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The effect of different live feed on the early growth and development of ballan wrasse (*Labrus bergylta* Ascanius, 1767) larvae and its organs

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Marine Coastal Development
Submission date: August 2012
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Acknowledgements

This Master's thesis was written at the Department of Biology, at the Norwegian University of Science and Technology (NTNU), under guidance of Professor Elin Kjørsvik at the Department of Biology NTNU, Research Manager Gunvor Øie at SINTEF Fisheries and Aquaculture, and Postdoctoral Fellow Per-Arvid Wold at the Department of Hydraulic and Environmental Engineering NTNU. The experimental work was carried out at the NTNU Centre of Fisheries and Aquaculture (Sealab) and in the laboratories of SINTEF Fisheries and Aquaculture Ltd from 24th of June until 23th of August 2011. The experiment was part of the research program "Produksjon av berggylte" (900554) funded by the Fishery and Aquaculture Industry Research Fund (FHF), which is a collaboration between SINTEF, Nofima, the Institute of Marine Research, NTNU and stakeholders in the production of ballan wrasse.

First, I would like to thank Elin Kjørsvik for excellent guidance and encouragement, Gunvor Øie for invaluable help through the planning and execution of the experiment, and Per-Arvid Wold for always being helpful and lending me your binder. Thanks to Werner Storøy, Marthe Schei and Kari Attramadal for your help during the experiment and to Tora Bardal for great guidance and assistance through my laboratory work. Maria Sørøy, Martin Almli and Martin Berg, thanks for cooperating with me during the experimental and statistical work. And thanks to Dag Altin for being a great company and for always having a solution when unforeseen situations occurs at lab outside of working hours.

Thanks to LAKS and all fellow students at Sealab for good talks and laughs through those long lunches (and huge amount of cake); you have contributed to making these two years both fun and memorable! Thanks Maria, for great friendship and support through these last five years spent at NTNU, and for invaluable talks and discussions regarding statistics and life in general over a cup of chilli coffee.

I would also like to express my gratitude towards Mads and Ingvild for ensuring that I have been well fed and groomed, and for keeping me company through many long hours while studying. And at last, an enormous thanks to my family for always being just a phone call away if ever in need of uplifting words and consolation.

Trondheim, August 2012

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Abstract

Salmon lice (*Lepeophtheirus salmonis*) have been an increasing problem for the Norwegian aquaculture industry in recent years, and several chemicals have been used by the industry to get rid of the louse. However, resistance and reduced sensitivity towards a large quantity of these has resulted in a blooming interest for the use of wrasse as a biological method of sea louse control. The ballan wrasse (*Labrus bergylta*) is considered the most promising species for aquaculture, but problems with poor survival, growth and skeletal deformities suggest that a suboptimal first feeding practice may be used.

At present, commercial farmers first feed the ballan wrasse larvae on enriched rotifers from 4 to approximately 30 dph, followed by *Artemia sp.* until weaning on formulated feed is successful. Copepods are considered the natural prey of most marine fish larvae, and usually a greater larval growth, survival and development are observed when they are used as first feed instead of rotifers. This has been attributed to the copepods high fraction of essential fatty acids in their polar lipid fraction, in addition to their great amounts of protein and free amino acids. The present study was conducted to evaluate the effect of using intensively reared copepods (*Acartia tonsa* naupliii) as early live feed for the ballan wrasse larvae on the larval growth and survival, and early organ growth and development, compared to using rotifers (*Brachionus ibericus*). Four different feeding regimes were used, varying in the live feed provided during the first 30 days. Larvae from the “Copepod”-treatment were fed exclusively with *A. tonsa* during this period. Larvae from the “Cop7”-treatment were fed *A. tonsa* from 4 to 10 dph, with a transition to enriched rotifers. Fish larvae from the “RotMG”-treatment were fed enriched rotifers the whole period, while the “RotChl”-treatment had a diet consisting of unenriched rotifers. All treatments had a transition to *Artemia* from 24-30 dph, and were weaned to formulated feed from 40-50 dph.

Results from the present study indicated that intensively reared *A. tonsa* was more suitable as early live feed for ballan wrasse larvae compared to enriched or unenriched rotifers. Increased growth rates were obtained while feeding the larvae with copepods, and it resulted in larvae with significantly higher dry weight at the end of the experimental period (61 dph). No difference in larval growth was observed when feeding with enriched or unenriched rotifers, however larvae fed unenriched rotifers had a significantly lower survival than larvae from all other treatments. Higher organ volume growth rates were observed when copepods were used as feed, and the organ volumes were found to relate to the larval standard length. At 21 dph, the Copepod larvae had a significantly higher proportion of musculature than larvae from the other treatments, and the intestine appeared to be more developed and mature.

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Abbreviations

ARA	Arachidonic acid (20:4*n-6*)
CNS	Umbrella term for the nervous tissue of brain and spinal cord
Cop7 larvae	Larvae fed *Acartia tonsa* from 4-10 dph with a transition to enriched rotifers up to 30 dph
Copepod larvae	Larvae fed *Acartia tonsa* as first feed up to 30 dph
DHA	Docosahexaenoic acid (22:6*n-3*)
Dph	Days post hatch
DW	Dry weight (mg/larvae)
DWI	Daily weight increase
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid (20:5*n-3*)
FAA	Free amino acids, not bound to proteins
g	Allometric growth coefficient
HE staining	Hematoxylin and eosin tissue stain, colours basic structures red or pink and acidic structures purplish blue
MH	Myotome height, measured perpendicular to the axial skeleton right behind the anus
PFA	Paraformaldehyde, fixative
PL	Phospholipid
PUFA	Polyunsaturated fatty acid, fatty acid with two or more double bonds
RotChl larvae	Larvae fed unenriched rotifers as first feed up to 30 dph
RotMG larvae	Larvae fed enriched rotifers as first feed up to 30 dph
RV	Relative volume, % of total tissue volume
SGR	Specific growth rate
SGR_T	Specific growth rate of tissue volume
SL	Standard length, measured from the tip of the upper lip to the end of the vertebrae
TAG	Triacylglycerid
V_T	Tissue volume

1. Introduction

1.1 Ballan wrasse in aquaculture

Salmon lice (*Lepeophtheirus salmonis*) have been an increasing problem for the Norwegian aquaculture industry in recent years. Costello (2009) reported that sea lice control had an estimated cost of €131 million for Norway's salmonid farming industry in 2008. Also other countries have recorded financial loss caused by sea lice infestation, and in 2008 a total loss of €305 million was estimated for several countries (including Norway) where sea lice were reported as a problem (Costello, 2009). The greatest costs were caused by the purchase of parasiticides, equipment and labour, while reduced fish growth and food conversion efficiency came in second and third (Johnson et al., 2004 ; Costello, 2009).

The salmon louse is a caligid copepod that is an ectoparasite of salmonids in seawater. They are carried by the water current into the sea cages, where they settle and reproduce on salmonids. A stress response, caused by increased blood levels of cortisol and glucose, has been recorded both at high and low levels of salmon louse infections on Atlantic salmon (Grimnes & Jakobsen, 1996 ; Nolan et al., 1999 ; Bowers et al., 2000). The preadult and adult stages set off the response, and prolonged infections with large numbers of lice can develop into chronic stress which amongst others leads to increased susceptibility of secondary infections (Mustafa et al., 2000). They also cause lesions, anaemia and osmoregulatory failure (Grimnes & Jakobsen, 1996 ; Wagner & McKinley, 2004 ; Wagner et al., 2008), and is known to cause fish death when appearing on fish in large numbers (Grimnes & Jakobsen, 1996). Since the 1980s, several chemicals have been used by the aquaculture industry to get rid of the louse problem. However, resistance and reduced sensitivity have been observed among a large quantity of these, e.g. organophosphates (Jones et al., 1992), pyretoides (Sevatdal & Horsberg, 2003), and emamectin benzoate (Slice) (Lees et al., 2008). This has resulted in a blooming interest for using wrasse (*Labridae*) as a biological method of sea louse control, meant to keep the sea louse population inside the cages at a minimum.

Cleaning activity among north temperate wrasse species was first reported in 1973 (Potts, 1973), and the use of wrasse as a delousing agent in Norway was initiated by the Institute of Marine Research in the late 80's (Espeland et al., 2010). However, the amount used has been relatively low up until recent years. According to numbers from the Norwegian Directorate of Fisheries (updated 01.12.2011), 1-2 million individuals per year were used by the Norwegian aquaculture industry in the productions of salmonids between 2001 and 2008. This number increased to 4.9 millions in 2009, and 11 million by the end of 2010. The wrasses are wild caught and a mixture of different species. The quality is known to vary

depending on catch method and transportation time (Kvenseth et al., 2003b ; Grøntvedt, 2011). An increased interest has developed towards rearing of wrasse (Treasurer, 2002 ; Skiftesvik & Bjelland, 2003) to establish a year round supply of cleaner fish of good quality and the right size(Skiftesvik & Bjelland, 2003 ; Espeland et al., 2010). The number estimates of the wrasse population along the Norwegian coast are uncertain, and the increasing catches for use in the aquaculture industry may put a strain on the wild population (Skiftesvik & Bjelland, 2003 ; Nodland, 2009). Ballan wrasse (*Labrus bergylta*) is considered the most promising species for aquaculture (Kvenseth et al., 2003a ; Ottesen et al., 2008 ; Kvenseth & Øien, 2009), and commercial producers have started to appear on the Norwegian marked, e.g. Marine Harvest Labrus and Nordland leppefisk. It is found to be an effective cleaner of louse from larger salmon (3-7 kg) and to have a big appetite (Ottesen et al., 2008). In addition it is active at lower temperatures than other wrasse species (Kvenseth & Øien, 2009 ; Skiftesvik, 2009).

As with the production of other marine fish, the first feeding period is considered a bottleneck. The larvae are small and need a feed of adequate size and nutritional composition. While affecting both growth rate, survival and stress tolerance (Coutteau et al., 1997), the diet is also known to affect the skeletal development, and malnutrition increase the occurrence of e.g. scoliosis, lordosis and jaw deformations in marine fish (Cahu et al., 2003). Poor survival, growth and skeletal deformities are normal problems in the intensive cultivation of ballan wrasse (Grøntvedt, 2010 ; Helland et al., 2012), suggesting that a suboptimal first feeding practice is being used, not fulfilling the larval dietary requirements.

1.2 Nutritional requirements of marine fish larvae

Proteins are important for the developing fish larvae as growth primarily is an increase in body muscle mass by protein synthesis and accretion, and it make up between 60 and 80 % of the larval dry weight (Kjørsvik et al., 2004). Specific growth rates during the larval phase can be high, and a daily rate close to 30 % has been measured for the Atlantic cod (*Gadus morhua*) reared at 14 ºC (Otterlei et al., 1999). Amino acids are the building blocks of proteins, and they are also considered a major energy source during larval development (Fyhn, 1989 ; Finn & Fyhn, 1995 ; Rønnestad et al., 1999 ; Rønnestad et al., 2003). They are provided through the diet incorporated in proteins and as free amino acids (FAA), with especially the FAA being rapidly and efficiently absorbed by fish larvae (Rust et al., 1993 ; Rønnestad et al., 2000 ; Applebaum & Rønnestad, 2004). The protein and FAA amount may vary depending on the live feed organism, life stage and rearing conditions of the live feed (Helland et al., 2003 ; Olsen, 2004 ; van der Meeren et al., 2008). Proteins are quantitatively

the most important dietary component. However, having the right amount and composition of lipids are an equally important part of the larval diet (Rainuzzo et al., 1997 ; Sargent et al., 1999 ; Olsen et al., 2004).

Lipids are grouped as either neutral or polar, depending on their polarity. Storage lipids as triacylglycerids (TAG) and wax esters (WE) are neutral lipids and function as a major source of energy. Phospholipids (PL) are polar lipids, and besides serving as a source of energy, they are important structural and functional components of cell membranes and brain and eye tissue (Sargent et al., 1993 ; Watanabe & Kiron, 1994 ; Furuita et al., 1998 ; Sargent et al., 2002). The larval PL biosynthesis does not take place at a sufficient rate to meet the PL requirement in the fast growing larvae (Geurden et al., 1995 ; Tocher et al., 2008). A sufficient supply of PL through the diet is therefore important, and the larval stages are sensitive towards PL deficiency and require higher levels of dietary PL than juveniles (Geurden et al., 1995 ; Coutteau et al., 1997). Compared to TAG the PLs are more easily digested (Olsen et al., 1991 ; Tocher et al., 2008), and their presence may enhance digestion of other lipids in addition to being a key component of the lipoproteins transporting nutrients after uptake by the enterocytes (Coutteau et al., 1997 ; Tocher et al., 2008).

The PLs and neutral lipids are composed of fatty acids, e.g. *n-3* polyunsaturated fatty acids (PUFA), some of which are essential to ensure optimal larval growth and development (Watanabe & Kiron, 1994 ; Furuita et al., 1998 ; Izquierdo et al., 2000 ; Evjemo et al., 2003). Since these essential fatty acids (EFAs) cannot be synthesized by the fish larvae *de novo*, they need to be provided through the diet (Bell et al., 2003). The PUFAs docosahexaenoic acid (22:6*n-3*; DHA), eicosapentaenoic acid (20:5*n-3*; EPA) and arachidonic acid (20:4*n-6*; ARA) are considered as the most important EFAs for marine fish larvae (Sargent et al., 1999 ; Bell et al., 2003). They can be supplied as part of the PLs, or incorporated in the neutral lipid fraction. In addition to dietary PLs having a positive effect on growth, survival and development in itself (Coutteau et al., 1997 ; Tocher et al., 2008 ; Cahu et al., 2009), the EFAs are more beneficial and readily digested when incorporated in the PL-fraction as opposed to the neutral lipid fraction (Izquierdo et al., 2000 ; Gisbert et al., 2005 ; Kjørsvik et al., 2009 ; Wold et al., 2009). Studies on Atlantic cod found that incorporation of the EFA in the PL-fraction lead to better larval growth, more developed digestive organs, earlier ossification and increased levels of DHA in the tissue (Kjørsvik et al., 2009 ; Wold et al., 2009). The amount and ratio of which the EFAs are provided is also of great importance, with a varying optimum from species to species (Sargent et al., 1999). Commonly, the demand is greater during larval stage compared to juveniles and adults (Coutteau et al., 1997).

There is little information available regarding the protein and lipid requirements of ballan wrasse. It has an altrical development (Balon, 1979), with the intestine being a straight, undifferentiated tube and lacking a stomach at time of first feeding (Dunaevskaya, 2010). This is a common feature among other marine fish larvae (e.g. Atlantic cod (Kjørsvik et al., 1991), Atlantic halibut (*Hippoglossus hippoglossus*) (Luizi et al., 1999), turbot (*Scophthalmus maximus*) (Segner et al., 1994), Senegalese sole (*Solea senegalensis*) (Ribeiro et al., 1999), common dentex (*Dentex dentex*) (Santamaria et al., 2004)). The need for nutrition and dietary restrictions is likely similar to other marine fish larvae with altrical development of the digestive system, which emphasises the need for proteins and lipids that are easily digestible at time of first feeding. Recent studies has discovered that ballan wrasse lacks a stomach also as an adult (Hamre & Sæle, 2011), which needs to be taken into consideration when developing formulated feed further.

1.3 What is the optimal first feed for ballan wrasse larvae?

Live feed is commonly used in first feeding of marine fish larvae (Conceicao et al., 2010), and are the preferred choice by larvae when presented in combination with inert diets (Le Ruyet et al., 1993 ; Fernández-Díaz et al., 1994 ; Conceicao et al., 2010). The movement of the live feed keeps it suspended in the water column and may help to stimulate feeding behaviour. Also, the high water content (normally above 80%) and availability in varying sizes make them appealing and ideal as feed in early stages of rearing of marine fish larvae (Turingan et al., 2005 ; Conceicao et al., 2010). The most commonly used live feed is rotifers (*Brachionus* sp.) and brine shrimp (*Artemia* sp.), due to the existence of standardized cost-effective protocols for their mass production (Lubzens, 1987 ; Sorgeloos et al., 2001 ; Southgate, 2003 ; Conceicao et al., 2010). These species are also used as first feed in the cultivation of ballan wrasse (Skiftesvik et al., 2011). Exogenous feeding occurs from 4 dph, where up to approximately 30 dph the ballan wrasse larva is fed rotifers. This is followed by *Artemia* until weaning on formulated feed is successful. Rotifers and *Artemia* do not fulfil the fish larval requirements for EFAs, and are therefore enriched to get a more proper nutritional quality (Lubzens, 1987 ; Evjemo & Olsen, 1997 ; Øie et al., 1997 ; Sorgeloos et al., 2001 ; Conceicao et al., 2010). When enriched the EFAs are incorporated into the neutral lipid fraction (Rainuzzo et al., 1994a ; Rainuzzo et al., 1994b ; Nerhus, 2007), where they are less available to the fish larvae (Izquierdo et al., 2000 ; Gisbert et al., 2005 ; Kjørsvik et al., 2009 ; Wold et al., 2009). The skeletal deformities and low survival obtained when first feeding ballan wrasse larvae on rotifers and *Artemia* (Grøntvedt, 2010 ; Helland et al., 2012) suggests that the current first feeding practice is suboptimal and may be improved further.

Naturally harvested or intensively reared copepods have proven to be suitable as live prey for marine fish larvae, often resulting in better growth, pigmentation and survival compared to feeding with rotifers and *Artemia* (Næss et al., 1995 ; Evjemo & Olsen, 1997 ; Luizi et al., 1999 ; Shields et al., 1999 ; Imsland et al., 2006 ; Eidsvik, 2010 ; Koedijk et al., 2010 ; Busch et al., 2011 ; Kortner et al., 2011). When harvested from the wild there are seasonal variances of the catch, resulting in an unpredictable supply of copepod species and biomass. In addition, there is a possibility of transferring diseases and parasites from the copepods to the reared fish larvae (van der Meeren & Naas, 1997). Effort has been put into developing intensive rearing of copepods for use in start feeding of marine fish fry (Støttrup, 2000 ; Ajiboye et al., 2011 ; Drillet et al., 2011), and the calanoid copepod *Acartia tonsa* fed a monoalgal diet of *Rhodomonas baltica* has been successfully used in first feeding experiment with the result of increased growth, survival and higher quality for Atlantic cod larvae (Eidsvik, 2010 ; Hansen, 2011 ; Norheim, 2011). However, the upscale of copepod cultures to commercial levels is still a challenge (Ajiboye et al., 2011). They are considered the natural prey of many species of fish larvae (Hunter, 1981), and information about their nutritional value is important when making improvements of live feed enrichment emulsion or formulated feed used during larval and early juvenile stages in marine fish culture (Evjemo & Olsen, 1997 ; Hamre et al., 2008 ; van der Meeren et al., 2008).

Copepods are naturally rich in both DHA and EPA incorporated in their PLs (Evjemo & Olsen, 1997 ; Evjemo et al., 2003 ; Drillet et al., 2006 ; van der Meeren et al., 2008 ; Overrein et al., 2010), making it easy accessible for marine fish larvae with an immature digestive system. They have also been found to have a higher protein and FAA content than rotifers and *Artemia* (Næss et al., 1995 ; Evjemo et al., 2003 ; Helland et al., 2003 ; van der Meeren et al., 2008). The digestive system of fish larvae has initially a high assimilation capacity towards FAA and a low protein digestibility which increases as the proteolytic capacity matures (Rønnestad et al., 2000 ; Cahu & Zambonino Infante, 2001 ; Rønnestad et al., 2003 ; Applebaum & Rønnestad, 2004). According to this, feeding ballan wrasse larvae with copepods will result in a higher dietary amount of both proteins and FAA, in addition to providing a more beneficial fatty acid supply than first feeding with rotifers. This may have a positive effect on larval growth, survival and development. The diet has also been known to impact the tissue structure of different digestive organs (Fontagne et al., 1998 ; Wold et al., 2008 ; Wold et al., 2009), development of an efficient brush border (Cahu & Zambonino Infante, 2001) and fatty acid composition in different body tissue (Bell et al., 1995 ; Shields et al., 1999 ; Kjørsvik et al., 2009), which emphasises the importance of right nutrition early in larval development.

1.4 Early organ development and allometric growth

At time of hatching, many marine fish larvae have immature organs and nutrition is provided endogenously through the yolk sac (Osse et al., 1997 ; Yufera & Darias, 2007). The main organs and organ systems become functional by the time of first feeding, and differentiate during the larval stage and metamorphosis (Falk-Petersen, 2005 ; Yufera & Darias, 2007). During this period, the larva directs the available resources into developing those organs most needed to enhance further growth and survival, and the organ differentiation has to take place over a relatively short period of time (Osse & van den Boogaart, 2004). After opening of the mouth, a quick growth and differentiation of the digestive organs is necessary to reinforce the digestion and nutrient absorption (Yufera & Darias, 2007). This has been recorded for turbot (*Psetta maxima*) and common dentex (*Dentex dentex*) , where the digestive organs increased in volume at a relative faster rate than the other organs observed before switching to exclusive exogenous feeding occurred (Sala et al., 2005). This priority of growth is a common feature in larval development, and the resulting unequal growth of the different systems is called allometric growth (Alami-Durante, 1990 ; Osse & van den Boogaart, 2004 ; Sala et al., 2005). Allometric growth of outer structures also occurs during larval development, and a higher growth rate of the head and tail region, as opposed to the trunk, is a common feature (Fuiman, 1983 ; Osse et al., 1997 ; van Snik et al., 1997 ; Peña & Dumas, 2009). This results in an increased ability to capture prey, in addition to being beneficial when escaping predators and reducing the energy expenditure during locomotion (Osse et al., 1997). Generally, there is a much greater amount of allometry within a fish during its early life than later, representing more sharp changes during the larval period. However, through adulthood, the fish growth becomes more isometric. The growth then inflicts no change on the structures or organs proportion relative to each other (Fuiman & Higgs, 1997). Few reports are available on the growth pattern of internal organs, and they are often limited to a single organ group. Information about the growth of these structures may help in understanding critical points throughout the early development of the fish larvae (Sala et al., 2005). Nutrition is one of the extrinsic factors regulating growth (Osse & van den Boogaart, 2004), and different nutritional requirements may occur during different periods of fish larval development.

1.5 Aim of study

If aquaculture of ballan wrasse is to be successful, the fish produced need to be of good quality and have a high rate of growth and survival. The present study was conducted to evaluate the effect of using intensively reared copepods (*Acartia tonsa* nauplii) as early live feed for the ballan wrasse larvae on the larval growth and survival, and early organ growth and development, compared to using rotifers (*Brachionus ibericus*). Copepods were fed to the larvae for a short-term period from 4 to 10 dph followed by enriched rotifers up to 30 dph, or for the whole period from 4 to 30 dph. The larvae fed rotifers received either enriched or unenriched rotifers through the whole period from 4 to 30 dph. All treatments had a transition to *Artemia* from 24-30 dph, and were weaned to formulated feed from 40-50 dph. The experiment lasted up to 61 dph.

Hypothesis: Copepods are more suitable than rotifers as first feed for the ballan wrasse larvae, increasing the larval growth and survival and resulting in better organ growth and development.

The allometric growth and nutritional effect on the volume of nine different organs was evaluated; intestine, liver, pancreas, heart, gills, muscle, central nervous system (brain + spinal cord), eye and notochord. The histological analysis was performed on 4, 8 and 21 days old larvae to evaluate the direct effects of the live feed on the development, and a special focus was given to the tissue structure and development of organs associated with digestion. Analysis was performed on light microscopy sections. Histology was used as a tool to describe the development of the digestive organs while stereology was used to determine organ volumes in addition to the total larval volume.

2. Materials and methods

2.1 The start feeding experiment

The first feeding experiment was performed on ballan wrasse larvae (*Labrus bergylta*). Four different feeding regimes were used (table 2.1), and there were three replicates of each treatment. The treatments varied in type of live feed provided during the first 30 days. Larvae from the "Copepod"-treatment were fed exclusively with *Acartia tonsa* nauplii. Larvae from the "Cop7"-treatment were fed *A. tonsa* nauplii from 4 to 10 dph, with a transition to rotifers (*Brachionus ibericus,* Cayman) enriched on Multigain (BioMar AS, Norway) (table 2.2). Fish larvae from the "RotMG"-treatment were fed enriched rotifers the whole period, while the "RotChl"-treatment had a diet consisting of unenriched rotifers.

