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The effect of different live feeds and water qualities on growth, survival, ossification and skeletal anomalies of ballan wrasse *(Labrus bergylta)* larvae

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Abstract

Salmon lice *(Lepeophtheirus salmonis)* infections is one of the major problems in Atlantic salmon *(Salmo salar)* aquaculture. This has led to an increasing interest in the cultivation of ballan wrasse *(Labrus bergylta)*, for cleaner fish purposes. The bottleneck in ballan wrasse production is the live feed period, where the main challenges include survival, growth and skeletal anomalies, factors known to be influenced by both dietary and environmental conditions. The dietary requirements for ballan wrasse larvae are generally unknown, but the use of copepods as live feed is widely acknowledged as beneficial for marine pelagic fish larval growth, survival and development, particularly due to their high content of highly saturated fatty acids incorporated in the phospholipid fraction.

This study consists of two start feeding experiments of ballan wrasse; a live feed experiment and a water quality experiment. Larvae from the live feed experiment were fed either enriched rotifers *(Brachionus plicatilis)* followed by enriched *Artemia franciscana*, or cultivated copepods *(Acartia tonsa)* of increasing sizes until 45 days post hatch (dph). Larvae from the water quality experiment were reared in flow-through systems including a regular biofilter. One treatment received microbially matured water conditioned to the carrying capacity (CC) of the larval rearing tanks, to minimize the bloom of opportunistic bacteria following the large gap in CC between intake water and tank water. The larvae were fed enriched rotifers and *Artemia* until 41 dph, and formulated feed until 70 dph. One additional group was fed 50 % rotifers and 50 % preserved copepods (Planktonic) during the rotifer period.

The use of copepods as live feed for ballan wrasse larvae resulted in significantly higher growth throughout the entire live feed experiment, and this was also the case for larvae reared in microbially matured water in the water quality experiment. Larvae reared in microbially matured water showed an unusually high mortality, probably due to an accidental release of H_2S. The larvae fed live copepods had a significantly lower occurrence of skeletal anomalies than all other groups of both experiments, indicating that copepods were the optimal feed organisms for the ballan wrasse larvae. No differences in skeletal anomaly occurrence were found when rearing the ballan wrasse in microbially matured water.

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Trondheim, May 2015 **Oda Skognes Høyland**

Table of Contents

1 Introduction

1.1 Ballan wrasse in aquaculture

Production of Atlantic salmon *(Salmo salar)* is an important industry in Norway. In 2012, the salmon production was 1,2 million tons round weight, and the value of slaughtered fish that year was 28 billion NOK (Fiskeridirektoratet, 2013). One of the main challenges associated with the salmon industry is the problem with diseases and parasites (Fiskeri-og-kystdepartementet, 2009), especially parasitic infections with salmon lice *(Lepeophtheirus salmonis)*. The salmon louse is a small ectoparasitic copepod species that infects salmon in seawater. It can cause anemia, lesions and osmoregulatory problems, as well as both short term and long term stress that weakens the salmon's immune system (Grimnes & Jakobsen, 1996; Bowers et al., 2000; Wagner et al., 2008). Traditionally, chemical treatments have been used to deal with the salmon lice problems, but the lice have developed resistance and reduced sensitivity to many of the chemical agents. For this reason, there is an imminent need to find more effective and sustainable solutions (Denholm et al., 2002; Fallang et al., 2004; Burridge et al., 2010). This has led to a large interest in other delousing methods, particularly the use of cleaner fish such as wrasses (*Labridae*) and lumpfish (*Cyclopterus lumpus*) (Bjordal, 1988; Karlsbakk et al., 2014).

The use of cleaner fish as biological sea lice removal is already in use throughout Norway (Espeland et al., 2010). The number of wrasse individuals used in Norwegian salmon cages increased from 1-2 millions in 2008 to 14 million in 2012 (Skiftesvik & Nedreaas, 2014). Although there are some producers that cultivate wrasse and lumpfish, most of the fish are taken from natural stocks. Norwegian fisheries of wrasse is dated back to 1988 (Darwall et al., 1992b). The use of wild caught wrasse is unpredictable for the fish farmers due to the variability in landings of wrasse in terms of species, sizes, diseases and parasites, as well as a lack of continuous year-round supply (Fiskeridirektoratet, 2015). As the biology and distribution of wrasse species is not well known, the use of wild caught cleaner fish is a possible threat to the wild stocks (Skiftesvik & Bjelland, 2003; Espeland, et al., 2010). Removal of dominant

territorial or nest-guarding males can affect social structures and reduce the egg survival (Darwall et al., 1992a). The fish are often transported over long distances, which can cause stress and injuries that lead to reduced fish health and high mortalities in the net cage (Espeland, et al., 2010; Mortensen & Skiftesvik, 2013). Questions about sustainable fisheries, fish welfare, ethics and potential diseases contribute to causing a bad reputation for the salmon industry (Mortensen & Skiftesvik, 2013; Skiftesvik & Nedreaas, 2014; Langørgen, 2015). The need for a more stable, predictable and sustainable supply of cleaner fish has led to a large interest in interest in cultivation of wrasse.

Ballan wrasse (*Labrus bergylta*) is a robust and efficient cleaner fish (Skiftesvik & Bjelland, 2003), active at lower temperatures than other wrasse species, which can be beneficial at exposed localities (Skiftesvik & Nedreaas, 2014). Cultivated ballan wrasse are equally efficient as wild ones when it comes to delousing capacities and rates, and a ratio of 5 % wrasse to salmon can keep the lice loads at very low levels (Skiftesvik et al., 2013). Marine fish species like ballan wrasse generally have fragile pelagic larvae with high mortalities in their natural environment (May, 1974), and the main challenges of cultivating marine fish species are larval feeding, growth, survival (Planas & Cunha, 1999) and skeletal anomalies (Boglione et al., 2013). Growth and survival are obviously important factors for the marine fish production, but skeletal anomalies also greatly impact the economic and biologic viability, as well as ethical issues including fish welfare (Boglione, et al., 2013).

The bottleneck of the ballan wrasse production is the live feed period and until around 30 g of size (Hamre & Sæle, 2011). Although specific knowledge on cultivated ballan wrasse larvae is still limited, the importance of early larval nutrition has been shown. Øie et al. (in press) found that feeding larvae with cultivated copepods for a short time during the live feed period increased both survival and growth, compared to feeding with rotifers. From the same study, Sørøy (2012) reported on the positive effects of copepods on occurrence of skeletal anomalies. This is the only known study that involves copepods as live feed through the entire start feeding period, and the first to relate exclusive feeding with cultivated copepods to skeletal anomaly occurrence in ballan wrasse. Environmental factors, like water turbulence has shown effects on the occurrence of skeletal anomalies in cod *(Gadus morhua)* (Helland et al., 2009).

Attramadal et al. (submitted) further demonstrated that selecting for certain bacterial communities resulted in better growth of ballan wrasse larvae.

1.2 Nutrition of ballan wrasse larvae

In order to produce ballan wrasse of good growth, high survival and few anomalies, the nutritional requirements need to be met. Ballan wrasse and other marine fish larvae have high requirements for highly unsaturated fatty acids (HUFA), especially docosahexaenoic acid (DHA, 22:6 n-3) and eicosapentaenoic acid (EPA, 20:5, n-3). A dry weight proportion of 0,5-1 % HUFA is required for normal growth in marine fish larvae and juveniles, and the requirements for rapid growth are probably higher (Sargent et al., 1997; Whalen et al., 1998). The traditional live feeds for marine fish larvae are rotifers like *Brachionus plicatilis* and brine shrimp *Artemia franciscana* (hereafter *Artemia*). They are cheap and easy to produce, but are nutritionally deficient in terms of dietary requirements for marine fish larvae. They especially lack marine HUFA, and are therefore enriched with high-HUFA diets. Even after enrichment, the HUFA are mainly found in the neutral lipids like triacylglycerol, which is not the optimal form for the fish larvae (Gisbert et al., 2005; Cahu et al., 2009). The enriched rotifers may also have suboptimal levels of vitamins and minerals including thiamine and manganese (Hamre et al., 2008).

Copepods are the natural feed marine fish larvae prey on in the wild, which indicates that they meet the nutritional requirements of the fish larvae. Several studies show that the use of extensively harvested copepods is more beneficial for marine fish larvae when it comes to growth, survival and normal development (Shields et al., 1999; Bell et al., 2003; Cahu, et al., 2009), when comparing with the use of rotifers. The supply of wild copepods is, however, seasonal and unpredictable. Copepods from natural stocks can also carry pathogens that may spread diseases in the hatcheries (Marcogliese, 1995). This has led to an increasing interest in using cultivated copepods, but the production has been small (Støttrup, 2000) and only a few commercial hatcheries use copepods as live feed. Co-feeding marine fish larvae with rotifers and preserved, harvested copepods has resulted in better growth, survival and stress tolerance in compared to only feeding with rotifers (sea bream *(Sparus aurata)*; (Piccinetti et al., 2014), and sole *(Solea solea)*; (Piccinetti et al., 2012)

Unlike rotifers and *Artemia*, copepods are rich in omega 3 HUFA and store them in the phospholipid fraction (as opposed to neutral lipids), which is the optimal form for utilization in the fish larvae (Shields, et al., 1999; Bell, et al., 2003; Evjemo et al., 2003). In addition, the copepods do not lose their nutritional value upon starvation, which both rotifers and *Artemia* do (Evjemo, et al., 2003). Marine fish larvae require a high proportion of free amino acids, as opposed to peptides or protein bound amino acids (Støttrup, 2003). Copepods have larger amounts of free fatty acids compared to both rotifers and *Artemia* (Dabrowski & Rusiecki, 1983), which means the amino acids of copepods are more easily digested and utilized for growth in the fish larvae (Conceição et al., 2010). Live, cultivated copepods can be supplied in increasing sizes throughout the whole live feed period of ballan wrasse (Hagemann, 2013), as well as other marine fish larvae. *Acartia tonsa* Dana is a calanoid copepod that can be intensicely cultivated for larval rearing of several marine fish species (Støttrup et al., 1986).

1.3 Environmental conditions

In addition to nutritional factors, environmental conditions also impact the growth, survival and occurrence of skeletal anomalies in marine fish larvae (Houde, 1989). Fish larvae are in direct contact with their physical and chemical environment. Survival, growth and a wide variety of skeletal anomalies are contributed to environmental factors like temperature (Houde, 1989), water turbulence (Chatain & Ounais-Guschemann, 1990), salinity (Tandler et al., 1995), photoperiod (Hart et al., 1996) and microbial interactions (Vadstein et al., 1993). A fish farmer generally knows little to nothing about which bacteria species are present in the fish tanks. While the specific species composition might not be crucial to know of, the fish larvae always benefit from being reared in tanks with a low share of fast-growing opportunistic bacteria. This can, to a large extent, be controlled through water treatment procedures (Vadstein et al., 2004).

r- and K-selection are important terms when classifying bacterial communities, and was described already in 1967 (MacArthur & Wilson). r-strategists are opportunistic bacteria with rapid growth and reproduction, which thrive in unstable systems with

high amounts of food (organic matter) per individual. K-strategists are developing slower, but are favoured by systems with low amounts of food per individual and high competition. There is a continuous distribution between the two, and in a given system, K-selection is favoured over time (Pianka, 1970). The gap in carrying capacity between the disinfected intake water and the rearing water of the fish tank is an opening for opportunistic bacterial blooms in the fish tank. In a flow-through system, maturing the intake water in a biofilter fed with fish feed will increase the carrying capacity and hence decrease the gap in carrying capacity between intake- and tank water (Vadstein, et al., 1993; Salvesen et al., 1999; Attramadal et al., 2012a).

Intensive production of marine fish species, as well as their traditional live feeds, requires feed distribution at high concentrations, which is ideal for heterotrophic bacteria. This might include opportunistic r-strategists, and can be highly variable between live feed species, production methods, culture conditions, over time and even between tanks (Verschuere et al., 1999). The procedure of maturing the water in a high-capacity biofilter results in a more stable microbial community and a low tank-to-tank variation (Skjermo & Vadstein, 1999; Attramadal, et al., submitted). A positive effects on growth has been documented for ballan wrasse larvae reared in microbially matured water compared to regular flow-through (Attramadal, et al., submitted). No studies related to microbially matured water and skeletal anomalies are known.

1.4 Skeletal development and anomalies

The causative factors for the different skeletal anomalies are not fully understood, but they are largely contributed to genetics (Aulstad & Kittelsen, 1971; Izquierdo et al., 2010), rearing environment (Divanach et al., 1996) and nutrition (Halver, 1957; Cahu et al., 2003b; Zambonino Infante & Cahu, 2010). For ballan wrasse specifically, nutritional composition of the live feed has been shown to affect the skeletal development and occurrence of anomalies (Sørøy, 2012).

Skeletal anomalies are found in all marine aquaculture fish species, and is a problem of which there is still limited knowledge. The occurrence of skeletal anomalies varies between species and cultivation conditions, but also within hatcheries. The most

apparent effect of skeletal anomalies is related to abnormal external morphology, which lowers the price and reputation of the aquaculture product (Boglione, et al., 2013). The occurrence of skeletal anomalies may negatively affect the larval quality and welfare, due to increased sensitivity to environmental factors (Paperna et al., 1980), lower growth rates (Koumoundouros et al., 1997), higher mortalities (Andrades et al., 1996; Koumoundouros et al., 2002) and abnormal behavior (Basaran et al., 2007).

There is a lack of standard routines for assessing skeletal anomalies in fish farms. Different diagnostic methods (X-ray, palpation, external observation, bone staining etc.) give different results of anomaly occurrence and severity (Roo et al., 2009; Grini et al., 2011), but the largest diagnostic challenge is that the anomaly identification is largely subjective, as there is a lack of standard classification protocol and terminology for skeletal anomalies in teleost fish (Boglione, et al., 2013). According to Boglione et al. (2013), almost all known potentially lethal anomalies in European reared fish species are skeletal or related to skeletal defects. An uninflated swim bladder for instance, can induce mechanical overload on pectoral fins and pre-hemal vertebrae which may result in axis deviations and vertebrae anomalies (Kitajima et al., 1981; Kranenbarg et al., 2006).

Skeletal anomalies are found in all parts of reared fish species, from skull to vertebrae and fins. Skull anomalies are mainly sublethal, and include anomalies of the gill cover (reduction or folding of the opercular plate), jaws (cross-bite, reduction or elongation of the lower jaw, pugheadness) and dentary. Vertebrae anomalies include vertebral column curvatures such as lordosis, kyphosis and scoliosis (and even a combination of all three; LSK syndrome (Afonso et al., 2000)), anomalies of the vertebrae (Hattori et al., 2004; Sawada et al., 2006) neural and hemal arches (Satoh et al., 2008) and ribs (Komada, 1980). Fin anomalies include lateral bending, duplication, “saddleback syndrome” and severe anomalies like dislocation, supernumerary fins or lack of fin (Boglione, et al., 2013).

2 Aims

There were two aims of this study on ballan wrasse larvae, where growth, survival, ossification and skeletal anomalies were used as quality parameters.

The primary aim was to determine the effects of using cultivated, live copepods of continuously increasing sizes as live feed, when compared to feeding with rotifers and *Artemia*. It was expected that ballan wrasse larvae fed exclusively on copepods would have better growth, survival, earlier ossification and fewer skeletal anomalies, than larvae fed on rotifers and *Artemia*.

The secondary aim was to determine the effects of microbially matured rearing water adjusted to the fish tank carrying capacity, compared to a regular flow-through system with water maturation at low carrying capacities. Rearing ballan wrasse larvae in microbial matured water was expected to result in better growth, survival, earlier ossification and fewer skeletal anomalies, compared to using a regular flow-through system. The effects of co-feeding with rotifers and preserved copepods were also to be assessed, where the use of preserved copepods in combination with rotifers was expected to be more beneficial than using rotifers alone.

3 Materials and methods

Two start feeding experiments were conducted at the experimental facilities of Norwegian University of Science and Technology (NTNU), Centre of Fisheries and Aquaculture (Sealab), and in SINTEF Fisheries and Aquaculture's laboratories, in Trondheim.

The first experiment named **live feed experiment** was designed to investigate the effects of two different live feeding regimes on growth, survival and skeletal anomalies of ballan wrasse larvae. These larvae were reared on either copepods of increasing sizes or the traditional live feeds rotifers and *Artemia* nauplii. The main emphasis of this thesis lies on the live feed experiment, where I took part in the planning and execution.

The **water quality experiment** was the second experiment, designed to investigate the effects of microbially matured flow-through rearing water for ballan wrasse larvae fed rotifers. The maturation process took place in a biofilter of high carrying capacity. In addition, the effects of substituting 50 % of the rotifers with preserved copepods were assessed. People at NTNU responsible for the execution of this experiment provided information, dry weight (DW) data and fixed larvae.

3.1 General rearing conditions

The seawater used in the fish tanks of both experiments was pumped at 70 m depth in Trondheimsfjorden, 800 m outside of Brattørkaia. Particles of >40 µm was removed via two sand filters, and the water was then matured according to Skjermo et al. (1997). A degasser of low atmospheric pressure was used to avoid gas supersaturation (N_2 in particular), and the water was heated to 12 ^{o}C. The water exchange rate in the fish tanks was increased gradually from 2 to 8 times day^{-1} during the experiments. Ceramic clay (Vingerling K148, WBB Fucs GmbH, Germany) was dissolved in seawater and added manually to the tanks to enhance the visual contrast and reduce the bacterial load in the larval tanks (Attramadal et al., 2012b).

Upon arrival, the ballan wrasse larvae were transferred to a lightly aerated acclimatizing tank (250 L, 12 ºC). At 3 dph, larval density was measured before the larvae were equally distributed to 100 L tanks with flow-through water. The tanks were lightly aerated, and the oxygen saturation was measured daily and kept >80 %. The water temperatures were measured daily and increased gradually from 12-16 ºC. Salinity was measured weekly. Debris and dead fish were removed every second day from 11 dph. From 15 dph, the number of dead larvae was counted for mortality estimates. The larvae were fed at constant feed densities in the fish tanks. Each experiment is described in further detail in the following sections.

3.2 Live feed experiment

Ballan wrasse larvae were supplied from Nofima (Sunndalsøra, Norway) at 2 days post hatch (hereafter dph). At 3 dph, they were distributed equally to six tanks, at approximately 8200 larvae per tank. Three tanks were then reared on copepods *(Acartia tonsa)* of increasing sizes as live feed, and three tanks were reared on enriched rotifers *(Brachionus plicatilis)* and *Artemia* nauplii. The three tanks fed copepods were named **Cop**, and received copepod nauplii from the onset of exogenous feeding, 4 dph, to 15 dph, copepodites from 16-35 dph and a mix of adults and smaller copepods until the experiment was terminated at 45 dph. The three tanks fed on traditional live feed organisms were named **Rot**, and received rotifers from 4-40 dph, a co-feeding period with rotifers and *Artemia* nauplii from 30-40 dph, and *Artemia* until 45 dph. The live feeds were harvested daily and distributed manually into the fish tanks. The water exchange rate was adjusted to from 2 to 4 times day^{-1} at 10 dph due to high salinity in the fish tanks. It was set back to 2 times day^{-1} the following day. Temperature, water exchange rate, clay addition, sampling (DW, SL, MH) and live feed densities were as described in table 3.1.

