0.43± 0.33	0.17± 0.09	0.23± 0.13	0.22± 0.12
C16:0 (Palmitic acid)	19.38 ±0.55	17.46 ±0.34	18.31 ±0.46	18.03 ±0.68	18. 03± 0	19.52 ±0.8	17.19 ±0.08	18.54 ±0.42	14.76 ±0.25	17.84 ±1.43	18.71 ±0.41	17.13 ±0.55	18.99 ±0.01	14.45 ±0.61	17.67 ±0.71	18.9± 1.11	17.82 ±0.39	18.7± 0.39	19.72 ±1.34	18.16 ±0.45
C16:1n5 (Hexadecenoic acid)	0.21± 0.19	0±0	0.24± 0.02	0±0.1 1	0±0	0.31± 0.03	0±0	0.13± 0.11	0.06± 0.11	0.08± 0.14	0.2±0 .17	0.08± 0.14	0.22± 0.02	0.15± 0.13	0±0	0.1±0 .17	0.06± 0.1	0.19± 0.01	0.13± 0.12	0.08± 0.14
C16:1n7 (Palmitoleic acid)	1.34± 0.09	1.97± 0.06	2.68± 0.37	1.98± 0.25	1.9 8±0	1.27± 0.02	1.67± 0.14	2.25± 0.12	4.27± 0.11	1.82± 0.03	1.25± 0.04	2.74± 0.16	2.74± 0.11	4.33± 0.06	1.81± 0.15	1.24± 0.08	1.76± 0.14	2.15± 0.06	3.19± 0.45	1.96± 0.05
C16:1n9 (Hypogeic acid)	1.64± 0.2	0.84± 0.02	0.57± 0.21	0.36± 0.32	0.3 6±0	1.51± 0.01	0.74± 0.09	0.48± 0.01	0.45± 0.24	0.39± 0.1	1.54± 0.05	0.98± 0.09	0.76± 0.04	0.47± 0.22	0.46± 0.03	1.52± 0.1	0.75± 0.09	0.39± 0.09	0.4±0 .08	0.31± 0.06
C17:0 (Heptadecanoic acid)	0.53± 0.04	0.55± 0.05	0.55± 0.07	0.41± 0.15	0.4 1±0	0.5±0 .04	1.43± 0.15	1.78± 0.04	1.06± 0.08	0.63± 0.22	0.49± 0.04	0.62± 0.02	0.71± 0.05	1.08± 0.14	0.59± 0.01	0.48± 0.03	1.5±0 .05	1.33± 0.69	0.95± 0.3	0.42± 0.03
C17:1n7 (Heptadecenoic acid)	0.17± 0.17	0.39± 0.04	0.07± 0.11	0.36± 0.01	0.3 6±0	0.26± 0.11	0.43± 0.03	0.35± 0.3	0.78± 0.53	0.44± 0.32	0.23± 0.08	0.39± 0.17	0.46± 0.03	0.83± 0.53	0.51± 0.06	0.12± 0.2	0.35± 0.19	0.4±0 .24	0.41± 0.36	0.24± 0.21

Diet	ExpCir					Cop-Art					Rot-Art					Cop-cir				
Time (DPH)	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**
C18:0 (Stearic acid)	7.53±0.21	10.17±0.32	9.86±0.63	7.22±0.36	7.22±0	8.05±0.52	9.26±0.12	8.54±0.34	7.59±0.45	7.83±1.26	7.58±0.12	9.91±0.15	10.22±0.07	7.38±0.64	7.78±0.37	7.85±0.61	9.2±0.52	8.85±0.49	8.4±0.63	6.8±0.3
C18:1n7 (Vaccenic Acid)	2.31±0.02	4.02±0.1	5.01±0.96	3.12±0.06	3.12±0	2.3±0.03	2.25±0.02	2.36±0.01	8.04±0.7	3.64±0.58	2.33±0.02	2.98±0.04	2.79±0.05	8.81±0.78	3.5±0.17	2.31±0.02	2.27±0.03	2.41±0.15	7.01±1.37	3.22±0.24
C18:1n9 (Oleic acid)	11.34±0.1	10.06±0.24	9.17±1.14	12.29±0.1	12.29±0	11.33±0.33	7.51±0.61	6.06±0.1	13.25±0.88	12.52±0.46	11.52±0.14	11.08±0.1	10.45±0.05	14.07±0.8	12.44±0.28	11.44±0.19	7.28±0.21	6.32±0.15	10.58±0.84	12.77±0.31
C18:2n6 (Linoleic acid, LA)	3.88±0.05	2.08±0.1	2.04±1.2	17.35±0.35	17.35±0	3.83±0.12	4.57±0.35	5.2±0.04	4.48±0.27	14.26±4.93	3.85±0.07	3.02±0.05	3.09±0.03	4.56±0.2	16.07±1.07	3.82±0.14	4.71±0.05	5.41±0.51	2.57±0.9	19.05±0.26
C18:3n3 (Alpha linolenic acid ALA)	0.36±0.08	0.31±0.14	1.07±1.43	1.57±0.12	1.57±0	0.32±0.02	2.85±0.55	2.86±0.06	3.12±0.27	1.44±0.21	0.3±0.01	0.63±0.07	0.49±0.04	3.37±0.06	1.51±0.03	0.3±0.01	3.1±0.11	3.01±0.51	0.93±0.24	1.71±0.05
C18:3n6 (Gamma linolenic acid)	0.36±0.21	0.17±0.02	0.19±0.04	0.91±0.05	0.91±0	0.11±0.1	0.29±0.04	0.32±0.04	0.29±0.05	0.24±0.23	0.25±0.21	0.15±0.01	0.15±0.01	0.29±0.05	0.32±0.28	0.18±0.31	0.33±0.03	0.31±0.05	0.26±0.06	0.37±0.23
C18:4n3 (Stearidonic acid)	0.23±0.03	0.49±0.03	0.39±0.34	0.95±0.03	0.95±0	0.21±0.04	0.97±0.22	0.92±0.01	0.29±0.25	0.33±0.31	0.2±0.01	0.24±0	0.2±0.02	0.33±0.28	0.58±0.04	0.2±0.02	1.13±0.03	0.59±0.53	0.41±0.36	0.56±0.48
C20:0 (Arachidic acid)	0.17±0	0.2±0	0.2±0	0.16±0.01	0.16±0	0.18±0.01	0.21±0.01	0.19±0.01	0.18±0.01	0.24±0.05	0.17±0.01	0.16±0	0.15±0.01	0.18±0.01	0.2±0.01	0.17±0.01	0.22±0.03	0.19±0.01	0.22±0.01	0.2±0.01
C20:1n7 (Eicosenoic Acid)	0.05±0.1	0.63±0.04	0.62±0.57	0.38±0.04	0.38±0	0.15±0	0.18±0.01	0.18±0.02	0.14±0.12	0.17±0.15	0.1±0.09	0.31±0.27	0.5±0.02	0.15±0.13	0.24±0	0.09±0.08	0.13±0.11	0.11±0.1	0.33±0.58	0.23±0.2
C20:1n9 (Gondoic acid)	1.35±0.01	1.79±0.12	1.83±0.64	1.42±0.03	1.42±0	1.33±0.09	0.74±0.1	0.48±0.01	0.57±0.01	1.15±0.16	1.37±0.01	1.61±0.05	1.52±0.03	0.65±0.09	1.18±0.03	1.34±0.03	0.73±0.08	0.46±0.01	1.04±0.46	1.48±0.05
C20:2n6 (Eicosadienoic acid)	0.96±0.03	0.77±0.05	0.74±0.3	0.59±0.13	0.59±0	0.94±0.04	1.42±0.05	1.66±0.01	0.36±0.02	0.54±0.1	0.97±0.01	0.8±0.01	0.65±0.01	0.3±0.04	0.57±0.01	0.97±0.01	1.4±0.01	1.71±0.11	0.81±0.09	0.67±0.04

Diet	ExpCir					Cop-Art					Rot-Art					Cop-cir				
Time (DPH)	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**
C20:3n3 (Eicosatrienoic acid)	0.18±0	0.12±0.02	0.17±0.14	0.22±0.04	0.22±0	0.18±0.01	0.47±0.06	0.62±0.02	0.24±0.02	0.15±0.01	0.17±0.01	0.26±0.01	0.22±0.01	0.18±0.12	0.14±0	0.17±0	0.49±0.01	0.63±0.05	0.29±0.02	0.16±0.01
C20:4n3 (Eicosatetraenoic acid)	0.21±0.02	0.16±0.02	0.12±0.11	0.49±1.41	0.49±0	0.19±0	0.33±0.04	0.33±0.01	1.99±3.03	0.19±0.19	0.2±0.02	0.63±0.04	0.69±0.01	2.74±4.02	0.19±0.01	0.23±0.06	0.36±0	0.22±0.19	2.17±3.15	0.32±0.28
C20:4n6 (Arachidonic acid, ARA)	3.79±0.07	3.83±0.04	3.4±0.25	2.03±0	2.03±0	3.76±0.15	3.22±0.28	2.46±0.05	6.35±0.47	4.71±1.74	3.89±0.03	4.58±0.03	4.8±0.04	6.81±0.41	4.06±0.21	3.89±0.14	3.05±0.04	2.6±0.17	2.48±0.5	1.75±0.11
C20:5n3 (Eicosapentaenoic acid, EPA)	10.29±0.13	11.62±0.47	13.35±1.21	7.56±0.42	7.56±0	10.2±0.27	9.22±0.25	9.03±0.18	9.35±1.95	6.5±0.38	10.33±0.14	5.58±0.11	4.24±0.1	9.8±3.36	6.52±0.3	10.38±0.36	9.23±0.02	8.99±0.77	9.93±2.66	7.88±0.58
C22:0 (Behenic acid)	0.08±0.01	0.07±0.01	0.14±0.07	0.19±0.16	0.19±0	0.09±0.04	0.18±0.03	0.2±0.04	0.4±0.08	0.32±0.07	0.08±0.02	0.09±0.01	0.08±0.03	0.44±0.08	0.31±0.04	0.07±0.04	0.2±0.03	0.22±0.05	0.18±0.01	0.18±0.02
C22:1n9 (Erucic acid)	0.07±0.02	0.07±0.01	0.09±0.02	0.09±0.14	0.09±0	0.05±0.01	0.03±0	0.04±0.02	0.03±0	0.07±0.01	0.05±0.01	0.09±0	0.1±0.01	0.03±0	0.07±0	0.05±0.01	0.05±0.01	0.06±0.03	0.1±0.02	0.08±0
C22:5n3 (Docosapentaenoic acid)	1.62±0.05	1.21±0.01	4.97±6.15	1.09±0.18	1.09±0	1.63±0.04	1.11±0.03	1.03±0.01	2.64±3.4	3.56±4.51	1.68±0.07	1.5±0.03	1.64±0.03	2.92±3.79	1.04±0.04	1.61±0.03	1.11±0.05	4.37±5.81	3.62±4.49	0.66±0.57
C22:5n6 (Docosapentaenoic acid, DPA)	0.45±0.02	0.46±0.03	0.16±0.23	0.79±0.12	0.79±0	0.28±0.25	0.87±0.04	1.17±0.01	3.59±3.11	2.26±2.16	0.44±0.01	4.55±0.12	6.24±0.11	3.65±3.15	2.8±0.15	0.44±0.01	0.89±0.04	0.77±0.63	0.67±0.61	0.57±0.5
C22:6n3 (Docosahexaenoic acid, DHA)	30.01±0.84	29.23±0.4	22.42±4.91	18.74±0.96	18.74±0	30.09±0.48	31.83±0.81	31.48±0.25	14.6±3.41	17.37±4.56	30.79±0.05	28.45±0.28	26.04±0.26	10.91±3.87	18.05±0.09	30.86±0.74	30.32±1.12	28.37±5.63	21.55±2.04	18.69±0.75