A. tonsa nauplii (stage NIII-V, 170-210µm) and *B. Ibericus* (adult lorica length: 180 µm) are equivalent in size (Nesse, 2010 ; Penglase et al., 2010 ; Alver et al., 2011), resulting in a similar biomass being available to the fish regardless of feeding regime. Addition of live feed started at day 4 post hatch. Rotifers and copepods were fed manually to the fish larvae three times a day (8AM, 16PM and 23PM) at a density of 12 000 L^{-1} until 19 days post hatch (dph). Thereafter, the fish larva were fed manually three times a day (9AM, 3PM and 9PM) and once a day (3AM) by a Storvik feeding robot (Storvik Aqua AS, Norway), and the feed density was increased to 17 500 L^{-1} in the tanks fed enriched rotifers. This adjustment was based on food density calculations by an automatic counter with water intake located approximately 10 cm below the surface (Alver et al., 2007 ; Alver et al., 2011), where the measurements suggested that less food was available to the fish larvae in these tanks. The robots feed reservoirs were filled each evening at 9PM. All treatments had a co-feeding period with *Artemia franciscana* from 24 to 30 dph, before being fed *Artemia* exclusively up to 40 dph. The doses were adjusted to the fish larval appetite, keeping the food density at 3000 L^{-1}. Weaning to formulated feed (Nofima, Appendix 1) occurred between 40 and 50 dph, and the formulated feed was distributed by the feeding robot. After weaning (from 51 dph), the fish larvae were fed formulated feed (10 g per tank day^{-1}) once every 30 minutes (feed size 300-600 µm) before this was increased to once every 15 minutes (feed size 600-800 µm). The experiment was terminated 61 dph.

Table 2.1

Experimental setup and parameters for the start feeding experiment of ballan wrasse larvae. Sampling dates for different measurements are listed at the below the different parameters. Dph = days post hatch.

Dph	**1**	**2**	**3**	**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**	**34-39**	**40**	**41**	**42**	**43**	**44**	**45**	**46**	**47**	**48**	**49**	**50**	**51-60**	**61**
Day degrees	12	24	36	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	492-572	588	604	620	636	652	668	684	700	716	732	748	764-908	924
Water exchange rate	2 times day^{-1}														4 times day^{-1}											6 times day^{-1}								8 times day^{-1}													
Temperature (°C)	12							13						14	15											16																						
Clay (g tank^{-1} day^{-1})	0			5				10							13.33				16.7							20							25													0
Sieve	**200 µm**														**500 µm**																																**750 µm**
Copepod				**Copepods**																														**Formulated feed (Nofima)**												
3 tanks																								**Artemia (enriched with Multigain)**																						
Cop7				**Copepods**							**Rotifers (enriched with Multigain)**																								**Formulated feed (Nofima)**												
3 tanks																								**Artemia (enriched with Multigain)**																						
RotMG				**Rotifers (enriched with multigain)**																														**Formulated feed (Nofima)**												
3 tanks																								**Artemia (enriched with Multigain)**																						
RotChl				**Rotifers (not enriched)**																														**Formulated feed (Nofima)**												
3 tanks																								**Artemia (enriched with Multigain)**																						
Sampling																																															
CN/Dry weight		x		x				x				x			x						x						x						x		x						x					x
Mortality														x		x		x		x		x		x		x		x				x		x		x		x		x		x	x	x	x
SL / MH				x				x													x							x					x												x	
Histology				x				x													x																										

Table 2.2

Overview of age, stage and proportion of nauplii fed to the Cop7 and Copepod treatments at different days post hatch (dph)

Cop7 treatment								**Copepod treatment**			
DPH	4-7	8	9	10	11	12-23		4-9	10	11	12-23
Copepod nauplii (%)	100	67	67	100	33	0		100	100	100	100
Rotifers (%)	0	33	33	0	67	100		0	0	0	0
Age of nauplii (days)	3	3	3	3	4	-		3	4	4	5
Stage of nauplii	III-IV					-		III – IV			V (180 µm)

2.2 Larval rearing

The ballan wrasse larvae (2 dph) were supplied from Marine Harvest LABRUS (Øygarden) and distributed at an estimated density of 8400 fish larvae per tank (100 litres). The water exchange rate was gradually increased from two times a day at 2 dph to eight times a day on 30 dph. Temperature and O_2-concentration was measured daily (Traceable ® VWR ® Digital Thermometer, VWR, USA; pH/mV-meter, WTW ph 315i, Germany), the O_2-level was kept above 80 % and the temperature was gradually increased from 12 to 16 ºC (table 2.1). Until 4 dph, the fish tanks were kept in darkness, after which the larvae were reared in continuous illumination (daylight fluorescent tubes, Philips MASTER TL-D 90 Graphica, 18W/965). From the day of first feeding (4dph), clay (Vingerling K148, Sibelco, Germany) was mixed with water and added to each tank (Tøndel, 2009 ; Attramadal et al., 2012) continuously using a peristaltic pump. The amount was gradually increased from an addition of 5 grams clay day^{-1} to each tank, to 25 grams clay day^{-1} by 30 dph (table 2.1). Clay was added until the end of weaning period.

Sea water (34 ppt) was treated with a sand filter and filtered through a 1 µm mesh before being heated and microbially matured based on descriptions from Skjermo et al. (1997). During the maturation process, the water was continuously treated with a degasser and a 1 µm filter, before being ready to enter the larval rearing tanks.

Dead fish and debris was removed by siphoning the bottom every second or third day from 13 dph, and every day from the beginning of dry feed period (40 dph). Each tank was aerated at the bottom of the cone and equipped with a surface skimmer. The water outlet was situated in the tank middle, and the sieve was cleaned daily from the beginning of dry feed period. Mesh size on the sieve were increased from 200 µm at the beginning of the experiment, to 750 µm at the end of the experiment (table 2.1).

2.3 Live feed production

2.3.1 Cultivation and harvesting of microalgae (*Rhodomonas baltica*)

Plexiglas cylinders containing 160 and 200 litres (40 cm in diameter) were used to grow *Rhodomonas baltica* (Clone NIVA 5/9 Cryptophycea: Pyrenomonadales), in addition to 300 litre plastic bags in periods of increased production. The cylinders were washed, chlorinated (10-15% NaOCl, 0.25 ml per litre, no aeration, >5 hours) and dechlorinated ($Na_2S_2O_3$, 3 gram per 100 litres, aeration, >5 hours) between cultivation (Hoff & Snell, 1987). Prior to usage, the sea water (34ppt) was treated with a sand filter, heated to 20 ºC and filtered through a 1 µm mesh, before chlorinating and dechlorating it. The pH was kept between 7.5 and 8.5 (pH/mV-meter, WTW ph 315i, Germany) and air with an addition of 1-2% CO_2 was added. The cultures were continuously illuminated by 6 fluorescent tubes (GE Polylux XL 830 F58W) on three different sides, and the light intensity inside an empty cylinder was measured to 400 µEinstein m^{-2} s^{-1}.

Algae provided from intermediate cultures (10 L round laboratory glass flasks, 2-3 x 10^6 cells mL^{-1}) was used when starting a new culture, and constituted a minimum 10 % of the total volume in the cylinder. 1 ml Conwy medium (modified from Walne (1974), Appendix 2) was added per litre sea water. After reaching the stationary phase (approximate cell density of 1.2 mill ml^{-1} in the cylinders and 800 000 ml^{-1} in the plastic bags), the cultures were run as continuous cultures with 40-50% of the volume was harvested daily. 1 ml Conwy medium was added per litre sea water when diluting the culture, and they were run for two weeks after reaching stationary phase.

2.3.2 Cultivation of copepods (*Acartia tonsa*)

Egg production

Acartia tonsa (clone DFH.AT1) was cultivated under constant light in two cylindrical tanks (1000 and 1600 litres), with flow through of sea water (sand filtered, heated to 20 ºC and filtered through 1 µm mesh) at a water exchange rate of once per day. Temperature and oxygen saturation were measured daily (pH/mV-meter, WTW ph 315i, Germany), and the oxygen saturation was kept above 60 % and the temperature held at 19-22 ºC. Pure oxygen was added when the oxygen level dropped below 80 %. Between each production cycle the tanks were cleaned and disinfected using Virkon S (Lilleborg Profesjonell, Ski) in the 1000L-tank and 70 % ethanol in the 1600L-tank.

Rhodomonas was fed continuously (Nesse, 2010) at an amount keeping the cell concentration at 15-30 000 cells ml^{-1} (measured by a Meckman Multisizer^{TM}3 Coulter Counter®, capillary diameter 100 µm, considering all particles with a diameter in the range 5.5-9.5 µm as algal cells) (Skogstad, 2010). The sieve covering the outlet was cleaned daily, and eggs were collected once each day by siphoning along a harvesting arm. The water containing the eggs was first filtered through a 120 µm sieve to eliminate dead *Acartia* and other waste, and thereafter filtered through a 64 µm sieve to collect the eggs. The eggs were cleaned with salt water and transferred to NUNC EasyFlasks^{TM} (Nunc A/S, Denmark) for storing in sea water at 2 ºC (SANYO Pharmaceutical Refrigerator MPR-311D (H), Japan).

Production of copepod nauplii for feed

Based on the calculated hatching success (Appendix 3), the needed amount of eggs was collected from the refrigerated NUNC flasks, rinsed with sea water, and transferred to 100 litre tanks for hatching (gentle aeration, 19-22 ºC) at a maximum density of 120 nauplii ml^{-1}.They were kept there for three days, changing 50 % of the water daily. The nauplii were fed *Rhodomonas* (10-30 000 cells ml^{-1}). From 4 dph the nauplii were transferred to, and stored in, a 300 litre reservoir. The density was estimated upon transferring by stirring the water column, collecting a small water sample and fixating it with fytofix (Lugol's solution). The amount of nauplii in 3 ml of the sample was counted and used to estimate the density. Before transferring to the larval tanks, the nauplii were concentrated.

2.3.3 Cultivation, harvesting and enrichment of rotifers (*Brachionus ibericus*, Cayman)

Brachionus ibericus (Cayman) was cultivated in four tanks with conical bottoms (250 litres) in sea water (34 ppt) at a temperature of 19-23 ºC (measured with a Traceable ® VWR ® Digital Thermometer). The water exchange rate was kept at 1-1.5 times a day, and the oxygen level above 80 %. Washing of the cultures, followed by transfers to clean tanks, occurred once a week.

DHA *Chlorella* (Chlorella industry co. Ltd, Japan) was fed to the rotifers (feed ratio of 2.5 ml per million rotifers day^{-1}) continuously. Dilution (25-40%) of the cultures occurred at densities above 750 ml^{-1}, and debris was removed daily by flushing for 5 seconds from an outlet at the bottom of the cone. Culture density and egg ratio was measured daily by counting the number in 12 samples (each 50 µl), and cancelling the highest and lowest count. Short-time enrichment before feeding to fish larvae (0.15 g Multigain (Biomar) per million rotifers) was done once a day for two hours, with a maximum density of 400 ml^{-1}. Enriched rotifers were

stored at 8 ºC prior to usage (maximum 24 hours). Before being fed to the fish larvae, both unenriched and enriched rotifers were concentrated and washed using a sieve. A loss of 20 % during transfer and cleaning was estimated.

2.3.4 Cultivation of *Artemia* sp.

Prior to decapsulation, the *Artemia franciscana* cysts (EG ® INVE Aquaculture, Belgium) were hydrated in fresh water (4.9 litre water for 450-500g cysts, 15-25 ºC) with heavy aeration for one hour. The cysts were decapsulated according to Sorgeloos et al. (1977), weighed and transferred to a refrigerator for storage (max 6 days).

Two days prior to feeding, the necessary amount of decapsualted cysts was put up for hatching in sea water (25-28 ºC, pH 8-8.5) with a maximum density of 2 g cysts liter^{-1}. Heavy aeration was kept at all times to keep the O_2-level above 2.5 mg litre^{-1}(Hoff & Snell, 1987). After 24 hours, the hatched *Artemia* was washed and concentrated using an *Artemia*-washer, before transferring them to new tanks (100-300 nauplii per ml). During the next 24 hours enrichment of the *Artemia* occurred twice (10g Multigain (Biomar) per 60 litre), before they were washed and concentrated (200 ml^{-1}), and stored in a cool area (8 ºC, maximum 20 hours (Evjemo et al., 2001)). The amount of *Artemia* needed was estimated before each feeding, and concentrated in as small a volume as possible.

2.4 Larval sampling and fixation

Larvae were randomly sampled and anesthetized using tricaine methanesulfonate (MS-222 Finquel®, Agent Chemical Laboratories Inc., USA) before rinsing them in distilled water and treating them further. Up to 4 dph all larvae were treated identical, and it was not distinguished between treatments at samples from 2 and 4 dph.

For measurements of standard length (SL) and myotome height (MH), and for the histological analyses, the collected fish larvae were fixated in 4% paraformaldehyde (PFA) in phosphate buffered saline (pH 7.4, Apotekproduksjon AS; Norway) and stored cold (4 ºC) in glass vials.

2.5 Larval survival and growth

2.5.1 Dry weight and daily weight increase

Samples for dry weight (DW) were collected on 11 different days: 2, 4, 8, 12, 15 and 21 dph (n = 12 from each tank), 28 dph (n = 24 from each tank), 33, 40 and 47 dph (n = 30 from each tank), and 61 dph (n = 50 from each tank). Larvae from 2 to 28 dph were analyzed for carbon content by using an Elemental combustion analyzer (Costech Analytical Technologies Inc., USA) using acetanilide as standard (analyses conducted by Marthe Schei, SINTEF), and the result was used to determine the larval dry weight according to equation 2.1 (Reitan et al., 1993).

$$DW = (\mu g\ carbon\ larvae^{-1}) * 2.34 \quad [2.1]$$

Larvae from 33 to 61 dph were transferred to individual, pre-weighed capsules and dried at 60 ºC for a minimum of 24 hours, after which they were weighed (Mettler-Toledo microgram balance UMX2 automated-s ultra-microbalance, and UM3 precision single-pan balance, Switzerland). Specific growth rate (SGR) and daily weight increase (%DWI) was further calculated using equations according to Ricker (1958) (equation 2.2 and equation 2.3), where W_1 and W_2 are the dry weight at time t_1 and t_2 respectively.

$$SGR = \frac{\ln W_2 - \ln W_1}{t_2 - t_1} \quad [2.2]$$

$$\%DWI = (e^{SGR} - 1) * 100\% \quad [2.3]$$

2.5.2 Standard length and myotome height

Measurements of standard length (SL) and myotome height (MH) were performed on fish larvae from 6 different days: 4, 8 and 21 dph (n = 12 from each treatment) and 28, 39 and 58 dph (n = 30 from each treatment). The measurements were performed with the software CAST 2 (Olympus Inc., Denmark) on pictures of the fish larvae taken through a stereo microscope (Leica M205C, Leica Microsystems, Germany; Nikon digital sight DS-SM, Nikon Corporation, Japan). SL was measured from the tip of the upper lip to the end of the vertebrae pre flexion and to the peduncle (root of the caudal fin) post flexion. MH was measured perpendicular to the axial skeleton, right behind the anus (figure 2.1).

Figure 2.1
Ballan wrasse larvae 15 days post hatch. The standard length was measured from the tip of the upper lip to the end of the notochord (horizontal line) while the myotome height was measured perpendicular to the axial skeleton right behind the anus (vertical line).

2.5.3 Survival

Estimated number of fish larvae at the beginning of the experiment was 8400 per tank (100 L). All sampling was registered, and from 13 dph dead fish were regularly counted and removed. The data was corrected for sampled larvae and larvae accidentally removed during cleaning of tanks. The mortality from 2-13 dph was assumed to be linear. At the end of the experiment, all fish larvae were counted.

2.6 Histology

Histological analysis was performed with a light microscope on larvae from 4 (n = 6), 8 and 21 dph (n = 6 per treatment). The fish larvae were embedded in paraffin (Tissue-Tek® III Embedding wax, Sakura, UK) (Appendix 4), cut into 4 µm thick longitudinal sections (Jung Autocut 2055, Leica Microsystems, Germany), and stained with Mayer's hemalum solution (Merck, Germany) and Eosin Y-solution 0.5% aqueous (Merck, Germany) (HE-staining, Appendix 5).

2.6.1 Stereological analysis with LM

Sections were studied using a Zeiss Axioskop 2 plus microscope (Zeiss Inc., Germany) equipped with a JVC TK-C1381 colour video camera (JVC, Japan). Volume was estimated by the Cavalieri method (figure 2.2, modified from Howard and Reed (1998)) using CAST 2 (Olympus Inc., Denmark) to apply a point grid (Jørgen et al., 1981 ; Michel & Cruzorive, 1988 ; Mayhew, 1991 ; Howard & Reed, 1998). The tissue volume (V_T) of ten different organs was determined: eye, muscle, intestine, liver, central nervous tissue (CNS) (brain + spinal cord), notochord, heart, gills, pancreas and "other" tissues, consisting of all tissues not covered by the previous categories (e.g. cartilage, kidney, fin fold, oesophagus and swim bladder). Together these 10 categories covered all different tissue throughout the fish larvae, and the whole tissue volume of the fish would function as the reference volume (Howard & Reed, 1998). When the point grid was applied, points touching any tissue were registered as hits in its respective category. Points not touching any tissue or touching the lumen of the buccopharyngeal cavity, digestive tract and swim bladder, were not registered as hits (Sala et al., 2005).

On 4 dph every third section was analysed (on average 26 sections per larvae) at 16x magnification, using 144 points in each grid. On 8 dph every fourth section was analysed (on average 24 sections per larvae) at 10x magnification, using 144 points in each grid. On 21 dph every fifth section was analysed (on average 34 section per larvae) at 4x magnification, using 196 points in each grid. Each point represented and area of 3230.51 µm^2, 8356.48 µm^2 and 34597.96 µm^2 at 4, 8 and 21 dph respectively. The distance between each section studied was determined making sure that every organ would be represented in at least five studied sections if developed at the time of interest. Volume (V) was calculated from $V = \sum A(E+C)$, where A represented the summation of the measured area in the section, E the thickness of the section and C the distance between the measured sections.

Pictures were taken with a Nikon digital sight DS-SM camera (Nikon Corporation, Japan).

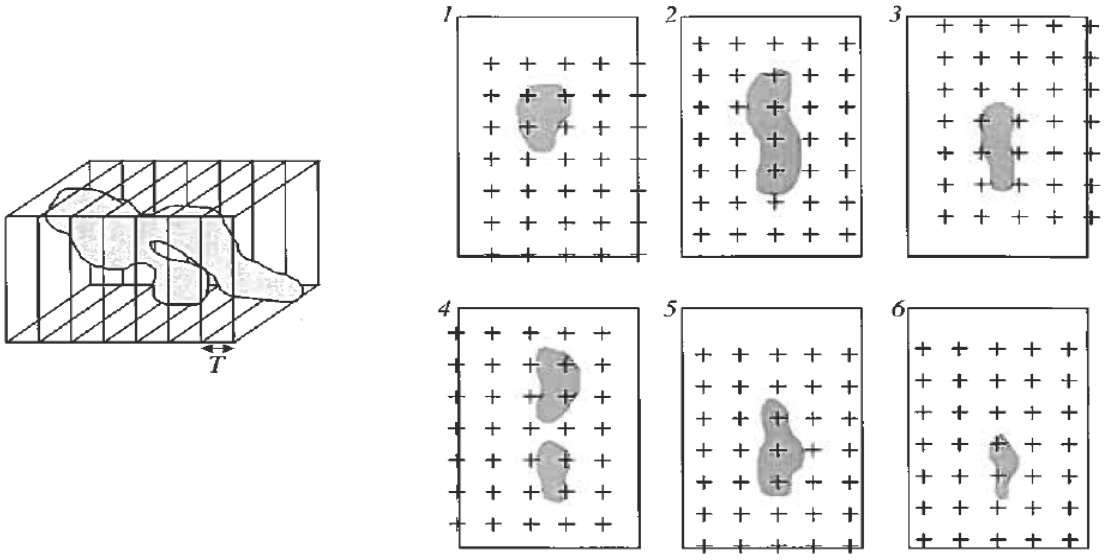

Figure 2.2
In the Cavalieri method a series of parallel sections is cut through the object. Cross sections at a fixed distance, T, are studied by randomly applying a point grid. A known areal is connected with each point, and the areal of tissue in a section is determined by counting the number of points hitting the tissue and multiplying with the point area. The volume is determined by multiplying the tissue areal in a section with the section thickness and T. The figure is modified from Howard and Reed (1998).

2.7 Analysis of nutritional value of live feed organisms

Samples of *Acartia tonsa*, rotifers (enriched on Multigain and unenriched) and enriched *Artemia* (Multigain) were collected. A sieve was used to rinse the samples with distilled water, before giving them a quick dry on a paper cloth and transferring them (approximately 10 ml) into individual sampling tubes. The samples were immediately stored at -80 ºC and freeze dried, before being shipped off for further analysis (table 2.3). Analyses were performed under supervision of Annbjørg Bøkevoll at NIFES using accredited methods: acid hydrolysis to determine the total amount of fat (principle after EU/EØS RD 98/64 part B), Dumas' method for estimation of nitrogen amount, and generation of methyl esters prior to GLC analysis to determine the fatty acid composition.

Table 2.3
Analyses of the nutritional value of different live feed used in the first feeding of ballan wrasse larvae. The amount of protein and lipids are measured in grams per 100 gram larval dry weight, and the % value of different essential fatty acids is from the total amount of lipids.

	Protein g/100g DW	Lipids g/100g DW	DHA %	EPA %	ARA %	DHA:EPA ratio
Acartia tonsa	67.8	8.8	22.4	4.2	0.8	5.3
Rotifers, unenriched	44.4	11.9	14.4	8.5	0.9	1.7
Rotifers, enriched on Multigain	42.3	11.6	19.8	6.5	0.9	3.0
Artemia, enriched on Multigain	34.9	27.6	21.5	5.3	2.5	4.0

2.8 Statistical analysis of data

Arc sine transformation was performed before statistical testing of percentage values and volume fraction values. A Shapiro-Wilk-test was used to test for normality of data (P<0.05), while a Levene test was used to test for homogeneity of variance (p = 0.05). All data were normally distributed, and difference of means was tested using One-way ANOVA (p = 0.05) followed by the post-hoc-tests Student-Newman-Keuls if there were homogeneity of variance and Dunnett T3 if there were not. Pearson correlation test (p = 0.01, 2-tailed) was used to test for correlation. Statistical analysis and graphs were performed with the software PASW Statistics v 19.0 (SPSS Inc., USA) for PC, with exception of allometric growth determination which were made in SigmaPlot 11.0 (Systad Software Inc., USA 2010). Tables were made in Microsoft Office Excel 2007.

Allometric growth was described as a power function of total tissue volume (V_T) using non-transformed data: $y = aV_T^g$; where y is the measured character (organ volume), a the intercept, and g the growth coefficient (Fuiman, 1983). A growth coefficient of 1 indicates isometric growth, while one greater or less than 1 indicates positive or negative allometric growth respectively when comparing volume to volume. When comparing volume to standard length, a growth coefficient of 3 equals isometric growth (Gisbert, 1999). Linear regressions were performed on log-transformed data in the statistical program SigmaPlot 11.0 (Systad Software Inc., USA 2010) by using the least squares method. These were further used to determine inflexion points in the development and the allometric growth functions (points where the growth coefficient changes). Inflexion points were determined by repeatedly plotting linear regressions in both directions from consecutive points in areas with possible changes in the growth coefficient. A t-test was performed to determine in which of these points the difference was significant. If the difference was significant in several points for the same inflection point, the one with the highest t-value was used (Müller & Videler, 1996 ; van Snik et al., 1997). The t-value was determined by equation 2.4, where g_1 and g_2 are the slope in equation 1 and 2 respectively, SD_1 and SD_2 is the standard deviation sample pre and post the inflection point (1 and 2 respectively), and n_1 and n_2 the sample size pre and post the inflection point (1 and 2 respectively).

$$t = \frac{(g_1 - g_2)}{\sqrt{\left(\frac{SD_1^2}{n_1} + \frac{SD_2^2}{n_2}\right)}} \qquad [2.4]$$

Correlation coefficients of the linear relationship describing the growth was determined with a Pearson correlation test (p = 0.05, 2-tailed).

