The Effect of Dietary Supplementation with Zooplankton or Fish Protein Hydrolysate on Atlantic Cod (Gadus morhua) Production Traits and Physiology

by

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A thesis submitted to the

School of Graduate Studies

in partial fulfillment of the requirements of the degree of

Master of Science in Aquaculture

Aquaculture Program

Memorial University of Newfoundland

St. John's, Newfoundland and Labrador

May 2014

Abstract

This thesis examined the effects of partial dietary supplementation with wild-zooplankton or fish protein hydrolysate on cod production traits, and how they related to the cod's physiology and the expression of growth and appetite regulating genes. Atlantic cod larvae were fed three different diets: enriched rotifers / Artemia (RA); RA + fish protein hydrolysate (RA-PH); and RA supplemented with 5-10% wild zooplankton (RA-Zoo). Partial supplementation with zooplankton significantly improved the dry weight at 60 dph (by approximately 4-fold), specific growth rate (by 2.5% day^{-1}) and the general development of cod larvae. In contrast, the protein hydrolysate enrichment did not improve growth, had a negative effect on survival, and increased the incidence of external deformities in 18 month post-hatch juveniles. Although the zooplankton fed cod were still larger at approximately 1.5 years of age, the growth advantage of this group decreased with age (the difference in wet mass decreasing from approx. 30% at 0.5 years old to 11% at 1.5 years old). Metabolic parameters, and pre- and post-stress cortisol levels, were similar in juvenile cod from the RA and RA-Zoo groups. Finally, the growth enhancement observed in the zooplankton fed larvae was not related to alterations in the mRNA expression of the main growth regulating genes [Insulin-Like Growth Factor 1 (IGF-1); IGF-2; Growth Hormone (GH); GH Receptor-1 (GHR-1); GHR-2; and myostatin] or appetite regulating hormones [(Cocaine and Amphetamine Regulated Transcript (CART) and Neuropeptide Y (NPY)]. This latter finding suggests that the transcript levels of these hormones and hormone receptors are not a valuable biomarker for growth in cod larvae.

Acknowledgements

First, I would like to thank my supervisor Dr. Kurt Gamperl for his continuous support of my M.Sc. research, for his patience, motivation and guidance, but on top of all for teaching me how to be more critical, and the difference between good and bad science. I would also like to thank Dr. Matthew Rise and Dr. Chris Parrish and their lab staff for their assistance throughout this project. Drs. William Driedzic and Iain McGaw for being on my supervisory committee and offering their feedback and encouragement, and Drs. Dr. Celine Audet and Cyr Couturier for serving as external examiners Gord Nash and Jennifer Hall for their significant and major guidance, which allowed me to finish this thesis. I would also like to thank my lab mates from the Gamperl lab, Danny Boyce and the staff from the Dr. Joe Brown Aquatic Research Building, as well as the entire Ocean Sciences Centre's staff, for their endless assistance. Last but not least, I would like to thank my parents: Yehiskel and Shosh Katan for their continuous support and for always being there for me. None of this would have been possible without them.

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List of Symbols, Nomenclature or Abbreviations

°C	temperature in degrees Celsius
µg	micrograms
µl	microliters
µm	micrometers
AA	arachidonic acid
AAT	aspartate amino transferrase
ACTH	adrenocorticotropic hormone
AMR	active metabolic rate
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ATP	adenosine triphosphate
b	scaling exponent
BAL	bile salt-activated lipase
BLs^{-1}	body length per second
Body weight day^{-1}	body weight per day
bp	base pairs
C	rate of energy consumption
C3	key component of the complement system
CART	cocaine and amphetamine regulated transcript
CCK	cholecystokinin
cDNA	complementary DNA
cm	centimeter
cm sec^{-1}	centimeter per second
CRH	corticotropin-releasing hormone
C_T	cycle threshold
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
DNAse	deoxyribonuclease

dNTPs	deoxynucleotide triphosphates
dph	days post-hatch
DTT	dithiothreitol
DW	dry weight
EDC	endocrine disruption compounds
ELISA	enzyme-linked immunosorbent essay
EPA	eicosapentaenoic acid
ESD	extreme studentized deviate
Fst	follistatin
g	grams
g liter^{-1}	gram per liter
GH	growth hormone
GHBP	growth hormone binding protein
GHR	growth hormone receptor
GLP	glucagon-like peptide
G_r	growth rate due to gonad (reproductive) development
G_s	somatic growth rate due to protein synthesis + lipid deposition
HSI	hepatosomatic index
HSD	honestly significant differences
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
IGFR	insulin-like growth factor receptor
IRC	immunoreactive corticosteroid
JBARB	Dr. Joe Brown Aquatic Research Building
K	condition factor
kg	kilograms
L	liters
LDH	lactate dehydrogenase
m	meters
M_a	metabolic rate increase due to activity