Diet	ExpCir					Cop-Art					Rot-Art					Cop-cir				
Time (DPH)	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**	**4**	**12**	**18**	**32**	**48**
C24:1 (Nervonic acid)	0.41± 0.01	0.44± 0.04	0.44± 0.05	0.41± 0.01	0.4 1±0	0.41± 0.05	0.31± 0.02	0.33± 0.02	0.11± 0.01	0.35± 0.02	0.44± 0.02	0.54± 0.01	0.62± 0.08	0.1±0	0.32± 0.03	0.42± 0.02	0.31± 0.03	0.31± 0.03	0.44± 0.06	0.36± 0.01
DHA/EPA	2.92± 0.12	2.52± 0.14	1.68± 0.39	2.48± 0.08	2.4 8±0	2.95± 0.04	3.46± 0.18	3.48± 0.07	1.56± 0.05	2.7±0 .84	2.98± 0.04	5.1±0 .08	6.14± 0.2	1.11± 0.06	2.77± 0.14	2.97± 0.07	3.28± 0.12	3.2±0 .83	2.25± 0.5	2.38± 0.08
EPA/ARA	2.72± 0.08	3.03± 0.15	3.94± 0.41	3.72± 0.23	3.7 2±0	2.71± 0.03	2.88± 0.33	3.68± 0.1	1.49± 0.4	1.51± 0.53	2.65± 0.03	1.22± 0.02	0.88± 0.03	1.46± 0.56	1.6±0 .01	2.67± 0.02	3.03± 0.05	3.46± 0.36	3.97± 0.31	4.51± 0.16
Σ Saturated fatty acids	28.76 ±0.58	29.32 ±0.59	30.24 ±0.32	27.31 ±0.47	27. 31± 0	29.32 ±1.42	28.99 ±0.19	30.24 ±0.47	25.02 ±0.58	27.82 ±0.16	27.88 ±0.62	28.8± 0.73	31.37 ±0.12	24.55 ±1.23	27.61 ±0.68	28.31 ±1.6	30.19 ±0.73	30.22 ±0.83	30.66 ±1.5	26.87 ±0.69
Σ Unsaturated fatty acids	71.24 ±0.58	70.68 ±0.59	69.76 ±0.32	72.69 ±0.47	72. 69± 0	70.68 ±1.42	71.01 ±0.19	69.76 ±0.47	74.98 ±0.58	72.18 ±0.16	72.12 ±0.62	71.2± 0.73	68.63 ±0.12	75.45 ±1.23	72.39 ±0.68	71.69 ±1.6	69.81 ±0.73	69.78 ±0.83	69.34 ±1.5	73.13 ±0.69
Σ n-3 fatty acids	42.9± 0.65	43.14 ±0.28	42.49 ±2.17	30.62 ±0.9	30. 62± 0	42.82 ±0.78	46.78 ±0.28	46.28 ±0.31	32.22 ±1.12	29.56 ±0.5	43.67 ±0.27	37.3± 0.46	33.53 ±0.06	30.25 ±0.47	28.03 ±0.19	43.76 ±1.03	45.74 ±1.02	46.19 ±1.01	38.9± 3.95	29.98 ±0.17
Σ n-6 fatty acids	9.44± 0.12	7.31± 0.12	6.53± 1.21	21.67 ±0.65	21. 67± 0	8.94± 0.4	10.38 ±0.21	10.82 ±0.08	15.06 ±2.41	22±1. 52	9.41± 0.27	13.11 ±0.17	14.93 ±0.12	15.61 ±2.55	23.82 ±1	9.3±0 .5	10.38 ±0.14	10.8± 0.09	6.79± 1.85	22.42 ±0.78
Total lipids (% of dry weight)	20.56 ±3	16.48 ±0.64	17.86 ±1.1	23.04 ±0.91	23. 04± 1	23.84 ±6.65	14.98 ±0.05	15.42 ±0.98	18.8± 0.68	14.94 ±1.26	19.66 ±2.06	14.66 ±1.03	16.47 ±1.97	20.45 ±0.22	15.78 ±2.67	22.18 ±7.03	18.6± 8.89	14.46 ±0.89	15.75 ±0.68	18.7± 1.2

Appendix 6: Principal component analysis (PCA) and hierarchical clustering of fatty acid composition in feed

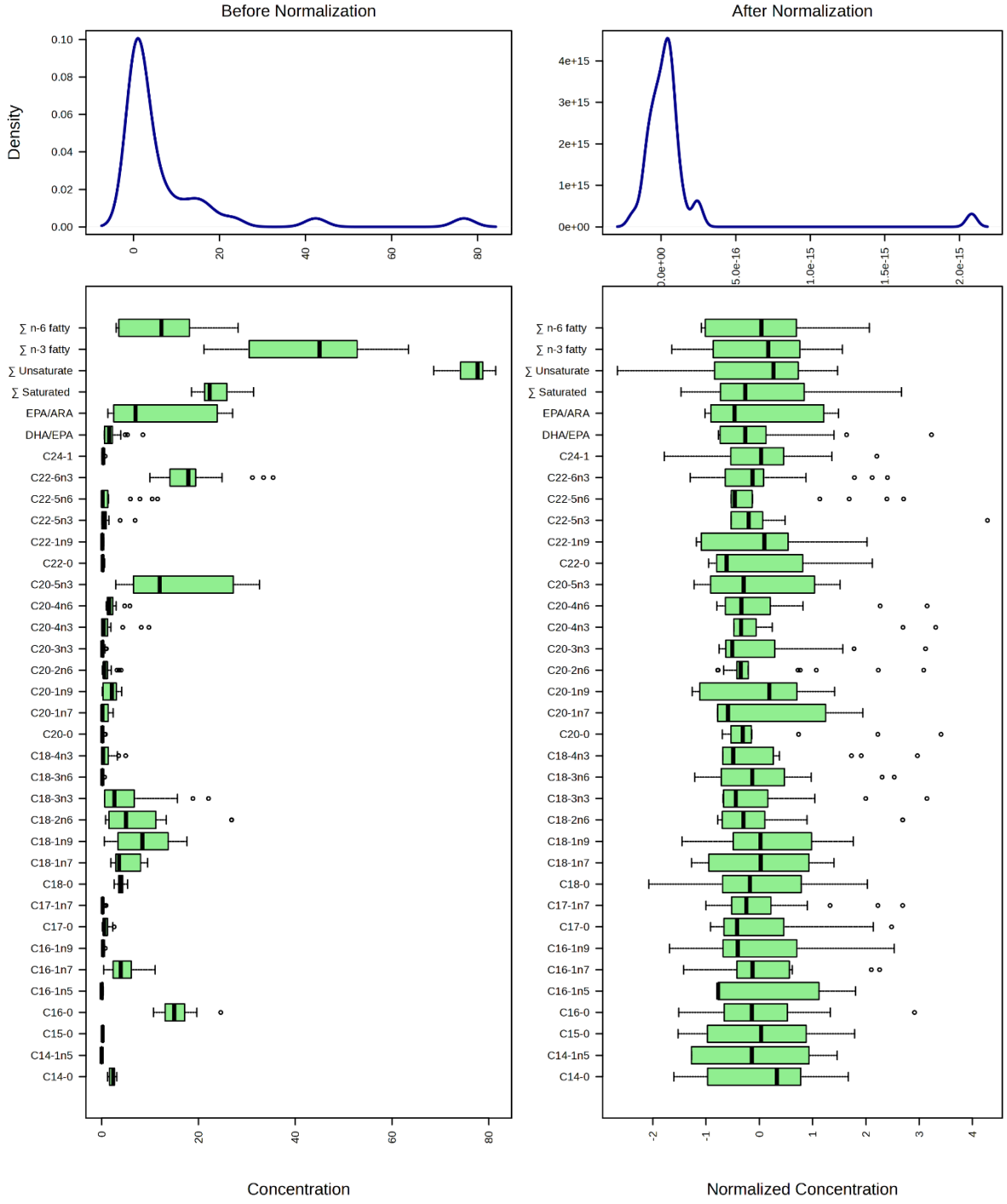


Figure A6a. Normalization of fatty acid composition of the feeds. Normalization performed using "autoscaling" in MetaboAnalyst 6.0 (Pang et al., 2021), corresponding to mean-centering and dividing by the square root of the standard deviation of each variable.