3. Results

3.1 General observations

Fish larvae fed copepods looked bigger and stronger than the fish larvae that only were fed rotifers. In addition, they appeared more quick, being difficult to catch during sampling. The pigmentation was more prominent and had a yellow tint the period copepods were supplied through the diet. The RotChl-larvae had very little pigmentation up until they were fed *Artemia*. Throughout the whole period they reacted more slowly, which made them easier to sample. In tanks where the density appeared high, the larvae gathered in clusters, regardless of treatment. This behaviour appeared from the *Artemia* period.

Fish larvae in one replica of the Copepod-treatment (Copepod 1) experienced high mortality and poor growth when compared to the other Copepod-larvae. The larval DW in Copepod 1 was significantly different from the two other replicates from 21 dph to the end of the experiment. It also deviated from the other treatments later in the experiment, having the lowest growth observed of all the tanks at 61 dph. A difference in total day degrees did not account for this variance. Values from this tank were omitted from the mean values of growth and survival (overview of survival, DW, %DWI and day degrees for the individual tanks in Appendix 6, 7, 8 and 9 respectively). Due to mixing of replicates during histological sampling, there was no way of omitting larvae from this tank from the histological analysis.

3.2 Larval survival and growth

3.2.1 Dry weight

The dry weight (DW) was significantly higher for Copepod- and Cop7-larvae at 8 and 12 dph compared to RotMG- and RotChl-larvae (figure 3.1). From 15 to 33 dph, larvae from the Copepod treatment continued having a significantly higher DW than larvae from the other treatments. A slower growth was observed for the Cop7 larvae after switching from copepods to rotifers, and while the DW of the Cop7 larvae still was higher than the other rotifer fed larvae at 21 dph, this difference was only significant between the Cop 7 and RotMG larvae. A slower growth was also observed for the Copepod-larvae from 33 to 40 dph (figure 3.2), which is after switching feed from copepods to *Artemia*. At 40 and 47 dph there were no differences between larvae from any of the treatments, but at 61 dph the Copepod- and Cop7-larval DW was significantly higher than the RotMG- and RotChl-larval DW. RotMG- and RotChl-larvae had similar growth throughout the experiment.

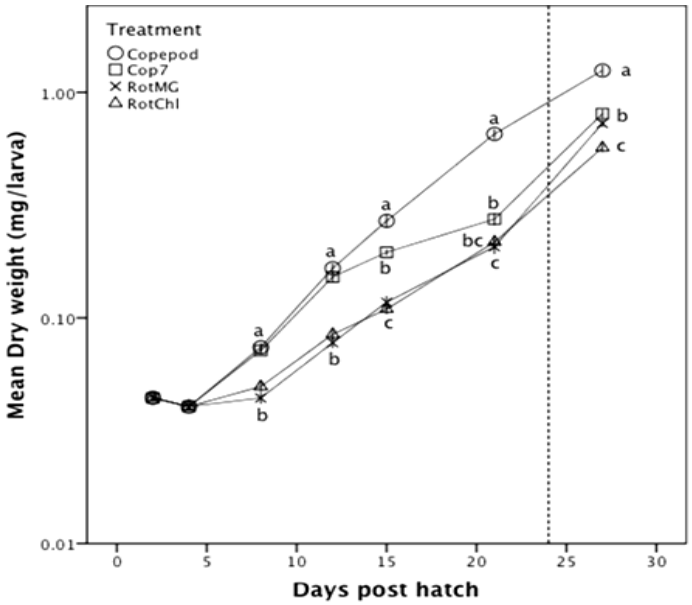

Figure 3.1
Mean dry weight (mg/larvae) of ballan wrasse larvae. Measurements were made on day 2, 4, 8, 12, 15, 21 (n = 36 for each treatment, except for the Copepod treatment where n = 24) and 27 post hatch (n = 72 for each treatment, except for the Copepod treatment where n = 48), and significant differences are denoted by letters a to c. The dotted line indicates the start of co-feeding with *Artemia*.

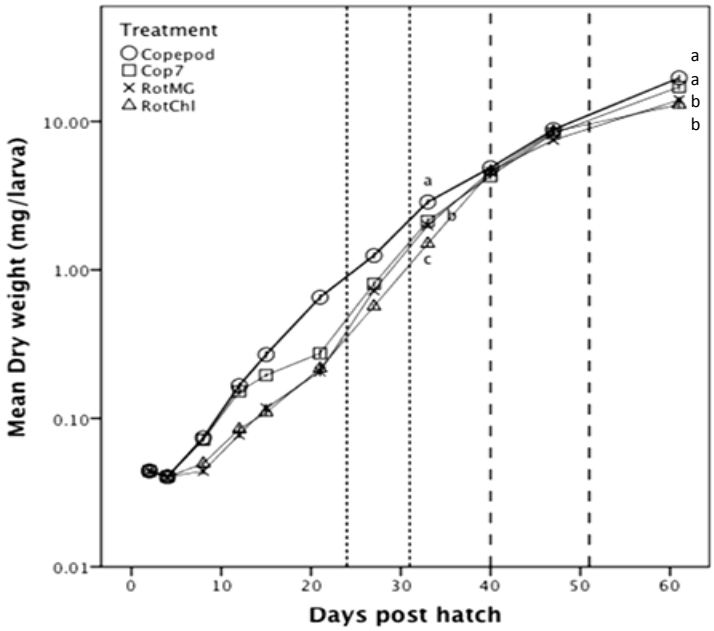

Figure 3.2
Mean dry weight (mg/larvae) of ballan wrasse larvae. Measurements were made on day 2, 4, 8, 12, 15, 21 (n = 36 for each treatment, except for the Copepod treatment where n=24), 27 (n = 72 for each treatment, except for the Copepod treatment where n=48), 33, 40, 47 (n=90 for each treatment, except for the Copepod treatment where n=60) and 61 post hatch (n=150 for each treatment, except for the Copepod treatment where n =100). The dotted line indicates the start of co-feeding with *Artemia*, and the transition to only feeding with *Artemia*. The dashed line indicates the start and stop of weaning.

3.2.2 Daily weight increase

The period from 2 to 4 dph was represented by endogenous feeding, and the mean % daily weight increase (% DWI) was negative (figure 3.3). Copepods resulted in a higher mean % DWI than feeding with rotifers. Both Copepod- and Cop7-larvae had significantly higher % DWI than RotMG- and RotChl-larvae from 4 to 8 dph, and increased their weight by 15-16 % daily compared to 2-5 %. The Copepod-larvae continued having a significantly higher % DWI from 8 to 21 dph. Switching from copepods to another feed had a negative effect on the larval % DWI. The Cop7 larvae had a % DWI similar to the other rotifer fed larvae from 8 to 21 dph, while it was significantly lower than the Copepod larvae which continued receiving copepods during this period. Also, a negative effect of switching from copepods to *Artemia* was observed for the Copepod larvae. During the *Artemia* phase the Cop7-, RotMG- and RotChl-larvae had a DWI of 16-18 %, which were significantly higher than the Copepod-larvae DWI of 11 %. From 40 dph and to the end of the experiment, no significant difference in larval % DWI between the treatments was registered. The DWI for the whole experiment was approximately 10 % for larvae regardless of treatment.

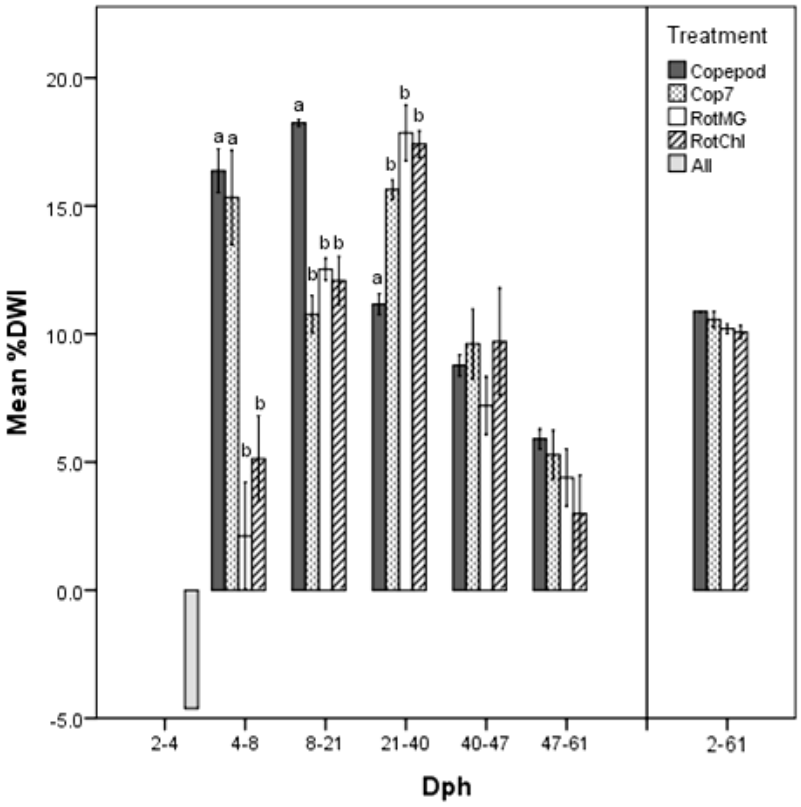

Figure 3.3
A comparison of mean % daily weight increase (% DWI) for ballan wrasse larvae from four different start feeding regimes. The period from 2-4 dph was represented by endogenous feeding. From 4-10 dph the Copepod- and Cop7-larvae received copepods while RotMG- and RotChl-larvae received enriched and unenriched rotifers. From 10-31 dph the Cop7 treatment switched live food from copepods to enriched rotifers, while the other treatments received the same live feed as they did previously. *Artemia* were distributed to all treatments from 24-40 dph, followed by a weaning period from 40-50 dph and a dry feed period from 50-61 dph.

3.2.3 Standard length and myotome height

The average mean larval standard length (SL) at 4 dph was 3.9 mm, and ranged from 13.1 mm in the RotChl treatment to 13.8 mm in the Cop7 treatment at the end of the experiment. At 8 dph there was a significant difference in SL between those larvae fed copepods and those fed enriched rotifers (figure 3.4). From 21 dph the Cop7 larvae were of equal length as the rotifer fed larvae, while the Copepod-larvae had a significantly higher mean SL than larvae from all other treatments at both 21 and 28 dph. At 39 dph a significant difference in SL were found only between Copepod larvae and RotChl larvae, and by 58 dph no difference in SL was detected between fish larvae from any of the treatments.

The same tendency was observed for the mean myotome height (MH), where both Cop7 and Copepod larvae had a significantly higher MH at 8 dph than larvae fed rotifers (figure 3.5). Only the Copepod larvae were significantly different at 21 and 28 dph, where they had a higher MH than larvae from all the other treatments. The Copepod and the RotChl larvae differed significantly at 39 dph, while the mean larval MH was equal regardless of treatment at 58 dph. The average larval MH at 4 dph was 0.2 mm, while it at the end of the experiment ranged from 2.7 mm in the Copepod treatment to 3.0 mm in the RotChl treatment.

The SL and MH were correlated (Pearsons correlation, $p<0.01$, 2-tailed) within the different treatments, with coefficients of 0.978, 0.970, 0.985 and 0.976 for the Copepod, Cop7, RotMG and RotChl larvae respectively.

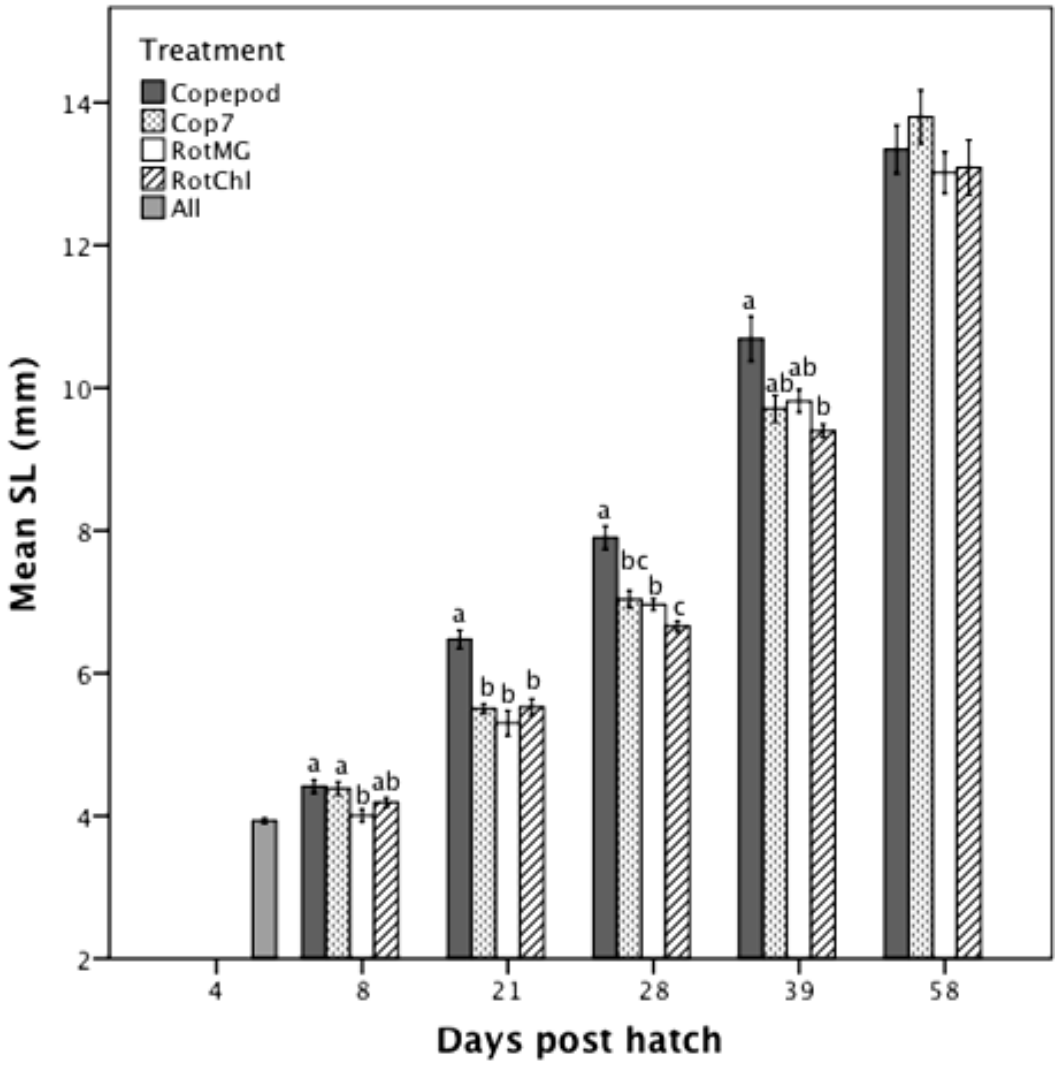

Figure 3.4
Ballan wrasse larval standard length (SL). Samples were taken at 4, 8 and 21dph (n=12 from each treatment), and 28, 39 and 58 dph (n=30 from each treatment, except the Copepod treatment where n = 20 on 28, 39 and 58 dph and RotChl where n = 20 at 58 dph).

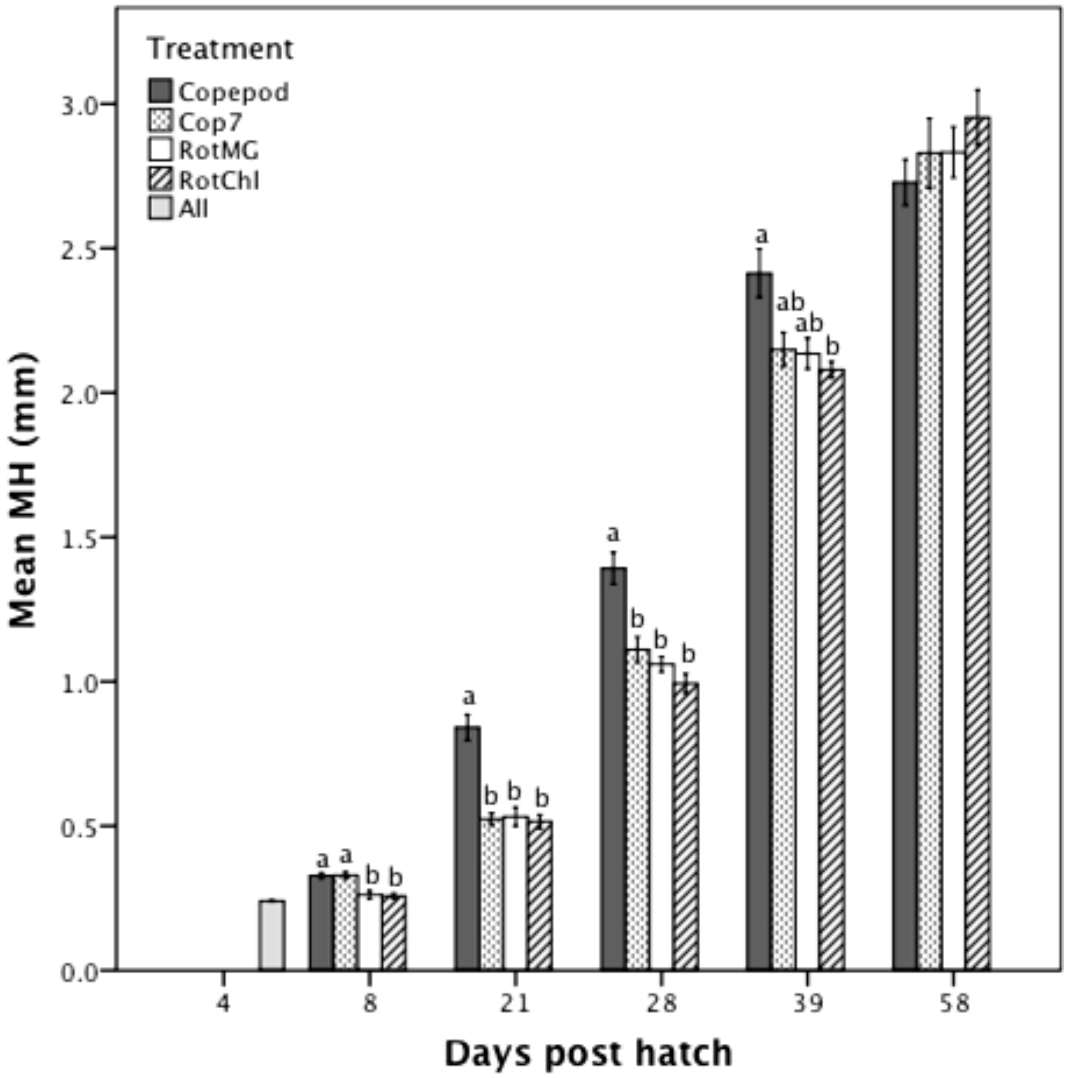

Figure 3.5
Ballan wrasse larval myotome height (MH). Samples were taken at 4, 8 and 21dph (n=12 from each treatment), and 28, 39 and 58 dph (n=30 from each treatment, except the Copepod treatment where n = 20 on 28, 39 and 58 dph and RotChl where n = 20 at 58 dph).

3.2.4 Survival

The greatest mortality occurred prior to 13 dph in all the treatments, followed by a slow decrease in survival up to 61 dph (table 3.1). No mortality was registered post 40 dph for the RotChl larvae, however this was the treatment with the lowest overall survival throughout the whole period. A difference in survival was first detected at 24 dph, where the Cop7-treatment had a significantly higher survival than the RotChl-treatment (20% for Cop7 compared to 7% for RotChl). At the start of weaning period, both Copepod and Cop7 larvae had a significantly higher survival rate than RotChl larvae, and at the end of the experiment this was also true for RotMG-larvae. The survival at 61 dph was 5% in the RotChl treatment, about half the larval survival from the other treatments. It was no significant difference in survival between the larvae fed copepods and the larvae fed enriched rotifers. One of the RotChl replicates was ended by day 40 post hatch due to no fish being left.

Table 3.1

The mean percentage survival ± standard error for ballan wrasse larvae on selected days. The Estimated number larvae in each tank were 8400 at the beginning of the experiment. n = 3 per treatment, except for the Copepod treatment where n = 2. The different days were selected by when transition from one diet to another occurred. The period 24 to 31 dph was the transition from early live feed to *Artemia*, while weaning occurred from 40 to 51 dph. Significant differences are indicated by different letters.

Dph	Copepod	Cop7	RotMG	RotChl
13	16 ± 2.6	24 ± 5.3	17 ± 2.7	12 ± 0.9
24	14 ± 1.5^{ab}	20 ± 4.1^{a}	13 ± 1.6^{ab}	7 ± 1.0^{b}
31	14 ± 1.6^{ab}	18 ± 3.8^{a}	12 ± 1.4^{ab}	6 ± 0.9^{b}
40	13 ± 1.4^{a}	16 ± 3.2^{a}	11 ± 1.5^{ab}	5 ± 0.6^{b}
51	13 ± 1.3^{a}	15 ± 2.8^{a}	10 ± 1.5^{ab}	5 ± 0.6^{b}
61	11 ± 0.5^{a}	12 ± 1.4^{a}	10 ± 1.3^{a}	5 ± 0.6^{b}

3.3 Histology

3.3.1 Structure of the digestive organs

The mouth and anus was open by 4 dph, and the alimentary canal was differentiated into the buccal cavity, oesophagus, pre- and postvalvular intestine and rectum (figure 3.6 A, postvalvular intestine and rectum not shown). The yolk sac was connected to the lower, anterior part of the liver (figure 3.6 B), and the intestine was visible as a straight tube. No histological differences were observed between the pre- and postvalvular intestine: both regions were lined by a simple columnar epithelium with medial nuclei (prevalvular intestine in figure 3.6 C). Simple folds were present throughout the whole intestine. A large portion of yolk still remained. The pancreas was visible caudally to the liver as a continuous structure packed around the anterior part of the intestine, and basic zymogen granules (stained red) was present. Endocrine pancreatic tissue, islet of Langerhans, was observed in some larvae. The gall bladder was located between the liver and pancreas. Inflation of the swim bladder had not occurred, and it was connected to the dorsal wall of the oesophagus through a pneumatic duct (figure 3.7).