MDH	malate dehydrogenase
mg	milligrams
mg O_2 $kg^{-0.8}$ h^{-1}	milligram of oxygen per kilogram$^{-0.8}$ per hour
mg O_2 l^{-1}	milligram of oxygen per liter
mg O_2 l^{-1} min^{-1}	milligram of oxygen per liter per minute
min	minutes
ml	milliliters
ml min^{-1}	milliliter per minute
mm	millimeters
M-MLV	moloney murine leukemia virus
MO_2	muscle oxygen consumption
M_r	standard metabolic rate
mRNA	messenger ribonucleic acid
MS-222	tricaine methane sulfonate
MSH	melanocyte-stimulating hormone
n	number
n-3 HUFA	omega 3 highly unsaturated fatty acid
ng	nanogram
ng g^{-1}	nanogram per gram
ng ml^{-1}	nanogram per milliliter
nM	nanomolar
NORA	Nordic Atlantic Cooperation
NPY	neuropeptide Y
OSC	Ocean Sciences Centre
P	probability
PBS	phosphate buffered saline
PIT	passive integrated transponder
ppm	parts-per-million
ppt	parts-per-thousand
QPCR	quantitative polymerase chain reaction

RCB	randomized complete block
RQ	relative quantity
RVM	relative ventricular mass
S	seconds
S.E.	standard error of the mean
SDA	metabolic rate increase due to specific dynamic action
SFA	saturated fatty acid
SGR	specific growth rate
SL	standard length
SMR	standard metabolic rate
TAE	tris-acetate-EDTA
TMB	tetramethylbenzidine
TG	triglycerides
TG:SL	triglyceride sterol
TGF-β	transforming growth factor beta
U_{crit}	critical swimming speed
UV	ultraviolet
w6DPA	omega six docosapentaenoic acid
w3	omega 3

Introduction

1.1. Background

A collapse of the Northern Atlantic cod (Gadus morhua) stocks occurred in several countries including Norway (Jakobsen, 1993) and Canada (Myers et al., 1996) during the late 1980s-early 1990s, and this had a devastating economic and social impact on the cod fishery. In 1992, a total moratorium on fishing was put in place on the Northern cod stocks in Canadian waters (NAFO Divisions 3JKL) (Hutchings and Myers, 1994). As a result, there was increased interest in developing a commercial cod aquaculture industry that would satisfy consumer demand while allowing wild stocks to recover (Svåsand et al., 2004). The impetus to develop the cod aquaculture industry was mainly based on the success of salmon aquaculture and the biological suitability of cod for culture (Tilseth, 1990; Brown et al., 2003; Rosenlund and Skretting, 2006).

The first mass production of Atlantic cod was achieved in 1983, when 75,000 juveniles were extensively cultivated in marine ponds at the Institute of Marine Research in Norway (Øiestad et al., 1985), and this was followed by several operations that used seawater enclosures for farming and enhancement of natural stocks (Svåsand, 1998). In 1989, this production reached a peak when approximately 900,000 juveniles were produced in seawater lagoons using extensive and semi-intensive methods (Svåsand et al., 2004). In the extensive method, the larvae are reared in lagoons or ponds that are first cleared of natural predators, and fed entirely on natural plankton that is produced in the system. In contrast, in the semi-intensive method, the larvae are reared in large tanks or plastic bags, and the food organisms (i.e. mainly copepods) are collected and