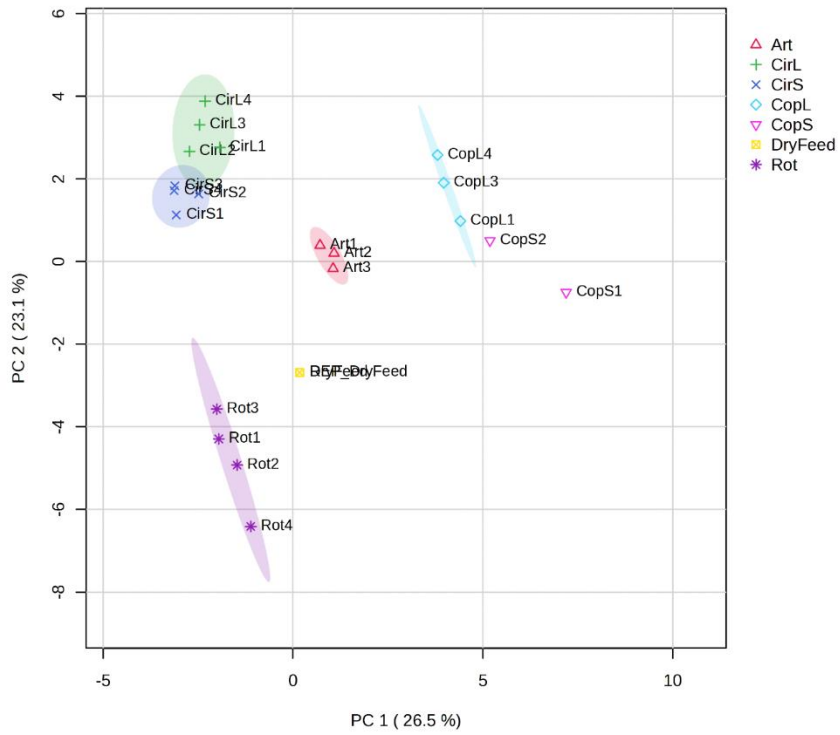


Figure A6b. PCA analysis of fatty acid composition in feeds. 2d-scores plot made using MetaboAnalyst 6.0 (Pang et al., 2021). Groups are indicated by color, and the shading corresponds to the 95% confidence interval for each group, calculated using bootstrap resampling. The explained variances for each principal component (PC) are shown in brackets (A). Feeds: Art (*Artemia*); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers). The number after the abbreviation indicates the replicate number.

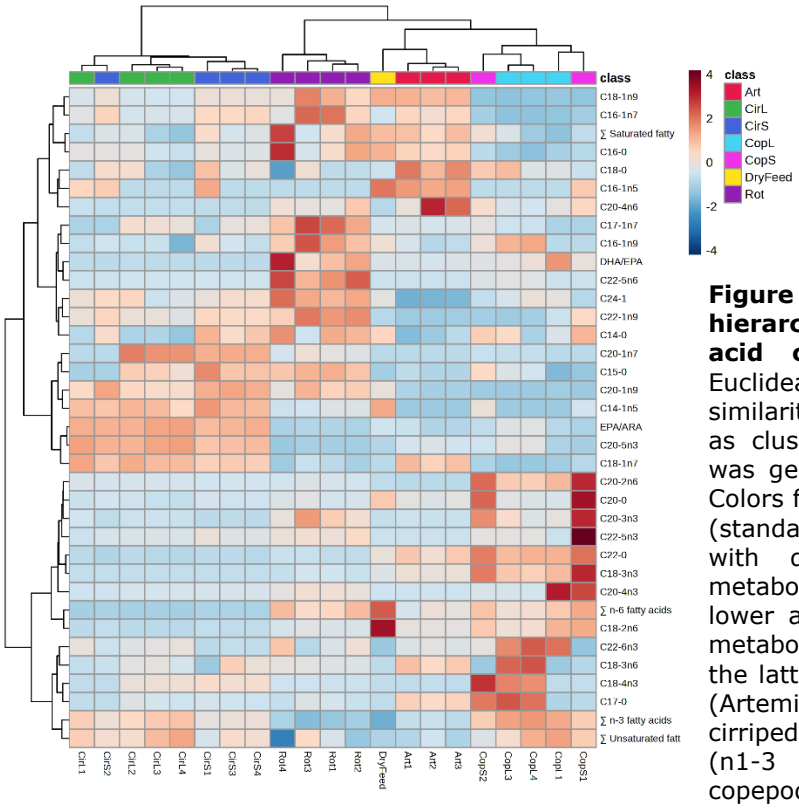


Figure A6c. Heatmap showing hierarchical clustering of the fatty acid composition of the feeds. Euclidean distance was used as similarity measure and Ward's linkage as clustering algorithm. The heatmap was generated using normalized data. Colors from red to blue indicate Z score (standard deviation from the mean), with deeper red reflecting higher metabolite abundance, and deeper blue lower abundance. Rows correspond to metabolites and columns to samples, the latter grouped by type of feed (Art (Artemia); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers), number indicates replicate.

Appendix 7: Principal component analysis (PCA) and hierarchical clustering of fatty acid composition in larvae

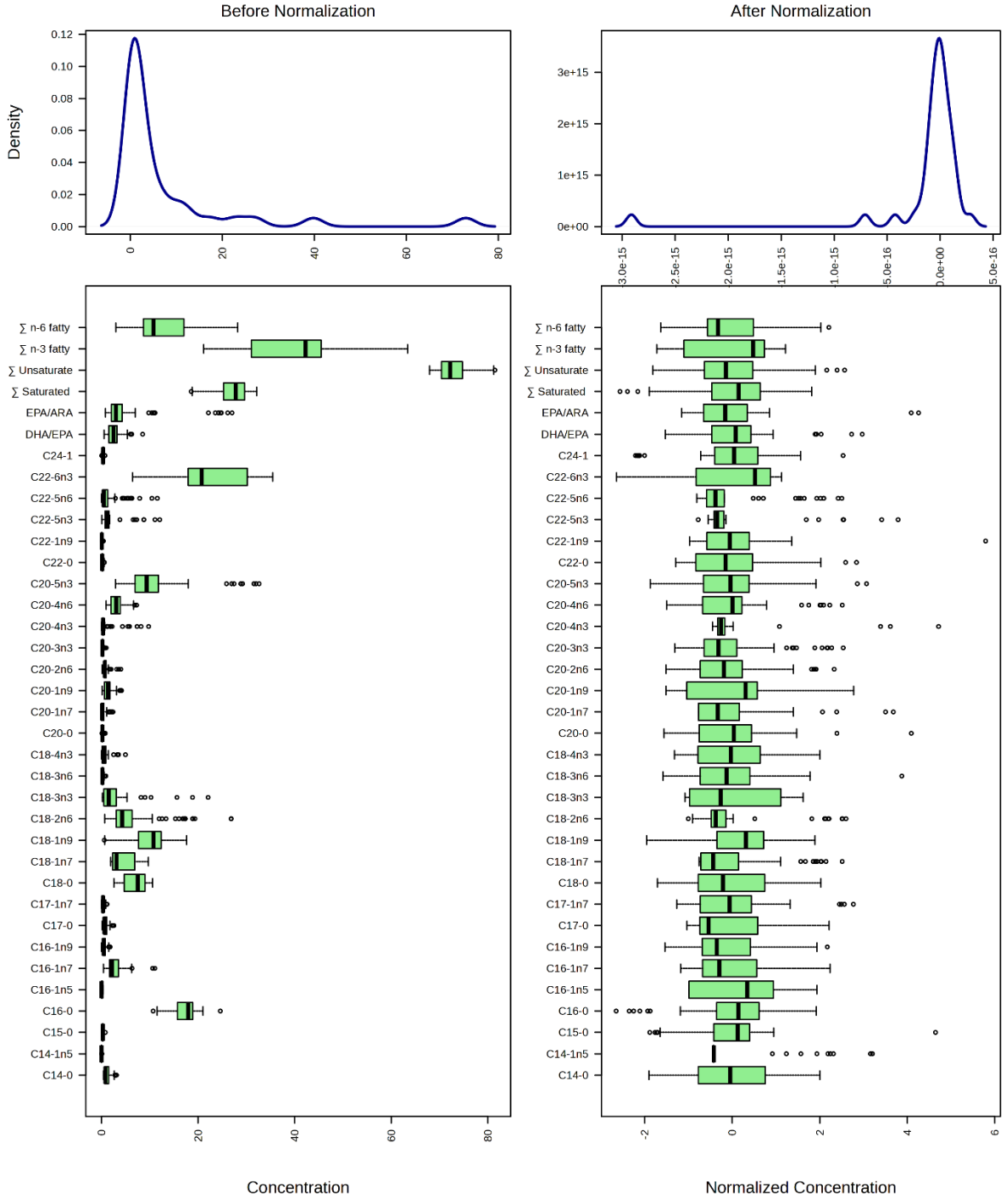


Figure A7a. Normalization of fatty acid composition of the larvae. Normalization performed using "autoscaling" in MetaboAnalyst 6.0 (Pang et al., 2021), corresponding to mean-centering and dividing by the square root of the standard deviation of each variable.