The feed densities were checked prior to each feeding. When there were an adequate amount of rotifers or copepods left in the fish tank, one feeding for that particular tank was skipped. Excess *Artemia* were removed from the tank prior to each feeding, as they rapidly lose nutritional value when starved (Evjemo et al., 1997; Evjemo et al., 2001; Figueiredo et al., 2009). The plan was to use copepods of steadily increasing

sizes throughout the experiment to match the sizes of the growing fish larvae. Due to unpredictable copepod hatching, there were some deviations to the original plan, but on an overall basis the fish were offered increasingly larger copepods.

Table 3.1 Experimental setup for the live feed experiment, showing day degrees, temperatures, water exchange rates, clay, sampling days (mortality, DW, SL, MH) and feed densities from 4-45 dph. Copepod stages are shown at bottom left.

Days post hatch (dph)	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16-21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**	**31**	**32-35**	**36**	**37**	**38**	**39**	**40**	**41**	**42**	**43**	**44**	**45**
Day degrees (°C)	48	60	73	86	98	110	123	136	149	162	175	188	202-272	287	302	317	332	347	362	377	392	407	422	437-482	497	512	528	544	560	576	592	608	624	640
Temperature (°C)	12						13						14	15													16							
Water exchange rate	2						4	2					4				6							8										
Clay (g tank^{-1} day^{-1})	3,2									4,8				7,5			8			8,5		9												
Mortality check												X	X	X		X			X		X		X	X	X		X			X		X		X
DW, SL, MH	X*				X							X								X					X									X

Cop	*Acartia tonsa*																																	
Density L^{-1} feeding^{-1}	5000																										10000							
Stage**	n												c			n	c	n	c								2/3 c 1/3 a					2/3 n 1/3 a			

Rot	*Brachionus plicatilis*																																	
Rotifer density L^{-1} feeding^{-1}	3500						5000						12000																					
																								Artemia franciscana									
Artemia density L^{-1} feeding^{-1}																								3000										

* sampled 3 dph

** Copepod stages	Age (dph)	Length (μm)
nauplii (n)	1-5	94-211
copepodite (c)	6-10	398-820
adult (a)	11+	1000-1500

3.3 Water quality experiment

Ballan wrasse larvae were supplied from Marine Harvest Labrus (Øygarden, Norway), and distributed equally to nine fish tanks, at approximately 6000 larvae per tank. Three tanks were used for each treatment.

Rot was fed enriched rotifers from 3-27 dph, co-feeding with rotifers and enriched *Artemia franciscana* from 23-27 dph and *Artemia* nauplii until 41 dph. **RotMC** had the same feeding regime as Rot, but was reared in microbially matured water. The water for this treatment was run through a separate 450 L fed biofilter (KMT Kaldnes K3 Media) of high carrying capacity, prior to entering the fish tanks. The biofilter was conditioned with 30 g pulverized fish feed day^{-1} (Gemma Micro Diamond 300, Skretting, Norway) 10 days prior to transferring fish larvae to the tanks, in order to reduce the gap in carrying capacity between intake water and rearing water. From 4 dph, fish feed was added daily in two doses (40 g at 4-14 dph and 60 g at 14-27 dph, 80 % Gemma Micro Diamond 300 and 20 % Aglonorse 5) (Attramadal, et al., submitted). **Rot50** was fed 50 % rotifers and 50 % preserved copepods (Planktonic diet of 100 - 200 µm, Planktonic AS, Norway) from 3-27 dph, followed by a co-feeding period of rotifers, Planktonic and *Artemia franciscana* from 23-27 dph, and *Artemia* until 41 dph. The live feeds were harvested daily and distributed to the fish tanks with a feeding robot (Storvik, Norway), as well as manually. Temperature, water exchange rate, clay addition, sampling for DW, and live feed densities were as described in table 3.2.

From 42 dph, the remaining fish larvae were combined into one tank per treatment (1000 each of Rot and Rot50 larvae, 553 of RotMC larvae). They were weaned onto dry feed (300-600 and 600-800 µm, Nofima, Norway; appendix 1), up to concentrations of 10 g per tank per day. The experiment was terminated at 70 dph.

Table 3.2. Experimental setup for the water quality experiment, showing day degrees, temperatures, water exchange rates, clay, sampling days (mortality, DW) and feed densities from 3-41 dph.

Days post hatch (dph)	**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**	**34-37**	**38**	**39**	**40**	**41**
Day degrees (°C)	36	25	38	51	64	77	90	104	118	134	140	155	160	186	201	216	232	248	264	280	296	312	327	342	357	373	389	405	421	437	453-501	517	533	549	565
Temperature (°C)	12				13					14	15							16	15		16															
Water exchange rate	2											4									6					8									
Clay (g tank^{-1} day^{-1})	16												32								48														
Mortality check					X			X			X		X		X			X		X		X		X		X		X		X		X		X
CN	X			X				X					X						X						X						X				X

Rot	*Brachionus sp.* (12 000 L^{-1} feeding^{-1})	
		Artemia franciscana (3000 L^{-1} feeding^{-1})
RotMC	*Brachionus sp.* (12 000 L^{-1} feeding^{-1})	
		Artemia franciscana (3000 L^{-1} feeding^{-1})
Rot50	50% *Brachionus sp.*, 50% Planktonic	
		Artemia franciscana (3000 L^{-1} feeding^{-1})

3.4 Production of live feed

3.4.1 Cultivation of microalgae *(Rhodomonas baltica)*

R. baltica was cultivated in 40 cm O polymethyl methacrylate cylinders of 160 and 200 L, as well as one 300 L plastic bag. From each cylinder, 40-50 % of the volume was harvested daily, and the tanks were refilled with seawater (20 ºC) and Conwy medium (1 ml L^{-1} seawater; modified from (Walne, 1979), appendix 2). The seawater (34 ppt) was sand filtered (1µm), heated to 20 ºC, chlorinated (NaOCl (10-15 %), 0.25 ml L^{-1}, no aeration, >5 hours) and dechlorinated (3 g $Na_2S_2O_3$ 100 L^{-1} seawater, heavy aeration, >5 hours). Each culture was harvested completely every 2 weeks, and the cylinder was cleaned, chlorinated and dechlorinated before new algae were started from intermediate cultures (10 L, 2-3 x 10^6 ml^{-1}), seawater and Conwy medium (1 ml L^{-1} seawater; (Hoff & Snell, 1989)). Air with 1-2 % CO_2 was supplied to the tanks, and the pH was kept between 7.5-8.5 by regulating the CO_2 supply. Each culture was under continuous illumination by 6 fluorescent tubes (GE Polylux XL 830, F58W, GE Lightning, US). The density of the harvested volumes was 1-1,4 million algae ml^{-1} seawater.

3.4.2 Cultivation of copepods *(Acartia tonsa)*

Copepods *(A. tonsa)* were cultivated in two continuously lit 1600 L tanks with flow-through seawater (sand filtered, 1µm; exchange rate 1 time day^{-1}). Temperatures were kept between 19-22 ºC, oxygen saturation >4 mg O_2 L^{-1}, pH from 7.6-8 and salinity 34-35 ppt (ProODO Optical Dissolved Oxygen Meter, YSI Inc., USA; LH-T28, China; pH/mV-meter, WTW ph 315i, Germany). The outlet filters (64 µm) were cleaned daily. Harvested *R. baltica* was kept in a 1000 L tank, and supplied continuously to the copepod tanks through an Electromagnetic Dosing Pump (AXS602, Seko, Italy), at concentrations of approximately 33 000 cells ml^{-1}.

Eggs and debris was siphoned daily from the bottom of the copepod tanks, filtered through two sieves of 120, 100 µm mesh size to remove dead copepods and other waste and finally collected on a 64 µm sieve. The eggs were cleaned with seawater, transferred to NUNC EasyFlasks™ (Nunc A/S, Denmark) and stored at 2 ºC (SANYO Pharmaceutical Refrigerator MPR-311D (H), Japan).

When the copepod cultures were started, eggs were incubated in 100 L plastic tanks with moderate aeration. The copepods were fed *R. baltica* manually at least three times day^{-1}, starting 24 hours after incubation (Nesse, 2010). The densities were calculated by fixing collected water samples with Lugol's solution and counting the number of copepods. Six 500 µl water droplets were used to estimate the density for each tank, canceling the highest and lowest count. The required amount of copepods were collected with a sieve and stored in 100 L tanks of 8 ºC for a maximum of 12 hours. The copepods were concentrated right before distribution to the fish tanks.

3.4.3 Cultivation of rotifers *(Brachionus plicatilis)*

Rotifers were cultivated semi-continuously in three 250 L cylindrical tanks with conical bottoms. Temperatures were held at 19-23 ºC, water exchange rate at 1-1.5 times day^{-1} and oxygen levels above 80 %. The tanks were flushed daily for debris collected at the bottom, and filters were cleaned. The rotifers were rinsed and the holding tanks were cleaned every 1-2 weeks. Feed was supplied continuously to the cultures through peristaltic pumps. The rotifers of the live feed experiment were fed baker's yeast (2.6 g million rotifers^{-1} day^{-1}) dissolved in seawater, and DHA Chlorella paste in the water quality experiment (2.5 µg rotifer^{-1} day^{-1}, Chlorella Industry Co., Ltd., Japan)). Multigain (0.14 g million rotifers^{-1} day^{-1}; BioMar A/S, Norway) dissolved in seawater added to the tanks twice day^{-1}.

Densities were calculated by collecting small water samples from each tank and counting individuals and eggs in 12 x 25 µl droplets fixed with Lugol's solution, canceling the highest and lowest count. Egg ratio was calculated to determine the growth of the culture (Øie & Olsen, 1997).

The required amount of rotifers was harvested, rinsed in seawater and transferred to a 100 L enrichment tank. The culture was moderately aerated and short-term enriched with Multigain (0.14 g million rotifers^{-1} day^{-1}; BioMar A/S, Norway) for two hours. The rotifers were rinsed and transferred to a 100 L aerated holding tank of 8 ºC for a maximum of 12 hours, and then concentrated right before distribution to the fish tanks.

3.4.4 Cultivation of *Artemia franciscana*

Artemia cysts (EG ® INVE Aquaculture, Belgium) were decapsulated according to Sorgeloos et al. (1997). Prior to decapsulation, the cysts were hydrated in fresh water (4.9 L water for 450-500 g cysts, 15-25 ºC) for one hour. The decapsulated cysts were weighed and stored in a refrigerator for a maximum of 6 days.

Two days prior to feeding, the required amount of *Artemia* cysts was transferred to a hatching tank with seawater (25-28 ºC, pH 8-8.5; <2 g cysts L^{-1}). The tank water was heavily aerated to maintain an oxygen level >4.5 mg L^{-1} (minimum 2.5 mg L^{-1}; (Hoff & Snell, 1989)). After 24 hours, the bottom was flushed for 2 seconds and the hatched *Artemia* were transferred to an *Artemia*-washer for rinsing and concentration. After 10 minutes the *Artemia* were transferred to an enrichment tank where Multigain (10 g 60 L^{-1}; BioMar, Norway) was added two times during the next 24 hours. The *Artemia* were rinsed and transferred to a 100 L aerated holding tank of 8 ºC for a maximum of 12 hours, and then concentrated right before distribution to the fish tanks.

3.5 Sampling and preservation

The larvae were sampled randomly from the tanks using a ladle. They were then anesthetized using an overdose of tricaine methanesulfonate (MS-222, Finquel®, Agent Chemical Laboratories Inc., USA) photographed for size measurements, and rinsed in distilled water to prepare them for CN-analyses. After rinsing, larvae sampled for skeletal analyses (total n=241) were fixed in 4 % paraformaldehyde (PFA) in phosphate buffer (pH 7,4; Apotekproduksjon AS, Norway) and stored at 4 ºC.

Larvae from the live feed experiment were sampled from each tank on 3 (n=20 from holding tank), 8, 15, 28, 36 (n=10-15 from each tank) and 45 dph (n=25-30 from each tank). Larvae from the water quality experiment were sampled on 3 (n=13 from holding tank), 6, 10, 15 (n=12 from each tank), 21, 27, 34 (n=15-30 from each tank) and 70 dph (n=15 from each tank).

3.6 Growth analyses of larvae

3.6.1 Dry weight

The carbon content of the larvae was analyzed using an Elemental Combustion Analyzer (Costech Analytical Technologies Inc., CA, USA) and acetanilide ($C_6H_5NH(COCH_3)$) was as standard (analysis conducted by Marte Schei, SINTEF). Based on the results, dry weight of the larvae was calculated according to equation 2.1 (Reitan et al., 1993).

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

Specific growth rate (SGR) and daily weight increase (%DWI) was calculated using equations 2.2 and 2.3, according to (Ricker, 1958).

$$SGR = (\ln W_2 - \ln W_1) / (t_2 - t_1) \quad [2.2]$$

Where W_1 and W_2 equals the dry weight at time t_1 and t_2, respectively.

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

3.6.2 Standard length and myotome height

The standard length (SL) and myotome height (MH) of the fish larvae were measured from pictures using a Nikon SMZ1000 stereomicroscope (Nikon Instruments Inc., USA) and Infinity 1-3C camera with Infinity Analyze software (Lumenera Corporation, Canada). SL was measured from the tip of the upper lip to the end of the notochord (preflexion larvae) or of the caudal peduncle (postflexion larvae). MH was measured perpendicular to the axial skeleton right behind the anus (measurements conducted by Stine Wiborg Dahle, SINTEF).

All fish larvae used for skeletal analyses were also measured for SL and MH after staining (figure 3.1), using a stereomicroscope, camera and Zen 2012 software (Leica MZ7.5, Leica Microsystems, Germany; AxioCam ERc5s, Carl Zeiss Microscopy GmbH, Germany; Carl Zeiss Microscopy GmbH, Germany). According to (Sørøy, 2012), the bone staining process does not alter the fish size, when comparing to other fixed specimens.

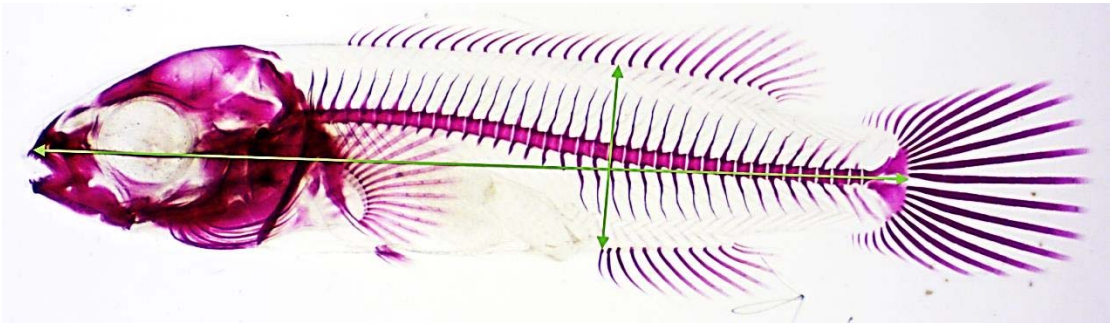

Figure 3.1. SL and MH measurements on stained larvae. SL measured from nose tip through the urostyle, and MH perpendicular to the axial skeleton right behind the anus. This is a 41 dph Rot larva from the water quality experiment, measuring 9,79 mm (SL) and 2,14 mm (MH). It has full ossification of the vertebral column and dorsal fin.

3.7 Skeletal analyses

Larvae from both experiments were sampled for bone staining and skeletal analyses with assessment of ossification, registration of skeletal anomalies, as well as of SL and MH measurements. Larvae from the live feed experiment were sampled at the termination day, 45 dph. Between 15-18 larvae were samples from each tank, a total of 45 larvae from Rot and 53 larvae from Cop. From the water treatment experiment, larvae were sampled at 41 and 70 dph. At 41 dph, 10-12 larvae were sampled from each tank, a total of 30 larvae from Rot, 36 larvae from RotMC and 32 larvae from Rot50. From 70 dph, 15 larvae were sampled from each treatment.

The larvae were subject to a bone staining procedure with Alizarin Red (adapted from (Kjørsvik et al., 2009)). The scales of larvae from 70 dph were removed after the bone staining, prior to preservation in glycerol. Full procedure is presented in appendix 3. The stained larvae were submerged in 40 % glycerol and photographed, before further preservation 100 % glycerol. Analyses of ossification and anomalies of the fish larvae were done by eye using the Leica MZ7.5 stereomicroscope, and through pictures using Adobe Photoshop (Adobe Systems Software Ireland Ltd.) and Preview (Apple Inc.). SL and MH of stained fish were measured using the software ImageJ v.1.47 for Mac OS X. In cases where the fish were too large to be photographed in one shot, Adobe Photoshop was used to merge multiple layer photographs of equal magnitude prior to measurements and analysis.

The emphasis of the skeletal studies lay on development of the vertebral column and dorsal fin, as well as skeletal anomalies. Relevant terminology is shown in figure 3.2.

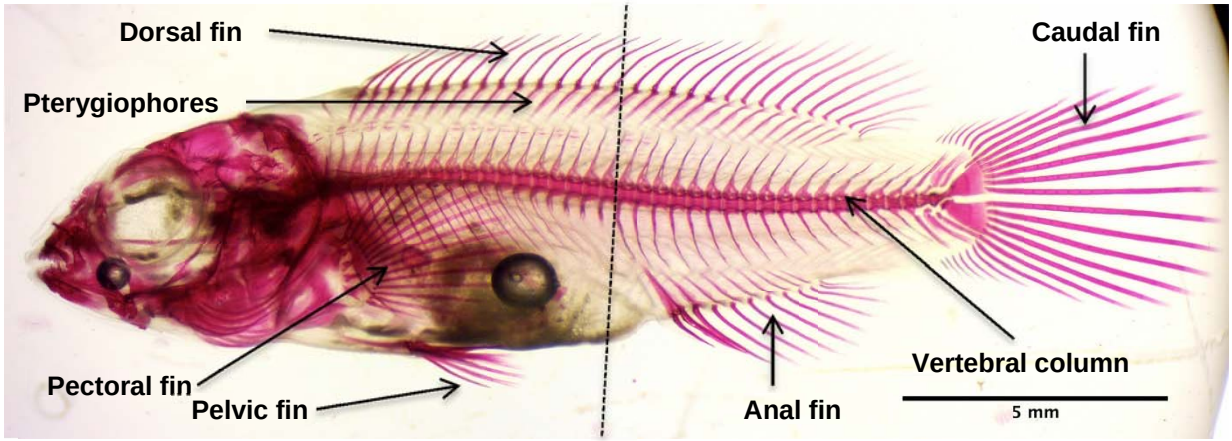

Figure 3.2 A stained RotMC fish at 70 dph measuring 17,82 mm (SL). Scales are removed on the facing side. The figure displays relevant terminology, including fins, pterygiophores (base of dorsal fin) and vertebral column. The vertical line marks the division between pre-hemal vertebrae (left) and hemal vertebrae (right). Both vertebral column and dorsal fin are fully ossified.

3.7.1 Vertebral column ossification

Ossification of the vertebral column was classified as transparent, compact or fully ossified, based on the color distribution and saturation of the bones (Eidsvik, 2010; Sørøy, 2012). The total number and the number of ossified vertebrae were counted in each larva.

3.7.2 Dorsal fin ossification

A scoring system of dorsal fin development was adapted from (Sørøy, 2012). The dorsal fin ossification was scored from 0 to 3, where 0 = no ossification, 1 = ossification of posterior parts, 2 = ossification of both anterior and posterior parts and 3 = ossification of the entire dorsal fin, including the pterygiophores. If any ossification was seen in each of the regions, the region was classified as ossified.