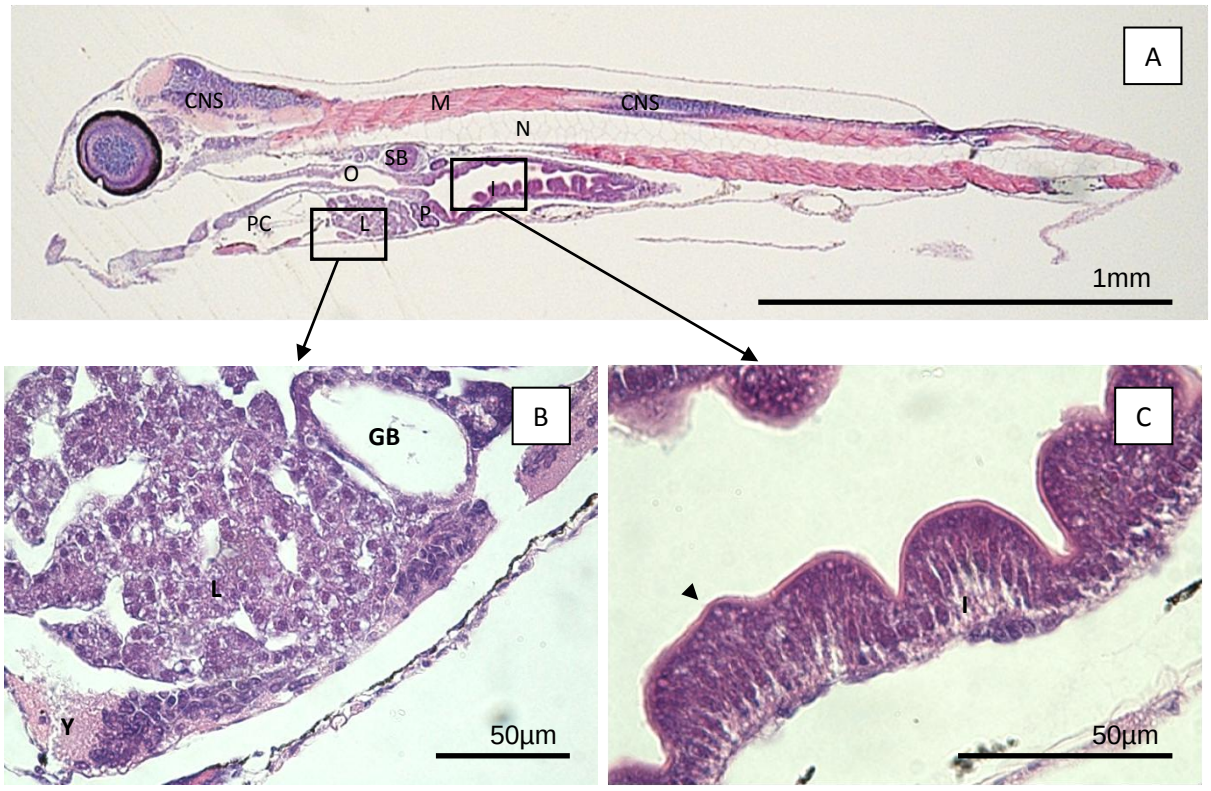

Figure 3.6
A: Ballan wrasse larva 4 dph stained with HE (4x magnification). The mouth and anus was open and the intestine (I) was a straight tube where folds had started to appear. The liver (L) and pancreas (P) were located below the oesophagus (O), and the swim bladder (SB) and pericardial cavity (PC) was visible. The notochord (N) passed straight through the body, enclosed by muscle (M) and nervous tissue from the brain and the spinal cord (CNS). **B**: Hepatocytes of the liver and the gall bladder (GB) (63x magnification). The yolk sac (Y) connected to the lower, anterior part of the liver. **C**: The prevalvular intestinal wall where the brush border was visible (small arrow) (100x magnification).

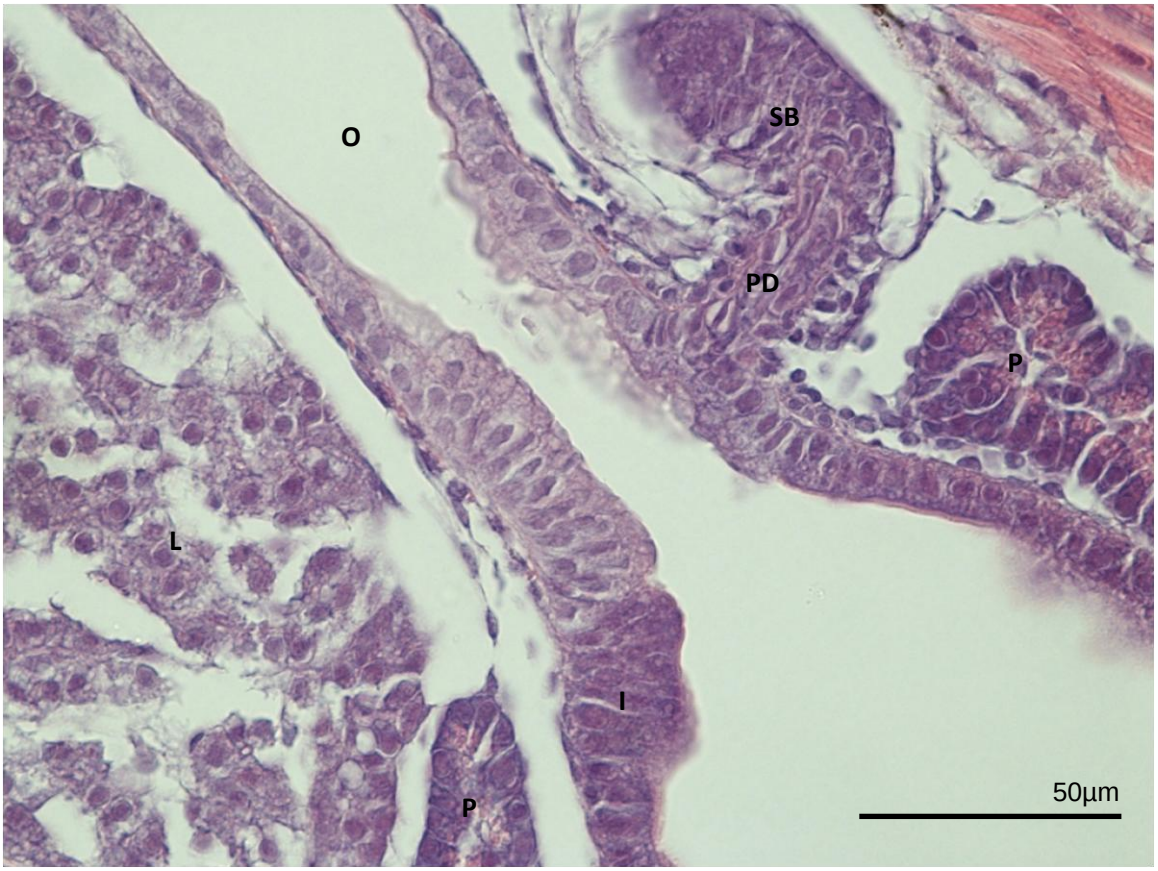

Figure 3.7
The ballan wrasse (4 dph) had a pneumatic duct (PD) connecting the swim bladder (SB) to the dorsal, posterior part of the oesophagus (O). The swim bladder was not yet inflated. HE-staining, 63x magnification. Abbreviations: I, intestine; L, liver; P, pancreas.

By 8 dph, thyroid follicles were observed in larvae from all treatments. They were located in the buccal cavity right beneath the cartilage of the lower jaw (figure 3.8 A). Mucus cells in the oesophagus had started to appear between 4 and 8 dph (figure 3.9 A). The intestinal wall appeared to be thicker than previously and with longer folds, this being more apparent in the Copepod and Cop7 larvae (figure 3.10 A - D). Supranuclear vesicles were prominent in the enterocytes of the postvalvular intestine, making it clearly distinguishable from the prevalvular intestine where none such vesicles were visible (figure 3.10). The yolk reserves were nearly depleted. Islet of Langerhans was observed in nearly all larvae (5 out of 6 of the Copepod and Cop7 larvae, and 4 out of 6 of the RotMG and RotChl larvae) in addition to the exocrine pancreatic tissue. The cytoplasm of the hepatocytes had a granular appearance indicating glycogen storage. There was observed a large variation in hepatocyte structure with regards to degree of vacuolisation and the size of the vacuoles (figure 3.11), but no relationship regarding SL or treatment was discovered. Also, no variation in hepatocyte structure within the same individual was observed. The swim bladder was inflated in approximately half of the fish larvae, and it appeared to occur independently of treatment.

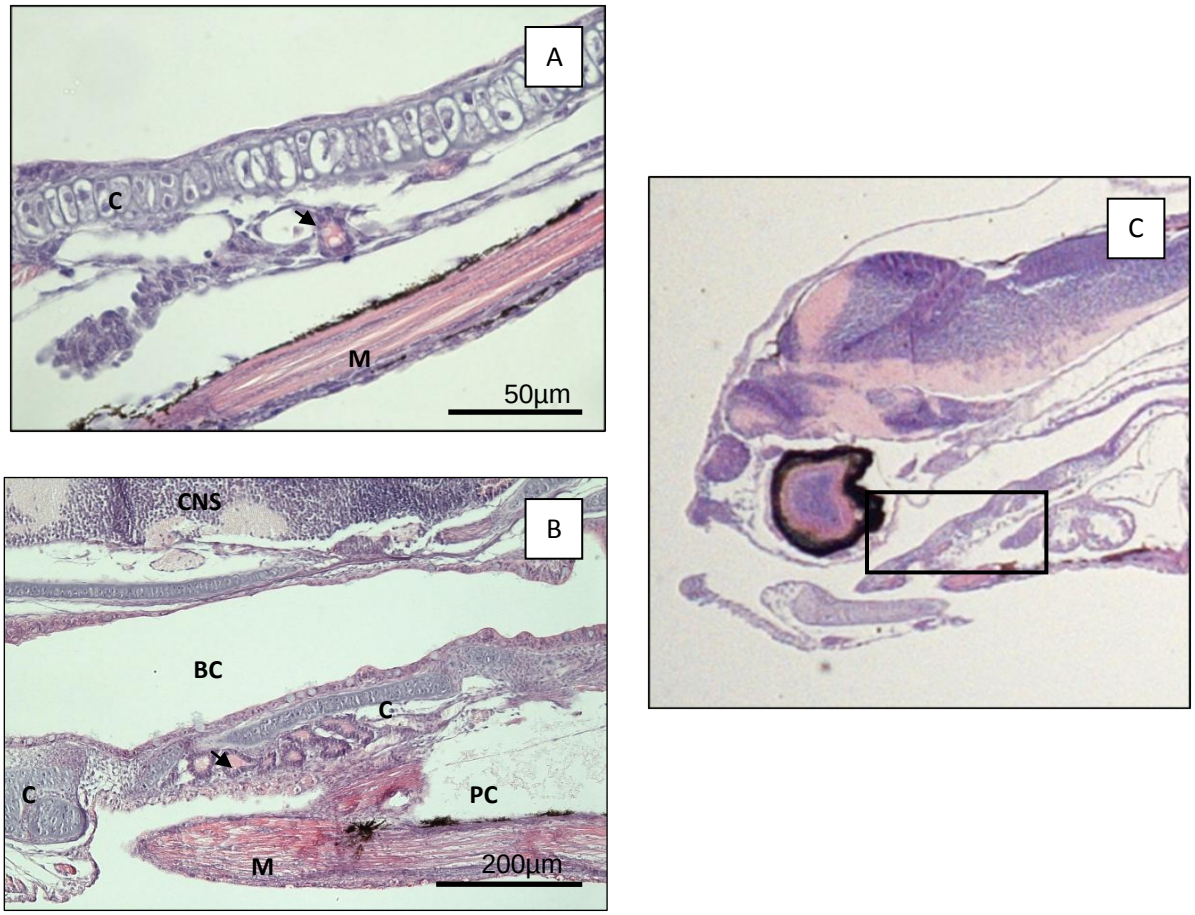

Figure 3.8
Early appearance of thyroid follicle below the lower jaw (**A**) in ballan wrasse larvae 8 dph, and more developed thyroid glands (**B**) at 21 dph (arrows). **C**: Cranial section of a ballan wrasse larvae 8 dph. All pictures are of larvae from the Copepod treatment. The square indicates the location of the thyroid. Sections are stained with HE, and pictures are taken at 63x (A), 14x (B) and 2.5x (C) magnification. Abbreviations: BC, buccal cavity; C, cartilage; CNS, nervous tissue from the brain and spinal cord; M, muscle tissue; PC, pericardial cavity.

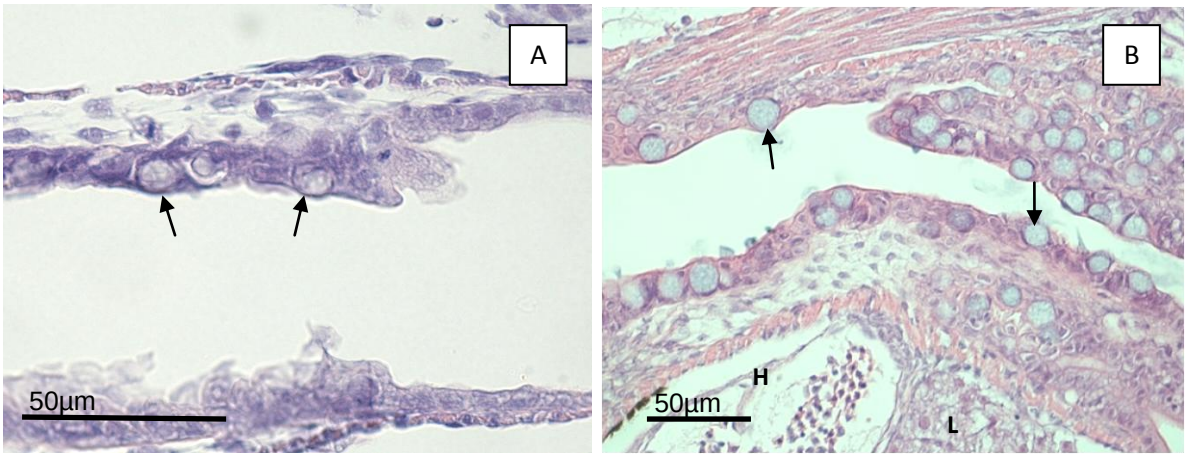

Figure 3.9
Mucus cells (arrows) increased in numbers in the oesophagus (OE) of larval ballan wrasse from 8 (**A**) to 21 dph (**B**), and contained an acidic mass by 21 dph. The pictures are taken from approximately the same place at the oesophageal beginning, in larvae from the Cop7 treatment. Sections are stained with HE, 63x magnification (A) and 40x magnification (B). Abbreviations: L, liver; H, heart.

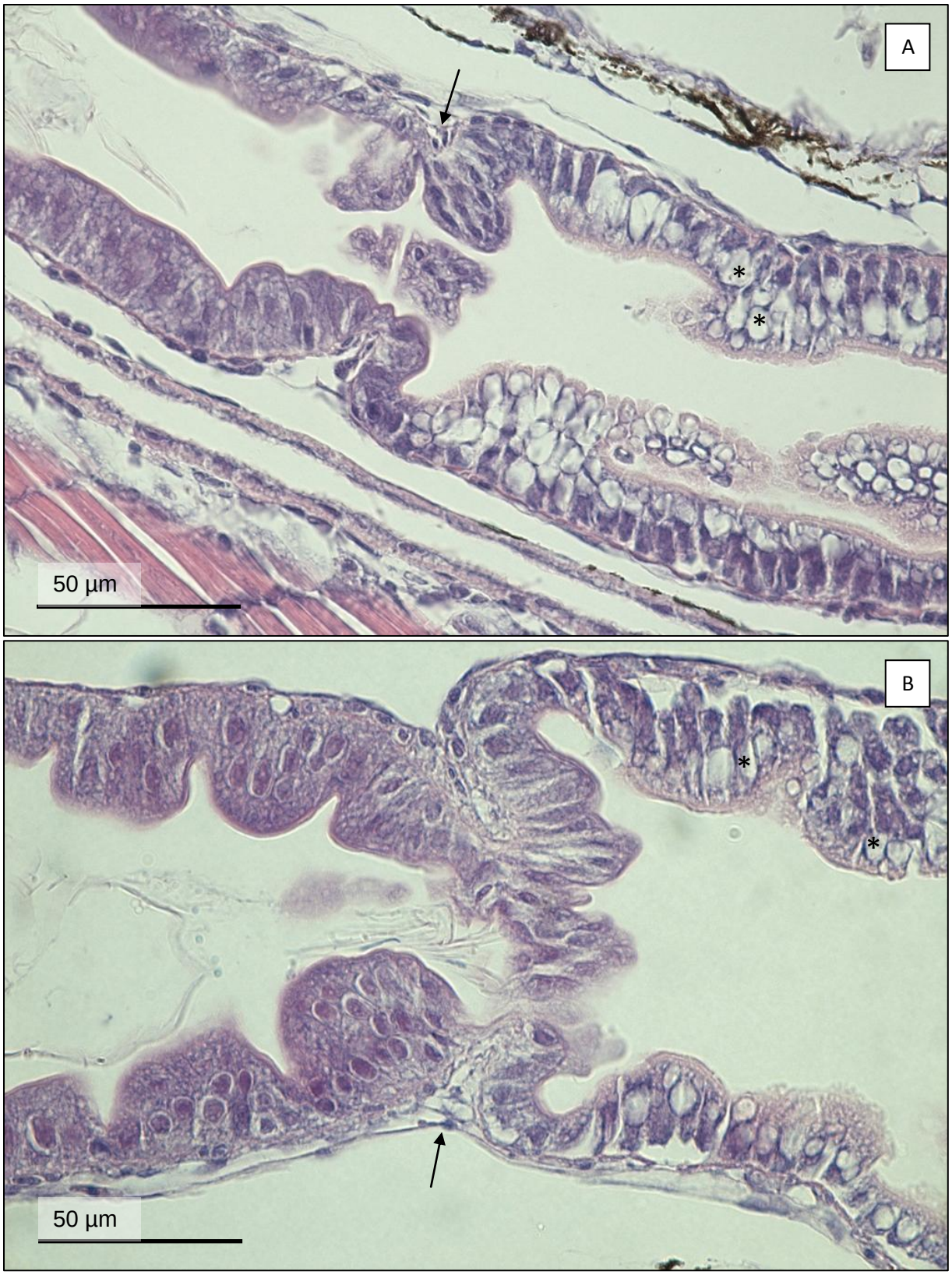

Figure 3.10
Longitudinal sections comparing the pre- (left) and postvalvular (right) enterocytes of ballan wrasse larvae (8 dph) from the Copepod (**A**), Cop7 (**B**), RotMG (**C**) and RotChl (**D**) treatment. The different parts of the intestine were separated by a valve (arrow). Pictures were taken at the same magnification (63x) and at approximately same location in each larva: close to having an open

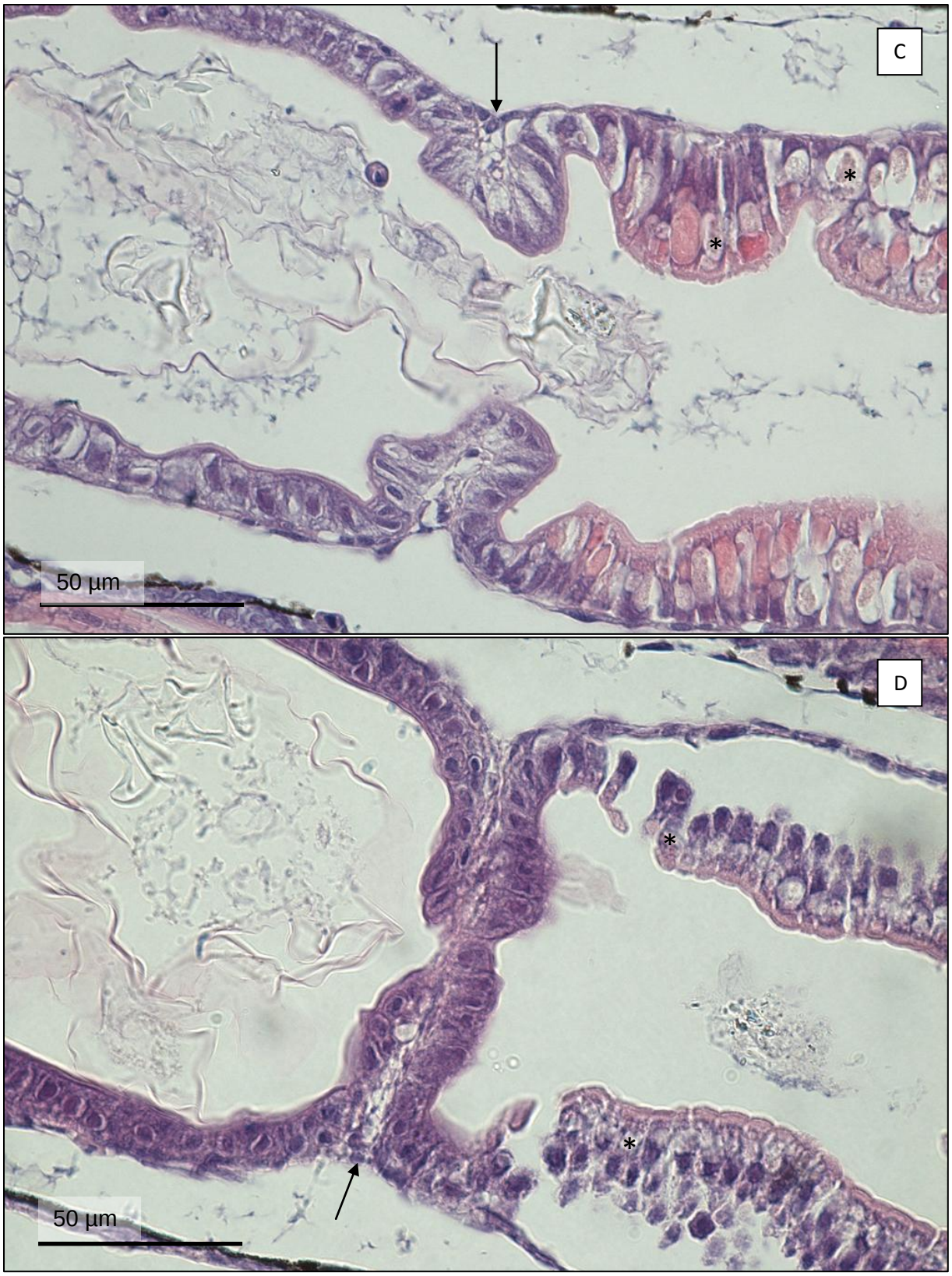

Figure 3.10 (cont.)
passage from the pre- to the postvalvular intestine. The two parts had a different appearance, with supranuclear vesicles being prominent in the postvalvular part of the intestine (*). The enterocytes appeared to have increased more in height in the Copepod and Cop7 larvae compared to the RotMG and RotChl larvae, especially in the prevalvular intestine. Sections are stained with HE.

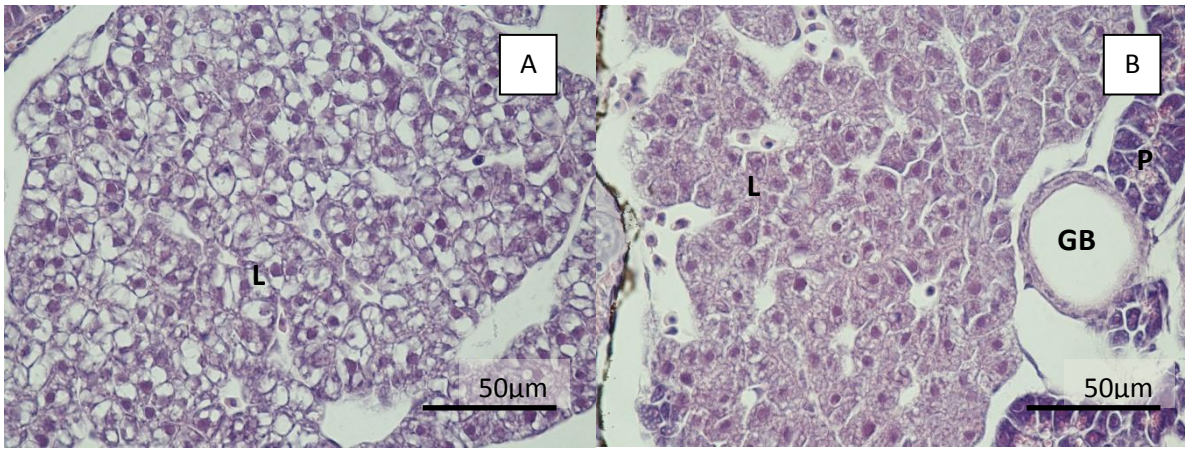

Figure 3.11
Hepatocytes of the liver of ballan wrasse larvae (8 dph) illustrating a high degree of cytoplasmic vacuolisation (**A**) and a less amount of vacuolisation (**B**). There were large variations within the different treatments regarding the tissue structure of the hepatocytes. Both of the pictures are of larvae from the Copepod-treatment. The sections are stained with HE and taken at 63x magnification. Liver (L), gall bladder (GB) and pancreas (P) is visible.