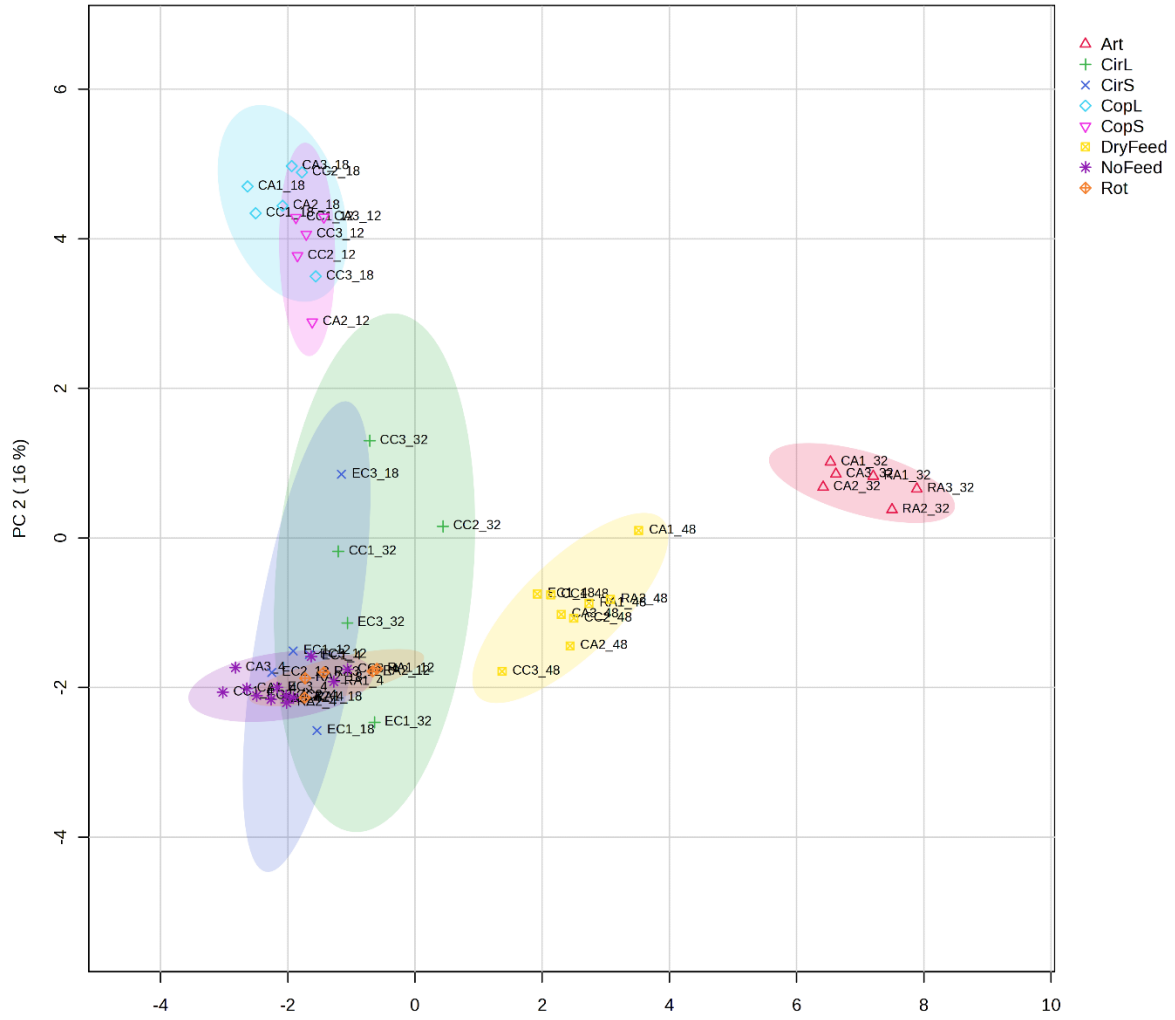


Figure A7b. PCA analysis of fatty acid composition in larvae. 2d-scores plot made using MetaboAnalyst 6.0 (Pang et al., 2021). Groups indicate last feed received, and the shading corresponds to the 95% confidence interval for each group, calculated using bootstrap resampling. The explained variances for each principal component (PC) are shown in brackets (A). Larval groups: EC (Experimental cirriped), CA (Copepod-*Artemia*), RA (Rotifer-*Artemia*), CC (Copepod-cirriped), first number indicates replicate; number after underscore indicates time (DPH)).

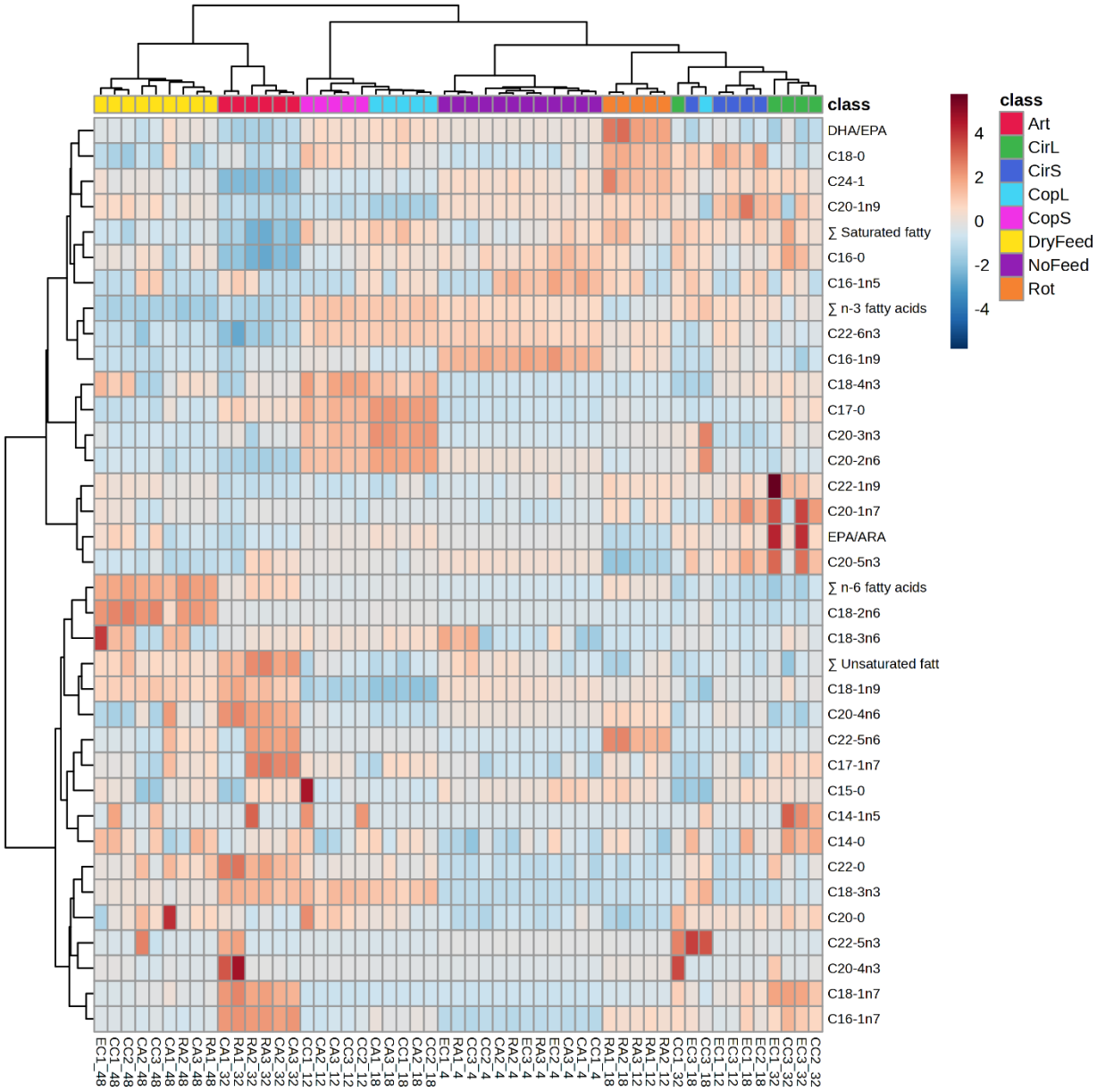


Figure A7c. **Heatmap showing hierarchical clustering of the fatty acid composition of the larvae.** Euclidean distance was used as similarity measure and Ward's linkage as clustering algorithm. The heatmap was generated using normalized data. Colors from red to blue indicate Z score (standard deviation from the mean), with deeper red reflecting higher metabolite abundance, and deeper blue lower abundance. Rows correspond to metabolites and columns to samples, the latter grouped by type of feed. EC (Experimental cirriped), CA (Copepod-Artemia), RA (Rotifer-Artemia), CC (Copepod-cirriped), first number indicates replicate; number after underscore indicates time (DPH).

Appendix 8: Fatty acid composition and total lipids of the larvae

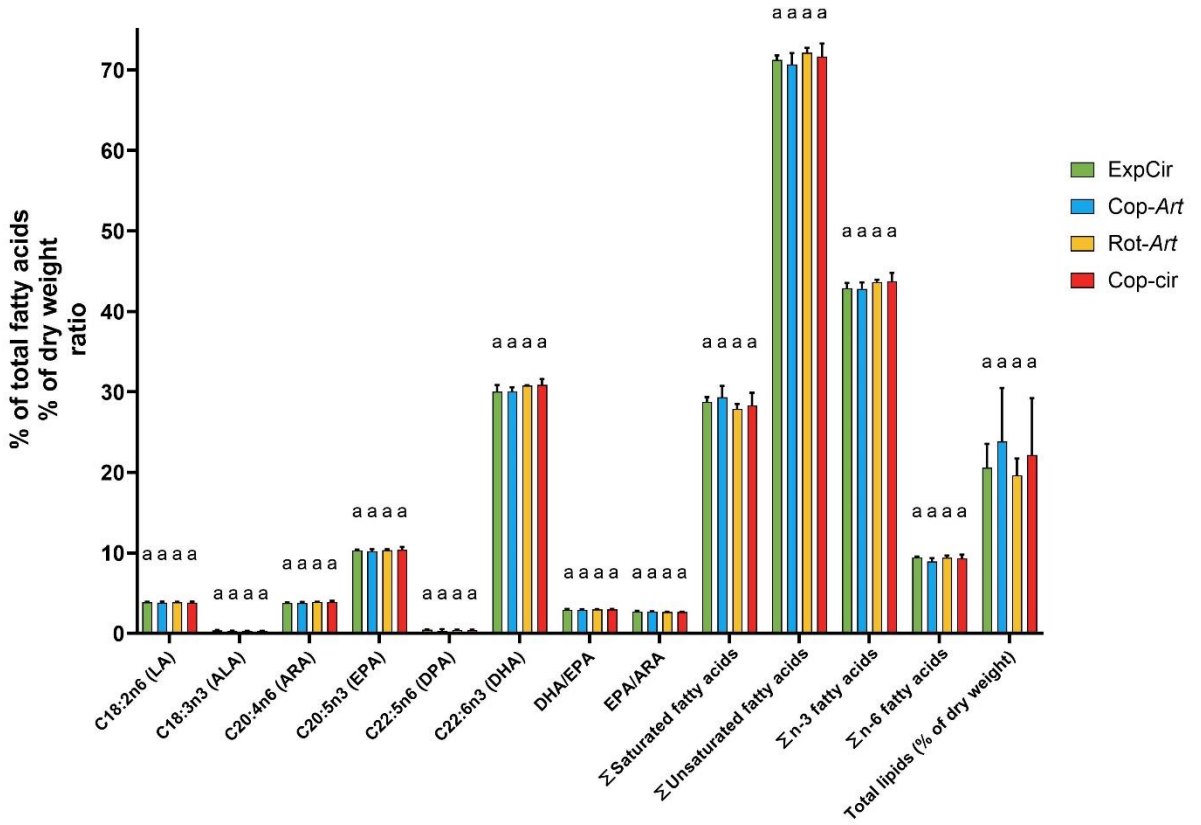


Figure A8. **Selected fatty acids or groups of fatty acids and total lipids of the larvae at 4 DPH.** Means and SD for each 3 tanks are shown. Colors refer to the diet that was supplied (Experimental cirriped (ExpCir), Copepod-*Artemia* (Cop-*Art*), Rotifer-*Artemia* (Rot-*Art*) and Copepod-cirripeds (Cop-cir)). Statistically significant differences ($p<0.05$, one-way ANOVA followed by Tukey's multiple comparisons test) within analytes are denoted by unequal letters above each bar.