3.7.3 Squamation

A scoring system for squamation was also adapted from (Sørøy, 2012), where numbers 0-3 were used to classify the squamation of the flank. Score 0 = no ossification of scales, 1 = beginning ossification along the midline, 2 = ossification of scales extending the midline up to

the base of the pterygiophores and 3 = full ossification of scales along the flank, including the pterygiophore area.

3.7.4 Skeletal anomalies

An anomaly was defined as a "major difference in the shape of a body part or organ compared to the average shape of that part" (Bæverfjord et al., 2009). Skeletal anomalies were detected through a Leica MZ7.5 stereomicroscope as well as images. The following vocabulary for anomaly identification was used (Boglione et al., 2001; Boglione, et al., 2013):

Abnormal arches: bent or abnormally shaped hemal and/or neural arches of the vertebral column. Excluding twisted arches.

Twisted arches: a spiral formation at the tip of the hemal and/or neural arches (described by (Izquierdo, et al., 2010) and (Lewis-McCrea & Lall, 2010)).

Fused vertebrae: a partial or total fusion of two or more vertebrae and/or associated hemal or neural arches.

Axis deviations: kyphosis (∧-shaped dorsal-ventral curvature), lordosis (∨-shaped dorsal-ventral curvature) or scoliosis (lateral curvature).

Blunt nose: anomalies of the pre-maxillary, palatine and mesethmoid bones that give the head a profile with a blunt nose.

3.8 Statistical analyses

Statistical analyses were conducted using Microsoft Excel for Mac OS X (Microsoft Office, USA), StatPlus Pro for Mac OS X (AnalystSoft Inc., USA) and SigmaPlot™ 12.5 (Sysat Software, Inc., USA). Tables were made in Microsoft Word and Excel 2011 for Mac OS X (Microsoft Office, USA). Graphs were made in SigmaPlot™ 12.5.

The data were examined for extreme outliers, but none were found. The difference of means between groups was assessed using one-way ANOVA. Student-Newman-Keuls post hoc t-test was applied to data of equal variance, to check for significant differences between the data. Data of unequal variance was assessed with a Dunnet T3 post hoc test. A significance level of $p<0,05$ was used. The results are presented as mean ± standard error of the mean (SE).

4 Results

4.1 Live feed experiment – growth and survival

4.1.1 Dry weight and daily weight increase

The larvae fed copepods had a significantly higher growth than the ones fed rotifers and *Artemia*. The difference lasted from after absorption of the yolk sac and throughout the entire experiment, for both DW, SL and MH. The DW was increasing exponentially, without any flattening of the growth curves. After a negative daily weight increase (%DWI) in both Rot and Cop from 3-8 dph, Cop showed a massive %DWI of 21 % from 8-15 dph, significantly higher than the Rot DWI of 11 %. The difference is visualized in figure 4.1, by the large gap between Cop and Rot DW at 15 dph, and figure 4.2 displaying the %DWI. Ballan wrasse larvae rely on the yolk sac for nutritional supply after hatching, and are ready to start exogenous feeding around 6 dph or 70 day degrees (Ottesen et al., 2012). The first period after hatching is therefore characterized by decreasing weight, as seen in the %DWI of both Rot and Cop (figure 4.2). The mean %DWI for 3-45 dph was similar for the two treatments: 11 ± 0 for Rot and 12 ± 0 for Cop, but the gap in DW due to the initial high %DWI of Cop was never closed.

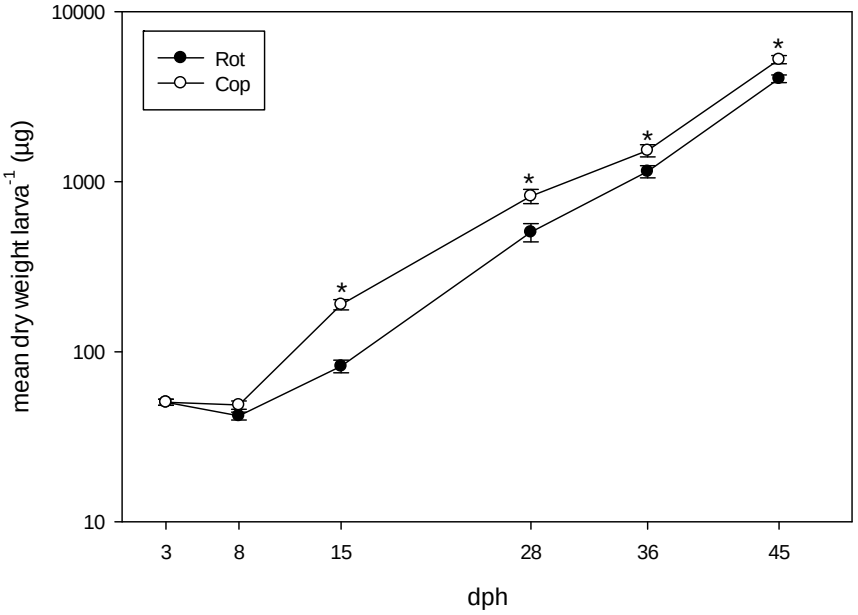

Figure 4.1 Mean dry weight (µg larva^{-1}) for Rot and Cop in the live feed experiment. Measurements were made on 3 dph (n=20 from holding tank), 8, 15, 28, 36 and 45 dph (n=35-45). Data are presented as mean values ± SE. Asterisks indicate significant differences between the treatments ($p<0,05$; one-way ANOVA).

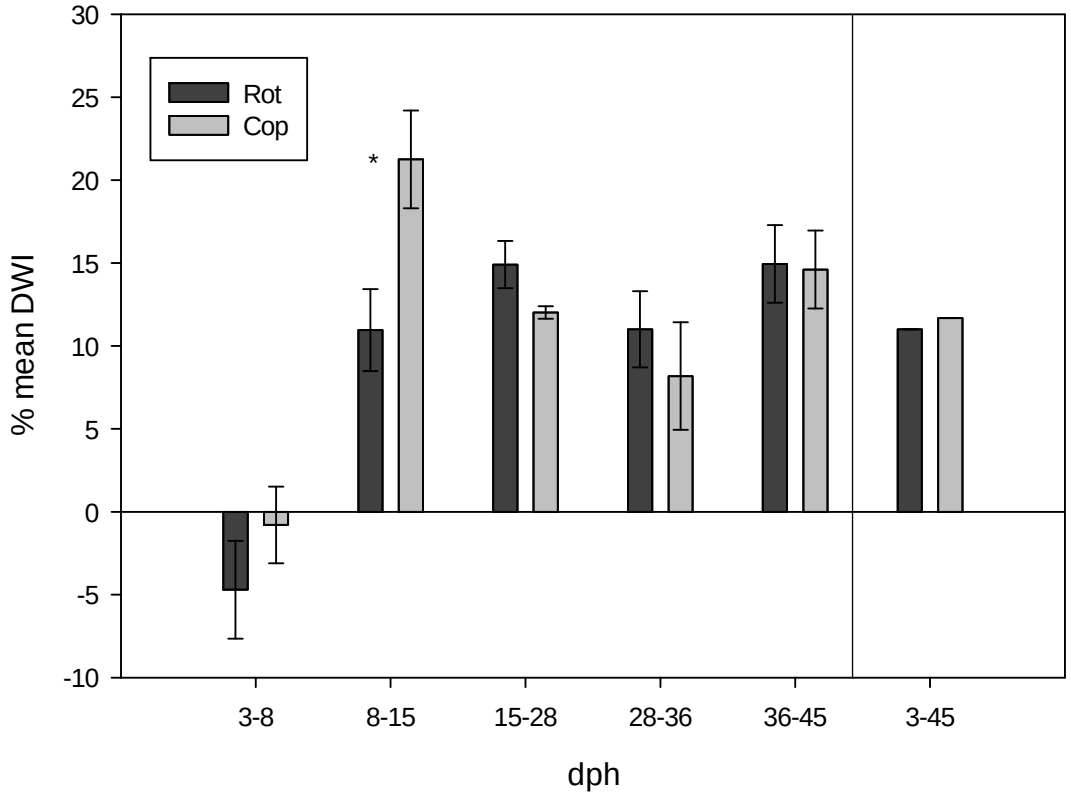

Figure 4.2 Mean percent daily weight increase (%DWI) for two start feeding regimes of ballan wrasse larvae; Cop was fed copepods and Rot was fed rotifers (*Artemia* from 30 dph). From 3-8 dph, the larvae are mainly in the yolk sac stage, which involved endogenous feeding. The values are based on mean %DWI from each fish tank (n=3), ± SE. Significant differences indicated by asterisks ($p<0,05$, one-way ANOVA).

4.1.2 Standard length and myotome height

The SL was significantly higher for Cop than Rot from 15 dph and throughout the experiment (figure 4.3), while the MH was significantly higher already from 8 dph (figure 4.4). Rot did not catch up with the increase in SL and MH of the Cop treatment. The correlation between SL and MH was >0,96 (Pearson correlation, $p<0,01$), showing that the fish larvae grew correspondingly in length and height (figure 4.5).

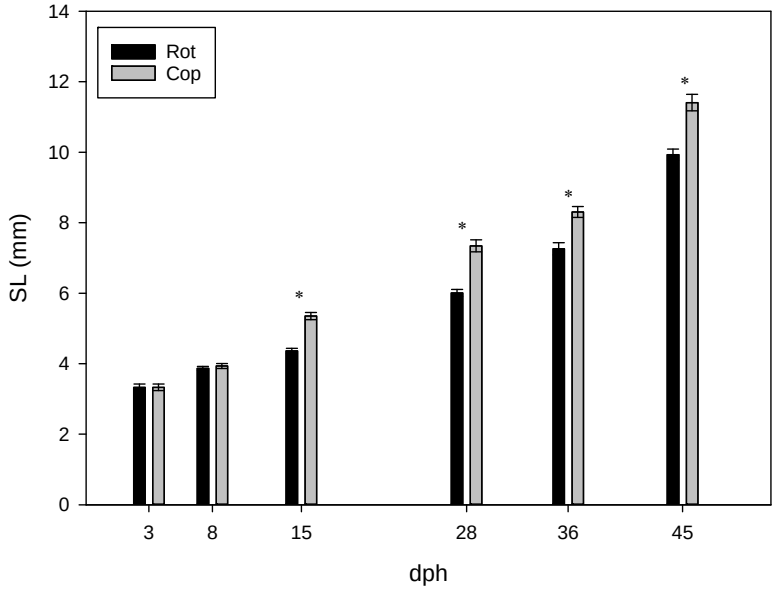

Figure 4.3 Mean standard length (SL) of ballan wrasse larvae fed rotifers and *Artemia* (Rot) or copepods (Cop). The values are means ± SE (n=35-45, except at 3 dph where n=20). Asterisks indicate significant differences between the treatments ($p<0,05$; one-way ANOVA).

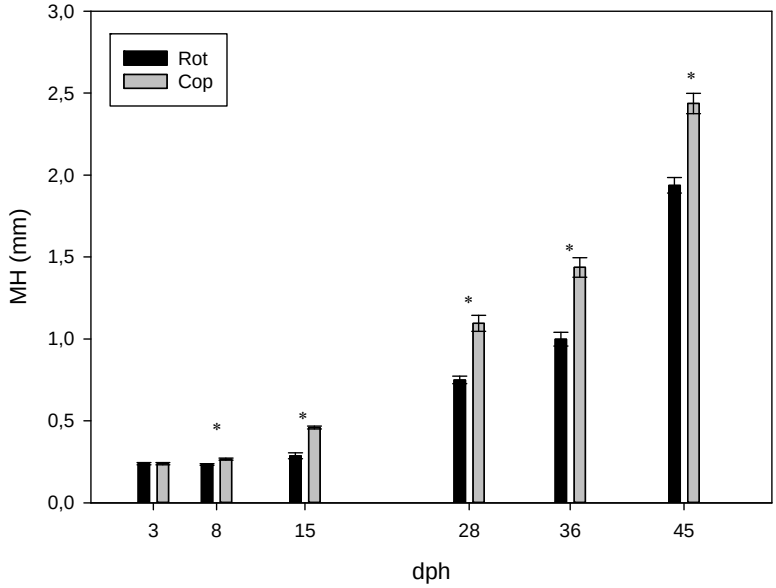

Figure 4.4 Mean myotome height (MH) of ballan wrasse larvae fed rotifers and *Artemia* (Rot) or copepods (Cop). The values are means ± SE (n=35-45, except at 3 dph where n=20). Asterisks indicate significant differences between the treatments ($p<0,05$; one-way ANOVA).

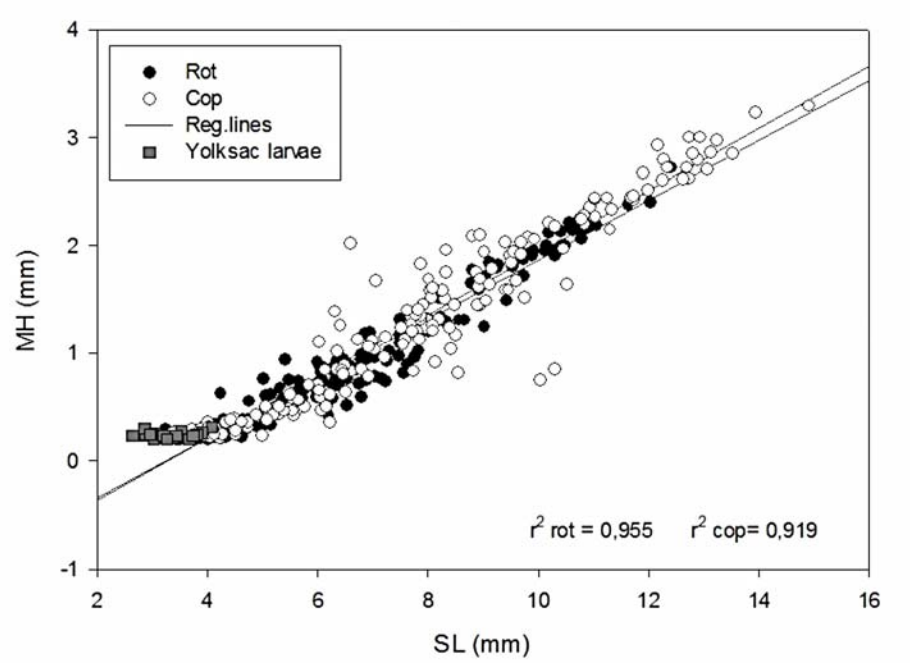

Figure 4.5 Standard length (SL) plotted against myotome height (MH) for ballan wrasse larvae fed rotifers and *Artemia* (Rot) or (Cop). Larvae are between 3-45 dph, the ones from 3 dph are marked as yolksac larvae. r^2 values are displayed. Pearson correlation coefficients for Rot: 0,977 and Cop: 0,966 ($p<0,01$).

4.1.3 Survival

The mortality of the ballan wrasse larvae was highest at the beginning of the experiment, before 15 dph. A slow decrease in survival then followed until 45 dph (table 4.1) when the experiment was terminated. Cop had a slightly higher survival than Rot at the end of the experiment, although no significant differences were found.

Table 4.1 Mean survival (%) ± SE of ballan wrasse larvae fed rotifers and *Artemia* (Rot) or (Cop). Values are based on mean % survival from each fish tank (n=3) between 15-45 dph. Initial larvae numbers were approximately 8200 per tank.

Survival (% ± SE)		
dph	**Rot**	**Cop**
15	15 ± 1	15 ± 1
20	13 ± 0	13 ± 2
29	11 ± 1	12 ± 2
37	9 ± 1	12 ± 2
45	9 ± 1	11 ± 2

4.2 Water quality experiment – growth and survival

4.2.1 Dry weight and daily weight increase

The RotMC reared in microbially matured water had a significantly higher DW than Rot and Rot50, lasting from after yolk absorption and throughout the experiment until the last DW measurement at 34 dph. All three treatments showed the same shape of the curves, with steady growth from 6-34 dph (figure 4.6). The DWs of Rot and Rot50 were similar for almost the entire period, while the RotMC DW was significantly higher from 10-34 dph. The higher DW started with a slightly, but not significantly larger %DWI of the RotMC between 6-10 dph (figure 4.7), which created a gap that neither Rot nor Rot50 closed at any point. The first period after hatching was characterized by decreasing weight, as was also true for the live feed experiment. RotMC also had the least negative %DWI from 3-6 dph.

The mean %DWI did not reveal any significant differences between the groups (n=2 for Rot, n=3 for RotMC and Rot50). The mean %DWI for 3-34 dph was 11 ± 0, 12 ± 1 and 11 ± 0, for Rot, RotMC and Rot50 respectively.

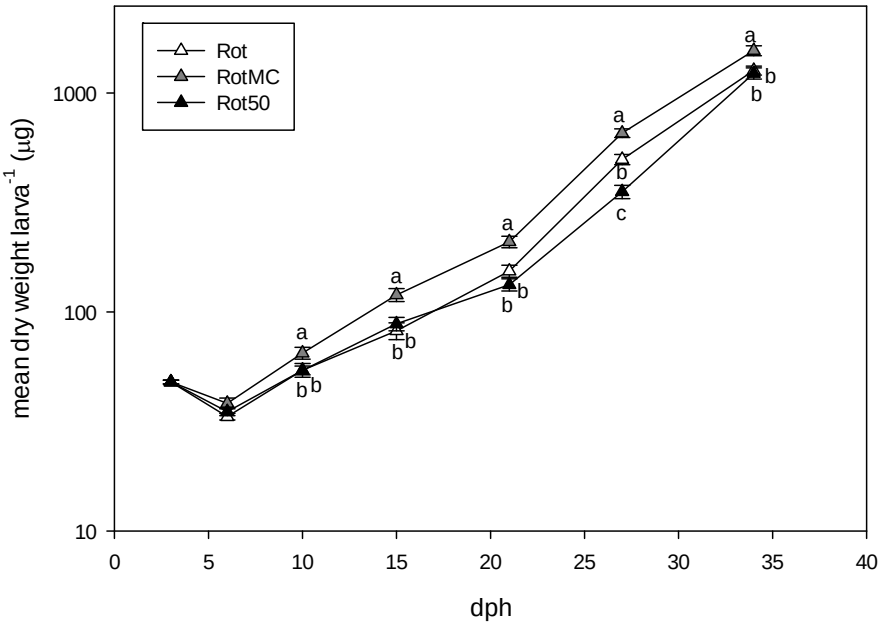

Figure 4.6 Mean dry weight (µg larva^{-1}) for Rot, RotMC and Rot50 in the water quality experiment. Measurements were made on 3 dph (n=13 from holding tank), 6, 10, 15 (n=24-36), 21 (n=49-60), 27, and 34 dph (n=60-90). Data are presented as mean values ± SE. Variation in letters indicates significant differences between the treatments for each dph ($p<0,05$; one-way ANOVA).