The thyroid glands were more developed and had increased in number by 21 dph (figure 3.8 B), and vacuole-like spaces was present in the colloid. Acidic mucus cells had appeared in large numbers in the epithelium covering the floor and roof of the buccal cavity, in addition to having increased in number in the oesophagus (figure 3.9 B). A transition from straight to coiled intestine took place at a SL of about 5.4 mm, and the Copepod treatment were the only treatment where coiling had occurred in all larvae observed. The intestinal folds had increased in amount and length, and they occupied most of the intestinal lumen both pre- and postvalvular (figure 3.12). Supranuclear vesicles were present in the intestine, however in reduced numbers compared to what was observed earlier. They were located both pre- and postvalvular, with still a higher frequency in the postvalvular part. No difference between the treatments was detected regarding the amount of vesicles. The liver and pancreas appeared to have increased in size from 8 dph, and in the largest fish larvae (found mainly in the Copepod treatment) the pancreas had spread throughout the whole abdominal cavity, filling all “empty” spaces (figure 3.12). The variation previously observed in the liver tissue was not observed at 21 dph, the liver appearing similar in structure and degree of vacuolisation from individual to individual (figure 3.13).

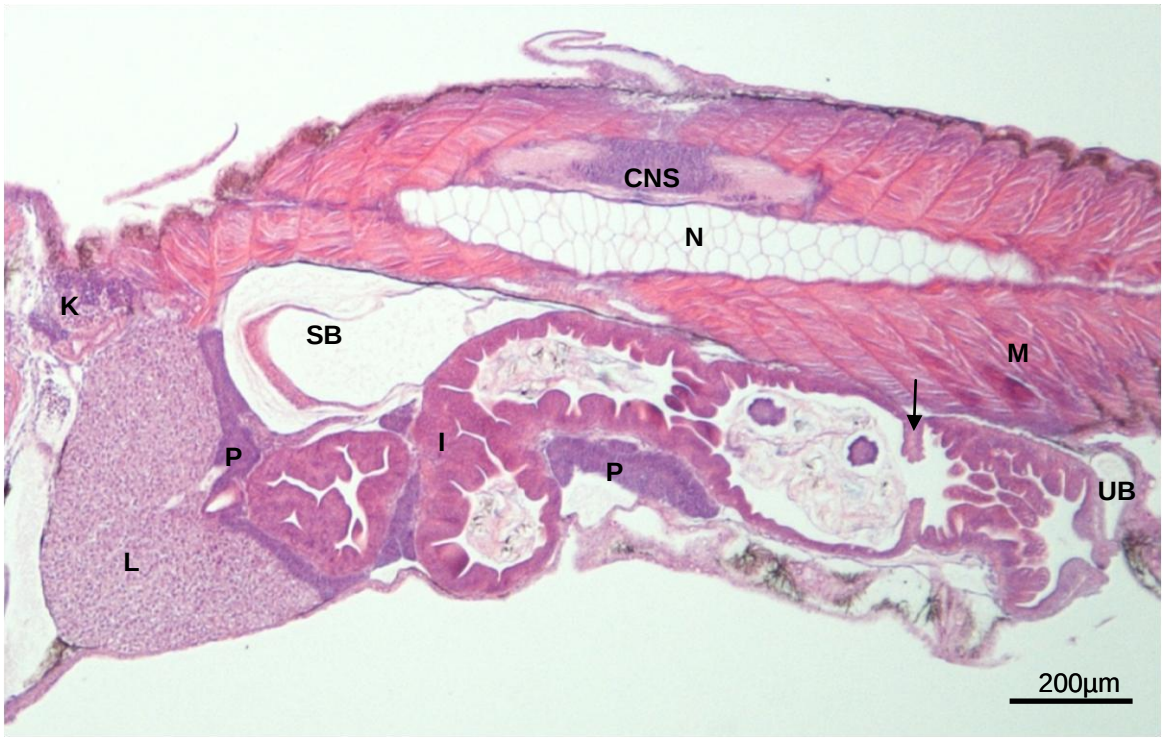

Figure 3.12
Ballan wrasse larvae 21 dph (Cop7 treatment). Coiling of the intestine has occurred, and the pancreas (P) has spread throughout the abdominal cavity. The intestinal folds have increased in amount and length, both pre- and postvalvular (valve marked by arrow). The section is stained with HE, 4x magnification. Abbreviations: CNS, nervous tissue of the brain and spinal cord; I, intestine; K, kidney; L, liver; M, muscle tissue; N, notochord; SB, swim bladder; UB, urinary bladder.

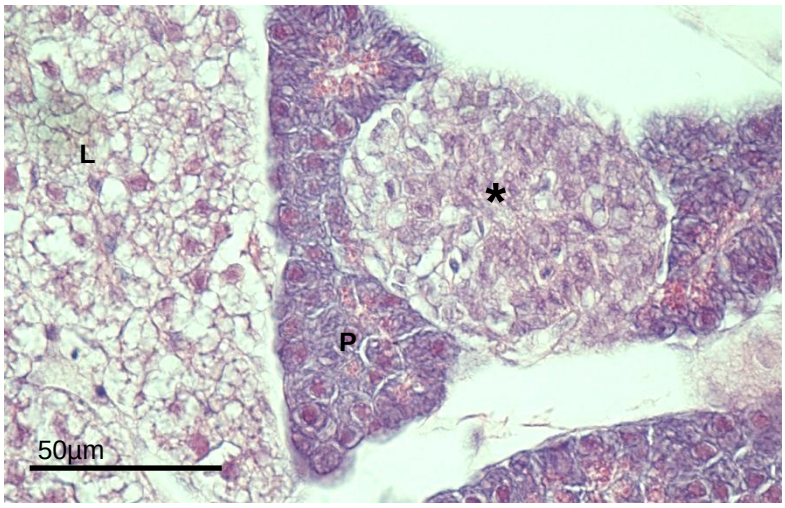

Figure 3.13
Longitudinal section of liver (L), and endocrine (*) and exocrine (P) pancreatic tissue in ballan wrasse larvae 21 dph (Cop7 treatment). HE staining, 63x magnification.

3.3.2 Organ growth

At 4 dph the total mean volume of tissue (V_T) in the fish larvae measured 0.075 ± 0.003 mm^3 (table 3.2). The larvae fed copepods more than doubled their total V_T by 8 dph, and had significantly more tissue mass than the RotMG and RotChl larvae (figure 3.14). The specific growth rate of the whole larval V_T (SGR_T) between 4 and 8 dph was above 20 % daily for the ones fed copepods (table 3.3). This was about twice as high as the RotChl larvae, and over four times higher than the RotMG larvae. By 21 dph the Copepod larvae had a V_T of 1.522 ± 0.217 mm^3 for the larva as a whole, which was significantly larger than the larvae fed rotifers (figure 3.14 and table 3.2). There was no significant difference in the total V_T between the Cop7, RotMG and RotChl larvae at 21 dph, it being respectively 0.693, 0.592 and 0.683 mm^3.

The intestine, liver and pancreas had a V_T of respectively 0.0038, 0.0012 and 0.0005 mm^3 at 4 dph, and were, together with heart and gills, the smallest organs in the fish larvae (table 3.2). Between 4 and 8 dph, the liver, pancreas, gills and heart were the organs increasing in volume the fastest, having a SGR_T between 30 and 40 % for larvae fed copepods and close

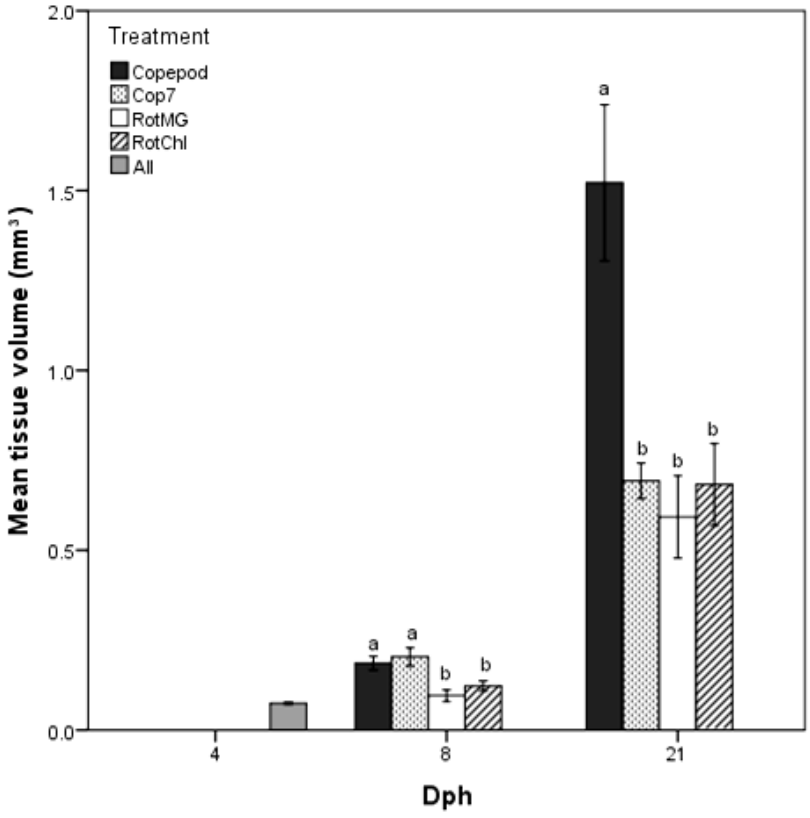

Figure 3.14
Mean volume per larva of all tissue in ballan wrasse larvae at 4, 8 and 21 days post hatch. Significant differences between treatments are indicated by letters (n=6). Error bars: ±1 SE.

to 20 % for RotChl larvae (table 3.3). The RotMG larvae experienced less growth than larvae from the other treatments during this period, which resulted in lower SGR_T values for these organ groups. The SGR_T for the liver and pancreas was higher in the Copepod larvae from 8 to 21 dph than in larvae from the other treatments, while the SGR_T for intestine, heart and gills were similar for Copepod, RotMG and RotChl larvae. At 21 dph, the Copepod larvae had two to three times larger V_T of intestine, liver and pancreas than larvae fed rotifers, however the difference was only significant for the intestine (table 3.2). The liver increased on average 53 times and the pancreas 51 times in V_T from 4 to 21 dph in larvae from the Copepod treatment. To compare, larvae fed rotifers increased the volume of the liver 14-18 times and the pancreas 12-14 times during the same period. The heart and gills were of significantly larger V_T in the Copepod larvae than the larvae fed rotifers at 21 dph. The different organs SGR_T were generally lower for the Cop7 larvae than for larvae from the other treatments after transition to enriched rotifers (table 3.3).

Muscle and CNS were the two largest organs in the fish larva, with a V_T of 0.0142 and 0.0151 mm^3 respectively at 4 dph (table 3.2). By 21 dph the Copepod larvae had on average increased their muscle V_T to 0.3888 mm^3, a 27 times increase from 4 dph. The Cop7 and RotMG larvae on average increased their muscle mass 8 times by 21 dph, while the RotChl larvae increased 11 times in muscle mass, ending up with a volume of muscle tissue of 0.1217, 0.1182 and 0.1562 mm^3 respectively. The SGR_T of muscle and CNS were higher when the larvae were fed copepods, the difference being more prominent in the period from 4 to 8 dph than from 8 to 21 dph (table 3.3).

In general, the Copepod larvae had higher SGR_T values for all the different organ types for the period 4 to 21 dph compared to Cop7, RotMG and RotChl larvae. The difference was most prominent for the intestine, liver, pancreas, muscle, heart and gills.

Table 3.2
The mean volume per larvae of different organs ± standard error of ballan wrasse larvae for the different start feeding regimes on 4, 8 and 21 dph. Significant differences between treatments at the same sampling day are indicated by letters (n=6). CNS = brain + spinal cord

			Volume of organ tissue (mm^3)									
dph	**Treatment**	**Total volume (mm^3)**	**Intestine**	**Liver**	**Pancreas**	**Heart**	**Gills**	**Muscle**	**CNS**	**Eye**	**Notochord**	**Other**
4	All	0.075 ± 0.003	0.0038 ± 0.0003	0.0012 ± 0.0002	0.0005 ± 0.0001	0.0004 ± 0.0001	0.0008 ± 0.0001	0.0142 ± 0.0015	0.0151 ± 0.0005	0.0062 ± 0.0006	0.0048 ± 0.0006	0.0275 ± 0.0010
8	Copepod	0.186 ± 0.019^{a}	0.0112 ± 0.0011^{ab}	0.0050 ± 0.0007	0.0018 ± 0.0003^{a}	0.0013 ± 0.0001^{a}	0.0035 ± 0.0004^{a}	0.0402 ± 0.0051^{a}	0.0295 ± 0.0031^{ac}	0.0149 ± 0.0011^{a}	0.0109 ± 0.0011^{ac}	0.0681 ± 0.0067^{a}
	Cop7	0.204 ± 0.025^{a}	0.0135 ± 0.0022^{a}	0.0063 ± 0.0014	0.0019 ± 0.0004^{ab}	0.0012 ± 0.0002^{a}	0.0030 ± 0.0003^{ab}	0.0413 ± 0.0059^{a}	0.0335 ± 0.0038^{a}	0.0154 ± 0.0011^{a}	0.0129 ± 0.0008^{a}	0.0745 ± 0.0096^{a}
	RotMG	0.095 ± 0.016^{b}	0.0059 ± 0.0015^{b}	0.0025 ± 0.0009	0.0006 ± 0.0001^{b}	0.0005 ± 0.0001^{b}	0.0022 ± 0.0003^{b}	0.0195 ± 0.0039^{b}	0.0164 ± 0.0029^{b}	0.0090 ± 0.0007^{b}	0.0071 ± 0.0014^{b}	0.0316 ± 0.0046^{b}
	RotChl	0.123 ± 0.014^{b}	0.0072 ± 0.0011^{b}	0.0033 ± 0.0008	0.0011 ± 0.0001^{ab}	0.0008 ± 0.0002^{ab}	0.0022 ± 0.0002^{b}	0.0244 ± 0.0032^{b}	0.0209 ± 0.0023^{bc}	0.0115 ± 0.0006^{b}	0.0085 ± 0.0007^{bc}	0.0431 ± 0.0047^{b}
21	Copepod	1.522 ± 0.217^{a}	0.0848 ± 0.0115^{a}	0.0638 ± 0.0123	0.0254 ± 0.0052	0.0135 ± 0.0017^{a}	0.0389 ± 0.0049^{a}	0.3888 ± 0.0704^{a}	0.2293 ± 0.0303^{a}	0.0933 ± 0.0099^{a}	0.0452 ± 0.0053^{a}	0.5389 ± 0.0748^{a}
	Cop7	0.693 ± 0.495^{b}	0.0433 ± 0.0056^{b}	0.0220 ± 0.0014	0.0067 ± 0.0008	0.0058 ± 0.0008^{b}	0.0157 ± 0.0017^{b}	0.1217 ± 0.0073^{ab}	0.1309 ± 0.0108^{b}	0.0586 ± 0.0055^{b}	0.0286 ± 0.0034^{b}	0.2594 ± 0.0181^{b}
	RotMG	0.592 ± 0.114^{b}	0.0456 ± 0.0090^{b}	0.0170 ± 0.0037	0.0061 ± 0.0015	0.0059 ± 0.0010^{b}	0.0163 ± 0.0046^{b}	0.1182 ± 0.0239^{b}	0.1078 ± 0.0226^{b}	0.0445 ± 0.0083^{b}	0.0259 ± 0.0039^{b}	0.2051 ± 0.0418^{b}
	RotChl	0.683 ± 0.114^{b}	0.0637 ± 0.0129^{ab}	0.0203 ± 0.0037	0.0070 ± 0.0014	0.0068 ± 0.0014^{b}	0.0181 ± 0.0030^{b}	0.1562 ± 0.0322^{ab}	0.1207 ± 0.0187^{b}	0.0528 ± 0.0067^{b}	0.0264 ± 0.0025^{b}	0.2108 ± 0.0350^{b}

Table 3.3
The daily specific tissue growth rate (% increase in tissue) of different organ systems in ballan wrasse larvae for the different start feeding regimes (n=6). Numbers are based on volume measurements from 4, 8 and 21 dph. CNS = brain + spinal cord

		Specific growth rate of organ tissue (% day^{-1})										
Dph	**Treatment**	**Total**	**Intestine**	**Liver**	**Pancreas**	**Heart**	**Gills**	**Muscle**	**CNS**	**Eye**	**Notochord**	**Other**
4-8	Copepod	22.2	26.6	37.8	31.5	32.1	35.0	25.5	15.9	22.0	21.3	22.0
	Cop7	24.1	29.7	40.2	31.4	28.0	31.3	25.5	19.0	22.8	25.9	23.8
	RotMG	4.7	7.0	11.5	5.2	3.1	23.3	6.5	0.4	9.5	9.1	2.3
	RotChl	11.9	14.8	24.2	20.4	18.1	24.0	13.0	7.3	15.8	15.4	10.6
8-21	Copepod	16.0	15.4	19.0	20.0	17.6	18.6	17.2	15.8	14.0	10.9	15.8
	Cop7	9.7	9.4	11.0	10.4	12.2	12.7	8.8	10.6	10.2	5.9	9.9
	RotMG	13.4	16.2	16.8	16.2	19.8	13.8	13.5	13.9	11.8	10.2	13.9
	RotChl	12.7	16.1	14.5	13.5	16.1	15.5	13.5	13.1	11.4	8.6	11.7
4-21	Copepod	17.5	18.0	23.4	22.7	21.0	22.4	19.2	15.8	15.9	13.4	17.3
	Cop7	13.1	14.1	17.9	15.4	15.9	17.1	12.7	12.6	13.2	10.6	13.1
	RotMG	11.6	14.1	15.5	13.6	15.9	16.1	11.9	10.7	11.2	9.9	11.1
	RotChl	12.5	15.8	16.8	15.1	16.6	17.5	13.4	11.8	12.4	10.2	11.5

3.3.3 Relative volume of different organ systems

The organs associated with digestion, intestine, liver and pancreas, made up a small proportion of the whole larval V_T at 4 dph (figure 3.15 – 3.18). The intestine was proportionally the largest of them, with a relative volume (RV) (% of total V_T) of 5.1 % (table 3.4). By 21 dph, the intestine made up 5.6 and 6.2 % of Copepod and Cop7 larvae total V_T respectively, which was a significantly smaller proportion than the 7.8 and 8.9 % of the RotMG and RotChl larvae. Larvae from all treatments had a significant increase in the relative liver volume from 4 to 21 dph (figure 3.15 – 3.18), which went from on average representing 1.5 % of the whole larval V_T at 4 dph, to 4.1 % in the Copepod larvae and 3.2, 2.8 and 3.0 % in the Cop7, RotMG and RotChl larvae on 21 dph (table 3.4). Copepod larvae had a significant increase in RV of pancreatic tissue from 8 to 21 dph, which was not observed in larvae from any of the other treatments (figure 3.15).

A significant increase in RV of heart tissue was observed from 8 to 21 dph in all treatments (figure 3.15 – 3.18). At 8 dph it represented 0.5-0.7 % of the larval total V_T, while the proportion had increased to 0.8-1.0 % for all treatments by 21 dph (table 3.4). The RV of gills increased in all treatments from 4 dph to 21 dph, representing 2.6 % of the Copepod, RotMG and RotChl larvae total V_T and 2.2 % of the Cop7 larvae total V_T at 21 dph. No significant difference in RV of heart and gills were observed between the treatments.

Muscle and CNS were the two largest organ groups in the fish larvae at 4, 8 and 21 dph, making up the largest proportion of the total V_T (table 3.4). The RV of nervous tissue was 20.5 % at 4 dph, and had decreased significantly in all treatments by 8 dph (figure 3.15 – 3.18). At 21 dph a significant difference in RV of nervous tissue was observed between Copepod larvae, where the CNS represented 15.3 % of the total V_T, and Cop7, RotMG and RotChl larvae, where the CNS represented 18.8, 17.7 and 18.0 % of the total V_T respectively (table 3.4). Close to 20 % of the larvae total V_T was muscle tissue at 4, 8 and 21 dph, with exception of Copepod larvae at 21 dph: The proportion of musculature in the Copepod larvae increased significantly from 8 to 21 dph, and at 21 dph muscle tissue represented 24.9 % of the whole Copepod larvae V_T which was a significantly higher proportion than in larvae from the other treatments (table 3.4).

The eyes had a relative volume of 8.4 % at 4 dph (table 3.4). The RV stayed the same for larvae from the Cop7, RotMG and RotChl treatment up to 21 dph, while eye tissue at the same time on average represented 6.3 % of the Copepod larvae total V_T. A significant decrease in relative volume of notochord was observed for larvae from all treatments from 8 to 21 dph, representing respectively 3.1, 4.1, 4.7 and 4.3 % of the Copepod, Cop7, RotMG and RotChl larval total V_T (figure 3.15 – 3.18).