Appendix 9: Normalization of lipidomics data prior to PCA, sPLS-DA and hierarchical clustering

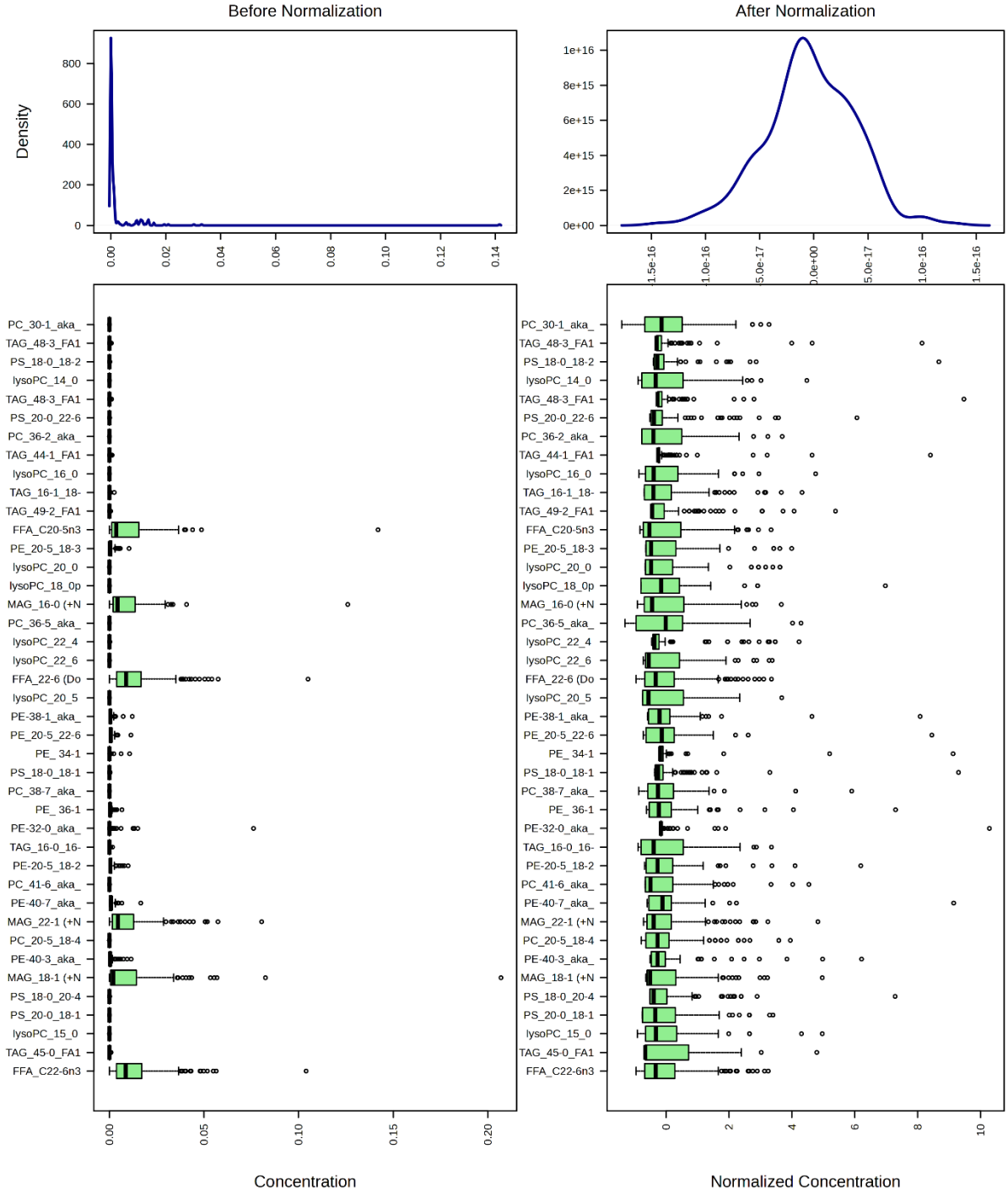


Figure A9a. Normalization of lipidomics data of larvae and feed. Normalization performed using "autoscaling" in MetaboAnalyst 6.0 (Pang et al., 2021), corresponding to mean-centering and dividing by the square root of the standard deviation of each variable.

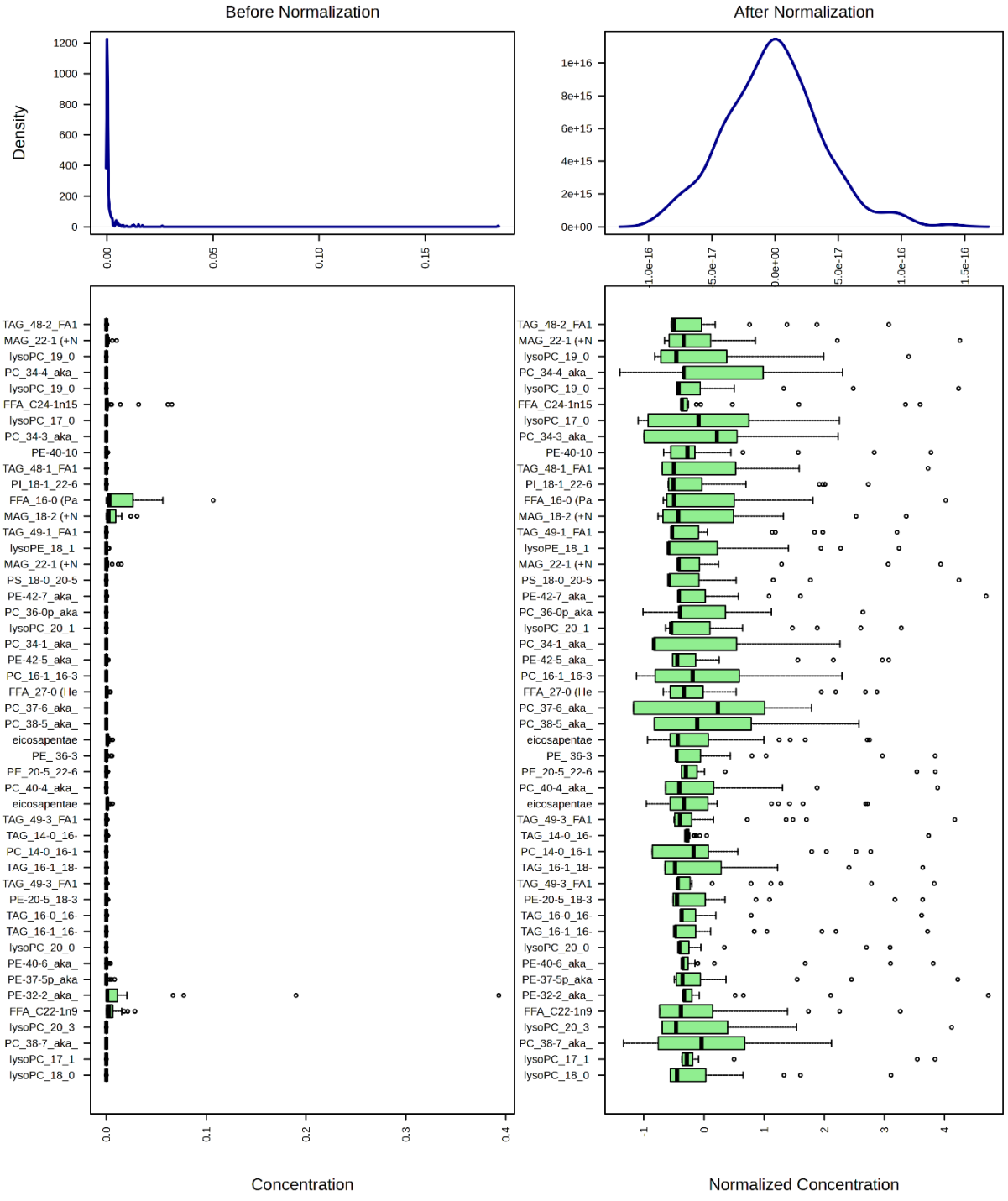


Figure A9b. Normalization of lipidomics data of feed. Normalization performed using "autoscaling" in MetaboAnalyst 6.0 (Pang et al., 2021), corresponding to mean-centering and dividing by the square root of the standard deviation of each variable.