concentrated from extensive lagoon or pond systems before being fed (Van der Meeren and Naas, 1997; Hamre, 2006) (see Figure 1.1). Both of these methods were not commercially viable due to several limitations such as: low stocking densities, the seasonality of plankton production, and the fact that there is less control over the rearing environment (Svåsand et al., 2004; Busch et al., 2010). Therefore, the production of juveniles for cod aquaculture shifted to land-based intensive systems. In these production systems, there is more precise control over environmental factors that are crucial for growth and development (e.g., temperature, photoperiod and light intensity, salinity and water oxygen levels), high stocking densities can be achieved, lower volumes of live feed are required, and juveniles can be produced year-round (Shields, 2001; Brown et al., 2003). Intensive cod farming based on hatchery-reared juveniles and sea-cage growout is a relatively young industry, only beginning in Norway in the mid-1990s (Howell, 1984; Rosenlund et al., 1993). Further, the growth of the industry was slow due to challenges such as insufficient numbers of fry and low market prices for cod. It was only in the early 2000s that the industry gained a foothold, with some 20-30 companies involved in juvenile rearing / production at large-scale hatcheries (Rosenlund and Skretting, 2006; Gardner Pinfold Report, 2010). The large increase in intensive production was largely achieved because of a significant research effort (e.g., in Canada, Norway and Scotland) that led to improved production protocols, favourable economic conditions for cod (Brown et al., 2003; Puvanendran et al., 2006; Fletcher et al., 2007; Busch et al., 2010), and the prevailing view that the key technical factors affecting production economics had been / would be resolved in the near future (Gardner Pinfold Report, 2010).

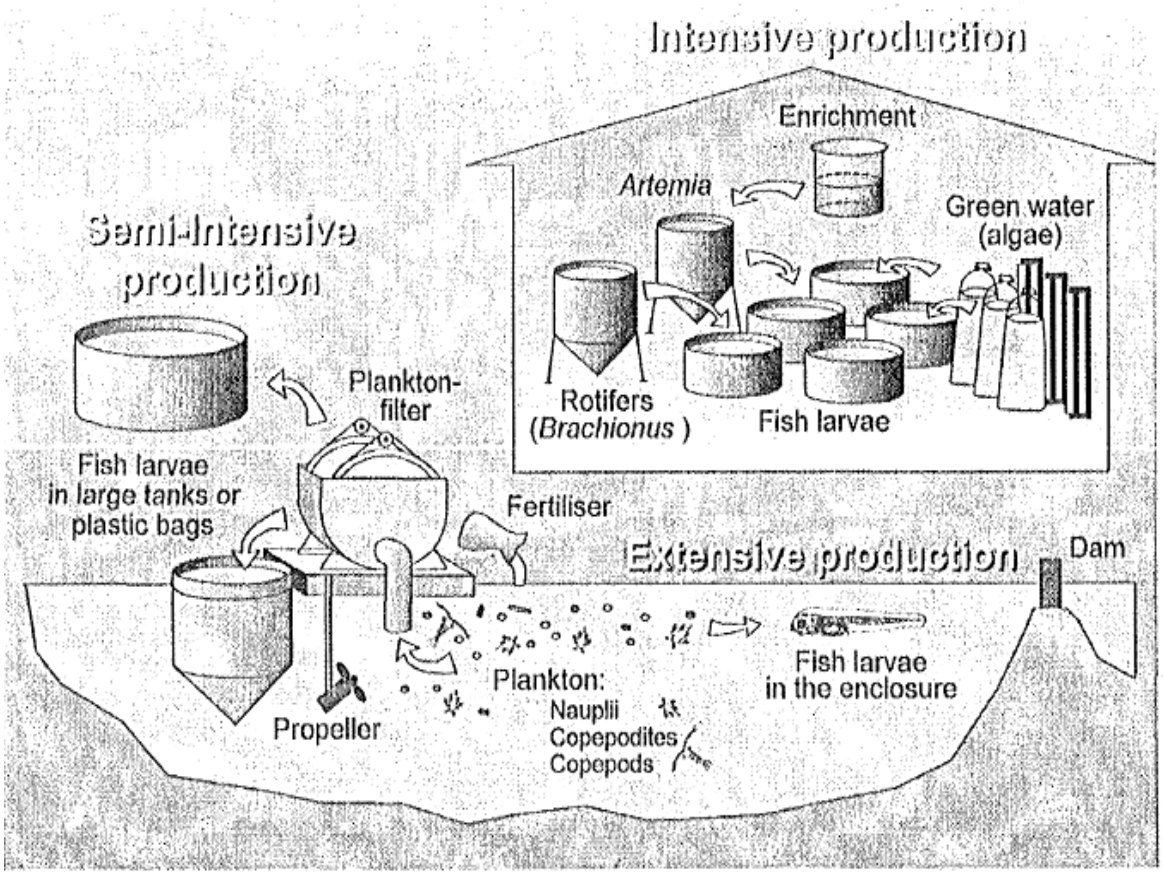


Figure 1.1. Schematic illustration of intensive, semi-intensive and extensive rearing methods (from Van der Meeren and Naas, 1997).