Table 3.4
The mean relative volume of organ tissue (% of the total volume of all body tissue) ± standard error for the different start feeding regimes of ballan wrasse at 4, 8 and 21 dph. Significant differences between groups at the same sampling day are denoted by letters (n=6). CNS = brain + spinal cord

		Mean relative volume of total volume (%)									
Dph	**Treatment**	**Intestine**	**Liver**	**Pancreas**	**Heart**	**Gills**	**Muscle**	**CNS**	**Eye**	**Notochord**	**Other**
4	All	5.1 ± 0.3	1.5 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	1.1 ± 0.1	18.8 ± 1.3	20.5 ± 1.2	8.4 ± 0.9	6.3 ± 0.6	37.0 ± 0.6
8	Copepod	6.0 ± 0.3	2.6 ± 0.1	0.9 ± 0.1	0.7 ± 0.0	1.9 ± 0.1	21.2 ± 0.6	15.8 ± 0.3	8.2 ± 0.4	5.9 ± 0.2	36.7 ± 0.7^{a}
	Cop7	6.4 ± 0.4	2.9 ± 0.4	0.9 ± 0.1	0.6 ± 0.1	1.5 ± 0.1	19.9 ± 1.0	16.6 ± 0.3	7.9 ± 0.6	6.8 ± 0.8	36.6 ± 0.5^{a}
	RotMG	5.8 ± 0.7	2.2 ± 0.5	0.7 ± 0.1	0.5 ± 0.1	2.5 ± 0.3	20.0 ± 0.7	17.1 ± 0.3	10.1 ± 0.8	7.4 ± 0.6	33.7 ± 0.9^{b}
	RotChl	5.8 ± 0.4	2.5 ± 0.4	0.9 ± 0.1	0.6 ± 0.1	1.8 ± 0.1	19.5 ± 0.6	17.0 ± 0.5	9.7 ± 0.6	7.1 ± 0.4	35.1 ± 0.5^{ab}
21	Copepod	5.6 ± 0.5^{a}	4.1 ± 0.6	1.6 ± 0.2	0.9 ± 0.0	2.6 ± 0.1	24.9 ± 1.2^{a}	15.3 ± 0.7^{a}	6.3 ± 0.4	3.1 ± 0.3^{a}	35.6 ± 0.6^{a}
	Cop7	6.2 ± 0.6^{a}	3.2 ± 0.3	1.0 ± 0.1	0.8 ± 0.1	2.2 ± 0.2	17.7 ± 0.4^{b}	18.8 ± 0.5^{b}	8.4 ± 0.4	4.1 ± 0.3^{ab}	37.5 ± 0.8^{a}
	RotMG	7.8 ± 0.5^{b}	2.8 ± 0.1	1.0 ± 0.2	1.0 ± 0.1	2.6 ± 0.4	19.7 ± 0.8^{bc}	17.7 ± 0.8^{b}	8.1 ± 1.0	4.7 ± 0.4^{b}	34.6 ± 1.1^{a}
	RotChl	8.9 ± 0.6^{b}	3.0 ± 0.2	1.0 ± 0.1	1.0 ± 0.1	2.6 ± 0.1	21.9 ± 1.3^{c}	18.0 ± 0.5^{b}	8.2 ± 0.6	4.3 ± 0.6^{ab}	31.1 ± 0.9^{b}

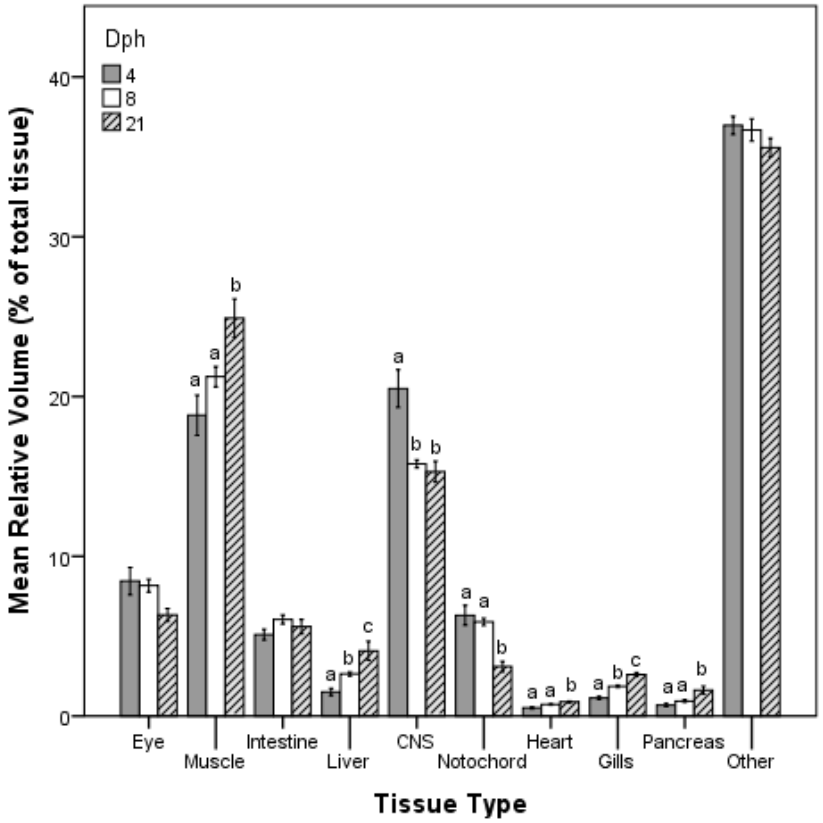

Figure 3. 15
The mean relative organ volume (% of the total volume of all body tissue) at 4, 8 and 21 dph for larvae from the Copepod treatment. Significant differences between days are denoted by letters (n=6). Error bars: ± 1 SE. CNS = brain + spinal cord

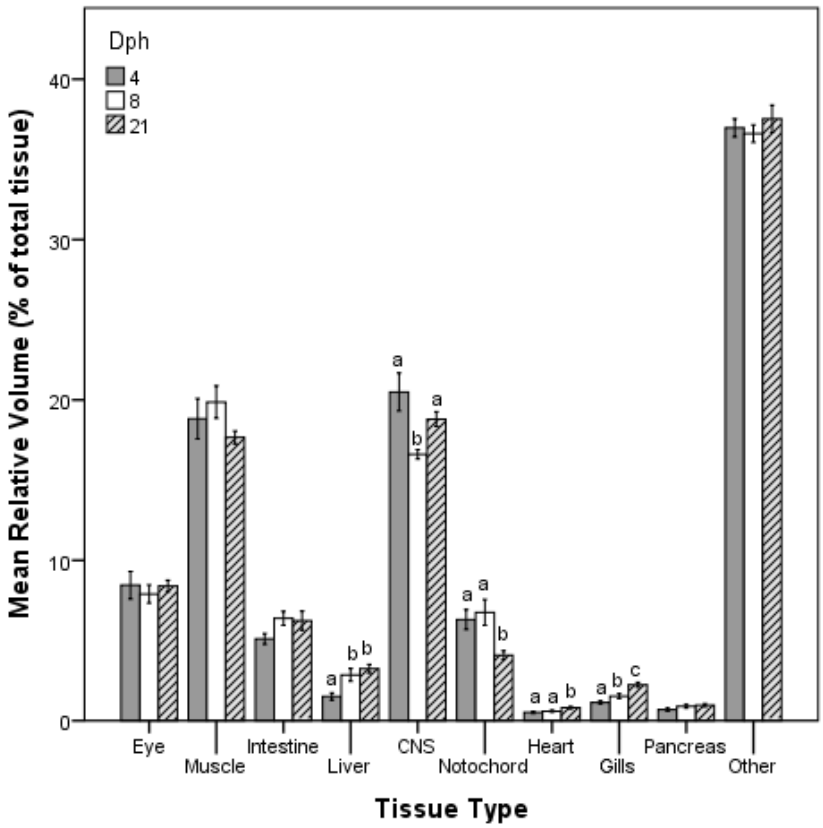

Figure 3.16
The mean relative organ volume (% of the total volume of all body tissue) at 4, 8 and 21 dph for larvae from the Cop7 treatment. Significant differences between days are denoted by letters (n=6). Error bars: ± 1 SE. CNS = brain + spinal cord

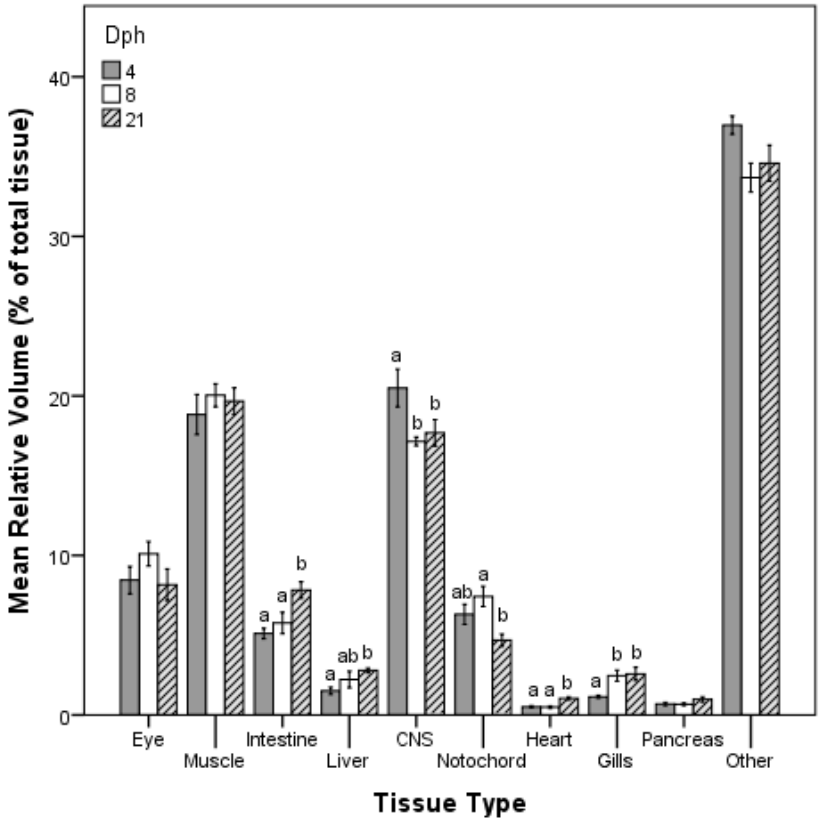

Figure 3.17
The mean relative organ volume (% of the total volume of all body tissue) at 4, 8 and 21 dph for larvae from the RotMG treatment. Significant differences between days are denoted by letters (n=6). Error bars: ± 1 SE. CNS = brain + spinal cord

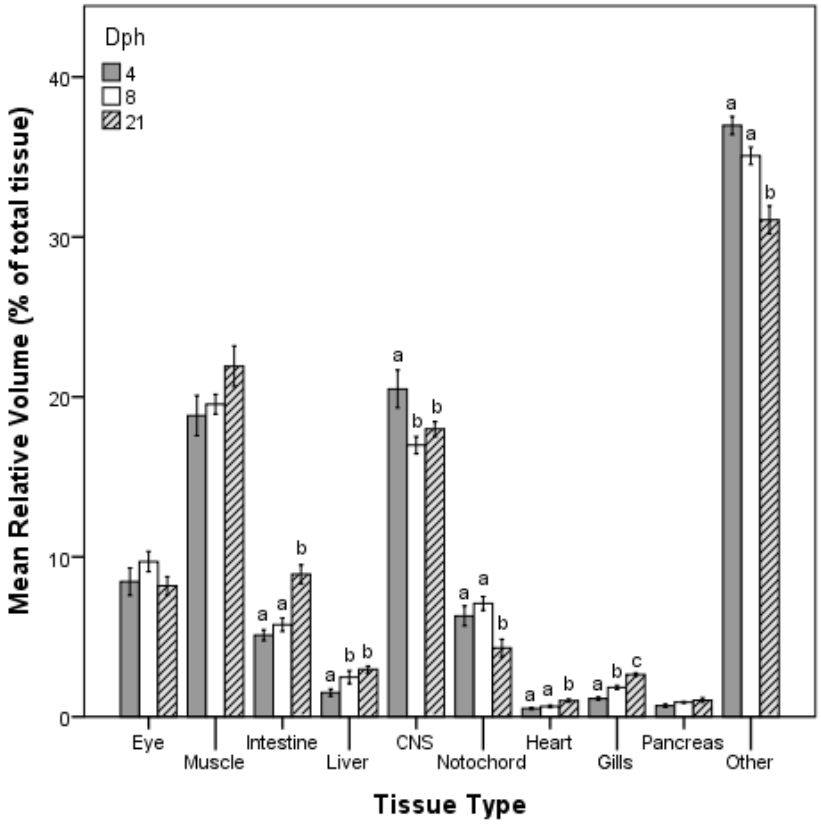

Figure 3.18
The mean relative organ volume (% of the total volume of all body tissue) at 4, 8 and 21 dph for larvae from the RotChl treatment. Significant differences between days are denoted by letters (n=6). Error bars: ± 1 SE. CNS = brain + spinal cord

3.3.4 Growth coefficients

The total V_T was correlated to the larval SL in all treatments (Pearsons correlation, $p<0.01$, 2-tailed), and when trend lines were applied the relationship was the same for the different treatments (figure 3.19). The correlation of the total V_T was also tested for all the organ groups with similar results (Appendix 10). Volume data from all the different treatments was therefore pooled for the calculation of growth coefficients.

There was a positive allometric increase in total V_T with increasing larval standard length with a growth coefficient (g) of 5.48. The $\log_{10}$-transformed data were following a linear relationship (figure 3.20).

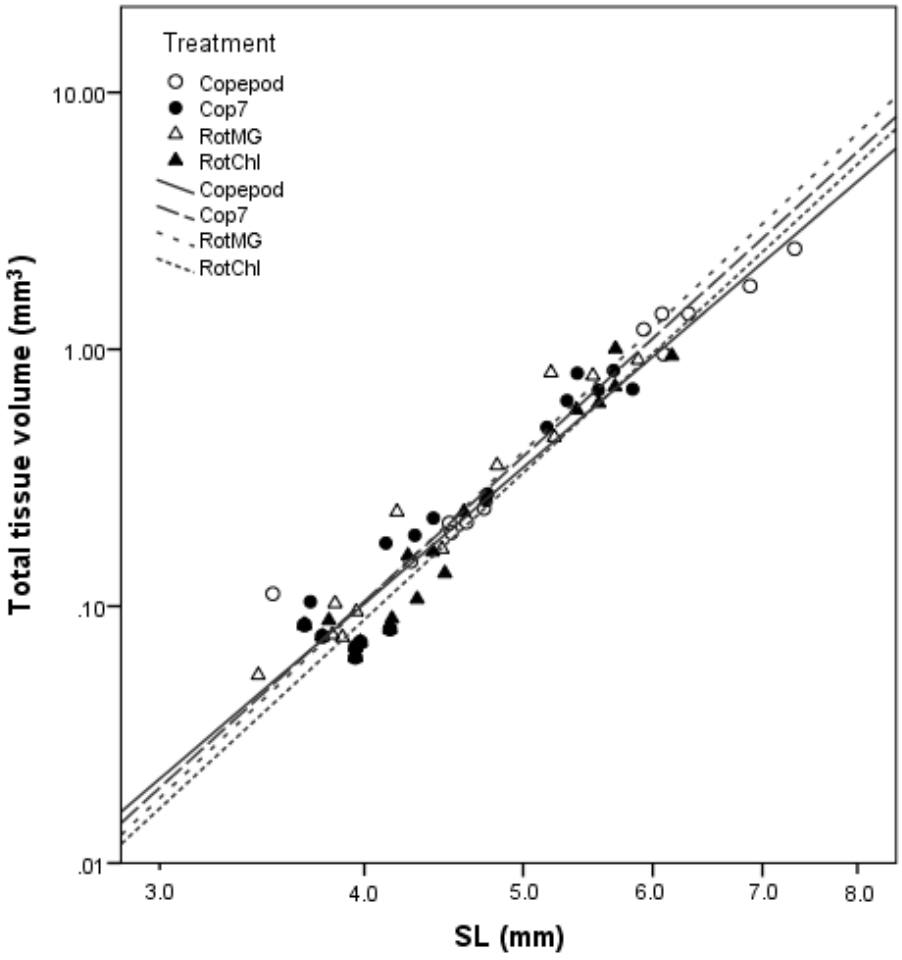

Figure 3.19
The relationship between standard length (SL) and total volume of all tissue (mm3) in ballan wrasse larvae from four different start feeding regimes. The data are sampled on 4, 8 and 21 dph, and each point represents values from individual larvae. The correlation coefficients were 0.974, 0.958, 0.955 and 0.971 for the Copepod-, Cop7-, RotMG- and RotChl-larvae respectively (Pearson correlation, $p<0.01$, 2-tailed).

A positive allometric growth was observed for the digestive organs from 4 dph (g > 1) (figure 3.21 A-C). The intestine had a growth coefficient of 1.48, and a point of inflexion occurred when the fish larval V_T reached 0.112 mm^3, or a standard length of roughly 4.0 mm (table 3.5). The standard length was estimated by using the equation $y = 5.49*10^{-5}x^{5.48}$ (figure 3.20), where y is the V_T (mm^3) and x is the standard length of the ballan wrasse larvae. The inflexion point of the liver was also located around 4.0 mm (table 3.5), where it changed from having a highly positive allometric growth with a growth coefficient of 2.62, to a growth coefficient close to isometric (g = 1.13). The intestine had an isometric growth after reaching the inflexion point. The pancreatic tissue had a growth coefficient of 1.21 up to the V_T of 0.812 mm^3, which equals approximately 5.8 mm. After this, the growth coefficient increased to 1.87, and the allometric growth became more positive.

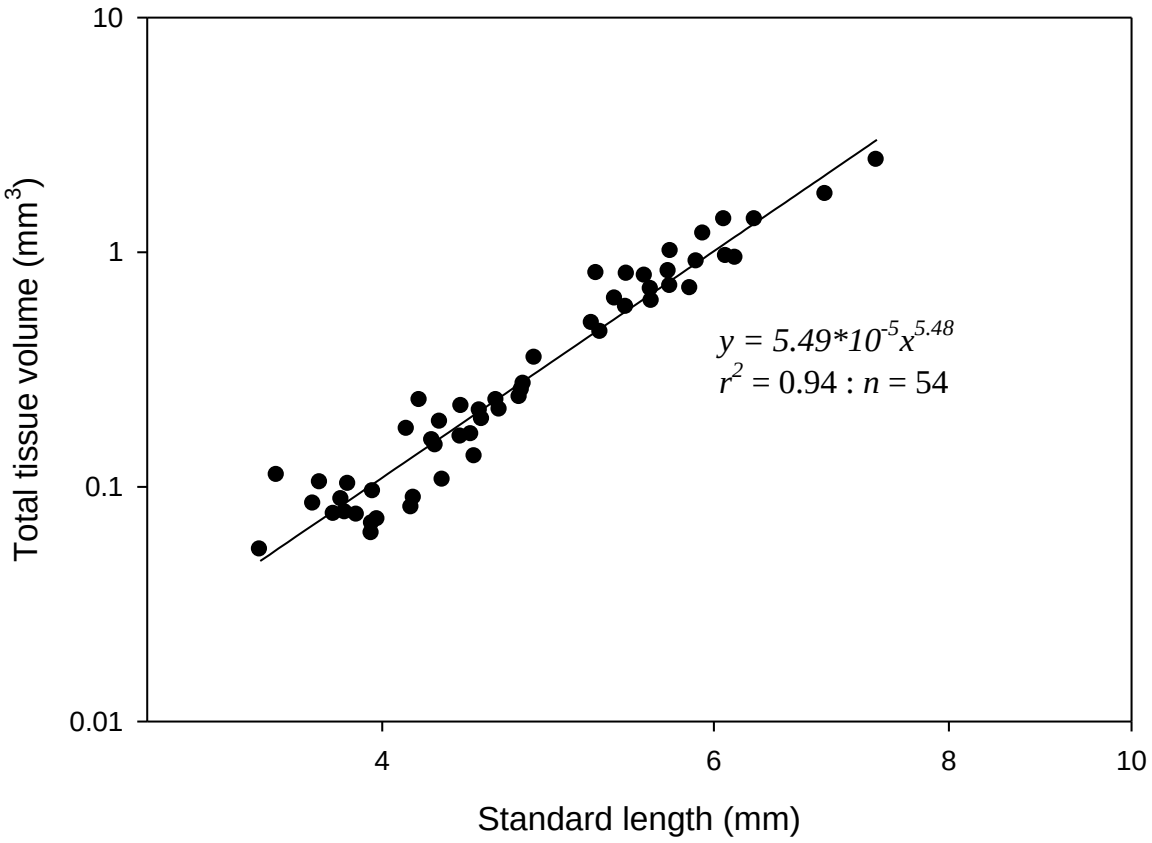

Figure 3.20
The relationship between the volume of all tissue and standard length in ballan wrasse larvae plotted on a logarithmic x- and y-axis, with each point representing values from individual larvae. Data from different treatments are pooled. A growth coefficient (g) of 5.48 indicates a positive allometric growth in volume (g > 3). Data collected from 4, 8 and 21 days old fish larvae. Correlation is tested with Pearson correlation test ($p<0.05$, 2-tailed).

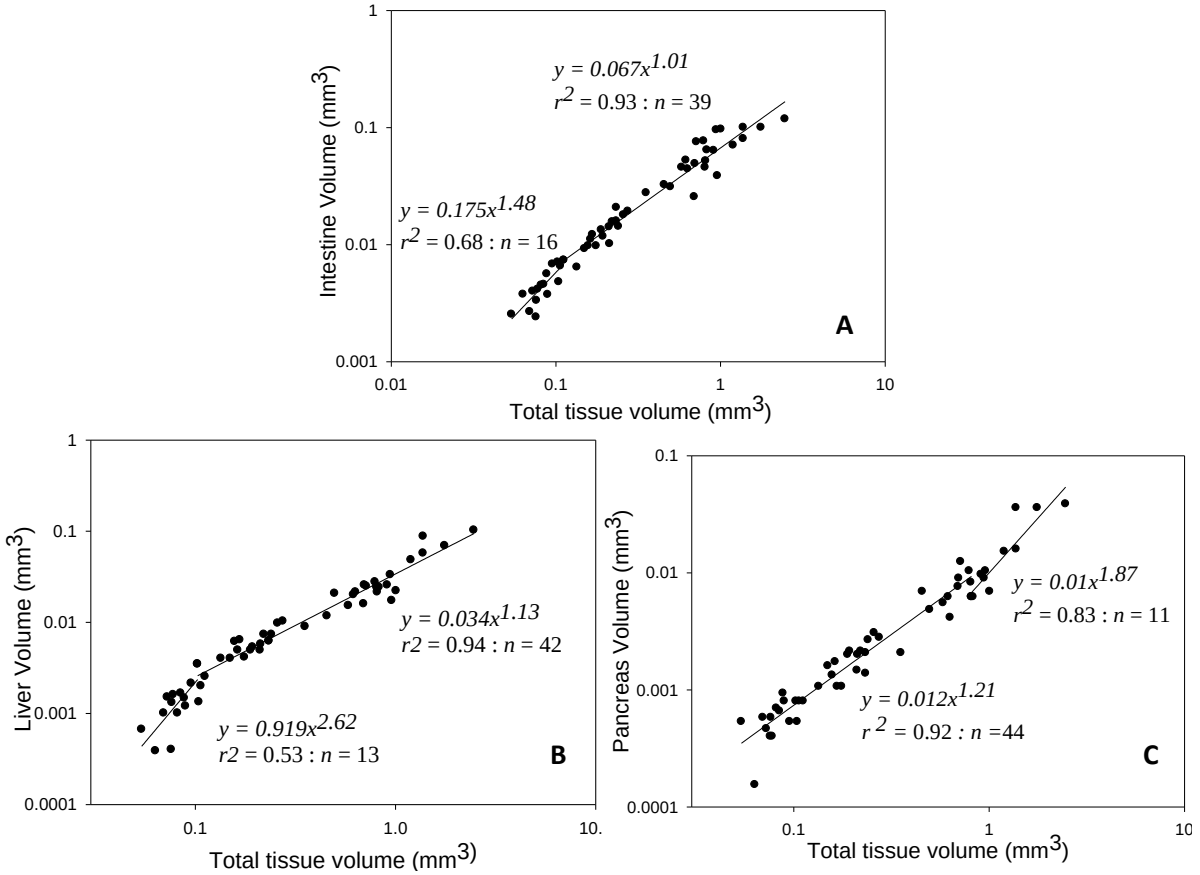

Figure 3.21

Allometric growth equations and relationship between the total volume of ballan wrasse and the volume of organs associated with digestion during early stages of development, plotted on logarithmic scales. Both the intestine (A), liver (B) and pancreas (C) had positive allometric growth in early stages, with growth coefficients of respectively 1.48, 2.62 and 1.21. Data from all treatments are pooled, each point is representing an individual larvae. Correlation is tested with Pearson correlation test ($p<0.05$, 2-tailed).

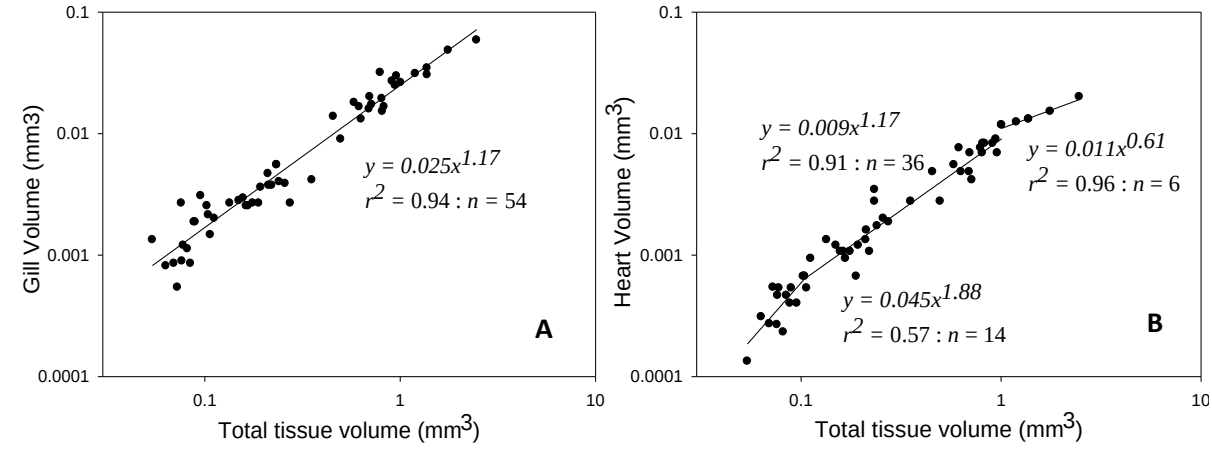

Figure 3.22

Allometric growth equations and relationship between the total volume of ballan wrasse and the volume of gills (A) and heart (B) during early stages of development, plotted on logarithmic scales. Gill development was constant and could be described by a linear function, while two inflexion points were detected for heart development. Data from all treatments are pooled, each point is representing an individual larvae. Correlation is tested with Pearson correlation test ($p<0.05$, 2-tailed).