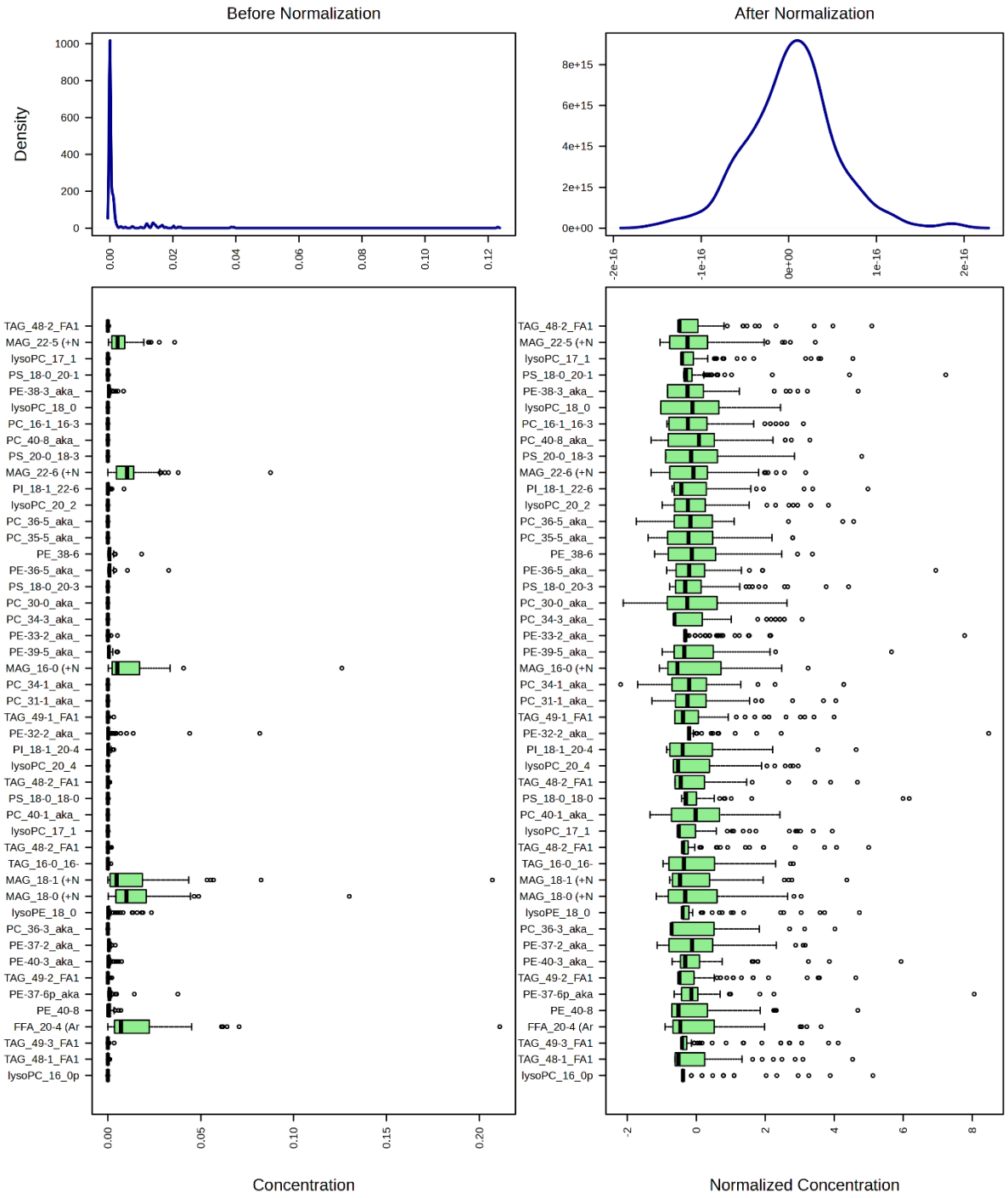


Figure A9c. Normalization of lipidomics data of larvae. Normalization performed using "autoscaling" in MetaboAnalyst 6.0 (Pang et al., 2021), corresponding to mean-centering and dividing by the square root of the standard deviation of each variable.

Appendix 10: Hierarchical clustering of lipidomics data in feed and larvae

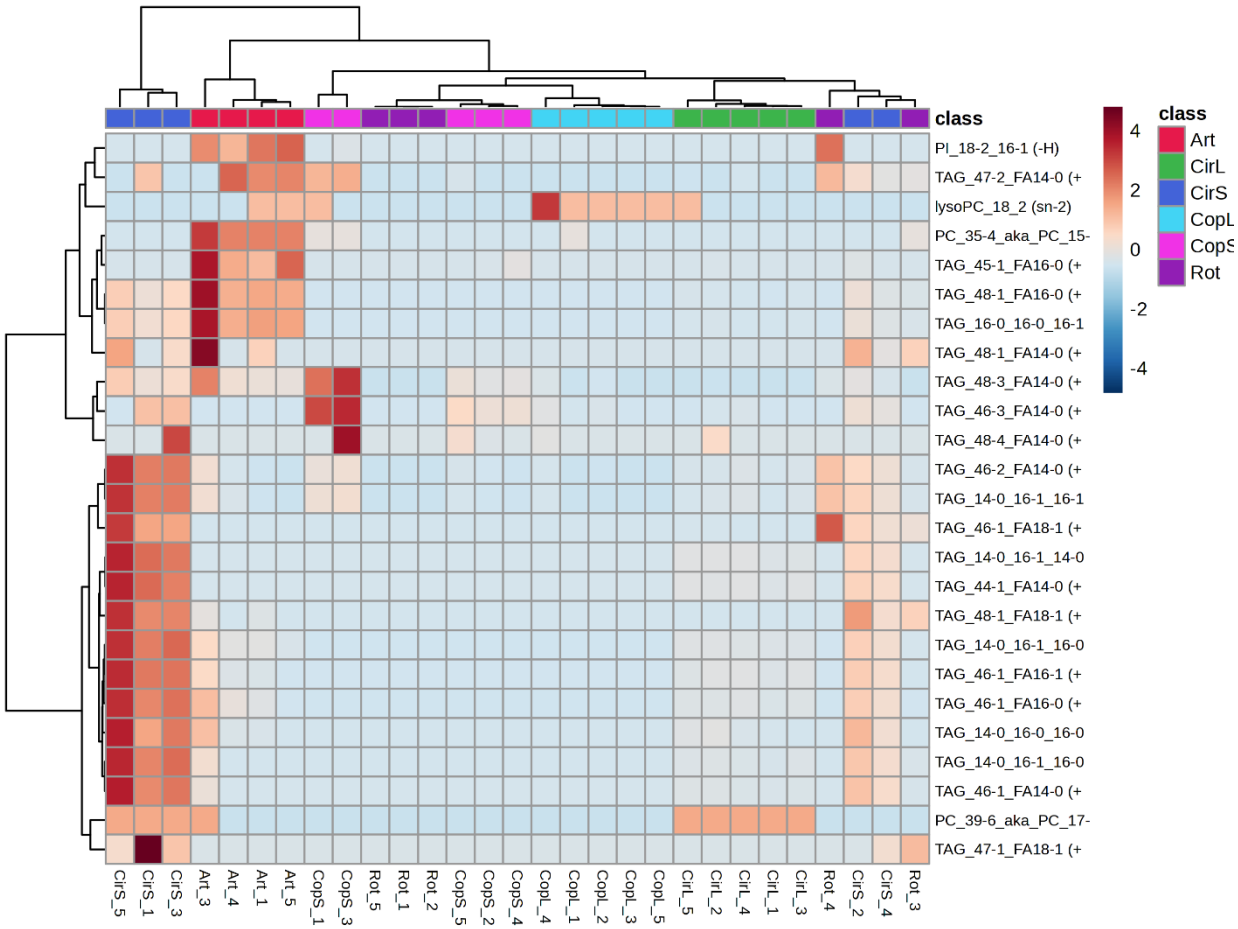


Figure A10a. **Heatmap showing hierarchical clustering of the lipidomics profiles of the feeds.** Euclidean distance was used as similarity measure and Ward's linkage as clustering algorithm. The heatmap was generated using normalized data. Colors from red to blue indicate Z score (standard deviation from the mean), with deeper red reflecting higher metabolite abundance, and deeper blue lower abundance. Rows correspond to metabolites (top 25 features with highest differences according to a t-test or ANOVA are shown) and columns to samples, the latter grouped by type of feed (Art (Artemia); CirS (experimental small cirripeds), CirL (large cirripeds), CopS (n1-3 copepods); CopL (n4-6 copepods), Rot (rotifers), number indicates replicate.