1.1.1. Challenges facing cod aquaculture

Attempts at commercial-scale cod aquaculture have not been very successful due to many factors, including: slow growth (Hamre, 2006); early maturation (Karlsen et al., 1995; Hansen et al., 2001); infectious diseases (Samuelsen et al., 2006); skeletal malformations (Hamre, 2006; Lein et al., 2009); cannibalism during the larval and early juvenile stages (Brown et al., 2003; Puvanendran et al., 2008); less than favourable sea-cage conditions (Pérez-Casanova et al., 2008), and low market price in the past few years (Gardner Pinfold Report, 2010). A solution to many of these issues is to identify molecular markers that can be used to select broodstock with favourable commercial traits such as rapid growth, a robust immune response and resistance to environmental stressors. Some progress has been made in this area (Feng et al., 2009; Hori et al., 2012). Further, molecular/functional genomics tools [e.g., the 20K microarray (Booman et al., 2011)] have been developed through the Atlantic Cod Genomics and Broodstock Development Project (CGP, http://codgene.ca), and the entire cod genome has now been sequenced (Star et al., 2011). These initiatives should lead to further advances in our understanding of cod molecular biology, physiology and health, and thus, improve the economics of cod aquaculture. However, a major bottleneck to the commercialization of cod aquaculture remains the development and optimization of diets to make them cost-effective (Tilseth, 1990; Rosenlund and Skretting, 2006), reduce mortalities during the start-feeding period, and to allow cod to reach their growth potential (Bell et al., 2003; Brown et al., 2003; Hamre, 2006).

1.1.2. Feeding in the early developmental stages

Normal rearing protocols at intensive cod hatcheries rely on the use of rotifers, followed by Artemia nauplii, as live prey for feeding during the larval stages, as both organisms can be cultured all year around and in large quantities (Olsen, 1997; Rosenlund and Halldorsson, 2007). However, rotifers and Artemia cannot fulfill all of the known nutritional requirements of marine fish larvae and they have to be enriched prior to feeding (Dhert et al., 2001; Hamre et al., 2008b).

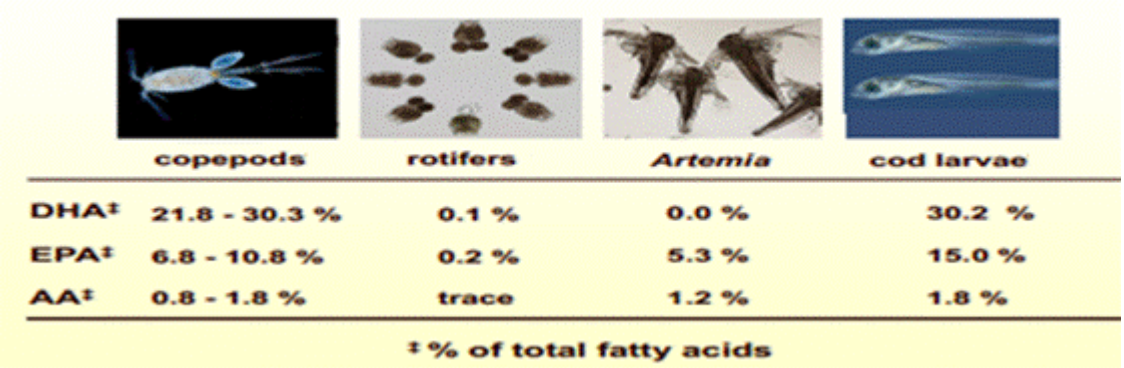
Different enrichments and bioencapsulation methods have been used to increase the level of essential highly unsaturated fatty acid (HUFA) in these live feeds, including DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid) and AA (arachidonic acid) (Devresse et al., 1994; Olsen, 1997; Dhert et al., 2001). These HUFA's are extremely important for larval growth, survival and health (Sargent et al., 1999; Bell et al., 2003; Faulk et al., 2005).