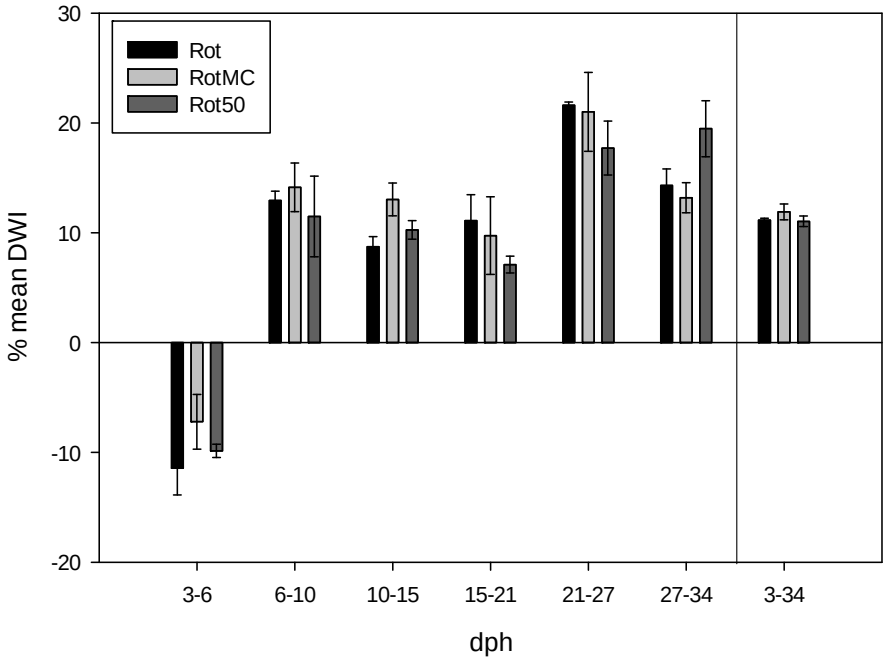

Figure 4.7 Mean percent daily weight increase (%DWI) for Rot, RotMC and Rot50 from the water quality experiment. From 3-6 dph, the larvae are mainly in the yolk sac stage, which involved endogenous feeding. The values are based on mean %DWI from each fish tank (n=3 for RotMC and Rot50, n=2 for Rot), ± SE.

4.2.2 Survival

As for the live feed experiment, the mortalities were highest in the beginning, resulting in a survival of 20 % or less at 27 dph (table 4.2). RotMC had the lowest survival by far, significantly lower than both other groups at 27 and 40 dph. Due to an accident with the biofilter, the RotMC fish tanks were probably provided with water containing H_2S at first day after transferal to the tanks (3 dph). This was detected by H_2S-smell coming from the rearing tanks. The tanks were therefore emptied, and the larvae transferred to an intermediate holding tank. Half of the water in the biofilter was exchanged with new water, but parts of the water had to be kept in order to maintain the microbially mature bacterial community. After one additional day of maturation in the biofilter, the MC fish tanks were refilled with water where H_2S was diluted, and the larvae were transferred back. H_2S traces in the water may have increased the mortality in these tanks (Attramadal, et al., submitted). Personal observations were not made as the experiment was conducted prior to the master thesis work.

The survival decreased substantially in all treatments after weaning to dry feed from 40 dph.

Table 4.2 Mean survival (%) ± SE of ballan wrasse larvae from the water quality experiment. Values are based on mean % survival from each fish tank (n=3) between 27-73 dph. No SE is provided for 73 dph, as there was only one tank per treatment. Initial larvae numbers were approximately 6000 per tank. Significant differences are indicated by letters a and b (p<0,05, one-way ANOVA).

Survival (% ± SE)			
dph	**Rot**	**RotMC**	**Rot50**
27	20 ± 2 **a**	4 ± 1 **b**	15 ± 2 **a**
40	15 ± 2 **a**	3 ± 0 **b**	12 ± 2 **a**
73	5	0,6	2,2

4.3 Comparing Rot groups

Rot groups from the two experiments received comparable diets, and are compared using day degrees to correct for differences between sampling days and in temperature. The curves show remarkably similar growth throughout the experiments, with a DW decrease at the beginning and then steady growth (figure 4.8). No significant differences were found when comparing DWs of similar day degrees.

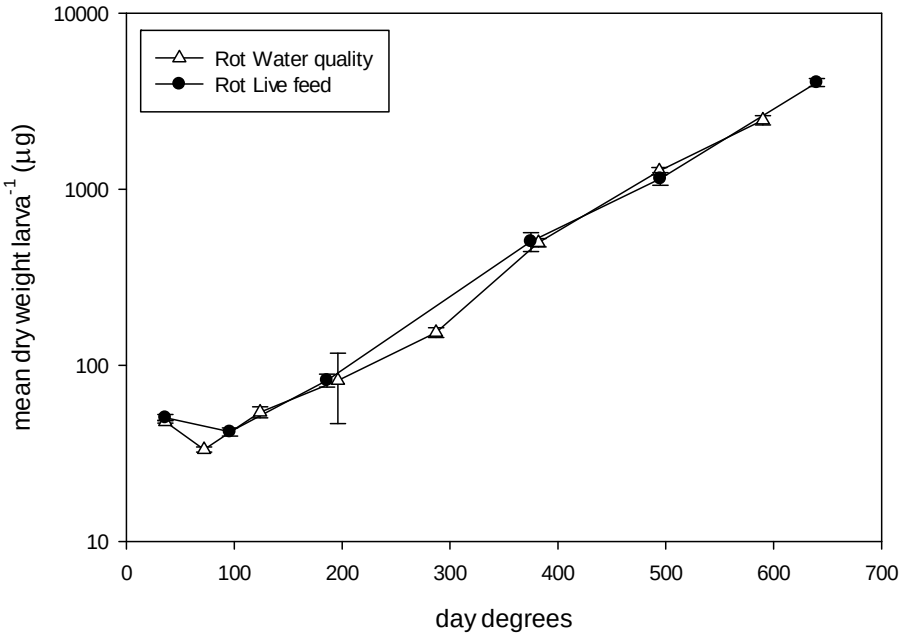

Figure 4.8 Mean dry weight (µg larva^{-1}) for Rot groups from the live feed and water quality experiment, both fed rotifers and *Artemia*. Measurements were made between 3-45 dph (n=12-60). Data are presented as mean values ± SE.

4.4 Standard length and myotome height of stained larvae

RotMC larvae were significantly longer than both Rot and Rot50 at 41 dph (figure 4.9). The RotMC MH was higher than Rot50, but similar as Rot (figure 4.10). There were no significant differences in the SL and MH between Rot, RotMC and Rot50 at 70 dph (figure 4.11).

For the live feed experiment, Cop had a significantly higher SL and MH than Rot, which was also the case for un-stained larvae (figures 4.3 and 4.4). There were no differences in the SL and MH measured on live fish or fixed fish after bone staining.

The Pearson correlation coefficient for SL and MH of stained larvae was 0,990 ($p<0,01$), showing that larvae from both experiments had similar relationships between SL and MH of the fish (4.12).

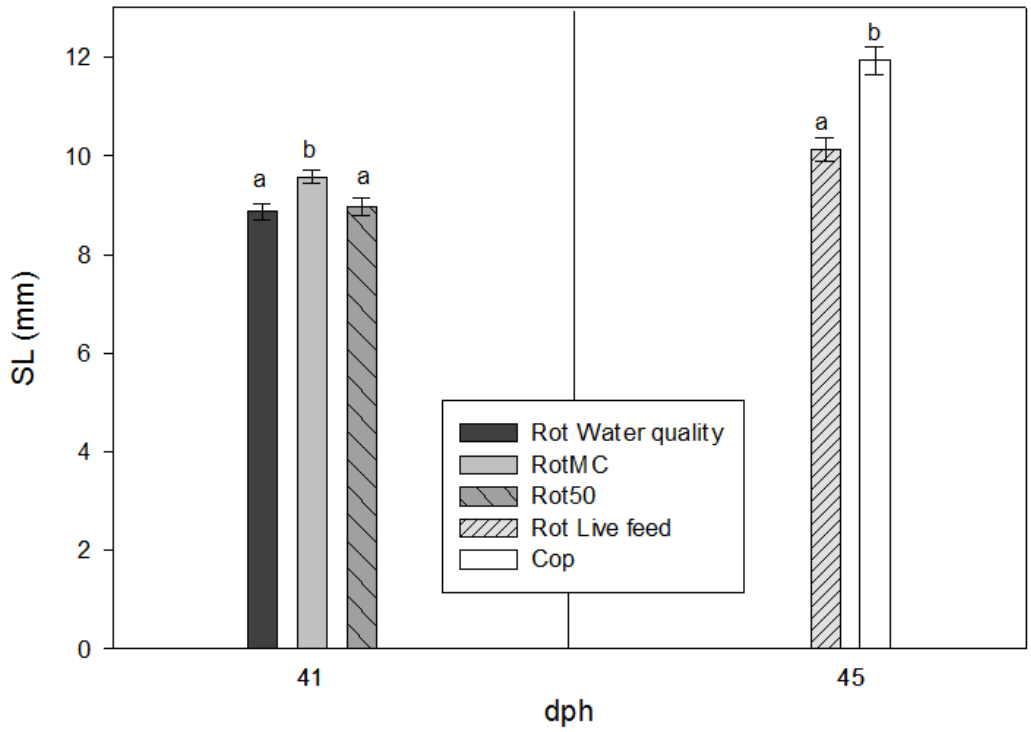

Figure 4.9 Mean standard length (SL) of bone stained larvae from both water quality and live feed experiments. Larvae were sampled at the end of the live feed period, at 41 dph (water quality, n=30-36) and 45 dph (live feed, n=45-53). The values are means ± SE. Significant differences within each experiment are indicated by letters a and b ($p<0,05$, one-way ANOVA).

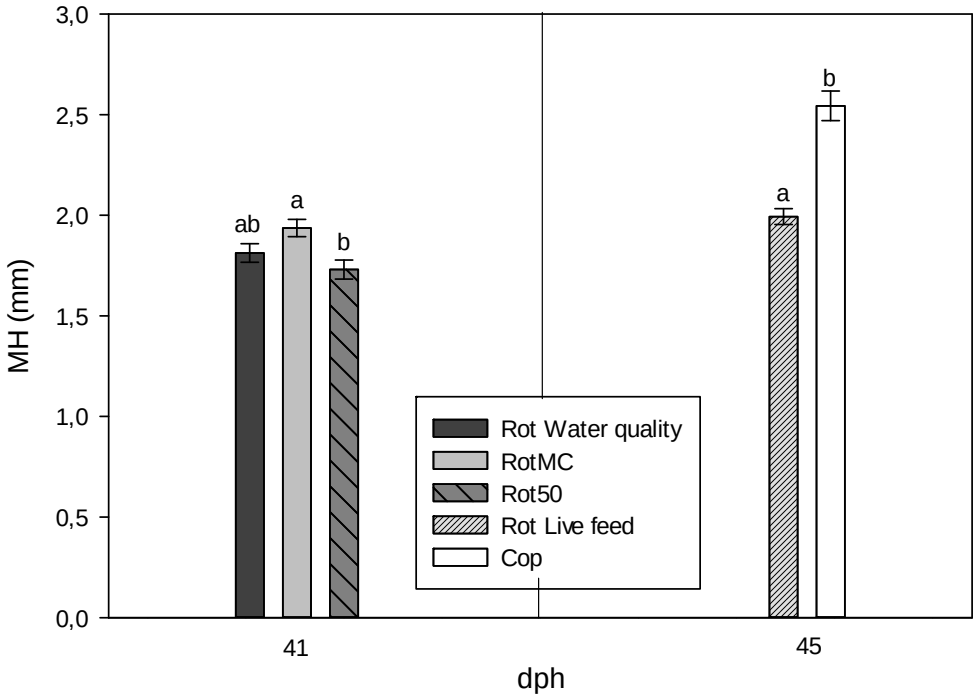

Figure 4.10 Mean myotome height (MH) after bone staining of larvae from both water quality and live feed experiments. Larvae were sampled at the end of the live feed period, at 41 dph (water quality, n=30-36) and 45 dph (live feed, n=45-53). The values are means ± SE. Significant differences within each year are indicated by letters a and b ($p<0,05$, one-way ANOVA).

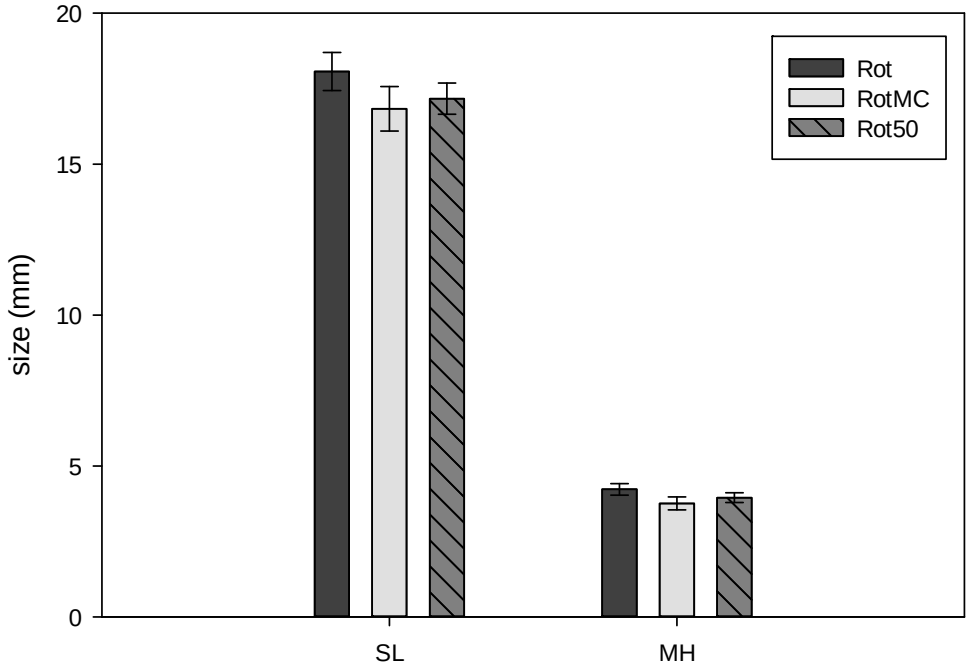

Figure 4.11 Mean SL and MH (mm) of 70 dph larvae from the water quality experiment, after bone staining. The values are means ± SE (n=15).

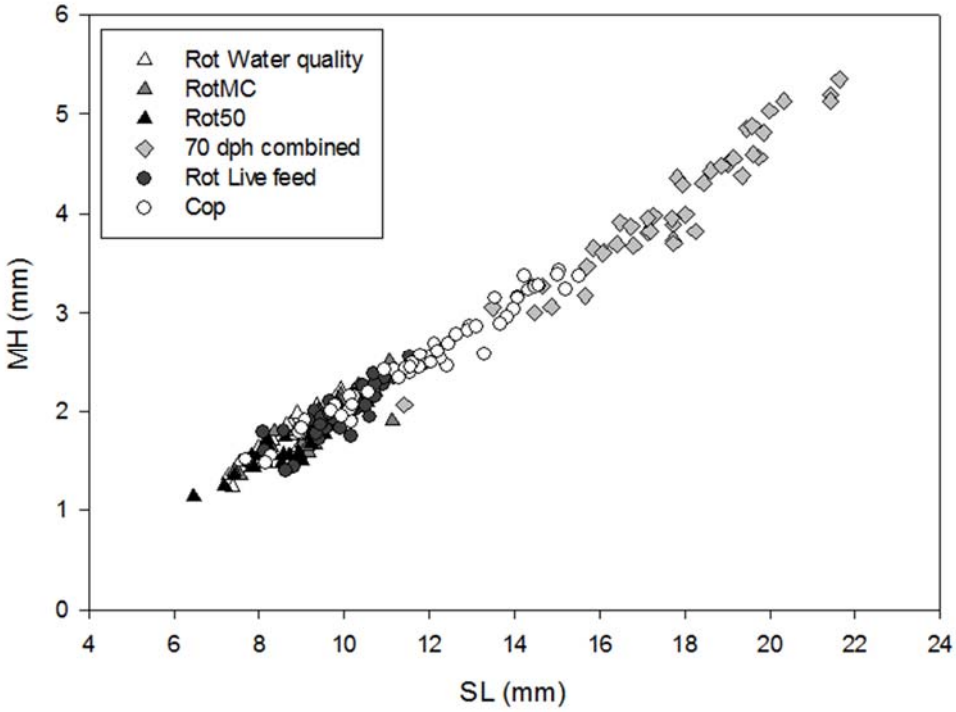

Figure 4.12 Standard length (SL) plotted against myotome height (MH) for ballan wrasse larvae from both water quality and live feed experiments. Larvae are between 41-70 dph, the groups were of similar sizes at 70 dph and are therefore combined. Pearson correlation ($p<0,01$) for all data combined: 0,990.

4.5 Skeletal analyses

4.5.1 Vertebral column

The vertebral column ossification was more dependent on fish size, than on chronological age (figure 4.13). When relating the ossification to standard length of the larvae, there were no differences between the treatments. Almost all fish were classified as having fully ossified vertebrae, only 6 ± 3 % had what was classified as a compact ossification. All fish at 70 dph had full ossification, as well did the fish of the Cop group, which were larger than the fish from the other groups (figures 4.9 and 4.10). The fish with compact vertebrae measured between 6,5 and 8 mm, with an average of 7,6 ± 0,2 mm.

Each fish larva had between 35 and 37 vertebrae, with an average of 36,1 ± 0,1. Rot from the live feed experiment had significantly fewer vertebrae than all other groups (35,7 ± 0,1). The difference lay in the pre-hemal region, where Rot had an average of 17,4 ± 0,1 vertebrae and the other groups had 18,0 ± 0,0. Appendix 6 shows all mean numbers of vertebrae.

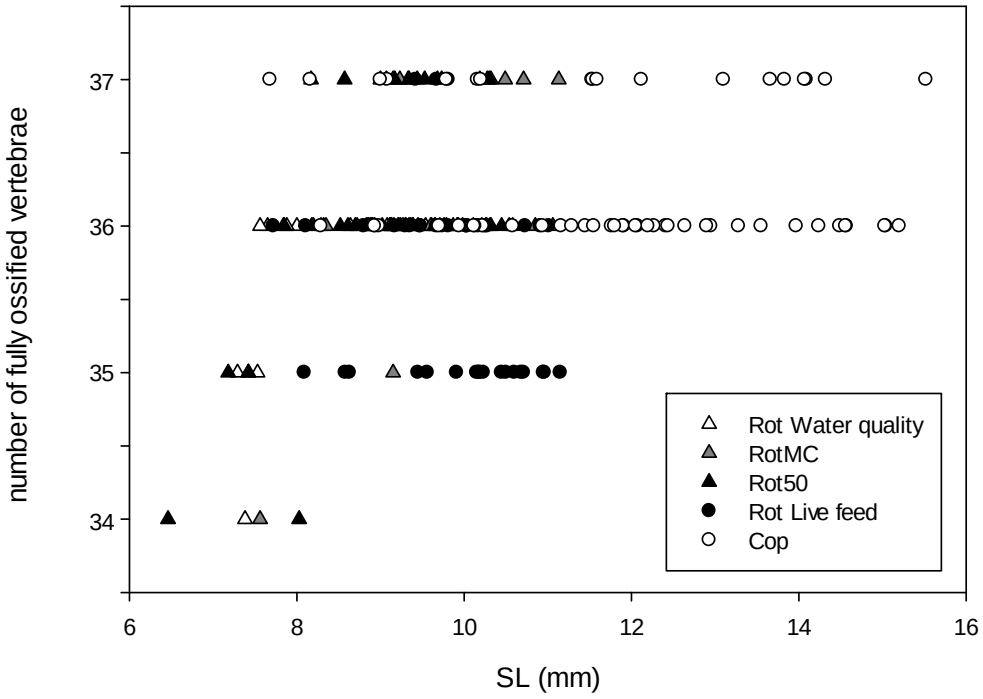

Figure 4.13 The number of fully ossified vertebrae plotted against standard length (mm), for individual fish larvae of 41 dph (water quality experiment) and 45 dph (live feed experiment). All fish at 70 dph were fully ossified, and are not included in the plot. The total number of vertebrae in each larva ranged from 35-37.