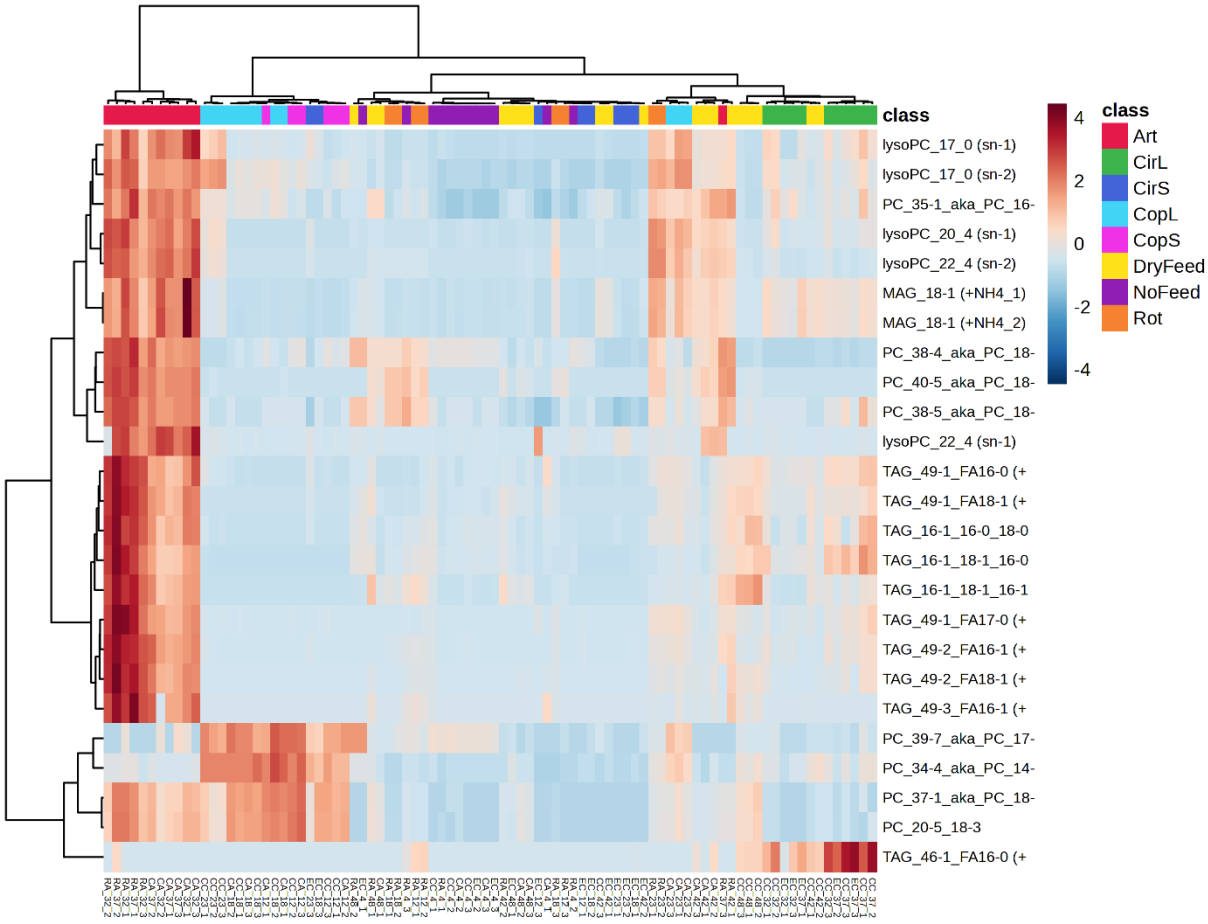
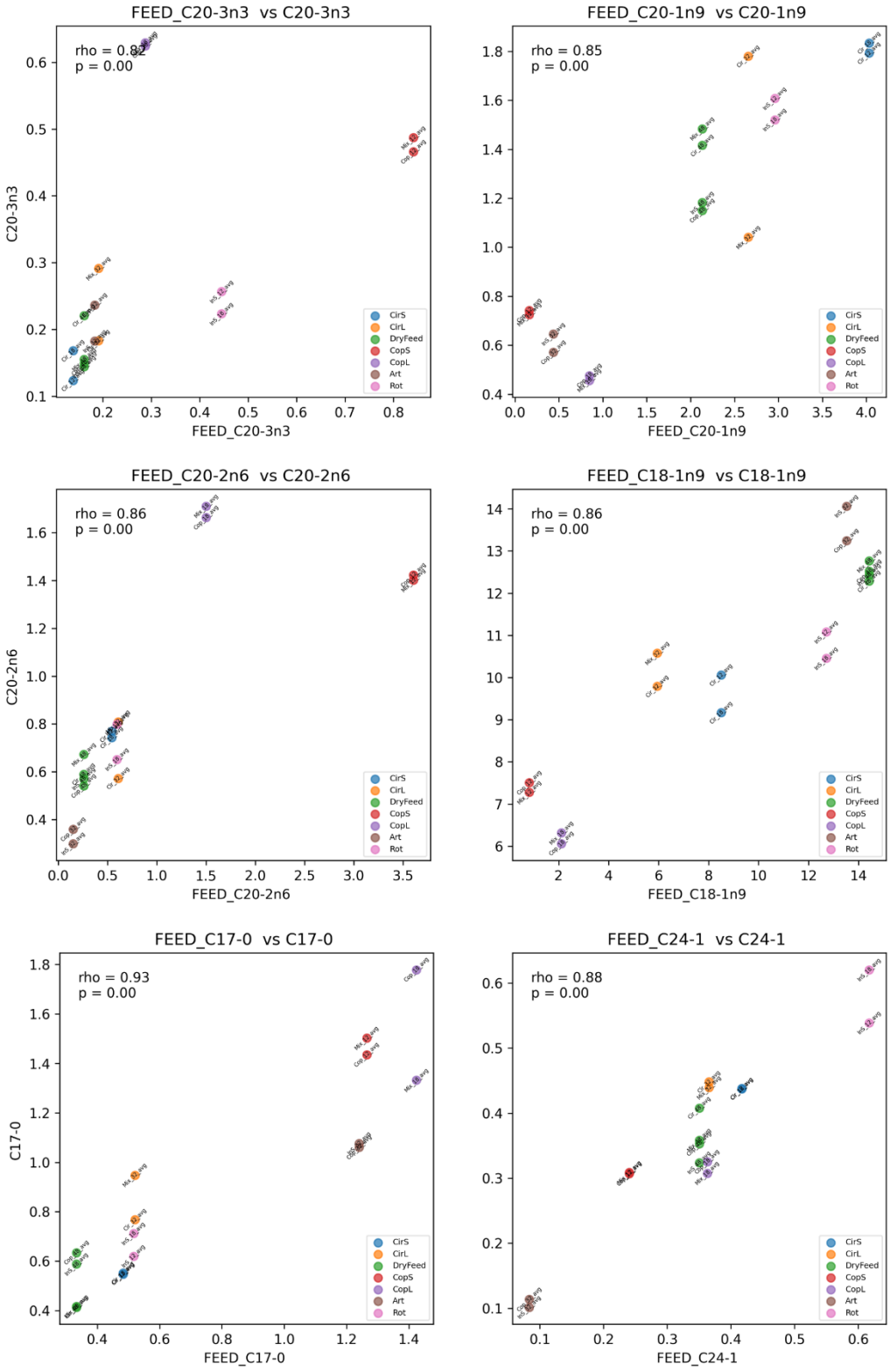


Figure A10b. **Heatmap showing hierarchical clustering of the lipidomics profiles of the larvae along the experiment.** Euclidean distance was used as similarity measure and Ward's linkage as clustering algorithm. The heatmap was generated using normalized data. Colors from red to blue indicate Z score (standard deviation from the mean), with deeper red reflecting higher metabolite abundance, and deeper blue lower abundance. Rows correspond to metabolites (top 25 features with highest differences according to a t-test or ANOVA are shown) and columns to samples, the latter grouped by type of feed. EC (Experimental cirriped), CA (Copepod-*Artemia*), RA (Rotifer-*Artemia*), CC (Copepod-cirriped), first number indicates replicate; number after underscore indicates time (DPH).

Appendix 11: Correlations between selected fatty acids in the larvae and in the last feed they received



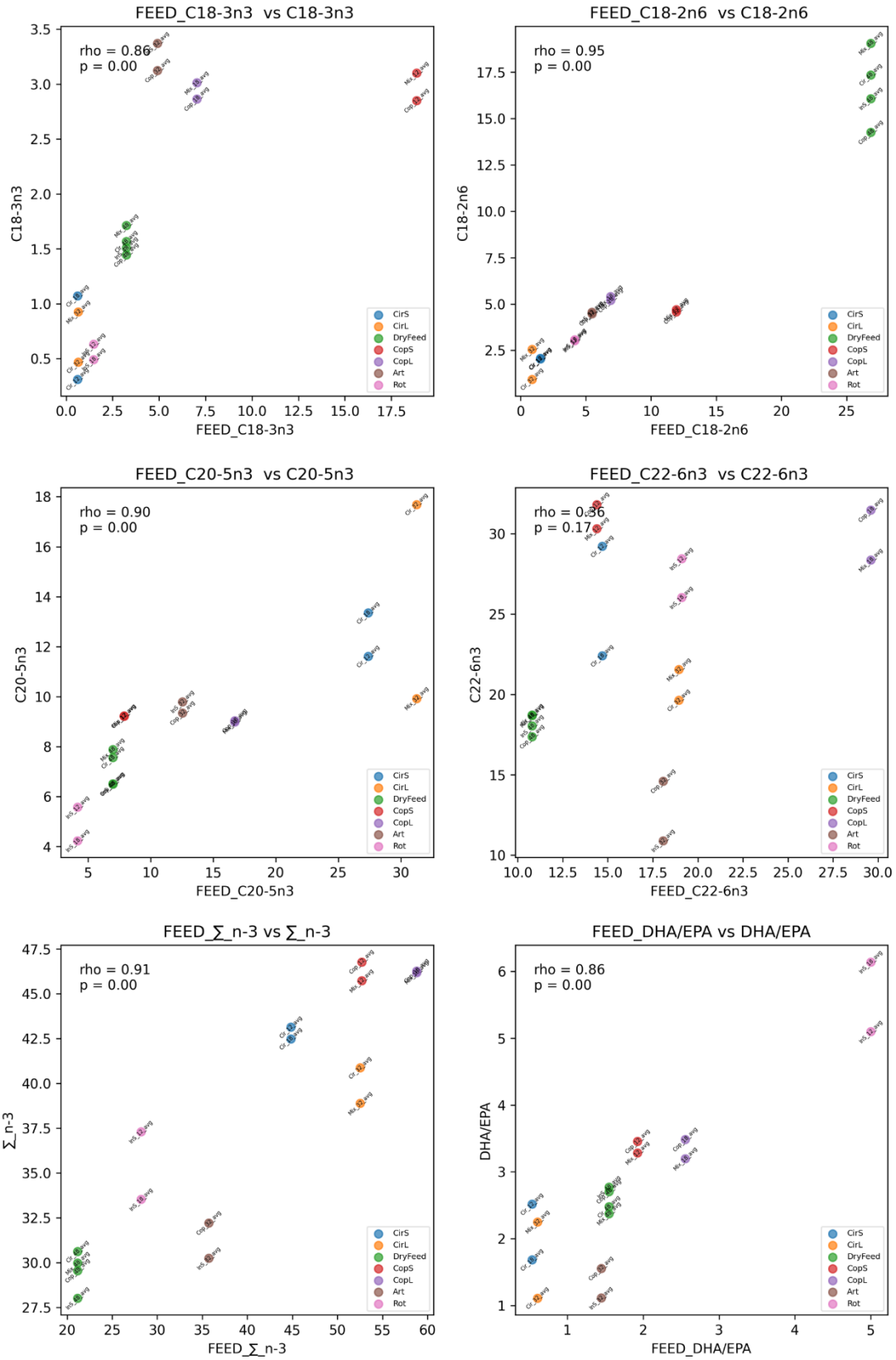
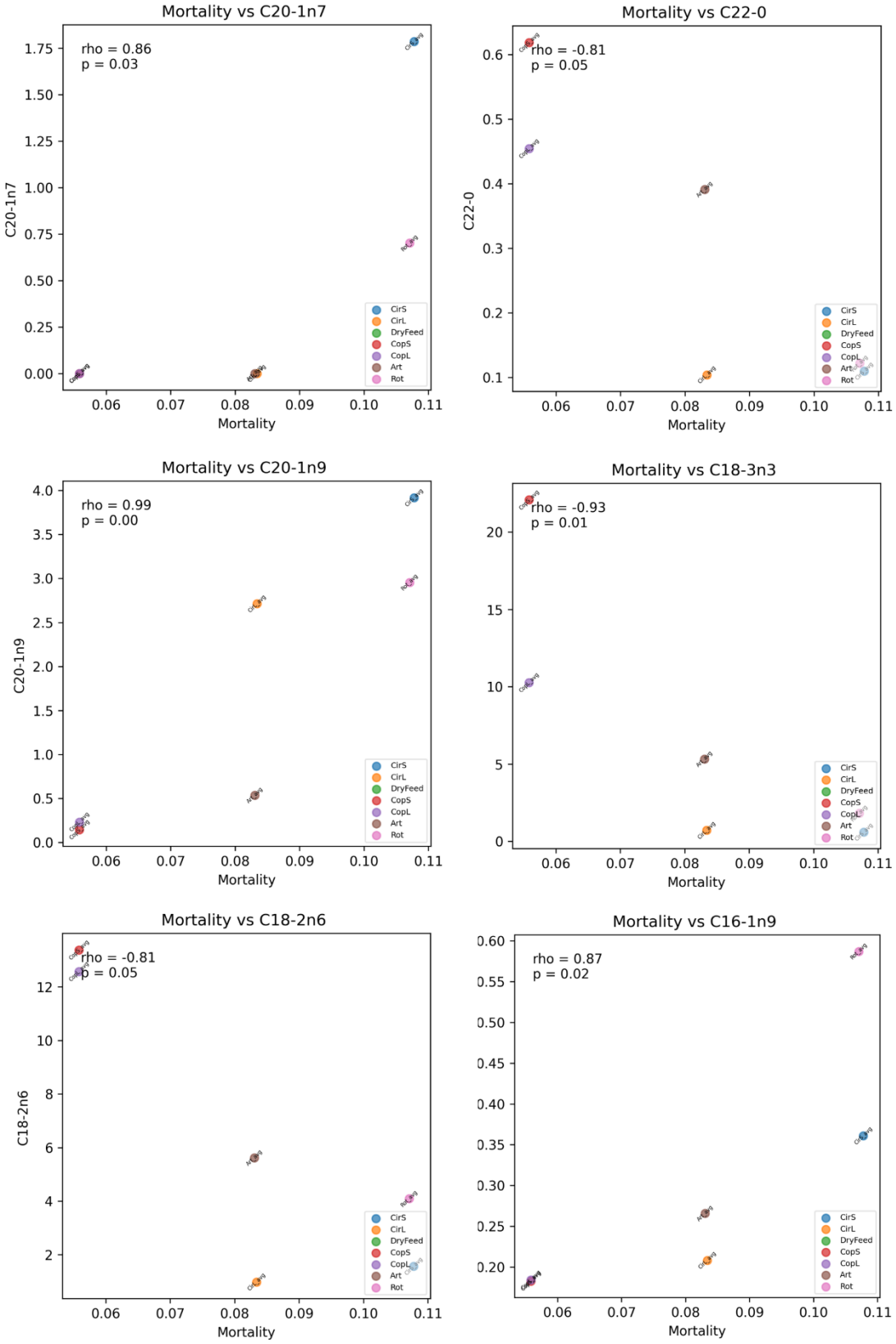