Despite these improvements, these diets still appear to be nutritionally deficient as problems such as abnormal pigmentation and deformities are very common (Støttrup, 2000; Hamre, 2006). In addition, there are large differences in the quality (i.e. incidence of deformities), survival rates and growth potential between cod reared at high density in tank systems (i.e. intensively) and those reared at low density in ponds or fjords (i.e. extensively) (Hamre, 2006; Busch et al., 2010). Further, research suggests that the difference in growth rates does not have to do with the rearing environment itself, but with the diet of the larvae. In extensive systems, the cod larvae are able to forage on a 'natural' diet including copepods of various life stages (nauplii, copepodites and adults),

as opposed to enriched rotifers and Artemia (Rosenlund and Halldorsson, 2007; Busch et al., 2010). Earlier studies have shown that the nutritional composition of these copepods is different from that of the rotifers and Artemia and more suited to fulfill the nutritional demand of cod larvae, especially with regards to the content and ratio of the essential fatty acids: DHA, EPA and AA (Sargent et al., 1999; Bell et al., 2003; Van der Meeren et al., 2008; Busch et al., 2010) (see Figure 1.2.). In addition, copepods contain a higher percentage of these HUFA's (mainly DHA and EPA) as phospholipids as compared to rotifers and Artemia (McEvoy et al., 1998), and it has been shown that when incorporated as phospholipids rather than neutral lipids, these HUFA's enhance the growth, survival and general development of cod, mainly due to higher absorption (Kjørsvik et al., 2009; Wold et al., 2009).

1.1.3. The use of zooplankton in aquaculture

Natural zooplankton has been used as live feed for cod in artificial production systems since the 1880s (Rognerud, 1887). These first experiments used natural environments such as ponds or lagoons for the purpose of fish stocks enhancement (Shelbourne, 1964; Solemdal et al., 1984). During the 1980s, when the first mass production of cod juveniles was achieved (i.e. through the use of extensive and semi-intensive methods), high levels of larval growth and survival (i.e. over 50% at the metamorphosis stage) were achieved, and many believed that this was due to the availability of a wide range of planktonic organisms such as copepods, phytoplankton and protozoans (Øiestad et al., 1985; Van der Meeren and Næss, 1993; Van der Meeren and Naas, 1997; Svåsand et al., 2004).



	copepods	rotifers	*Artemia*	cod larvae
DHA‡	21.8 - 30.3 %	0.1 %	0.0 %	30.2 %
EPA‡	6.8 - 10.8 %	0.2 %	5.3 %	15.0 %
AA‡	0.8 - 1.8 %	trace	1.2 %	1.8 %

‡ % of total fatty acids

Figure 1.2. Essential fatty acid composition (percent of total fatty acids) in cod larvae, unenriched rotifers and Artemia nauplii, and copepods of the species Eurytemora velox, Tisbe furcate and Acartia tonsa (from Bell et al., 2003).

However, relying on extensive methods was not commercialy viable, mainly due to the eventual depletion of live prey and the seasonality of the planktonic organisms (Folkvord, 1991; Van der Meeren and Naas, 1997) which led to cannibalism and losses of metamorphosed larvae. In order to overcome these problems and to achieve higher and more stable production levels, zooplankton (i.e. mainly copepods) were supplemented with Artemia and formulated diets as part of semi-intensive systems (Van der Meeren and Naas, 1997). However, the growth and survival of fish larvae reared in these systems were still not optimal (Berg, 1997; Evjemo et al., 2003), and on several occasions parasitic contamination associated with the copepods serving as an intermediate host was encountered (Bristow, 1990; Støttrup, 2000). As cod aquaculture production shifted to intensive culture on land, the target of producing eggs all year around was achieved, but a constant supply of copepods from the natural environment was still not possible (Støttrup, 2000). To solve the gap between the larvae's energetic needs and the number of available copepods, several attempts have been made to culture zooplankton intensively (Støttrup, 2003; Peck and Holste, 2006; Støttrup, 2006; Milione and Zeng, 2008), but most of these were small scale and of limited duration. Scaling-up of these systems has been very difficult and demands high economic investment into the development of methods / procedures for algal and copepod culture maintenance, the sorting of the copepods, egg harvesting etc.; all of which involve significant investment of human resources. In order to achieve large-scale production, it will be essential to use automated techniques / procedures and to develop new technologies for mass production (Støttrup, 2000; Drillet et al., 2011).