4.5.2 Dorsal fin

The dorsal fin ossification began in posterior parts of the fin (score 1), before extending to anterior parts (score 2) and finally the pterygiophores (score 3). A visualization of the process is presented in figure 4.14. All fish had some degree of dorsal fin ossification, and only a few of the smallest larvae from 41 dph of the water quality experiment were scored 1 (figure 4.15). When relating the dorsal fin ossification to standard length, no differences between treatments were found. The greater size of the Cop larvae therefore resulted in a higher ossification degree when comparing to the other treatments of 41 and 45 dph. All 70 dph larvae were fully ossified (score 3). The formation of a fully ossified dorsal fin occurred between 8,6 and 10,5 mm.

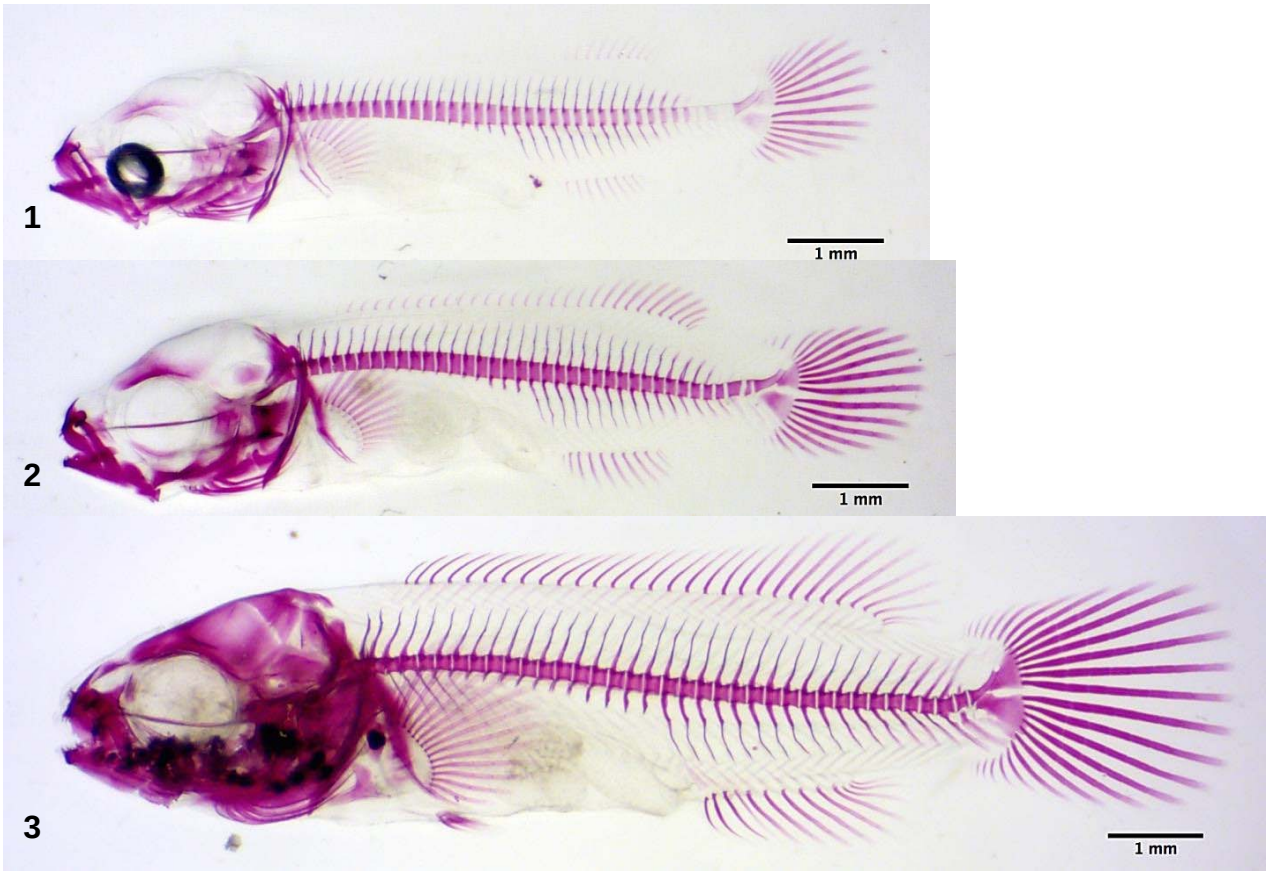

Figure 4.14 Classification of dorsal fin ossification based on distribution and saturation of color. 1) ossification of posterior parts, 2) ossification of anterior and posterior parts and 3) full ossification including the pterygiophores. The fish larvae are from Rot of the water quality experiment, and are comparable in size.

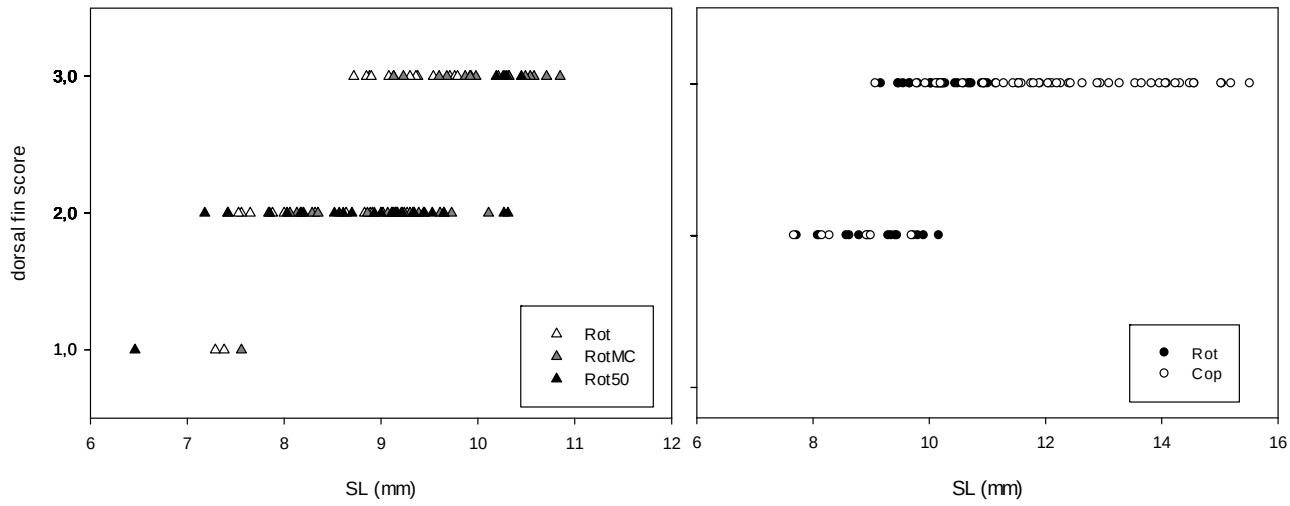

Figure 4.15 Dorsal fin score plotted against standard length (mm), for individual fish larvae of 41 dph (water quality experiment) and 45 dph (live feed experiment), respectively. 70 dph fish were all scored 3, and omitted from the figure. The score 1 = ossification of posterior parts, 2 = ossification of both anterior and posterior parts and 3 = full ossification including the pterygiophores.

4.5.3 Squamation

The ossification of scales started along the vertebral column of the fish larvae, and extended towards the pterygiophores (figure 4.16). All fish larvae of 70 dph in the water quality experiment had a squamation score of 2 or 3 (figure 4.17). No larvae at 41 dph had any ossification of scales; neither did the Rot fish of the live feed experiment. Five of the largest Cop fish (9 ± 4 %) had beginning ossification along the midline, scored 1. The size range of these fish was 14,2-15,5 mm, with an average of 14,9 ± 0,2 mm. No fish of any Rot treatment at 41 and 45 dph were close to that size. The ossification appeared to relate to standard length, although the 70 dph fish scored 2 were smaller than the Cop fish scored 1. The smallest fish at 70 dph measured 11-12 mm SL, and seemed to develop somewhat abnormally considering their age.

In order to determine the number of vertebrae and assess the skeletal anomalies of the 70 dph fish from the water quality experiment, the scales were removed from one side of the fish (figure 4.18).

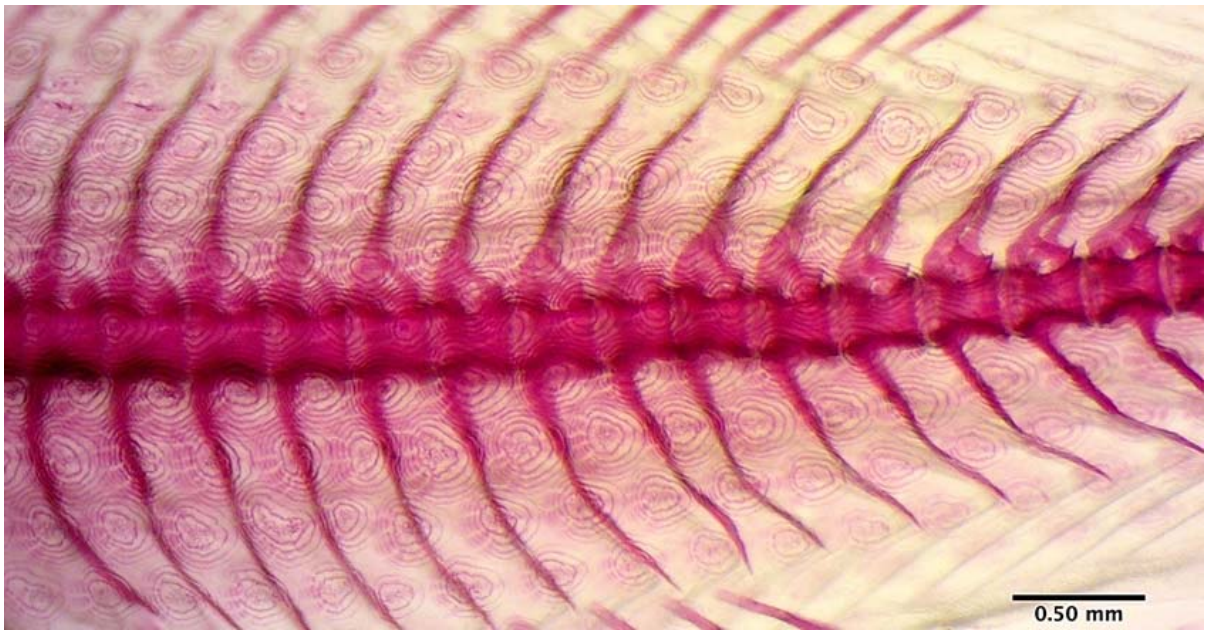

Figure 4.16 Scales of a 70 dph ballan wrasse larva from the water quality experiment, with SL 16,1 mm. Vertebrae, neural arches, hemal arches and pterygiophores are visible through the scales. The ossification of scales extended into the pterygiophore area resulting in score 3.

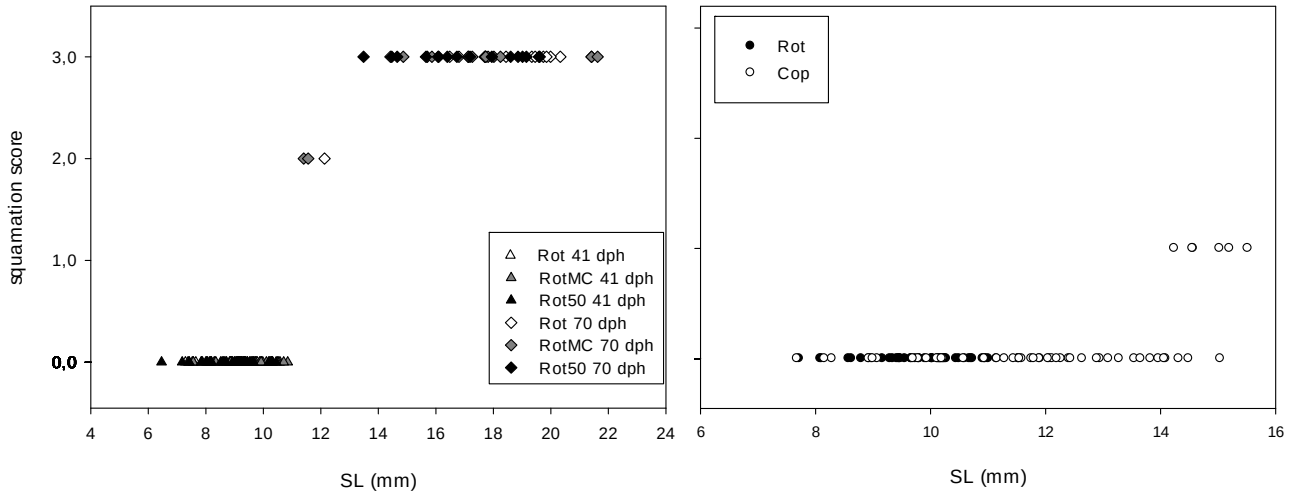

Figure 4.17 Squamation score plotted against standard length (mm), for individual fish larvae of 41 and 70 dph (water treatment experiment), and 45 dph (live feed experiment), respectively. Score 1 occurring in Cop-fish of SL 14,91 ± 0,23 mm (14,24 and 15,52 mm; 15,52 was the largest Cop specimen). Score 0 = no ossification, 1 = ossification along the midline, 2 = ossification of the flank up to the pterygiophore area and 3 = full ossification including pterygiophore area.

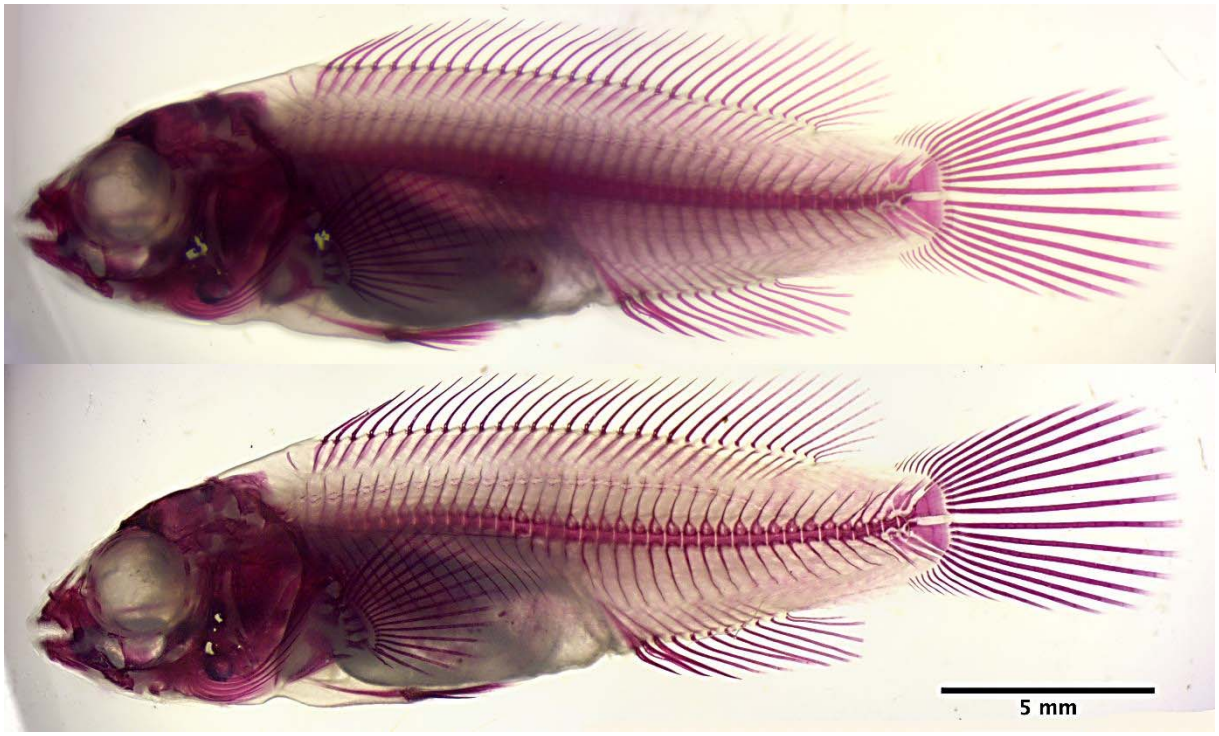

Figure 4.18 A Rot fish at 70 dph (water quality experiment), before and after removing the scales on one side, illustrating the effect of removing the scales when assessing the vertebral column.

4.6 Skeletal anomalies

Fish larvae from the Cop group had a significantly lower number of anomalies per larvae than any other group (figure 4.19), both when including and excluding twisted arches. All Rot groups had approximately 2 anomalies in each fish larva (including twisted arches), while Cop had an average of less than 1 anomaly per larva. The Cop fish had significantly lower occurrence of axis deviations and twisted arches than all other groups, and significantly less abnormal arches than all 41 dph groups of the water quality experiment. The occurrence of fused vertebrae and blunt nose were similar between all treatments. Mean numbers of each anomaly are presented in table 4.3. The occurrence of abnormal arches and blunt nose was reduced from 41 to 70 dph in the water quality experiment, but there was an increase in fused vertebrae and axis deviations.

The number of anomalies in one fish larva ranged from 0-4 (figure 4.20). Close to half the fish from the Cop group had 0 anomalies, whereas half the fish from all other group had 2 anomalies.

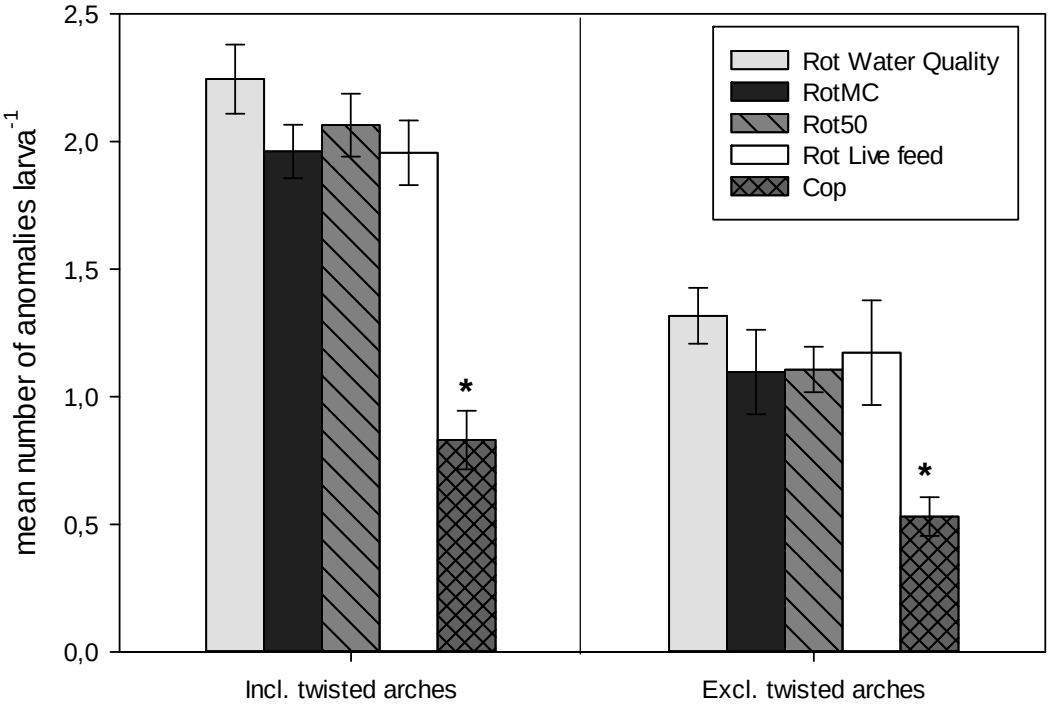

Figure 4.19 The mean number of anomalies per larva, for each individual treatment. There were no differences within treatments in 2011; 41 and 70 dph are therefore combined. n=45-53 for each treatment. The asterisks indicate significant difference from the other groups ($p<0,01$; one-way ANOVA).