Figure A11. Scatter plots showing correlations between selected fatty acids in the larvae and in the last feed they received. The 11 significant very strong correlations are represented ($p < 0.05$; Spearman's rho coefficient below -0.8 or above 0.8). Moreover, the correlation for C22:6n3 (DHA) is shown to illustrate the differences in larvae receiving *Artemia*. The y axis indicates fatty acid content in the larvae and the x axis in the feed. Spearman's rho coefficients and p values are shown for each correlation in the corresponding panel.

Appendix 12: Correlations between selected fatty acids in the feed and performance indicators in the larvae



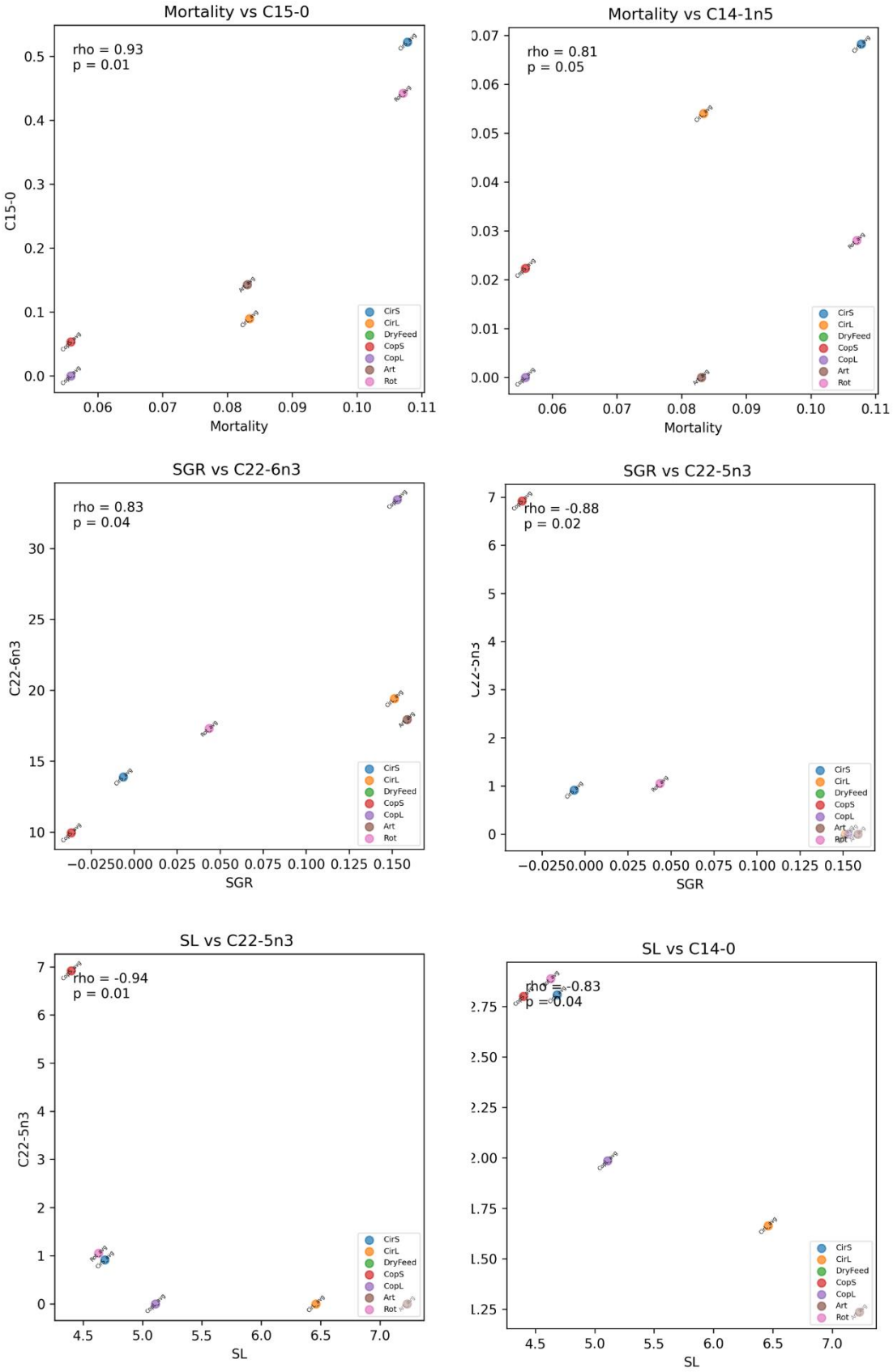
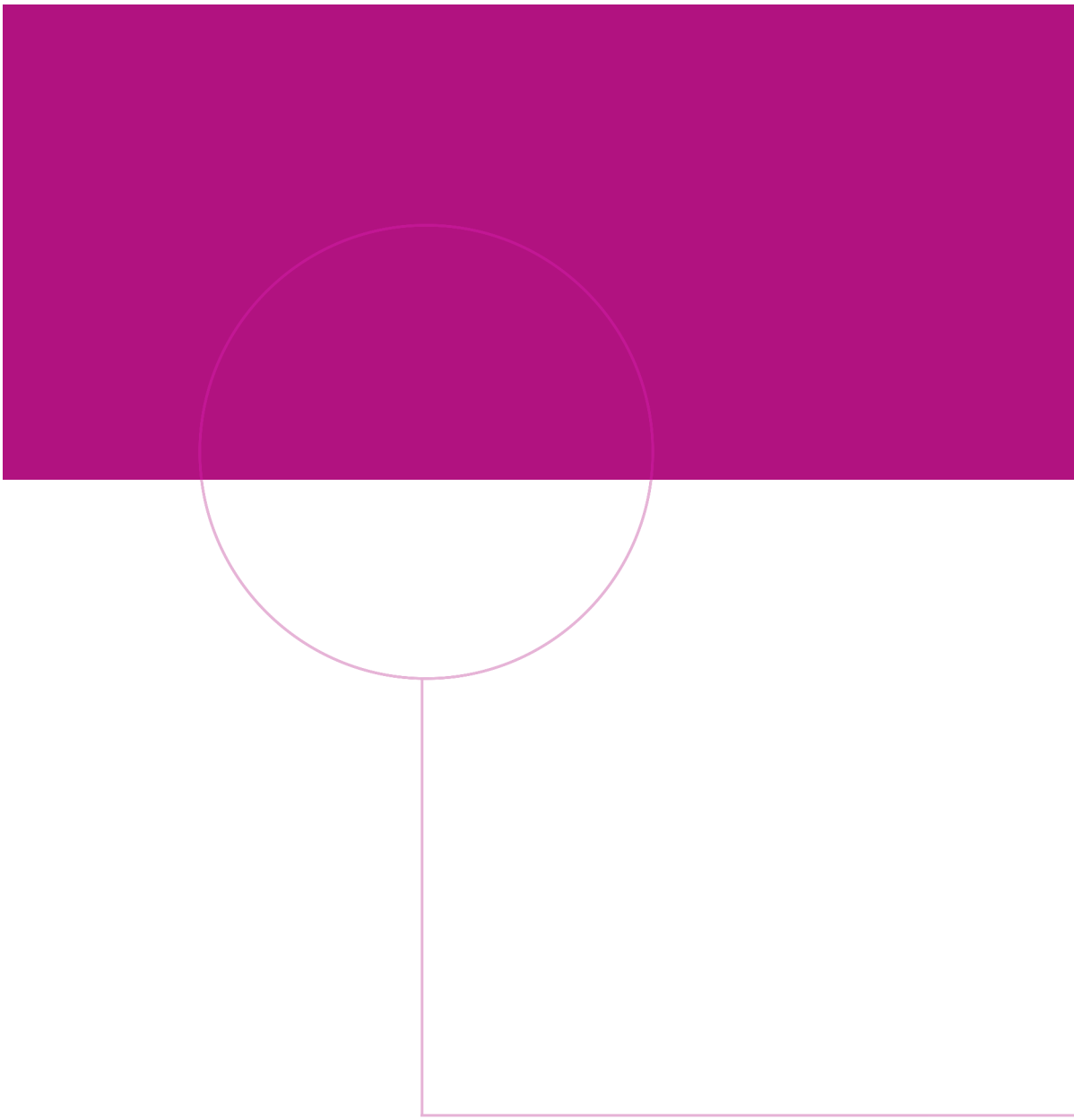


Figure A12. Scatter plots showing correlations between selected fatty acids in the feed and performance indicators in the larvae. Only significant very strong correlations are represented ($p < 0.05$; Spearman's rho coefficient below -0.8 or above 0.8). Spearman's rho coefficients and p values are shown for each correlation in the corresponding panel.



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