1.1.4. The use of fish protein hydrolysate in aquaculture

Marine fish larvae, including cod, have considerable growth potential (i.e. specific growth rates of more than 25% day^{-1} have been reported for cod) (Otterlei et al., 1999; Finn et al., 2002; Rosenlund and Halldorsson, 2007). Growth is mainly the result of protein synthesis and deposition, and earlier studies have shown that protein digestion and amino acid assimilation are extremely important throughout the larval stage to ensure optimal growth (Blier et al., 1997). However, larval digestion at first feeding is limited due to the lack of a functional stomach (absent until metamorphosis), and the capacity to digest complex proteins is restricted during this stage (Segner et al., 1994; Tonheim et al., 2004; Kvåle et al., 2009). To overcome these limitations, several attempts to replace a fraction of the protein content with prehydrolysed (i.e. more digestible) proteins have been made. These prehydrolysed proteins (i.e. protein hydrolysates) have been shown to have higher absorption efficiency as compared to intact protein (Tonheim et al., 2005) and have resulted in improved growth (Kotzamanis et al., 2007; Savoie et al., 2011). In addition, they can have beneficial effects on larval development (i.e. skeletal development) (Cahu et al., 2003), and for these reasons have potential as dietary additives for use in aquaculture production.

For example, common carp (Cyprinus carpio) and sea bass (Dicentrarchus labrax) larvae fed with a moderate concentration (around 20-25% of protein fraction) of fish protein hydrolysate had improved growth and survival (by 55% and 10%, respectively), and had higher intestinal digestive enzyme activity (Zambonino Infante et al., 1997; Cahu et al., 1999; Carvalho et al., 2004). Pollock protein hydrolysate used as a

live feed enrichment stimulated the production of lysozyme and C3 (a key component of the complement system) in Atlantic halibut (Hippoglossus hippoglossus L.) (Hermannsdottir et al., 2009). Finally, recent trials in Iceland with cod, conducted by our NORA (Nordic Atlantic Cooperation) partners, have shown positive results with regards to immune function, growth and the incidence of deformities when larvae are fed fish protein hydrolysate three days per week (Bjornsdottir et al., 2013).

1.1.5. Skeletal deformities

Skeletal malformations are considered a major problem in cod commercial hatcheries and often vary between 25 and 85% at the juvenile stage (Lein et al., 2006). The main types of vertebral deformations in hatchery-reared fish, including cod, are lordosis, kyphosis, scoliosis, fusion and stargazing (Boglione et al., 2001; Lewis et al., 2004; Lein et al., 2009), and this latter type is considered the most prevalent deformity in cod larvae (Hamre, 2006). In addition to the negative influence that deformities have with regards to fish welfare, they may also lead to reduced growth, survival and a lower market product value (Boglione et al., 2001; Gavaia et al., 2002; Koumoundouros, 2010). Earlier studies linked deformities to rearing conditions (Boglione et al., 2001; Cobcroft et al., 2001; Boglione et al., 2003; Helland et al., 2009; Georgakopoulou et al., 2010), to genetics (Gjerde et al., 2005; Kolstad et al., 2006; Bardon et al., 2009), and to dietary factors [e.g., suboptimal lipid (Sargent et al., 1999) and protein content (Cahu et al., 2003)], or the lack of specific vitamins and minerals (Lall and Lewis-McCrea, 2007; Baeverfjord et al., 2009). In addition, research has shown a lower incidence of skeletal deformities in fish that are start-fed on zooplankton as compared to rotifers and Artemia

(Hamre, 2006; Imsland et al., 2006). These data suggest that zooplankton have a nutritional composition that is much better for larval health / development (Hamre et al., 2002), however, what these factors are is still not fully understood.

1.1.6. Growth regulation in fish

Growth in fish is regulated through the integration of external environmental (i.e. photoperiod, temperature, season, salinity, etc.), internal (i.e. nutritional state) and humoral factors (i.e. neuroendocrine, endocrine, and autocrine-paracrine signals (Duan, 1997; Wood et al., 2005; Canosa et al., 2007; Chang and Wong, 2009; Reinecke, 2010), and results in the secretion of growth hormone releasing or growth hormone release-inhibiting factors from the hypothalamus. These factors influence the production and release of growth hormone (GH) from the pituitary, which binds to its receptor (i.e. GHR), and stimulates the synthesis of insulin-like growth factors (IGF) I and II in the liver and other tissues, and their release into the blood stream. IGF- I is responsible for cell differentiation and proliferation and leads to skeletal elongation and body growth (Duan, 1997; Bail et al., 1998), but can also inhibit GH synthesis through a negative feedback mechanism (Fruchtman et al., 2000) (see Figure 1.3). The effects of GH and IGFs are also mediated by a complex and interactive network of factors and hormones such as: GH- and IGF- binding proteins (GHBPs and IGFBPs, respectively) (Duan and Xu, 2005; Kelley et al., 2006), IGF receptors (IGFRs) (Gutiérrez et al., 2006), somatostatins (Very and Sheridan, 2002; Klein and Sheridan, 2008), and others (Schmid et al., 2003; Shved et al., 2008; Gahete et al., 2009). Moreover, it has been shown that nutritional status (i.e. quantity and quality of food) is the principal regulator of growth,