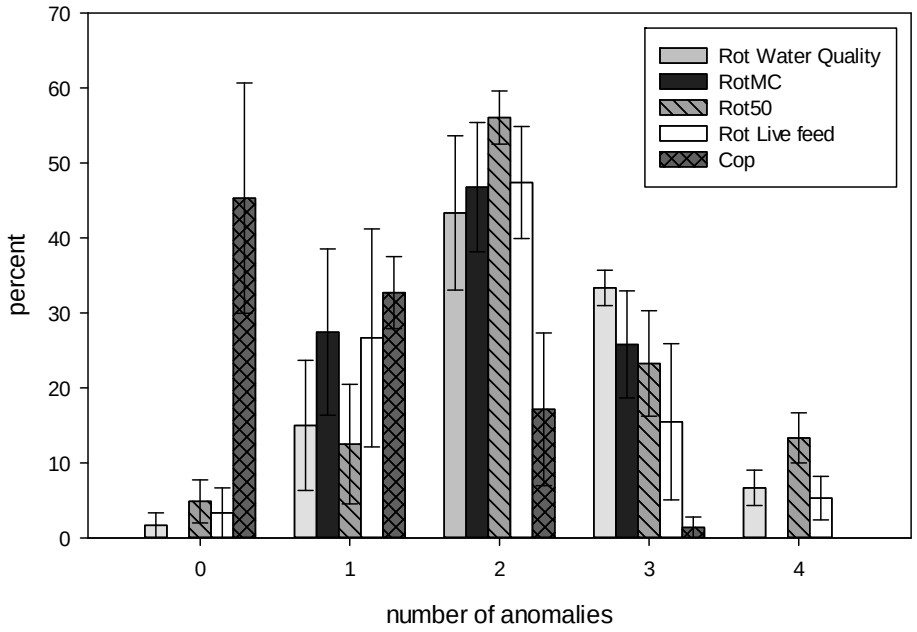

Figure 4.20 The proportion of each larvae group having a certain number of anomalies (0-4). There were no differences within treatments of the water quality experiment; 41 and 70 dph are therefore combined.

Table 4.3 Percent occurrence of abnormal arches, twisted arches, fused vertebrae, axis deviations and blunt nose on 41 dph, 70 dph (water quality experiment) and 45 dph (live feed experiment). The number of larva ranged from 15-53 per treatment (indicated in the n-column). Different letters indicate significant differences within each anomaly (n=3, p<0,05). 70 dph (2011) are omitted from the significant checks, due to only one tank per treatment (n=1).

			Skeletal anomaly occurrence (% larvae ± SE)				
dph	Treatment	n	Abnormal arches	Twisted arches	Fused vertebrae and/or arches	Axis deviations	Blunt nose
Water quality experiment							
41	Rot	30	97 ± 3 **a**	96 ± 3 **a**	7 ± 5 **a**	7 ± 5 **a**	17 ± 7 **a**
	RotMC	36	86 ± 6 **a**	85 ± 7 **a**	17 ± 6 **a**	11 ± 5 **a**	8 ± 5 **a**
	Rot50	32	81 ± 7 **ab**	93 ±5 **a**	6 ± 4 **a**	9 ± 5 **a**	22 ± 7 **a**
70	Rot	15	40 ± 13	93 ± 7	27 ± 12	40 ± 13	0 ± 0
	RotMC	15	7 ± 7	100 ± 0	27 ± 12	33 ± 13	0 ± 0
	Rot50	15	47 ± 13	100 ± 0	33 ± 13	20 ± 11	0 ± 0
Live feed experiment							
45	Rot	45	45 ± 13 **bc**	74 ± 6 **a**	45 ± 20 **a**	16 ± 5 **a**	0 ± 0 **a**
	Cop	53	30 ± 6 **c**	19 ± 6 **b**	6 ± 3 **a**	0 ± 0 **b**	4 ± 3 **a**

An anomaly not found described in the literature was encountered in three larvae in total (one each from Rot Water quality, Rot Live feed and Cop). A small partial vertebral segment including neural arch was found between segments 35-36 (figure 4.22c). The occurrence was 0,95 ± 0,5 in total. Fused segments and/or arches appeared mainly in 2 vertebrae of the caudal region, in the next to last vertebral segment (figure 4.22a). This anomaly was more frequently encountered at 70 dph compared to the same groups at 41 dph. Abnormal arches was less frequent at 70 dph compared to the same groups at 41 dph. Axis deviations were mild, and included 2-3 segments of the vertebral column, without causing major changes in the appearance. Visualizations of skeletal anomalies are presented in figures 4.21 and 4.22.

The most frequently encountered anomaly was twisted arches, occurring in 74 ± 3 % of the larva. The twisted arches always had a certain pattern of distribution, and a scoring system was made: 0 = no twisted arches, 1 = appearing in neural arches of the pre-hemal region, 2 = appearing in neural arches of both the pre-hemal and hemal region, and 3 = appearing in both neural and hemal arches all over the body. The anomaly never occurred in the hemal arches alone, and never occurred in neural arches of the hemal region without also occurring in the pre-hemal region. A wider distribution was generally associated with more intense twisting. The Cop group had significantly fewer twisted arches than all other groups, and the anomaly was significantly less prominent (mild spirals, rarely extending the pre-hemal region, score 1). Cop larvae had less widespread twisted arches than all water quality groups, and than Rot live feed. The occurrence of twisted arches and the mean score for each treatment is presented in table 4.4. Appendix 6 shows all mean values for each score (0-3).

Table 4.4 Percent occurrence of twisted arches ± SE in each treatment, and mean score (0-3). There were no differences within treatments in the water quality experiment; 41 and 70 dph are therefore combined. Different letters indicate significant differences between treatments ($p<0,05$; one-way ANOVA).

2011	**n**	**Occurrence of twisted arches (% ± SE)**	**Mean score (0-3) ± SE**
Rot	45	96 ± 3 **a**	2,1 ± 0,2 **a**
RotMC	51	86 ± 5 **a**	1,9 ± 0,2 **a**
Rot50	47	94 ± 4 **a**	2,0 ± 0,1 **a**
2012	**n**		
Rot	45	74 ± 6, **a**	1,3 ± 0,2 **b**
Cop	52	19 ± 6 **b**	0,4 ± 0,1 **c**

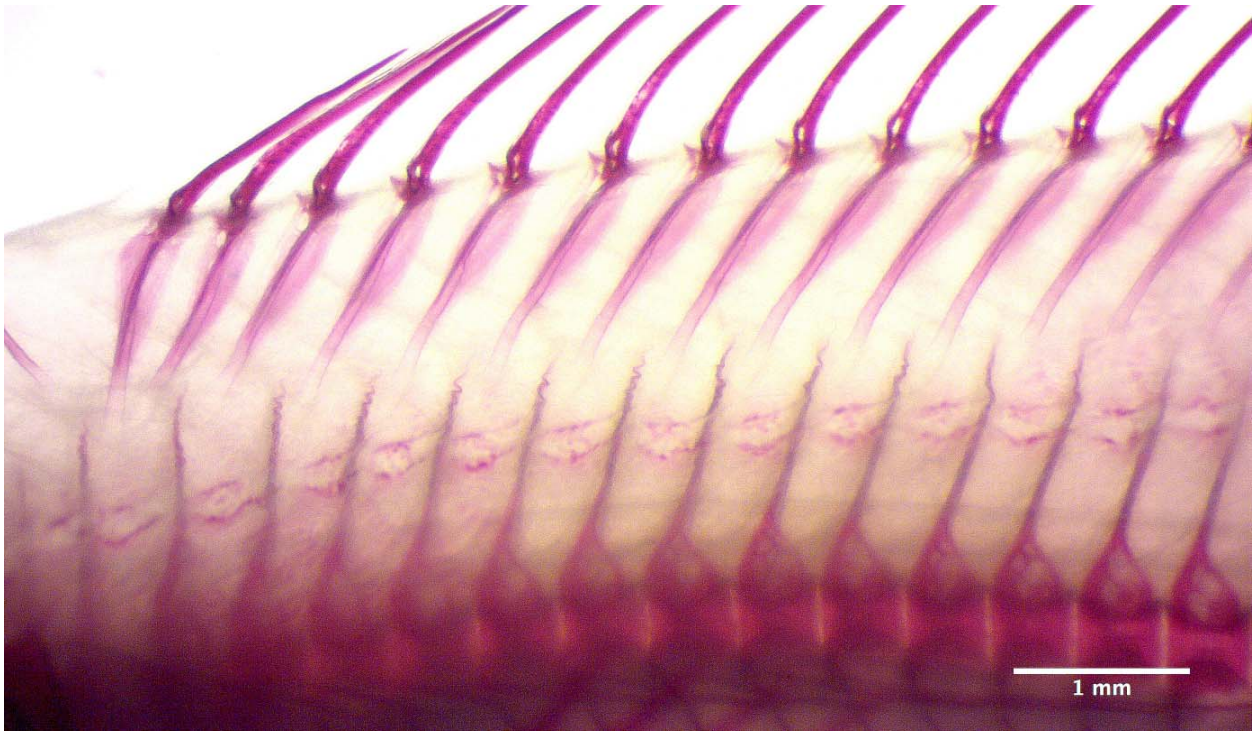

Figure 4.21 Twisted neural arches of the pre-hemal region in a 70 dph Rot fish of the water quality experiment. Dorsal fin rays and pterygiophores above the neural arches are fully ossified.

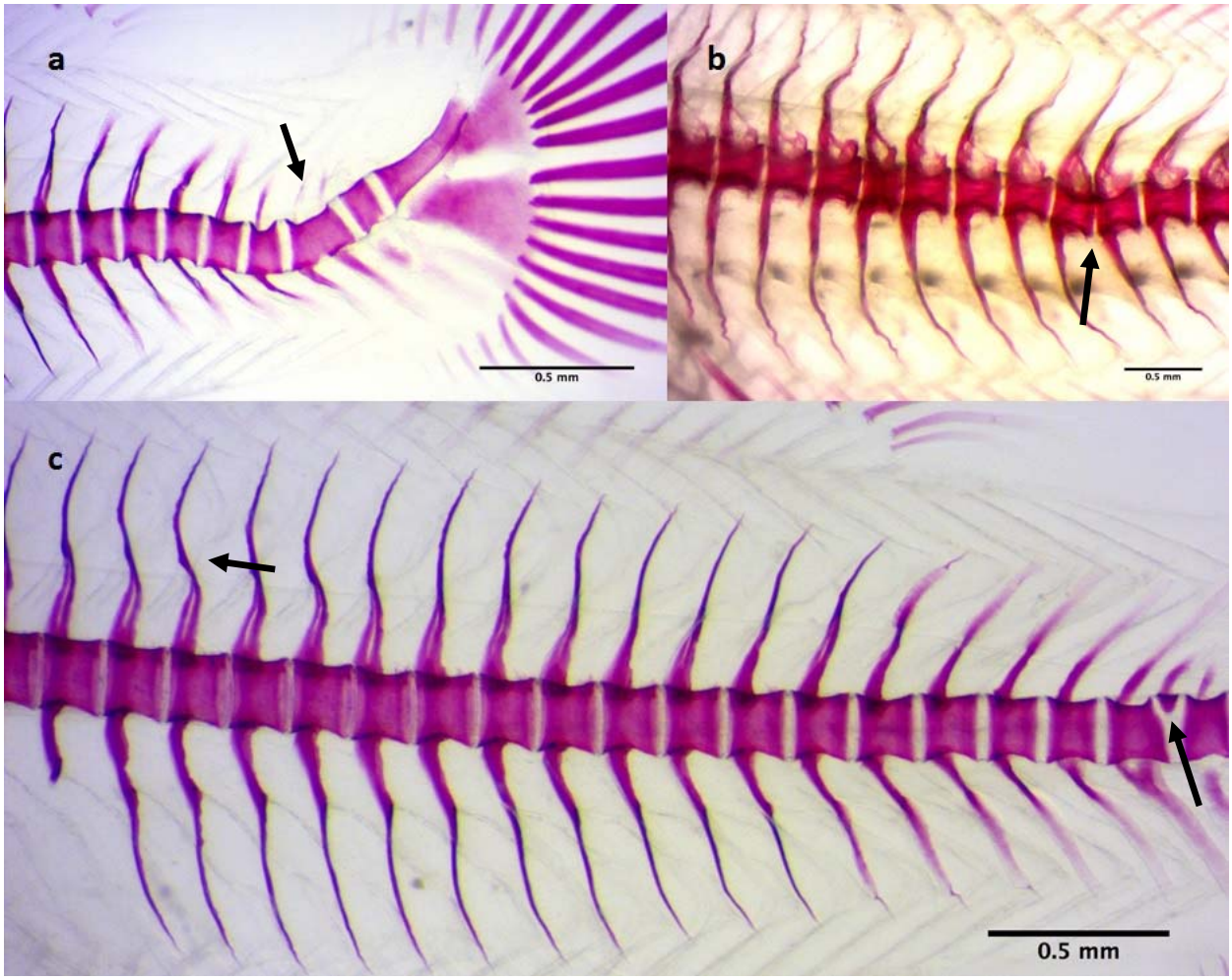

Figure 4.22 Skeletal anomalies in ballan wrasse fish larvae. All fish are oriented with the head to the left. **a)** Axis deviation and fusion of two vertebral segments in caudal region of fish larva. **b)** Axis deviation, abnormal arches and twisted arches both in the neural and hemal spines. **c)** Abnormal, bent arches. Notice the anomaly to the right, where a partial vertebral segment including neural arch is situated between segments 35-36 (indicated by arrow).

5 Discussion

As predicted, using cultivated copepods as live feed throughout the entire start feeding period had a clear positive effect on the growth and occurrence of skeletal anomalies in ballan wrasse larvae, when comparing to the use of traditional live feeds, rotifers and *Artemia*. The effect on growth was apparent from the onset of exogenous feeding and lasted to the end of the experiment, resulting in significantly larger fish larvae. In addition, the larvae fed copepods had significantly fewer skeletal anomalies than all larvae groups fed rotifers and *Artemia*. Co-feeding with rotifers and preserved copepods during the rotifer period of start feeding did not result in any differences on growth, survival or skeletal anomalies compared to using only rotifers. Rearing ballan wrasse larvae in microbially matured water adjusted to the carrying capacity of the fish tanks resulted in better growth than a regular flow-through system with maturation at low carrying capacity, but did not result in any differences in the skeletal anomaly occurrence. The survival of fish from microbially matured water was unexpectedly low, probably due to a H_2S-accident.

5.1 Dietary effects on growth and survival

The literature on ballan wrasse larval growth and development is limited to only a few publications. A study describing the development of ballan wrasse from hatching to metamorphosis (Ottesen, et al., 2012), provide SL measurements related to chronological and physiological age of larvae fed rotifers. When comparing those results to the ones of the current study, the SL reported by Ottesen et al. (2012) was always close to SL of the Rot group in the live feed experiment. Larvae from the Cop group however, were longer throughout the experiment, indicating a clear positive dietary effect of using copepods as live feed. When feeding ballan wrasse larvae with cultivated copepods from 4-10 and 4-30 dph, Øie et al. (in press) found significantly larger larvae when comparing to larvae fed rotifers. After switching from copepods to rotifers (10 dph) or *Artemia* (23 dph), the growth declined, especially in the group fed copepods for only 7 days. Larvae in the yolk sac- and preflexion stages could be vulnerable due to the development of new structural elements (Ottesen, et al., 2012), and early dietary changes to feed of lower nutritional quality seem to have a particularly negative effect on larval growth. When comparing the %DWI of larvae weaned

onto *Artemia* from 23 dph (Øie, et al., in press) and larvae fed copepods the entire live feed period (live feed experiment), the ones fed with only copepods showed a higher %DWI. The approximate %DWI from 30-45 dph, was 7 for the larvae weaned onto *Artemia*, and 11 for the larvae continuing on copepods. This indicates that using copepods throughout the entire live feed period could be more optimal for ballan wrasse larval growth than switching to *Artemia*. The critical duration of feeding with copepods could be studied further, to determine an optimal live feeding regime for ballan wrasse larvae. Considering that the production of copepods can have higher costs than using traditional live feeds, hatcheries might be interested in the optimal minimum period of which copepods should be used. For cod, the use of natural zooplankton in larval feeding has resulted in long-term positive effects on growth, survival and skeletal anomalies (Imsland et al., 2006; Koedijk et al., 2010). The long-term effects induced by early diet in ballan wrasse larvae should be investigated, for both partial and exclusive use of copepods in the live feed period.

Harvested, wild zooplankton has been recognized as superior to rotifers and *Artemia* for marine fish larval growth, survival and development (Shields, et al., 1999; Bell, et al., 2003; Cahu, et al., 2009). In this study, the use of cultivated copepods showed similar patterns regarding growth and development as wild copepods. Using cultivated copepods can be beneficial for continuous year-round supply and control over possible patogens (Marcogliese, 1995). Although production protocols for *Acartia tonsa* cultivation have been described decades ago (Støttrup, et al., 1986), the documentation on cultivated copepods in marine larviculture is scarce. This is probably the first study to involve the exclusive use of cultivated copepods for the entire live feed period of ballan wrasse, and the results encourage further work to optimize the feeding regimes for not only ballan wrasse, but other marine fish species as well.

The superiority of copepods as live feed is widely attributed to their HUFA composition and availability. Copepods store HUFA like DHA and EPA mainly in the phospholipid fraction of fatty acids. Supplying fatty acids via the phospholipids has proven beneficial for higher growth and survival, enhanced development, fewer anomalies and increased stress resistance in species like cod (Wold et al., 2007; Kjørsvik, et al., 2009), sea bass (*Dicentrarchus labrax)* (Cahu et al., 2003a; Gisbert, et al., 2005) and flatfishes (Bell, et al., 2003). Rotifers and *Artemia* that are enriched with high-HUFA diets can have very high levels of HUFA, but they are mainly found in the neutral lipids like triacylglycerol (Coutteau & Mourente, 1997; Bell,

et al., 2003). Not only is this suboptimal for growth in the fish larvae, but the HUFA of especially *Artemia* are readily metabolized under starvation (Olsen et al., 1993; Coutteau & Mourente, 1997; Evjemo, et al., 1997), for instance when awaiting distribution to the fish tanks. When staying in the fish tanks for long periods, *Artemia* would become more and more deficient in HUFA content, which is why excess *Artemia* was removed from the fish tanks prior to each feeding. Another reason for the superiority of the Cop group when regarding growth might be the higher protein, and especially free amino acid content of copepods, compared to rotifers (van der Meeren et al., 2008). Possible micronutrient deficiencies of enriched rotifers can further contribute to the overall suboptimal nutritional quality (Hamre, et al., 2008). The results of using copepods in the live feed experiment of this study, correspond to literature explaining the benefits of HUFA supplied through the phospholipids. Given the large amount of evidence on the superiority of copepods, the use should be considered in commercial hatcheries of ballan wrasse and other marine fish, to obtain higher quality larvae and juveniles.