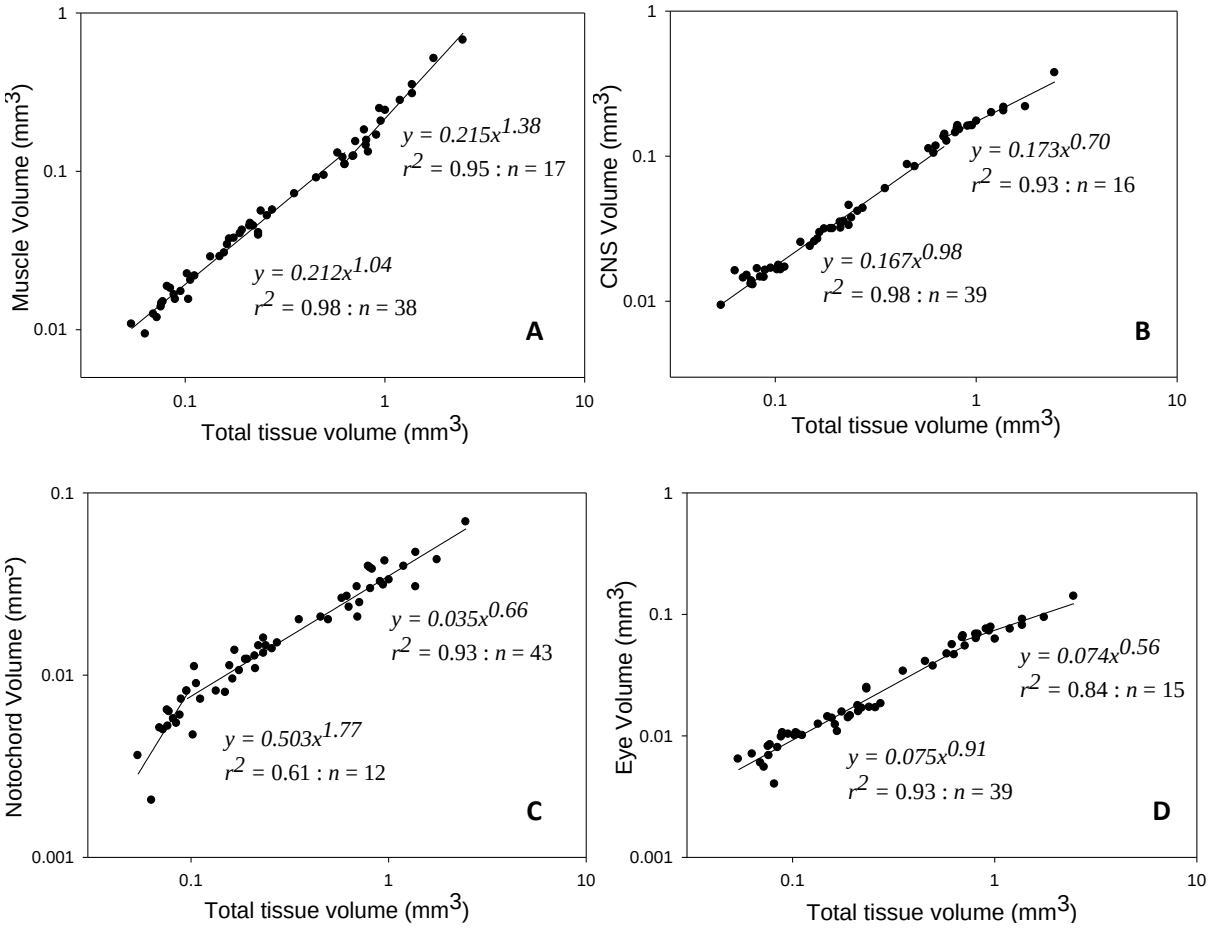

Figure 3.23
Allometric growth equations and relationship between the total volume of ballan wrasse and the volume of muscle tissue (A), nervous tissue (brain + spinal cord) (CNS) (B), notochord (C) and eye (D) during early stages of development, plotted on logarithmic scales. Data from all treatments are pooled, each point is representing an individual larvae. Correlation is tested with Pearson correlation test ($p<0.05$, 2-tailed).

The gills had a slightly positive allometric growth (g = 1.17), which was constant throughout the whole period (figure 3.22 A). The heart had three growth periods with different growth coefficients (figure 3.22 B). During early development up to approximately 4.0 mm (table 3.5), the larva had a highly positive allometric growth of heart tissue (g = 1.88). This was followed by a period of near isometric growth up to 6.0 mm, after which the growth became negatively allometric (g = 0.61).

Both muscle growth and growth of the CNS was nearly isometric during the earliest period, with a growth coefficient of 1.04 and 0.98 respectively (figure 3.23 A and B). The point of inflexion for muscle growth occurred when the V_T reached 0.6316 mm^3, or approximately 5.5 mm (table 3.5), after which there was a positive allometric growth (g = 1.38). The CNS had a negative allometric growth (g = 0.70) after the inflexion point around 5.6 mm. A highly

positive allometric growth of the notochord (g = 1.77) occurred from early on, but a negatively allometric growth (g = 0.66) takes place after the inflexion point is reached at approximately 3.9 mm (figure 3.23 C and table 3.5). The eyes had a negatively allometric growth during the whole period, with a slightly negative growth (g = 0.91) first, followed by highly negative growth (g = 0.56) at a SL of about 5.6 mm (figure 3.23 D and table 3.5).

Table 3.5
Summary of inflexion points of different organs in ballan wrasse larva. The inflexion points are given for larval total volume of tissue (V_T) (mm^3) and SL (mm), and the growth coefficients (g) ± standard error (SE) and r^2-values for the linear regressions used to determine g for the different organ groups are listed. The fish larva standard length (SL) at time of inflexion was determined by equation $y = 5.49*10^{-5}x^{5.48}$, where y is the volume (mm^3) and x is the SL (mm). A t-test was used to determine if the growth coefficients pre and post inflexion point (g_1 and g_2) were significantly different, and the correlation coefficient was determined for each line (Pearson correlation, $p<0.05$, 2-tailed). CNS = brain and spinal cord.

Organ group	Pre and post inflexion	g ± SE	r^2	V_T: Point of inflexion (mm^3)	SL: Point of inflexion (mm)	Significance between g_1 and g_2 (2-tailed)
Notochord	1	1.77 ± 0.448	0.61	0.095	3.9	0.0000
	2	0.66 ± 0.029	0.93			
Liver	1	2.62 ± 0.745	0.53	0.103	4.0	0.0007
	2	1.13 ± 0.045	0.94			
Heart	1	1.88 ± 0.471	0.57	0.104 1.007	4.0 6.0	0.0241 0.0009
	2	1.17 ± 0.062	0.91			
	3	0.61 ± 0.058	0.96			
Intestine	1	1.48 ± 0.271	0.68	0.112	4.0	0.0135
	2	1.01 ± 0.045	0.93			
Muscle	1	1.04 ± 0.026	0.98	0.632	5.5	0.0000
	2	1.38 ± 0.081	0.95			
CNS	1	0.98 ± 0.025	0.98	0.693	5.6	0.0000
	2	0.70 ± 0.050	0.93			
Eye	1	0.91 ± 0.042	0.93	0.693	5.6	0.0001
	2	0.56 ± 0.072	0.84			
Pancreas	1	1.21 ± 0.055	0.92	0.812	5.8	0.0004
	2	1.87 ± 0.281	0.83			
Gill	1	1.17 ± 0.040	0.94			Not significant

4. Discussion

4.1 Effects from first feeding ballan wrasse larvae with copepod nauplii on larval growth and mortality

The present study found that using intensively cultivated copepods as feed for ballan wrasse larvae during the first feeding period resulted in increased somatic growth compared to feeding with rotifers. This was observed when copepods were supplied only for a short period of time (4-10 dph) at the beginning of exogenous feeding, and when fed up until the *Artemia*-phase (4-30 dph). An immediate effect on the DW was evident at 8 dph, which continued for as long as the copepods were supplied through the diet. This was reflected in significantly higher % DWI values for the Copepod and Cop7 larvae: E.g. their increase was close to 15 % daily for the period 4 to 8 dph, compared to 2-5 % for the ones fed rotifers. Feeding with copepod also affected the SL and MH resulting in longer and thicker larvae, and the DW at the end of the experiment was found to be significantly higher for both the Copepod and Cop7 larvae compared to RotMG and RotChl larvae. Few studies are available on ballan wrasse larvae to compare the growth obtained during this study. Dunaevskaya (2010) measured the increase of SL on ballan wrasse larvae fed enriched rotifers (Red pepper). Compared with her results the SL measurements was similar up to 21 dph for larvae fed unenriched and enriched rotifers and for the Cop7 larvae, while the Copepod larvae had a greater growth. After 21 dph, the growth observed during the present study was higher regardless of treatment. Even though a part of this can be due to higher average temperature in our experiment (15 ºC versus 14 ºC), the larvae from all treatments appear to have had a good growth through the experimental period compared to the growth observed in Dunaevskayas study.

Increased larval somatic growth when feeding with copepods compared to other live feed has been reported for other species as well (Næss et al., 1995 ; Shields et al., 1999 ; Evjemo et al., 2003 ; Imsland et al., 2006 ; Eidsvik, 2010 ; Koedijk et al., 2010 ; Busch et al., 2011 ; Norheim, 2011). This has been attributed to a higher fraction of EFAs incorporated in the polar lipid fraction, and a greater amount of proteins and FAAs (Evjemo & Olsen, 1997 ; Bell et al., 2003 ; Evjemo et al., 2003 ; Tocher et al., 2008 ; van der Meeren et al., 2008). The superiority of dietary PLs is suggested to be caused by a greater ability to modulate phospholipase A2 expression than that of lipase in larval fish, which would give a more efficient capacity of utilizing PL than TAG (Cahu et al., 2009). Their presence is also found to enhance digestion of other lipids and to aid nutrient transport from the enterocytes by being a key component of lipoproteins (Coutteau et al., 1997 ; Tocher et al., 2008). Furthermore, EFAs appear to be utilized better when provided through the polar lipid fraction.

This has been observed for Atlantic cod and European sea bass, where EFA supplied through the PLs resulted in increased somatic growth, more developed digestive organs, earlier ossification and increased levels of DHA in the tissue (Gisbert et al., 2005 ; Kjørsvik et al., 2009 ; Wold et al., 2009).

Analysis of the live feed used in this experiment revealed a greater protein content in *A. tonsa* compared to the enriched and unenriched rotifers, and *A. tonsa* had the greatest amount of DHA and the largest DHA:EPA ratio. Based on observations from other studies (Evjemo & Olsen, 1997 ; van der Meeren et al., 2008), it is likely that the EFAs were incorporated in the copepods PLs. In a parallel study of the metabolites in the ballan wrasse larvae and different live feed taking part in this first feed experiment, the *A. tonsa* was found to contain both high levels of taurine and trimethylamine N-oxide (TMAO) compared to nothing in *B. Ibericus* (Martin Almli, pers. comm., thesis in prep.). These metabolites were also found to be present in a greater amount in the larvae fed copepods. Taurine has been proposed to stimulate increased growth rates in larval fish (Conceicao et al., 2010 ; Pinto et al., 2010), and providing TMAO through the diet had a positive effect on the growth performance of swine (Overland et al., 1999). The combination of higher levels of taurine and TMAO may, together with the greater amount of proteins and a more suitable FA composition, have contributed to the higher growth rates observed while copepods were fed to the larvae. While temperature is known to affect larval growth (Blaxter, 1991), the small difference in day degrees between the larval tanks are unlikely to have had an impact during this experiment. In addition, the rotifers and copepods used in the present study were of equal size (Nesse, 2010 ; Penglase et al., 2010), which excludes an impact of difference in feed size affecting the growth (Busch et al., 2011). This leaves variation in the live feeds' nutritional composition, combined with its ability to stimulate a feeding response, as a likely reason for the observed differences in growth.

While feeding with copepods had a positive effect on the larval somatic growth, changing feed from copepods to either rotifers or *Artemia* had a short-term negative effect on the larval growth. After transition to the *Artemia* phase the Copepod larvae had a significantly lower % DWI value compared to larvae from the other treatments, and in the DW figures this could be seen as a flattening of the growth curves which was not observed to the same extent for the other treatments. When Cop7 larvae switched feed from copepods to enriched rotifers, a similar reduction in growth was observed. Together, these incidents resulted in a gradual smoothing of the growth differences between all four treatments, leaving no significant difference in DW to be detected between the treatments at 40 and 47 dph.

During transition to *Artemia*, the negative effect observed on growth was more prominent for the Copepod larvae than larvae from the other treatments. This indicates an easier transition from rotifers to *Artemia* than from copepods to *Artemia*. A longer time may be needed to adapt when changing from a food source of high quality to a source of lower quality than when changing the other way around, and the Copepod larvae may have had trouble accepting the new food. Previous studies on Atlantic cod has observed the same tendency, where a short period of dietary change (22-36 dph) affected larval growth positively if changed from enriched rotifers to natural zooplankton, and negatively if the prey type changed *vice versa* (Koedijk et al., 2010). Dutton (1992) observed that prior experience to a prey type improved feeding success when the same prey was subsequently encountered, and a prolonging of the weaning period has previously given positive results on the growth of seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*) (Rosenlund et al., 1997). This suggests that an increased co-feeding period of copepods and *Artemia* during this experiment could have reduced the negative growth effect of switching feed by giving the larvae a longer period to adapt. Another possibility could be to skip the *Artemia* phase, and feed the larvae with copepods up to weaning.

After transition to formulated feed, higher growth rates were observed for Copepod and Cop7 larvae compared to larvae from the other treatments. Although the growth rates were not significantly higher, it resulted in a significantly higher DW for the Copepod and Cop7 larvae at 61 dph. This may be caused by a positive long term growth effect from receiving copepods as early feed, leading to an increased ability of utilizing formulated feed later on. Early diet has been found to have an effect on long term growth in Atlantic cod (Imsland et al., 2006 ; Koedijk et al., 2010), where fish in the groups first fed on zooplankton were 12-14 % larger than those first fed on rotifers after 17 months (Imsland et al., 2006). Imsland et al. proposed that this could be due to differences in the digestive tract caused by early diet, affecting the feeding capacity of the larvae. The Copepod larvae were of larger size (SL) at the time of weaning, which could have made the transition to formulated feed easier and affected the growth positively compared to larvae with a smaller SL. However, the positive effect was also observed for the Cop7 larvae, which were of similar length as the RotMG and RotChl larvae at the time of weaning. This makes it unlikely that a difference in larval length could have caused the observed differences in growth between the treatments after the transition to formulated feed. It is also uncertain whether the improved growth observed for the Copepod and Cop7 larvae post weaning would persist, and further research needs to be done before any long term effects from feeding with copepods can be determined.

Other studies have observed an improved growth in larvae fed enriched rotifers compared to larvae receiving unenriched rotifers (Øie et al., 1997 ; Baker et al., 1998 ; Copeman et al.,

2002). This was however not detected during this experiment. The nutritional composition of rotifers may vary depending on growth rate, type of feed and feeding ratio, in addition to whether or not they are enriched and the enrichment composition and procedure (Øie et al., 1994 ; Øie et al., 1997 ; Øie & Olsen, 1997 ; Copeman et al., 2002). Compared to the rotifers used in the studies mentioned above (Øie et al., 1997 ; Baker et al., 1998), the rotifers in the present study had a higher protein and a lower lipid content, both when enriched and not enriched. Furthermore, both the enriched and unenriched rotifers used in the present study had a similar total amount of lipids and proteins per 100 g DW. This may be a reason for the lack in growth difference observed between the RotMG and RotChl larvae, and why our study obtained other results than the studies mentioned above. In a similar first feeding experiment performed on Atlantic cod larvae, there were also found no difference in growth between those larvae fed enriched and those fed unenriched rotifers (Norheim, 2011). They used the same feed for their rotifer cultures (*Brachionus ibericus*) as during the present experiment (DHA Chlorella), and grew the cultures at similar densities. For Atlantic cod and ballan wrasse, this procedure may therefore obtain unenriched rotifers with a nutritional composition which is sufficient to sustain a larval growth similar as for larvae fed enriched rotifers. Even though the quantitative amounts of lipids and proteins were similar for the enriched and unenriched rotifers, the fatty acid content did vary with the enriched ones having a greater amount of EFAs and a higher DHA:EPA ratio. These EFA are located in the rotifers neutral lipid fraction (Rainuzzo et al., 1994a ; Rainuzzo et al., 1994b ; Nerhus, 2007), and may not have been available in a sufficient amount to sustain higher growth rates for the RotMG larvae compared to the RotChl larvae.

While no growth difference was obtained between the RotMG and RotChl larvae, a difference in survival was detected. This indicates that the inadequate nutritional value of the unenriched rotifers may affect other measurements criteria more negatively than the somatic growth of the larvae. At 61 dph, the survival ranged from 10 to 12 % in the Copepod, Cop7 and RotMG treatments, whereas a significantly lower survival was observed for the RotChl larvae compared to larvae from the other treatments, with only 5 % surviving to day 61 post hatch. The unenriched rotifers fed to the RotChl larvae were found to have the lowest amount of EFAs and DHA:EPA ratio of the analysed live feed. A deficiency in n-3 PUFA induces mortality (Izquierdo et al., 2000 ; Cahu et al., 2009), and the nutritional composition of the live feed may therefore have contributed to the difference observed in survival, as well as for the growth difference observed when feeding copepods as opposed to rotifers. The Copepod larvae were also found to tolerate stress significantly better than RotChl larvae in a parallel study performed on larvae from the present experiment. Fewer larvae died from the stress inflicted at 29 dph, and the live feed quality was suggested as an important factor for

the difference observed in mortality (Sørøy, 2012). Similar results were also observed in a first feeding experiment on Atlantic cod, where the larval response to handling stress at 37 and 58 dph was found to be affected by the treatment during the first 28 days, and not correlated to the SL. Larvae fed unenriched rotifers had the highest mortality after 24 hours followed by those fed enriched rotifers, while larvae fed copepods were the most hardy (Hansen, 2011).

During the first feeding experiment, most of the mortality occurred prior to 13 dph in all treatments and only 12-25 % of the larvae survived up to this point. Other factors beside the diet may have contributed to this early mortality, which emphasises the importance of a proper rearing environment during the early stages of development. The microbial environment in the rearing tanks has been suggested to have a big effect on the early mortality and growth of marine fish larvae (Skjermo et al., 1997 ; Alves et al., 1999 ; Skjermo & Vadstein, 1999). The specific immune system is still under development during the larval stage (Zapata et al., 2006), making the larvae vulnerable for infections. The poor results observed on survival and growth in the omitted Copepod parallel was visible from early on, which could point to a microbial infection. Other physical factors, such as heavy aeration or water flow, could also have contributed to an unfavourable rearing condition.

4.2 The effect of early live feed on ballan wrasse larval organ growth and development

No dietary effects were observed on the V_T of the different organ tissues investigated, and the growth correlated to the larval SL. So even though the different live feed affected the larval rate of the growth, once larvae from the different treatments had reached the same size, the organs made up a similar proportion of total V_T. In a study conducted by Wold et al. (Wold et al., 2009), the size of the liver and intestine of Atlantic cod was also found to relate to the larval size, and were not affected by different levels of neutral and polar lipids in the diet. The ontogeny of the ballan wrasse larval digestive system seemed to follow similar patterns as has been described previously in other marine fish species (Blaxter, 1988 ; Kjørsvik et al., 1991 ; Segner et al., 1994 ; Gisbert et al., 2004 ; Santamaria et al., 2004), and little variation in the tissue structure was observed between the different treatments. The alimentary canal was differentiated into buccopharynx, oesophagus, pre- and postvalvular intestine and rectum at 4 dph, which was also observed by Dunaevskaya (2010). The presence of supranuclear vesicles in the postvalvular intestine of larvae from all treatments at 8 dph indicated that pinocytotic activity and intracellular protein digestion took place, and that exogenous feed uptake occurred in all the studied larvae (Govoni et al., 1986). The

density of vesicles appeared to be reduced from 8 to 21 dph regardless of treatment, which might be connected to a decreasing intracellular digestion with the development of increased brush border membrane enzyme activity (Zambonino Infante & Cahu, 2001).

The measured results of V_T at 4, 8 and 21 dph coincided well with the larval DW measurements at the same days. By 21 dph, the total V_T of the Copepod larvae more than doubled that of larvae from the other treatments, and all organ groups investigated were of a larger volume. The higher total V_T observed for the larvae fed copepods was reflected by higher SGR_T values, both for the larvae as a whole and for the different organ groups. This difference was greatest from 4 to 8 dph, where organs as the liver, pancreas, heart and gills increased at a daily SGR_T between 30 and 40 % for larvae fed copepods. Osse and van den Boogaart (2004) proposed that the importance of the dietary and respiratory organs would result in them developing early, and that they would grow at a higher rate than the body as a whole. In accordance with this, the ballan wrasse larvae had a prioritised growth of the intestine, liver and heart at the transition to the mixed feeding period (both endo- and exogenous feeding), after which the growth became close to isometric, and positive allometric growth was also observed for the pancreatic tissue for the whole investigated period. Similar observations have previously been made for larvae of the common dentex and turbot, where the digestive organs had a fast relative growth during early development and the highest allometric growth coefficients of all organ systems studied (Sala et al., 2005). A prioritised volume increase of the pancreas and liver was also observed during early development of common carp larvae, where these organs, together with muscle tissue, were the only studied organs with positive growth coefficients (Alami-Durante, 1990).

The pancreas exhibited initial positive allometry in ballan wrasse larvae, which became even more positive up to the point where the histological investigations ended (21 dph). This sudden increase in pancreatic growth could be seen in the sections as a spreading of the pancreas throughout the abdominal cavity in the largest larvae at 21 dph. Most of these larvae belonged to the Copepod treatment, and a significant increase in RV of the pancreas was observed in this treatment from 8 to 21 dph. This was not observed for any of the other treatments. Acidophilic pancreatic zymogen granules were present at 4 dph, indicating that the exocrine pancreas was functional prior to first feeding. This is a common feature among marine fish larvae (Zambonino Infante & Cahu, 2001 ; Kjørsvik & Hoehne-Reitan, 2004), and has also been observed for ballan wrasse larvae previously (Dunaevskaya, 2010).

Due to the ballan wrasse lacking a stomach both as a larvae and an adult (Hamre & Sæle, 2011), protein hydrolysis into smaller units needs to be performed by alkaline proteases produced by the pancreas (Espe et al., 2001 ; Kjørsvik & Hoehne-Reitan, 2004). It is

therefore likely that the alkaline protease activity will continue to play an important role in the protein digestion also after the ballan wrasse larvae have metamorphosed. In common dentex and turbot larvae, which develop a functional stomach during the larval phase, the allometric growth of pancreatic tissue became negative early in the larval development (Sala et al., 2005). For turbot, this change from positive to negative allometric growth was found to coincide with proliferation of the gastric glands (Sala et al., 2005). Larvae of common carp, which similar to the ballan wrasse larvae lack a stomach, were found to have a continuous positive allometric growth of pancreatic tissue through the investigated larval period (Alami-Durante, 1990). Also, observation on the activity and production of alkaline proteases in whitefish coregonid hybrids (hybrids of *Coregonus wartmani* and *C. Lavaretus*), found that development of the stomach coincided with a lower activity and protease production. The stomachless roach (*Rutilus rutilus*) on the other hand were found to have a higher level of proteolytic activity (Lauff & Hofer, 1984). This emphasises the importance of pancreatic secretion in stomachless fish, and is likely the reason for the increased positive allometric growth observed when the ballan wrasse larvae reached a SL close to 5.8 mm.