The use of rotifers combined with 50 % preserved copepods did not result in any effect on growth or survival of ballan wrasse larvae, compared to larvae fed only rotifers. A positive effect has been reported for growth and survival in sole (Piccinetti, et al., 2012) and sea bream (Piccinetti, et al., 2014) fed preserved copepods, but this was not supported in the current study on ballan wrasse. There is little available research to describe why the results did not correspond with the hypothesis. (Dahle et al., 2013) suggested that introducing the preserved copepods too early, or suboptimal water circulation could be factors contributing to suboptimal conditions for the use of preserved copepods.

The effect of using live copepods that resulted in higher growth of the Cop larvae was not reflected in the survival numbers. The Cop larvae survival was slightly higher than for Rot larvae, but no significant differences were found. High mortalities are characteristic in the early stages of marine pelagic fish larvae (May, 1974), which was also experienced in this study, particularly in the first days before 15 dph. When assessing survival numbers in such an experiment, one should take into consideration that the numbers are estimates. The number of larvae distributed to the tanks at the beginning of the experiment was based on density estimations in the holding tank. Furthermore, mortality checks before 15 dph were not performed, as the small ballan wrasse larvae quickly disintegrated post mortem. Little is previously reported on the survival of ballan wrasse larvae, but the survival percent of this

experiment was only slightly lower than in the study of Øie et al. (in press). Between 13-61 dph, Øie et al. did not find any differences in the survival between larvae fed enriched rotifers or copepods for 30 days, which was also the case for Rot- and Cop larvae in this live feed experiment.

When comparing Rot groups from the live feed and water quality experiments, the DW was the only growth parameter available for analyses. As the chronological age for DW sampling was not similar in the two experiments, the physiological age (day degrees) was used. The two Rot groups had almost identical sizes and the growth curves were remarkably similar, with exponential growth as expected for marine fish larvae (Kjørsvik et al., 1991; Galloway et al., 1999; Wold et al., 2008; Garrido et al., 2012).

5.2 Water quality effects on growth and survival

The ballan wrasse larvae reared in microbially matured water adjusted to the carrying capacity of the fish tanks (RotMC) had higher growth during the live feed period, compared to larvae reared in flow-through systems equipped with only a regular biofilter. Only a small number of fish were assessed from each group at 70 dph (n=15), due to a limited number of fish larvae left in the tanks. A higher n could have revealed size differences between treatments, or confirmed that the groups were similar in size.

The use of microbially matured water obtained by non-selective reduction, or selective enhancement of bacteria can prevent opportunistic blooms of r-strategic bacteria that might be harmful to the fish larvae (Vadstein, et al., 1993). The initially higher growth of larvae in microbially matured water are supported in a previous study on cod (Attramadal, et al., 2012a). When assessing the microbiota of the water quality experiment, Attramadal et al. (submitted) found that the microbial composition of the RotMC water was more stable than in the system without microbial maturation to high carrying capacity, reducing the potential of opportunistic blooms. European sea bass juveniles with dietary probiotic supplements showed faster growth and lower stress levels compared to larvae that were not under any microbial control (Carnevali et al., 2006), further emphasizing the important role of microbial composition in the gut. Since both Rot and Rot50 were reared in water that had been treated in a regular biofilter, the tank water could have been favorable for higher variation in microbial communities and hence an increased chance of harmful opportunistic bacteria.

Larvae of RotMC had a significantly higher mortality than the other groups of the water quality experiment. A probable explanation for the very low survival can be found in the H_2S detected at the beginning of the experiment. H_2S is a highly toxic compound (Stine et al., 1976), and even low concentrations can be lethal to fish eggs, larvae and juveniles (Colby & Smith, 1967; Adelman & Smith, 1970). Exposure to sublethal doses of H_2S has been described as damaging for gills and liver in Atlantic salmon smolts (Kiemer et al., 1995), as well as respiratory organs, brain and gills in channel catfish (*Ictalurus punctatus,* (Torrans & Clemens, 1982)). Although the larvae were removed from the tanks immediately after smelling the H_2S, the initial exposure to an unknown H_2S-concentration is likely to have caused the high mortalities of the RotMC group. In order to maintain some of the microbial maturation that was established in the biofilter, parts of the water containing H_2S had to be kept, and the fish larvae were therefore reared in water with diluted concentrations of H_2S for the first period. The surviving larvae, although few in numbers, seemed to thrive well as seen in the superior growth results. The larvae could possibly have had an advantage of being fewer in numbers, due to reduced competition and increased amount of food. This is not very likely, however, because the larval density in the tanks was low for all groups, and the larvae were fed in excess on live prey. The chance of positive rearing conditions due to lower larval density in RotMC tanks is therefore low, but cannot be completely ruled out as no density-related investigations were conducted. If the microbial maturation had worked as planned, the survival rates for RotMC would probably have been higher, as was expected prior to the experiment.

5.3 Skeletal analyses

5.3.1 Ossification and squamation in relation to diet and water quality

Ossification and skeletal development was related to SL, more than age or treatment. This was also the case for ballan wrasse (Sørøy, 2012) and cod larvae (Hansen, 2011) of previous studies. A size-related development has been suggested for marine species (Sæle & Pittman, 2010) like sole (Amara & Lagardere, 1995) and cod (Wold et al., 2009), which also seems to apply to osteological development in ballan wrasse. The physiological age related to skeletogenetic events was clearly affected by the size variations of larvae induced by dietary composition and water quality. Cop- and RotMC larvae were the largest within each

experiment at 45 and 41 dph, and therefore had a higher degree of ossification of the vertebral column and dorsal fin.

The mean total number of 36,1 ± 0,1 vertebrae corresponded with the numbers from Sørøy (2012) and reported numbers from wild ballan wrasse (Helland et al., 2012). However, significant differences were found between groups. Rot larvae from the live feed experiment had significantly fewer vertebrae than both Cop larvae, and all three Rot-groups of the water quality experiment. The difference lay in the pre-hemal region, where Rot larvae from the live feed experiment had fewer vertebrae than larvae from the other treatments (appendix 6). This is the first study to report on variations in pre-hemal arches of ballan wrasse. The number of vertebral segments in marine fish larvae has been related to hatching temperature (halibut *(Hippoglossus hippoglossus)*; (Lewis et al., 2004)) and dietary effects like HUFA or retinoic acid status (sea bass; (Villeneuve et al., 2006)). For sea bass, diets high in retinoic acid slowed down the development, and hence lowered the number of vertebral segments, while diets high in HUFA in the phospholipid fraction seemed to induce a supernumerary vertebral segment (Villeneuve, et al., 2006). Dietary effects could have described the difference in vertebral segments between Cop- and Rot larvae of the live feed experiment, where Rot larvae had significantly fewer vertebrae than Cop larvae. However, the Cop larvae did not appear to have supernumerary vertebrae, as the mean of 36,3 ± 0,01 corresponded well with both the rotifer-fed groups of the water quality experiment, as well as previously reported vertebrae numbers. There are very few publications describing causative factors for variable vertebrae numbers, and none for ballan wrasse. The results from this study indicates a plausible dietary effect, as the only apparent difference between Rot- and Cop larvae were the live feed organisms.

Vertebrae and fin rays were fully ossified before scales appeared, which is consistent with results suggesting that squamation processes occur near or at the metamorphosis stage (Koumoundouros et al., 2001; Sire & Akimenko, 2004). This was true for the fish at 70 dph, which had morphological features similar to adult ballan wrasses. All fish of 70 dph had ossification of the scales, but a few fish had lower ossification scores than the rest. These fish were of substantially smaller sizes than what would be expected of ballan wrasse of that age (Ottesen, et al., 2012). They were almost comparable to fish from 41 dph in size, and this could indicate that the growth and development of these larvae was not normal. Of the younger larvae, only a few Cop larvae had any ossification of scales. These were the largest

fish of the Cop group, and had very early ossification of only a narrow band along the vertebral column. The Cop fish with squamation score 1 were larger than fish of 70 dph scored 2, which could further strengthen the idea of retarded growth and development in the smallest fish from 70 dph. Scales should be removed from larva with squamation scores 2-3, to enable assessment of skeletal anomalies.

5.3.2 Skeletal anomalies in relation to diet and water quality

Using copepods as live feed throughout the entire first feeding period had a large positive impact on the occurrence of skeletal anomalies, resulting in larger numbers of fish without anomalies, fewer anomalies per fish, and milder versions of the encountered anomalies, when comparing to fish fed with rotifers. The use of microbially matured rearing water (RotMC) had no effect on the occurrence of skeletal anomalies compared Rot and Rot50 larvae. Previous studies have suggested links between skeletal anomalies and rearing conditions such as water turbulence (Chatain & Ounais-Guschemann, 1990; Helland, et al., 2009) and temperature (Polo et al., 1991; Georgakopoulou et al., 2010). The rearing temperatures did not differ substantially between Cop and the other treatments, so this alone would not explain the higher incidence of anomalies in rotifer-fed larvae. Studies on the relationship between microbiota of the fish tanks and skeletal anomalies were not found, and this should be further investigated as microbial maturation has been beneficial for other quality parameters, like survival in cod larvae (Attramadal, et al., 2012a; Attramadal et al., 2014). The H_2S-incidence could possibly have had a negative effect on the skeletal anomaly occurrence. Without available research on neither H_2S nor microbiota in relation to skeletal anomalies in fish, further deductions would be nothing but speculations.

Dietary effects has been recognized as one of the key factors affecting skeletogenesis in marine teleost fish (Cahu, et al., 2003b; Lall & Lewis-McCrea, 2007). There are several studies directly connecting the larval nutrition to skeletal anomalies (Lewis-McCrea, et al., 2010; Sørøy, 2012; Zambonino Infante & Cahu, 2010), and the current study further emphasizes the importance of nutrition. The nutritional effects of HUFA, and especially DHA had a positive effect on skeletal anomaly occurrence in milkfish *(Chanos chanos)* (Cahu, et al., 2003b), but the exact roles of HUFA in bone lipid metabolism still needs further investigation in order to explain their role (Lall & Lewis-McCrea, 2007). Deficiencies in

phospholipids in particular, have been described as a causative factor for abnormal development (Finn et al., 2002; Hamre et al., 2002; Cahu, et al., 2003a; Kjørsvik, et al., 2009), which can explain why the fish fed copepods performed so much better than the ones fed rotifers and *Artemia*.

The larvae from this study generally had a larger mean number of anomalies than the larvae from Sørøy's study (2012). However, the relative differences in anomalies between larvae fed copepods or rotifers were similar between the two studies. The reduction of skeletal anomalies was >50 % in larvae fed copepods, compared to larvae fed rotifers and *Artemia*. The effect was apparent both when including and excluding twisted arches. Hansen (2011) found that there were no differences in skeletal anomalies of cod, when excluding twisted arches. The cod received the same dietary treatment as the ballan wrasse of Sørøy (2012), which could indicate that there are species specific responses in skeletal anomaly occurrence related to similar diets.

The most frequently observed anomaly was twisted arches, which was also the case in previous studies on cod (Hansen, 2011) and ballan wrasse (Sørøy, 2012). Cop larvae had a significantly lower incidence of twisted arches, and the score (reflecting distribution and severity) of twisted arches was significantly lower than all rotifer treatments (table 4.4). Not all anomalies have a negative effect on the appearance and functionality of the fish (Koumoundouros, 2010). While the twisted arches do not alter the external appearance of the fish, severe cases of twisted arches that include large numbers of neural and/or hemal arches can lower the fish performance, as they protect parts of the nervous and circulatory systems (Boglione, et al., 2013). Phosphorus deficiency in the diet has been correlated with vertebral malformations (Hamre, et al., 2008). A diet low in phosphorus has been shown to cause twisted neural and hemal arches in the pre-hemal and hemal vertebrae of red porgy *(Pagrus pagrus)* (Izquierdo, et al., 2010), and in the hemal region of halibut (Lewis-McCrea & Lall, 2010). Considering that even enriched rotifers might be deficient in phosphorus (Hamre, et al., 2008), this could explain why twisted arches were much more common in the larvae fed rotifers and *Artemia*, than in larvae fed copepods. Without having determined the exact nutritional composition of the live feed, conclusions regarding the amounts of specific nutrients cannot be drawn. What is clear, however, is the superior effect of copepods as live feed on skeletal anomalies in general.

Some skeletal anomalies might develop over time (Boglione, et al., 2013), which may explain why fused vertebrae and axis deviations appeared more frequently in fish at 70 dph compared to the same groups at 41 dph. The same development over time was seen in rotifer-fed larvae of this study and the study of Sørøy (2012); a decrease in abnormal arches, and a large increase in axis deviations. This is the opposite of both sea bass and sea bream, where abnormal arches generally increase with the size of the fish (Boglione, et al., 2013), emphasizing that conclusions drawn on one species should not be imposed on another. Red porgy showed variation in localization of fused vertebrae in intensive and semi-intensive rearing systems (Izquierdo, et al., 2010), indicating that environmental factors can influence distribution and occurrence of skeletal anomalies. In addition to environmental factors, dietary changes e.g. when weaning to dry feed of different nutritional composition than the live feed, may cause an increase or decrease in certain anomalies (Boglione, et al., 2013), like seen for ballan wrasse larvae reared on rotifers and *Artemia* in this study.

The larvae from the study of Sørøy (2012) were reared to juveniles in experimental facilities at NTNU, and then assessed for skeletal anomalies using radiography. The result was a very low occurrence of skeletal anomalies (Bæverfjord & Helland, 2014), which did not correspond to the number of anomalies recorded in the larvae up to 60 dph (Sørøy, 2012). This could indicate that the fish larvae with skeletal anomalies either die, or that some skeletal anomalies are reduced during time. The radiography study further reported that the juveniles were of good quality compared to ballan wrasse from commercial hatcheries (Bæverfjord & Helland, 2014), which could indicate that the fish larvae were also of good quality despite the number of skeletal anomalies reported by Sørøy (2012). Of the juveniles, the fish fed copepods were yet again superior to the ones fed rotifers, indicating a long-term effect of the early larval nutrition. The long-term effect should also be determined for fish reared on cultivated copepods the entire live feed period, to reveal whether or not a longer copepod-period has effects extending the larval period.

6 Conclusions and recommendations

The use of cultivated copepods of increasing sizes throughout the entire live feed period had superior effects on growth and reduced the incidence of skeletal anomalies, when compared to using rotifers and *Artemia*. *Acartia tonsa* as live feed fulfill nutritional requirements of the larvae that the use of traditional live feeds do not. The results from this study confirms the hypothesis that cultivated, live copepods are more optimal live feed organisms for ballan wrasse larvae than rotifers and *Artemia*. The survival was low in fish fed either diet, which could be contributed to other factors than nutrition, for instance genetics and egg quality. The use of preserved copepods however, did not show any effect on either growth, survival or skeletal anomalies compared to only using rotifers. Further emphasis should therefore lie on the use of live, cultivated copepods for a stable and predictable supply of high quality live feed.

Conditioning microbially matured water to the carrying capacity of the fish tanks resulted in higher growth of ballan wrasse larvae, but also resulted in significantly lower survival than fish reared in flow-through systems matured to low carrying capacities. The unexpectedly low survival was probably due to an accident releasing H_2S to the fish tanks at the beginning of the experiment. No effect on skeletal anomaly occurrence was observed when using microbially matured water, and other environmental factors should be investigated to determine an optimal rearing regime for ballan wrasse larvae. The H_2S release in itself could have contributed to the high degree of skeletal anomalies in larvae reared in the affected tanks.

The ossification of vertebrae and dorsal fin related more to the size of the fish than to age, hence the fish fed copepods had higher degrees of full ossification, and were also the only group that had ossification of scales at the end of the live feed period (45 dph). Only the copepod-fed larvae group stood out with having significantly less skeletal anomalies than all other groups fed rotifers (or rotifers and preserved copepods) and *Artemia*. Long-term effects of both early larval nutrition and rearing environment should be studied to determine the fish quality after the live feed period. Studies on ballan wrasse in recirculation aquaculture systems would be useful for further assessments of the water quality impact on fish larvae.

Due to the costs of using cultivated copepods throughout the entire live feed period, farmers can be reluctant to implement the use in commercial hatcheries. Determining the exact optimal time period for when copepods should be used as live feed would therefore be interesting, to obtain ballan wrasse of the best possible quality. Considering the superior larval quality obtained by rearing ballan wrasse on cultivated copepods, hatcheries should consider using copepods for at least parts of the live feed period.

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Appendix 1

Dry feed procedure and 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^{-1} 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.

Recipe for ballan wrasse compound diet

	% (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	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]Soylechitin GMO powder (20022), Agrosom, Mölln, Germany
[e]Cod fish powder, (0271), Seagarden, Avaldsnes, Norway
[f]Betafin S1, Danish Animal Nutrition, Helsinki, Finland
[g]Per kg: D3 3000 IE, K3 20 mg, C 500 mg, B1 20 mg, B2 30 mg, B6 25 mg, B12 5 µg, B5 60 mg, Folic acid 10 mg, Niacin 200 mg, Biotin 1 mg
[h]Per kg: Mn 30 mg, Mg 750 mg, Fe 60 mg, Zn 120 mg, Cu 6 mg, K 800 mg, Se 0,3 mg
[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 (1979), with the difference being a smaller amount manganese chloride than in the original recipe:

$NaNO_3$ (Sodium Nitrate)	100,0g
Na-EDTA (EDTA disodium salt)	45,0g
H_3BO_3 (Boric Acid)	33,6g
$NaH_2PO_4 \cdot 2H_2O$ (Sodium Phosphate, monobasic)	20,0g
$FeCl_3 \cdot 6H_2O$ (Ferric Chloride, 6–hydrate)	1,3g
$MnCl_2 \cdot 4H_2O$ (Manganous Chloride, 4–hydrate)	0,136g
Vitamin B_1 (Thiamin HCl)	0,1g
Vitamin B_{12} (Cyanocobalamin)	0,05g
Trace Metal Solution *	1 ml
Distilled water	1000 ml

Note: use 1 ml Conwy medium $liter^{-1}$ seawater

*Trace Metal Stock Solution:

$ZnCl_2$ Zinc Chloride	2,1g
$CoCl_2 \cdot 6H_2O$ (Cobalt Chloride, 6–hydrate)	2,1g
$(NH_4)_6Mo_7O_{24} \cdot 6H_2O$ (Ammonium Molybdate, 4–hydrate)	2,1g
$CuSO_4 \cdot 5H_2O$ (Copper Sulphate)	2,0g
Distilled water	100ml

Note: acidify with 1 M HCl until solution is clear

Appendix 3

Bone staining with Alizarin Red

Procedure after Balon 1985 (modified from Taylor 1967 and Dingerkus & Uhler 1977).

	<10mm	**10-20 mm**	**>20mm**
Step 1 - fixation			
- fix in 10 % neutral formalin 			
- rinse in distilled water	2 x 5’	2 x 10’	2 x 20’
Step 2 – rehydration and bleaching			
- 96 % ethanol	2 x 30’	2 x 1h	2 x 2h
- 50 % ethanol	30’	1h	2h
- 15 % ethanol	30’	1h	2h
- Distilled water	30’	1h	2h
- Sodium borate buffer (working solution)	Skip	Skip or store	Store overnight
- Bleach in 1:9 3 % H_2O_2:1 % KOH under strong light [1] (depending on the amount of pigments) *	10’-2 h	~6 h	10 h

* Bleaching under strong light: Watch every sample carefully, stop when ok. For small larvae, a less concentrated solution can be used, to be able to monitor better.

Clear in trypsin buffer (to almost transparent)	20 h	2 days	4 days renew solution daily

Step 3 – staining and scale removal

Stain bones in Alizarin working solution * renew solution once, or when necessary (pale water)	20 h	2 days	3 days *
Remove scales on one side, only for larger larvae. Clear in trypsin buffer if necessary (5-20 hours)			

Step 4 - preservation

- rinse in distilled water	5’	10’	20’
- rinse in 1 % KOH (2x or until surplus colour is gone). *Don't keep the samples in this solution for a long time, the alizarin staining can be washed away. Stable when glycerol is added*	2x	2x	2x
- 40 % glycerol in 1 % KOH	2 days	2 days	2 days
- Take pictures			
- 70 % glycerol in 1 % KOH	2-24h	1 day	2 days
- 100 % glycerol for long term storage			

Reagents:

Trypsin-buffer:

Saturated solution borate buffer:
Na-borate (NaB_4O_7• 10 H_2O), saturated solution is >1g to 100 ml d-H_2O at 25°C 

100 ml Working borate buffer solution:
70 ml distilled water + 30 ml of the clear upper part of a saturated solution

Trypsin-buffer 100 ml:
Working solution borate buffer
0,5 g trypsin 

H_2O_2/KOH for bleaching
1:9 3 % H_2O_2:1 % KOH
30 ml: 29,7 ml 1% KOH + 300 µl 30 % H_2O_2. 

Alizarin stock solution:
Alizarin red S (Sigma A-5533, C.I. 58005)
Make a saturated solution in ethanol (approx. 0,15 %; < 2,5 %) Filter if necessary.

Alizarin working solution:
Add 400 µl saturated solution to 10 ml 1 % KOH

For ballan wrasse larger than 20 mm (SL >15 mm): use 1000 µl saturated solution to 10 ml 1% KOH

Appendix 4

Mean dry weight for each tank

Mean dry weight (mg larva^{-1})											
Water Quality experiment						**Live feed experiment**					
dph	Treatment	Tank	Mean	SE	N	dph	Treatment	Tank	Mean	SE	N
3	All	All	0,0479	0,0010	13	3	All	All	0,0505	0,0021	20
6	Rot	1	0,0130^{a}	0,0006	12	8	Rot	1	0,0363^{a}	0,0021	12
		2	0,0154^{b}	0,0006	12			2	0,0355^{a}	0,0013	12
	RotMC	1	0,0142	0,0006	12			3	0,0564^{b}	0,0046	10
		2	0,0161	0,0019	12		Cop	1	0,0503^{a}	0,0045	12
		3	0,0187	0,0018	12			2	0,0383^{b}	0,0018	11
	Rot50	1	0,0149	0,0016	12			3	0,0571^{b}	0,0056	11
		2	0,0155	0,0007	12	15	Rot	1	0,0822	0,0080	12
		3	0,0145	0,0007	12			2	0,0835	0,0196	11
10	Rot	1	0,0206	0,0028	12			3	0,0811	0,0075	12
		2	0,0258	0,0017	12		Cop	1	0,1988	0,0246	13
	RotMC	1	0,0250^{a}	0,0024	12			2	0,2041	0,0235	9
		2	0,0234^{a}	0,0025	12			3	0,1690	0,0204	12
		3	0,0352^{b}	0,0031	12	28	Rot	1	0,3597	0,0419	10
	Rot50	1	0,0198^{a}	0,0020	12			2	0,5263	0,1319	11
		2	0,0248^{ab}	0,0059	12			3	0,6154	0,0112	11
		3	0,0300^{b}	0,0067	12		Cop	1	0,8915	0,1221	12
15	Rot	1	0,0326	0,0028	12			2	0,9017	0,1601	10
		2	0,0375	0,0055	12			3	0,6594	0,1375	10
	RotMC	1	0,0410^{a}	0,0034	12	36	Rot	1	1,0187^{ab}	0,1307	14
		2	0,0482^{ab}	0,0062	12			2	0,9239^{a}	0,0969	16
		3	0,0646^{b}	0,0062	12			3	1,5649^{b}	0,2200	13
	Rot50	1	0,0323	0,0032	12		Cop	1	1,4912^{a}	0,1547	14
		2	0,0382	0,0055	12			2	1,2057^{b}	0,1154	16
		3	0,0427	0,0045	12			3	1,9538^{ab}	0,3285	13
21	Rot	1	0,0696	0,0022	19	45	Rot	1	3,9644	0,4443	15
		2	0,0620	0,0043	20			2	3,9218	0,3423	15
	RotMC	1	0,0841	0,0062	20			3	4,2347	0,3278	14
		2	0,1070	0,0118	20		Cop	1	6,7012^{a}	0,4380	15
		3	0,0769	0,0087	20			2	4,3257^{a}	0,2774	15
	Rot50	1	0,0452^{a}	0,0045	20			3	4,6613^{b}	0,5562	14
		2	0,0560^{ab}	0,0053	19						
		3	0,0690^{b}	0,0077	20						
27	Rot	1	0,0091	0,0166	30						
		2	0,0088	0,0161	30						
	RotMC	1	0,2238^{a}	0,0198	29						
		2	0,2771^{a}	0,0184	29						
		3	0,3414^{b}	0,0217	29						
	Rot50	1	0,1455	0,0262	15						
		2	0,1599	0,0118	30						
		3	0,1457	0,0200	29						
34	Rot	1	0,0209	0,0382	30						
		2	0,0569	0,0341	30						
	RotMC	1	0,4706^{a}	0,0423	29						
		2	0,5925^{b}	0,0303	29						
		3	0,9328^{c}	0,0662	29						
	Rot50	1	0,4395^{a}	0,0379	30						
		2	0,4641^{a}	0,0271	30						
		3	0,6716^{b}	0,0757	30						

Significant differences between tanks of each treatment are indicated by different letters (p<0,05; one-way ANOVA)

Appendix 5

Mean % daily weigh increase for each tank

Water quality experment				Live feed experiment			
dph	Treatment	Tank	%DWI	dph	Treatment	Tank	%DWI
3-6	Rot	1	-13,94	3-8	Rot	1	-6,42
		2	-9,09			2	-6,80
	RotMC	1	-11,50			3	2,23
		2	-7,61		Cop	1	-5,37
		3	-2,89			2	2,50
	Rot50	1	-9,96			3	-0,11
		2	-8,80	8-15	Rot	1	12,40
		3	-10,87			2	12,98
6-10	Rot	1	12,09			3	5,31
		2	13,78		Cop	1	26,98
	RotMC	1	15,24			2	16,78
		2	9,69			3	21,71
		3	17,08	15-28	Rot	1	12,02
	Rot50	1	7,27			2	15,22
		2	12,45			3	16,87
		3	19,90		Cop	1	12,11
10-15	Rot	1	9,64			2	11,04
		2	7,78			3	12,23
	RotMC	1	10,39	28-36	Rot	1	13,90
		2	15,55			2	7,29
		3	12,91			3	14,43
	Rot50	1	10,27		Cop	1	3,79
		2	9,02			2	14,54
		3	7,33			3	6,64
15-21	Rot	1	13,46	36-45	Rot	1	16,30
		2	8,75			2	17,42
	RotMC	1	12,71			3	9,91
		2	14,23		Cop	1	15,25
		3	2,94			2	10,14
	Rot50	1	5,77			3	18,17
		2	6,57	3-45	Rot	1	10,95
		3	8,35			2	10,92
21-27	Rot	1	21,31			3	11,12
		2	21,91		Cop	1	11,18
	RotMC	1	17,72			2	11,37
		2	17,19			3	12,34
		3	28,20				
	Rot50	1	21,54				
		2	19,10				
		3	13,25				
27-34	Rot	1	12,79				
		2	15,81				
	RotMC	1	11,21				
		2	11,47				
		3	15,44				
	Rot50	1	17,11				
		2	16,44				
		3	24,40				

Table continues on the next page

Water quality experiment			
dph	Treatment	Tank	%DWI
3-34	Rot	1	10,97
		2	11,32
	RotMC	1	10,64
		2	11,47
		3	13,13
	Rot50	1	10,40
		2	10,59
		3	11,92

Appendix 6

Mean number of pre-hemal and total vertebral segments

The numbers were determined on all bone stained specimens. Statistical differences are indicated by letters ($p<0,05$; one-way ANOVA). Data presented as mean ± SE.

	Pre-hemal vertebrae	Total number of vertebrae
Water quality experiment – 41 dph		
Rot	18,00 ± 0,00 **a**	36,07 ± 0,05 **a**
RotMC	18,05 ± 0,05 **a**	36,19 ± 0,08 **a**
Rot50	18,00 ± 0,00 **a**	36,44 ± 0,09 **b**
Water quality experiment – 70 dph		
Rot	18,00 ± 0,00 **a**	36,20 ± 0,14 **ab**
RotMC	18,00 ± 0,00 **a**	35,93 ± 0,12 **ab**
Rot50	18,00 ± 0,00 **a**	36,20 ± 0,11 **ab**
Live feed experiment		
Rot	17,43 ± 0,11 **b**	35,71 ± 0,10 **c**
Cop	18,00 ± 0,00 **a**	36,32 ± 0,06 **b**

Appendix 7

Percent occurrence of twisted arches in each treatment

Including the scores: 0 = no twisted arches, 1 = appearing in neural arches of the pre-hemal region, 2 = appearing in neural arches of both the pre-hemal and hemal region, and 3 = appearing in both neural and hemal arches all over the body. Results presented as means ± SE. Significant differences are indicated by different letters.

		Occurrence of twisted arches (% ± SE)			
WQ	**n**	**Score 0**	**Score 1**	**Score 2**	**Score 3**
Rot	45	4,44 ± 3,11 **a**	28,89 ± 6,83	15,56 ± 5,46	48,89 ± 7,54 **a**
RotMC	51	13,73 ± 4,87 **a**	19,61 ± 5,61	27,45 ± 6,31	39,22 ± 6,90 **a**
Rot50	47	6,38 ± 3,60 **a**	25,53 ± 6,43	27,66 ± 6,60	38,30 ± 7,17 **a**
LF	**n**				
Rot	45	22,22 ± 6,27 **ab**	37,78 ± 7,97	28,89 ± 6,83	13,33 ± 5,12 **ab**
Cop	52	73,59 ± 6,17 **b**	16,98 ± 5,26	9,62 ± 4,13	0 ± 0 **b**

WQ = water quality experiment, LF = live feed experiment

Appendix 8

Individual SL, MH, total number of vertebrae, vertebrae score and dorsal fin score.

Vertebrae were either compact or fully ossified. Dorsal fin scores 1 = ossification of posterior parts, 2 = ossification of both anterior and posterior parts and 3 = full ossification including the pterygiophores. Asterisks next to fish of the Cop-treatment indicate observed squamation.

Water quality experiment - 41 dph

Rot					RotMC					Rot50				
SL	MH	# vert.seg	vert.score	dorsal fin	SL	MH	# vert.seg	vert.score	dorsal fin	SL	MH	# vert.seg	vert.score	dorsal fin
9,38	1,95	36	full	3	9,3	1,85	36	full	2	10,45	2,15	36	full	3
9,27	1,98	36	full	2	9,61	2	36	full	2	10,26	2,17	36	full	3
9,93	2,11	36	full	3	10,21	2,19	36	full	3	8,03	1,57	36	compact	2
9,15	1,9	37	full	2	8,15	1,58	36	compact	2	8,2	1,72	36	full	2
9,71	2,05	36	full	3	9,12	1,7	36	full	2	8,18	1,66	37	compact	2
8,89	1,95	36	full	2	9,92	2,16	36	full	3	6,46	1,13	36	compact	1
7,29	1,35	36	compact	1	9,02	1,68	36	full	2	7,42	1,35	37	compact	2
8,83	1,85	36	full	2	9,45	1,99	36	full	2	9,17	1,82	37	full	2
9,76	1,94	36	full	3	10,58	2,17	36	full	3	9,11	1,85	36	full	2
8	1,63	36	full	2	8,79	1,41	37	full	2	10,31	2,05	36	full	2
9,13	1,87	36	full	3	7,56	1,35	35	compact	1	9,65	1,9	36	full	2
8,88	1,9	36	full	3	9,07	1,86	37	full	2	9,22	1,67	36	full	2
8,85	1,75	36	full	3	9,98	1,99	36	full	3	9,65	1,85	36	full	2
8,72	1,87	36	full	3	8,29	1,63	36	full	2	9,34	1,81	36	full	2
8,9	1,97	36	full	3	10,54	2,09	36	full	3	9,13	1,75	36	full	2
8,64	1,85	36	full	2	9,84	2,5	36	full	2	7,84	1,55	36	full	2
7,88	1,43	36	full	2	9,87	2,09	36	full	3	8,93	1,57	36	full	2
9,92	2,21	36	full	3	9,33	1,66	37	full	2	7,18	1,24	37	compact	2
7,38	1,23	36	compact	1	9,13	1,65	36	full	3	9	1,5	37	full	2
9,36	2,05	36	full	3	10,11	2,14	36	full	2	10,27	2,08	36	full	2
9,79	2,14	36	full	3	9,39	2	36	full	2	10,3	2,04	37	full	3
8,91	1,63	36	full	2	9,73	2,06	37	full	2	8,7	1,55	36	full	2
9,54	1,87	36	full	3	8,35	1,79	36	full	2	9,53	1,77	37	full	2
9,3	1,92	36	full	3	10,49	2,14	37	full	3	10,28	2,06	37	full	3
7,65	1,48	36	full	2	9,16	1,9	36	full	2	8,52	1,47	36	full	2
7,56	1,43	36	full	2	8,86	1,8	36	full	2	8,57	1,56	37	full	2
9,08	1,79	36	full	3	10,32	2,26	36	full	3	8,61	1,74	36	full	2
10,32	2,09	37	full	3	9,23	1,92	37	full	3	9,44	1,94	37	full	2
7,53	1,48	36	compact	2	9,68	2,07	37	full	3	10,19	2,04	37	full	3
8,32	1,7	36	full	2	9,6	1,93	36	full	3	9,34	1,89	37	full	2
					10,85	2,39	36	full	3	7,85	1,44	37	full	2
					7,42	1,39	36	compact	2	8,17	1,47	37	full	2
					10,71	2,2	37	full	3					
					9,24	1,76	36	full	2					
					9,21	1,82	36	full	2					

Water quality experiment - 70 dph

Rot					RotMC					Rot50				
SL	MH	# vert.seg	vert.score	dorsal fin	SL	MH	# vert.seg	vert.score	dorsal fin	SL	MH	# vert.seg	vert.score	dorsal fin
17,73	3,89	36	full	3	11,41	2,06	36	full	3	16,4	3,69	36	full	3
16,48	3,91	35	full	3	15,7	3,47	36	full	3	17,19	3,82	37	full	3
17,7	3,95	37	full	3	16,1	3,61	36	full	3	14,47	3	36	full	3
16,8	3,68	36	full	3	15,86	3,65	36	full	3	14,66	3,27	36	full	3
12,13	2,58	36	full	3	14,87	3,06	36	full	3	16,08	3,6	36	full	3
14,42	3,27	36	full	3	11,56	2,54	36	full	3	13,49	3,05	36	full	3
19,34	4,38	37	full	3	17,27	3,98	37	full	3	15,66	3,17	36	full	3
17,13	3,81	36	full	3	17,13	3,95	36	full	3	19,02	4,5	37	full	3
19,45	4,85	37	full	3	18,02	3,99	35	full	3	18,6	4,43	36	full	3
18,44	4,31	36	full	3	18,25	3,82	35	full	3	18,86	4,48	36	full	3
20,33	5,13	36	full	3	17,73	3,73	36	full	3	17,95	4,29	36	full	3
19,99	5,03	37	full	3	21,42	5,13	36	full	3	19,59	4,88	36	full	3
19,74	4,56	36	full	3	17,82	4,36	36	full	3	19,15	4,55	36	full	3
21,42	5,19	36	full	3	21,63	5,35	36	full	3	19,61	4,59	37	full	3
19,85	4,81	36	full	3	17,74	3,7	36	full	3	16,73	3,87	36	full	3

Live feed experiment - 45 dph

Rot

SL	MH	# vert.seg	vert.score	dorsal fin
9,56	1,83	35	full	3
9,42	1,71	37	full	2
10,17	1,75	36	full	2
8,8	1,44	36	full	2
10,03	1,95	36	full	3
8,11	1,6	36	full	2
10,45	2,26	35	full	3
10,03	1,89	36	full	3
11,15	2,33	35	full	3
11,91	2,48	36	full	3
10,24	2,15	36	full	3
9,81	1,97	36	full	3
11,53	2,55	37	full	3
9,3	2	36	full	2
9,48	1,88	36	full	3
10,6	1,94	35	full	3
10,28	2,1	36	full	3
9,17	1,91	36	full	3
10,26	2,01	36	full	3
9,35	1,77	36	full	2
9,74	1,92	36	full	2
9,81	2,07	37	full	2
8,58	1,8	35	full	2
8,94	1,79	36	full	2
11,01	2,37	36	full	3
9,67	2,09	37	full	3
9,47	1,93	36	full	3
8,09	1,78	35	full	2
9,45	1,86	35	full	2
10,19	2,02	35	full	3
9,91	1,82	35	full	2
10,23	2,16	35	full	3
10,96	2,35	35	full	3
7,72	1,5	37	compact	2
8,63	1,4	35	full	2
10,5	2,05	35	full	3
10,91	2,27	36	full	3
10,04	1,94	36	full	3
10,95	2,34	35	full	3
9,67	1,97	36	full	3
10,18	2,05	35	full	3
10,73	2,14	36	full	3
10,15	1,91	35	full	3
10,71	2,27	35	full	3
10,68	2,38	35	full	3

Cop

SL	MH	# vert.seg	vert.score	dorsal fin	
13,55	3,15	36	full	3	
13,97	3,03	36	full	3	
15,52	3,37	37	full	3	*
12,95	2,86	36	full	3	
15,04	3,42	36	full	3	
14,09	3,16	37	full	3	
13,28	2,58	36	full	3	
11,9	2,55	36	full	3	
14,32	3,23	37	full	3	
9,79	2,05	37	full	3	
12,9	2,82	36	full	3	
15,2	3,24	36	full	3	*
13,83	2,96	37	full	3	
14,57	3,27	36	full	3	
11,16	2,42	36	full	3	
14,49	3,26	36	full	3	
14,07	3,15	37	full	3	
14,56	3,28	36	full	3	*
12,07	2,56	36	full	3	
13,66	2,89	37	full	3	
11,54	2,39	37	full	3	
12,41	2,46	36	full	3	
15,03	3,38	36	full	3	*
14,24	3,37	36	full	3	*
12,44	2,68	36	full	3	
8,29	1,55	36	full	2	
12,12	2,69	37	full	3	
10,22	2,15	36	full	3	
10,13	2,15	36	full	3	
10,16	1,89	37	full	3	
8,98	1,77	36	full	2	
10,12	2,01	36	full	3	
7,68	1,51	37	full	2	
8,16	1,48	37	full	2	
8,93	1,78	36	full	2	
12,64	2,78	36	full	3	
12,27	2,53	36	full	3	
13,1	2,86	37	full	3	
11,76	2,44	36	full	3	
9,7	2	36	full	2	
10,58	2,19	36	full	3	
12,2	2,6	36	full	3	
9,94	1,95	36	full	3	
10,94	2,42	36	full	3	
11,45	2,43	36	full	3	
11,59	2,48	37	full	3	
11,8	2,57	36	full	3	
9,08	1,9	37	full	3	
11,29	2,33	36	full	3	
12,05	2,49	36	full	3	
9	1,83	37	full	2	
10,2	2,06	37	full	3	
11,55	2,44	36	full	3	