In the present study, histological sections from the prevalvular intestine showed that the intestinal wall of the RotMG and RotChl larvae appeared to have increased less in height and looked less developed at 8 dph, when compared to the Copepod and Cop7 larvae. In addition, the intestinal folds appeared to be more developed in the larvae fed copepods. Measurements of the intestinal V_T from 8 dph support this observation, with the intestine being of greater volume in the larvae fed copepods. Suboptimal diets have been known to affect the enterocytes of the intestine, leading to a reduced height of the intestinal wall (Theilacker & Watanabe, 1989 ; Kjørsvik et al., 2011). However, it is likely that this difference in intestinal height and volume is due to the Copepod and Cop7 larvae being of greater size than the RotMG and RotChl larvae at the given time, rather a difference caused by the diet.

A thickening and increased folding of the intestinal epithelium, which appeared to be greater for the larvae fed copepods, has been found to coincide with enhanced membrane enzyme activity (Zambonino Infante & Cahu, 2001). The Copepod larvae also had the greatest V_T of intestine at 21 dph, and were the only treatment where all the larvae had a coiled intestine at this point. Coiling is caused by increased intestinal length, which would lead to an increased passage time of food (Blaxter, 1988). Together with the possibility of an enhanced membrane enzyme activity, this might imply that the digestive system matures at an earlier age in larvae fed copepods, caused by a more rapid larval growth.

The liver had a rapid growth from 4 to 8 dph in all treatments, and a significant increase in RV of liver was observed for Copepod, Cop7 and RotChl larvae for the same period. During histological investigations of the liver at 8 dph, a varying degree of vacuolisation and vacuole size was observed between different larvae. No apparent connections were discovered regarding the different treatments or the larval SL, however by 21 dph the difference in hepatocyte structure had disappeared. The period of mixed feeding and transition to exogenous food is a critical phase during fish larval development (Osse & van den Boogaart, 2004), and the low survival registered at 13 dph in the present study indicate that the mortality was high for the ballan wrasse larvae during this period. It might have been an individual variance in the amount of feeding and how quickly energy storages were deposited in the larvae. Such a difference in larval energy storage status might have caused the varying structure observed. Glycogen deposition was present in the liver cytoplasm in larvae from all treatments at 8 dph. Fish larvae have a high ability to mobilize energy stored as glycogen, which is a polymer of glucose (Hamre, 2001). High larval growth rates have been connected with having larger glycogen deposits in Atlantic cod larvae fed rotifers (Høvde, 2006). It was a tendency in the present study that the larvae with the greatest SL measurements at 8 dph, regardless of treatment, had among the largest degree of vacuolisation. These larvae would have had the highest growth rates, and according to the observations made by Høvde, this could indicate the presence of larger energy storages in these larvae.

Organs as the CNS and eyes went from having slightly negative to highly negative allometric growth through the investigated period. Other studies have also observed this negative allometry of nervous tissue and eyes through the larval development (Alami-Durante, 1990 ; Sala et al., 2005) and in adult fish (Oikawa et al., 1992 ; Schultz et al., 1999). Negative allometry of these organs is a well known feature in fish caused by the continuous growth throughout their lives (Kotrschal et al., 1998). Brain and eye tissue have a rapid development prior to hatching, and having highly developed sensory organs after hatching and at the time of first feeding is necessary for prey detection and capturing (Osse & van den Boogaart, 1999). Benevent (1971) (article on French, cited through Alami-Durante, 1990) proposed that tissues with prioritised development in prenatal life would experience the lowest relative growth speed after birth, which is in accordance with what was observed for the ballan wrasse larvae.

A high sensitivity towards the dietary EFA composition has been registered as nervous organs as the brain and eyes develop after hatching, and a deficiency has been found to effect the tissue development and larval vision (Bell et al., 1995 ; Furuita et al., 1998 ; Sargent et al., 1999 ; Shields et al., 1999). Feeding with zooplankton had a positive effect on

the number of rods in the retina of halibut larvae as opposed to feeding with *Artemia* (Shields et al., 1999), and larval herring (*Clupea harengus*) fed *Artemia* deficient in DHA experienced a loss of visual function resulting in less effective predation (Bell et al., 1995). Sørøy (2012) observed that ballan wrasse larvae from the present first feeding experiment were better predators and captured prey more efficiently if fed copepods during early development. Similar results have also been reported for Atlantic cod (Hansen, 2011). These findings may indicate that the first feed diet had an effect on the functionality of the sensory organs, or the interaction between these organs and the muscular tissue, which may inflict long term effects for the larvae. For a species where the intensive cultivation is dependent on the fish to be a good louse predator, determining whether the early diet has any long term effect on the ballan wrasse ability to capture prey should be of importance.

Tissue of muscle and CNS represented the major proportion of the ballan wrasse larval body tissue at all days investigated. Muscle accounted for close to 20 % of the total larval V_T at 4 dph, as did also the nervous tissue. This is similar to what is reported for common dentex and common carp. For these species, an increase in proportion of muscle and a decrease in proportion of nervous tissue were observed with increasing body mass (Alami-Durante, 1990 ; Sala et al., 2005). This was only observed for Copepod larvae during this study, which at 21 dph also had a significantly higher proportion of musculature and a significantly lower proportion of CNS compared to larva from the other treatments. The smaller size of the larvae from the other treatments may have accounted for this not being observed. Muscle growth first reached a higher positive allometric growth at a later stage of the development, when the larvae reached a SL of approximately 5.5 mm. While common carp and common dentex had an allometric growth of muscle tissue which was constantly slightly positive (k = 1.10) through the larval development (Alami-Durante, 1990 ; Sala et al., 2005), the turbot was found to have a similar biphasic growth pattern as was observed for the ballan wrasse. The growth, which initially was negatively allometric, changed to being positively allometric at a volume close to 1.0 mm^3 (Sala et al., 2005). This increased musculature growth were found to coincide with increased development of epiaxial and hypaxial musculature, resulting in a change in the growth pattern. In a parallel study on larvae from the same experiment, Copepod larvae were found to have a higher recruitment of muscle fibres, in addition to a larger degree of hyperplasia in existing fibres, compared to larvae from the other treatments at 21 dph (Martin Berg, pers. comm., thesis in prep.). The axial muscle cross section area (measured directly posterior to the gut) for the same day was found to be over twice as large for the Copepod larvae, and differed significantly from larvae from the other treatments. This is in accordance with the higher volume and muscle growth detected for the Copepod larvae during this study.

5. Conclusions

Using intensively reared *Acartia tonsa* nauplii as early live feed for the ballan wrasse larvae resulted in increased larval growth rates while the copepods were supplied through the diet compared to feeding with enriched or unenriched rotifers. It also resulted in larvae with a significantly higher DW at the end of the experimental period. This was observed when copepods were supplied only for a short period of time (4-10 dph) at the beginning of exogenous feeding, and when fed exclusively up until the *Artemia*-phase (4-30 dph). Higher organ volume growth rates were observed when copepods were used as larval feed, and the organ volumes were found to relate to the larval SL and not to the first feed diet. The Copepod larvae had a significantly higher proportion of musculature than larvae from the other treatments at 21 dph, and the intestine appeared to be more developed and mature. No difference in larval growth was observed when feeding with enriched or unenriched rotifers. Larvae fed unenriched rotifers did however have a significantly lower survival than larvae from all other treatments.

From these observations, copepods appear to have a nutritional composition more suited as early live feed for the rearing of ballan wrasse larvae compared to enriched and unenriched rotifers, resulting in increased larval somatic growth and organ growth, and earlier development and maturation of the intestine. Unenriched rotifers were the least suitable first feed, affecting the larval survival negatively. The higher DW observed for the Cop7 larvae at the end of the experiment indicate that feeding with copepods for a 7-days time period during early development may be sufficient to effect the growth positively. However, further research needs to be done to find a way to reduce the negative effect on growth observed when switching from copepods to another live feed.

At present, the availability of copepods is not adequate to support the amount that would be needed for a large scale intensive cultivation of ballan wrasse. There is need for a large-scale production of intensively cultured copepods, where the harvested eggs can be stored and shipped off to commercial buyers. The farmer could then hatch the needed amount in a similar manner as is common for the production of *Artemia* today. However, improvements of the *A. tonsa* culturing techniques must be made to make them more efficient, and a more predictable hatching rate is needed, before this can be commercially viable.

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Appendix 1

Feed production and feed recipe

The feed was produced at Nofima, Bergen, Norway. The ingredients were homogeneous mixed (Bjørn mixer) for a minimum of 20 minutes. The mixed ingredients were sieved through a sieve with a mesh opening of 0.6 mm (Allgaier 1200mm). The fraction with a particle size bigger than 0.6 mm was milled with a Retsch mill and thereby homogeneous mixed with the ingredients. The ingredient mixture was added 25 mg^{-kg} etoxiquin (0,05 g FEQ 500 per kg ingredient mixture). Etoxiquin was dissolved in 96 % ethanol and sprayed onto the mixture during continuous mixing. The feed was produced with a pilot scale twin-screw, co-rotating extruder (Wenger. The nozzle opening was 1.5 mm. After extrusion the diet was directly dried for 50-55minutes in a carousel dryer (GMBH) at 60 °C. Water content during drying was measured (HG 53 Halogen Moisture AnalyzerMettler Toledo). The feed was left overnight at ambient room temperature for cooling, before the feed was crushed/granulated on a Retsch mill and sieved (Allgaier) to the wanted particle sizes. The feed was packed in plastic bags and were stored at room temperature until transport.

Table A 1. Nutritional composition of ballan wrasse formulated feed

	% (DW)
Protein	60.1
Lipid	13.1
Carbohydrates	14.8
Ash	12.6

Table A 2. Recipe for ballan wrasse formulated feed

	% (WW)
Fish meal LT [a]	47,162
Shrimp meal[b]	24
Wheat[c]	17,8
Soy lecithin[d]	3
Cod Powder[e]	5
Betafin[f]	1,5
Vitamin mix[g]	0,31
Mineral mix[h]	0,52
Monosodiumphosphate (24% P)[i]	2
Carop. Pink (10%)[j]	0,03
Taurine[k]	0,2

[a]LT-Fishmeal, Karmsund Fiskemel AS, Norway
[b]Shrimp powder (7411), Seagarden AS, Avaldsnes Norway
[c]Wheat grain (510130), Norgesmøllene AS, Nesttun Norway.
[d]Soylecithin GMO powder (20022), Agrosom, Mölln Germany
[e]Cod fish powder, product code 0271, Seagarden, Avaldsnes Norway.
[f]Betafin S1, Danisco Animal Nutrition, Helsinki Finland.
[g] D3 3000 IE^{-kg}, E 160 mg^{-kg}, K3 20 mg^{-kg}, C 500 mg^{-kg}, B1 20 mg^{-kg}, B2 30 mg^{-kg}, B6 25 mg^{-kg}, B12 5 μg^{-kg}, B5 60 mg^{-kg}, Folic acid 10 mg^{-kg}, Niacin 200 mg^{-kg}, Biotin 1 mg^{-kg},
[h]Mn 30 mg^{-kg},, Mg 750 mg^{-kg}, Fe 60 mg^{-kg}, Zn mg^{-kg}, 120 mg^{-kg}, Cu 6 mg^{-kg}, K 800 mg^{-kg}, Se 0,3 mg^{-kg}.
[i]BOLIFOR® MSP, Yara AS, Norway
[j]Carophyll Pink (10 %),DSM, Basel Switzerland.
[k]Taurine, Sigma Aldrich

Appendix 2

Conwy medium

The algae medium is slightly modified from Walne, with the difference being a smaller amount manganese chloride than in the original recipe:

$NaNO_3$ (Sodium Nitrate)	100.0gr
Na-EDTA (EDTA disodium salt)	45.0gr
H_3BO_3 (Boric Acid)	33.6gr
$NaH_2PO_4 \cdot 2H_2O$ (Sodium Phosphate, monobasic)	20.0gr
$FeCl_3 \cdot 6H_2O$ (Ferric Chloride, 6–hydrate)	1.3gr
$MnCl_2 \cdot 4H_2O$ (Manganous Chloride, 4–hydrate)	0.136gr
Vitamin B_1 (Thiamin HCl)	0.1gr
Vitamin B_{12} (Cyanocobalamin)	0.05gr
Trace Metal Solution *	1ml
Distilled water	1 000ml

(Note: use 1 ml Conwy medium/litre of seawater)

Trace Metal Stock Solution *

$ZnCl_2$ Zinc Chloride	2.1gr
$CoCl_2 \cdot 6H_2O$ (Cobalt Chloride, 6–hydrate)	2.1gr
$(NH_4)_6Mo_7O_{24} \cdot 6H_2O$ (Ammonium Molybdate, 4–hydrate)	2.1gr
$CuSO_4 \cdot 5H_2O$ (Copper Sulphate)	2.0gr

Distilled water100ml

(Note: acidify with 1 M HCl until solution is clear)

Appendix 3

Estimation of hatching success (*Acartia tonsa* eggs) and amount of eggs

The hatching success of *A. tonsa* eggs stored cold (2 ºC, SANYO Pharmaceutical Refrigerator MPR-311D (H), Japan) was estimated one week prior to starting the experiment. The NUNC EasyFlask^{TM} Nunclon^{TM} cell culture bottles containing the eggs were shaken to get the eggs uniformly dispersed. By using a pipette, 50 µl of the sea water mixture were transferred to an eppendorph tube and diluted with sea water to 1 ml. 50 µl of this solution was further transferred from the eppendorph tube to a petri dish. The droplet were photographed with a stereo microscope (MZ-12.5, Leica Microsystems, Germany) equipped with a colour digital camera (DFW-SX900, Sony, Japan), and the number of eggs were counted. 10 mL sea water was added to the petri dish, before sealing it with parafilm and leaving the eggs to hatch for a period of 48 hours in constant light. After hatching, the tests were fixated using fytofix (Lugol's solution) and the hatching rate determined by counting the number of nauplii in the sample.

Each NUNC-flask was weighed. The volume (V) of sea water and eggs (cm^3) inside each individual flask was determined by subtracting the weight (g) of an empty NUNC-flask (B_0) from the weight (g) of a filled flask (B), and divide this by the density of sea water (1.028 g/cm^3)

$$V = (B - B_0) / 1.028$$

Number of eggs per cm^3 (X) was found during the hatching test, and by multiplying this with V, number off eggs inside each NUNC-flask (N) was estimated

$$N = X * V$$

Appendix 4

Paraffin embedding procedure

Standard length and myotome height were determined before the fixated fish went through a sequential process preparing it for embedding, starting with dehydration (using an increasing alcohol percent) before treatment with Tissue-clear (Tissue-Tek ® Tissue-Clear ® Xylene Substitute, Sakura, UK) and hot paraffin (Tissue-Tek® III Embedding wax, Sakura, UK). This process was automated (Leica TP1020, Leica Industries, Germany) and lasted 16 hours.

Paraffin wax were heated to keep it fluid (Leica EG1120, Leica Industries, Germany), and drained into a casting frame so that the bottom of the frame just were covered in paraffin. After positioning the fish correctly, the paraffin was cooled for a few seconds and hereby fixing the fish in its position. The frame was filled with more paraffin, covering the fish completely. Three fish were embedded in the same block, with fish number one orientated in the opposite direction from the others to keep them apart.

Appendix 5

HE-staining procedure

4 µm thick, longitudinal sections of ballan wrasse larvae (4, 8 and 21 dph)were stained with Mayer's hemalum solution (Merck, Germany) and Eosin Y-solution 0.5% aqueous (Merck, Germany) (HE-staining). Hematoxylin stains acidic structures a purplish blue, while eosin is an acidic dye which stains basic structures red or pink. Before staining, the sections were dried vertically over night at 37 ºC.

HE-staining sequence:

Tissue-clear	5 min
Tissue-clear	5 min
100 % ethanol	2 min
100 % ethanol	2 min
70 % ethanol	2 min
Distilled water	5 min
Mayer's Hematoxyline	3 min
Running tap water	3 min
Acid ethanol (1% HCl (12M) in 70% ethanol)	20 sec
0.5% Eosin	2 min
Tap water	dip
Distilled water	dip
70% ethanol	dip
100% ethanol	30 sec
100% ethanol	2 min
Xylen	2 min
Xylen	> 5 min

Cover slips were applied with Cytoseal^{TM} XYL (Richard Allan Scientific) and the sections were dried in a fume hood over night.

Appendix 6

Survival per tank

Table A 3. Measured survival for individual tanks at different days post hatch (dph). The numbers are corrected for sampled larvae and larvae accidentally removed during cleaning.

		% Survival at dph							
Treatment	**Tank**	**2**	**7**	**13**	**24**	**30**	**40**	**50**	**61**
Copepod	1	100.00	55.91	11.32	9.14	8.55	8.16	8.04	7.76
Copepod	2	100.00	57.45	13.78	12.33	11.86	11.53	11.43	10.68
Copepod	3	100.00	62.10	19.00	15.39	15.13	14.41	14.06	11.77
Cop7	1	100.00	56.60	13.11	11.69	10.80	9.78	9.74	9.40
Cop7	2	100.00	65.33	28.87	24.96	23.14	18.38	17.71	13.90
Cop7	3	100.00	67.79	29.16	22.42	21.15	20.02	18.96	13.49
RotMG	1	100.00	61.24	22.43	16.09	14.37	13.51	13.47	12.43
RotMG	2	100.00	59.40	15.64	11.66	10.05	9.29	9.27	8.91
RotMG	3	100.00	57.95	13.51	11.15	10.20	8.65	8.60	8.46
RotChl	1	100.00	55.42	10.78	8.48	7.34	6.09	6.07	6.07
RotChl	2	100.00	56.88	13.73	8.38	7.01	5.96	5.95	5.94
RotChl	3	100.00	55.89	11.72	5.35	4.58	4.24	4.24	4.24

Appendix 7

Mean dry weight table

Table A4. Mean dry weight, standard error and n from each tank per sampling day

Mean Dry weight (mg larva^{-1})											
Dph	Treatment	Tank	Mean	SE	Total N	Dph	Treatment	Tank	Mean	SE	Total N
2	All	All	0.0442	0.0016	12						
4	All	All	0.0404	0.00139	12						
8	Copepod	1	.0728	.0032	12	33	Copepod	1	2.4936	0.1941	30
		2	.0720	.0042	12			2	3.0184	0.2097	30
		3	.0762	.0055	12			3	2.7133	0.1886	30
	Cop7	1	.0797	.0033	12		Cop7	1	3.0452	0.1456	30
		2	.0639	.0043	12			2	1.5953	0.1372	30
		3	.0715	.0042	12			3	1.7662	0.1466	30
	RotMG	1	.0515	.0048	12		RotMG	1	1.9010	0.1404	30
		2	.0393	.0044	12			2	2.1472	0.1637	30
		4	.0417	.0040	12			4	1.9265	0.1167	30
	RotChl	1	.0434	.0043	11		RotChl	1	1.4104	0.0796	30
		2	.0531	.0033	12			2	1.5326	0.1141	30
		3	.0517	.0053	12			3	1.5598	0.1571	30
12	Copepod	1	.1395	.0130	11	40	Copepod	1	3.8792	0.2471	30
		2	.1482	.0131	12			2	4.9983	0.3742	30
		3	.1837	.0091	12			3	4.7666	0.4201	30
	Cop7	1	.1783	.0086	12		Cop7	1	4.9061	0.3827	30
		2	.1490	.0185	12			2	3.8585	0.2765	30
		3	.1281	.0138	12			3	4.0768	0.2860	30
	RotMG	1	.0674	.0093	12		RotMG	1	4.1793	0.1997	30
		2	.0821	.0056	12			2	4.6395	0.2901	30
		4	.0834	.0069	12			4	5.0101	0.2452	29
	RotChl	1	.0850	.0047	12		RotChl	1	4.7615	0.2233	30
		2	.0872	.0092	12			2	4.1584	0.2240	30
		3	.0815	.0110	12			3	4.8416	0.2321	22
15	Copepod	1	.2179	.0211	12	47	Copepod	1	7.3508	0.6550	30
		2	.2554	.0215	12			2	9.2463	0.8182	30
		3	.2840	.0259	11			3	8.3644	0.7993	30
	Cop7	1	.2198	.0056	12		Cop7	1	10.9654	0.7002	30
		2	.1779	.0108	12			2	7.1279	0.6523	30
		3	.1757	.0167	12			3	6.7709	0.4328	30
	RotMG	1	.1326	.0137	12		RotMG	1	7.1253	0.3696	30
		2	.1190	.0147	11			2	8.3293	0.4335	30
		4	.1005	.0126	12			4	7.0390	0.4243	30
	RotChl	1	.1236	.0091	12		RotChl	1	7.9587	0.3552	30
		2	.1120	.0095	12			2	9.0823	0.4469	30
		3	.0924	.0090	12	61	Copepod	1	7.7540	0.8175	50
21	Copepod	1	.4417	.0557	12			2	19.5940	1.7326	50
		2	.6246	.0474	12			3	19.6780	1.4085	50
		3	.6832	.0465	12		Cop7	1	21.5200	1.7276	50
	Cop7	1	.3471	.0247	12			2	12.0640	1.2437	50
		2	.2436	.0323	12			3	17.6620	1.1791	50
		3	.2313	.0215	12		RotMG	1	16.8380	1.4258	50
	RotMG	1	.2630	.0296	12			2	11.1260	1.1131	50
		2	.1713	.0204	12			4	13.8918	0.8810	49
		4	.1859	.0227	12		RotChl	1	14.7220	1.1531	50
	RotChl	1	.2364	.0279	11			2	11.1898	0.8785	49
		2	.2212	.0228	12						
		3	.1945	.0283	12						
27	Copepod	1	1.0103	.0942	24						
		2	1.2766	.0789	24						
		3	1.2227	.1071	24						
	Cop7	1	1.1754	.3014	24						
		2	.7766	.0423	24						
		3	.7570	.0678	24						
	RotMG	1	.7119	.0561	24						
		2	.7609	.0568	24						
		4	.7101	.0312	24						
	RotChl	1	.5563	.0322	24						
		2	.5754	.0511	24						
		3	.5720	.0429	24						

Appendix 8

Mean daily weight increase

Table A5. Mean % daily weight increase (%DWI) per tank for specific intervals during the first feeding experiment

Mean % daily weight increase per larvae							
Interval	Treatment	Tank	%DWI	Interval	Treatment	Tank	%DWI
2-4 dph	All		-4.6				
4-8 dph	Copepod	1	15.9	40-47 dph	Copepod	1	9.6
		2	15.5			2	9.2
		3	17.2			3	8.4
	Cop7	1	18.5		Cop7	1	12.2
		2	12.1			2	9.2
		3	15.4			3	7.5
	RotMG	1	6.2		RotMG	1	7.9
		2	-0.7			2	8.7
		3	0.8			3	5.0
	RotChl	1	1.8		RotChl	1	7.6
		2	7.2			2	11.8
		3	6.4	47-61 dph	Copepod	1	0.4
8-21 dph	Copepod	1	14.9			2	5.5
		2	18.1			3	6.3
		3	18.4		Cop7	1	4.9
	Cop7	1	12.0			2	3.8
		2	10.9			3	7.1
		3	9.5		RotMG	1	6.0
	RotMG	1	13.4			2	2.2
		2	12.0			3	5.0
		3	12.2		RotChl	1	4.5
	RotChl	1	13.9			2	1.5
		2	11.6	2-61 dph	Copepod	1	9.1
		3	10.7			2	10.9
21-40 dph	Copepod	1	12.1			3	10.9
		2	11.6		Cop7	1	11.1
		3	10.8			2	10.0
	Cop7	1	15.0			3	10.7
		2	15.7		RotMG	1	10.5
		3	16.3			2	9.9
	RotMG	1	15.7			3	10.2
		2	19.0		RotChl	1	10.3
		3	18.9			2	9.8
	RotChl	1	17.1				
		2	16.7				
		3	18.4				

Appendix 9

Total day degree

Table A6. Total amount of day degrees at the end of the experiment for individual tanks

	Total day degrees 61 dph (ºC)			
Tank	Copepod	Cop7	RotMG	RotChl
1	924	926	917	905
2	929	917	923	903
3	939	940	900	906

Appendix 10

Correlation of tissue volume and standard length

Figure A7. Correlating between tissue volume of the varying organ groups and standard length for the Copepod, Cop7, RotMG and RotChl treatments