and that this occurs through direct and indirect effects on the hormones of the GH-IGF system (see Figure 1.3) (Beckman et al., 2004; Ayson et al., 2007; Fox et al., 2009). Nonetheless, very few studies have investigated the effects of diet optimization on the expression of growth-regulating genes, and attempted to develop suitable molecular markers for growth in fish (Picha et al., 2008; Kortner et al., 2011a).

Plasma IGF I levels have been shown to be positively correlated with growth in cod (Davie et al., 2007), and GH mRNA levels were significantly increased in cod larvae whose diet was supplemented by 70% with copepods (i.e. Acartia tonsa) as compared with a rotifer diet (Kortner et al., 2011a). The mRNA expression of IGF I and II increased, while that of myostatin (a hormone that inhibits muscle growth) decreased, in copepod-fed (i.e. Centropages typicus) vs. enriched rotifer-fed yellowtail clownfish (Olivotto et al., 2008a). Finally, changes in the expression of these genes were also evident when clownfish were fed copepods (i.e. Tisbe sp.) at a 1:1 ratio with the traditional rotifer / Artemia based diet (Olivotto et al., 2008b); i.e. a 50% supplementation.

1.1.7. Appetite regulation in fish

Appetite in fish is primarily regulated by neuropeptides that are produced in the hypothalamus and act as stimulatory (orexigenic) or inhibitory (anorexigenic) factors (Schwartz, 2001; Sheridan, 2011). These neuropeptides interact with each other, and their production is influenced by factors circulating via the blood and by signals related to body energy stores. This allows for an appropriate balance between food intake and energy expenditure (Konturek et al., 2003; Wullimann and Mueller, 2004; Strader and

Woods, 2005). Several hormones are produced by peripheral tissues such as the gut, pancreas, interrenal gland and fat, and all can affect the activity of orexigenic and anorexigenic factors, and consequently food intake (Volkoff et al., 2005; Volkoff, 2006; Sheridan, 2011) (see Figure 1.4.). Several extrinsic and intrinsic factors such as temperature (Bendiksen et al., 2002; Sunuma et al., 2007), photoperiod (Bolliet et al., 2001), salinity (Imsland et al., 2001), life stage (Pankhurst et al., 2008), sexual maturity (Simpson et al., 1996), nutritional status (Boujard et al., 2000; Volkoff et al., 2009), and others (Hoskins and Volkoff, 2012), can stimulate or inhibit food intake in fish. Furthermore, these orexigenic and anorexigenic factors can regulate the secretion of growth hormone (GH) from the pituitary (see Figure 1.4.). Therefore, it is possible that somatic growth and development could be enhanced by modifying larval diets, due to alterations in the appetite regulatory pathways (MacKenzie et al., 1998; Kortner et al., 2011a).

Recent research has shown that central and peripheral injections of fish neuropeptide Y (NPY) increase food intake in goldfish (De Pedro et al., 2000; Narnaware et al., 2000) and channel catfish (Silverstein and Plisetskaya, 2000) in a dose dependent manner. In addition, fasting causes an increase in the hypothalamic expression of NPY in both goldfish (Narnaware and Peter, 2001) and salmon (Silverstein et al., 1999), and a decrease in cocaine and amphetamine regulated transcript (CART) expression in goldfish (Volkoff and Peter, 2001a) and cod (Kehoe and Volkoff, 2007), while refeeding reverses these effects (Narnaware and Peter, 2001). In cod, mRNA expression of both NPY and CART have been shown to undergo peri-prandial changes [(where NPY levels increase

during feeding and CART levels decrease at two hours post-feeding (Kehoe and Volkoff, 2007)], and the NPY mRNA expression of larvae was influenced by first feeding regime (Kortner et al., 2011a). This research suggests that the expression of these two neuropeptides is indicative of the appetite and energetic status of fishes, including cod, and may be useful for evaluating the effectiveness of various diets in improving fish growth and production traits.

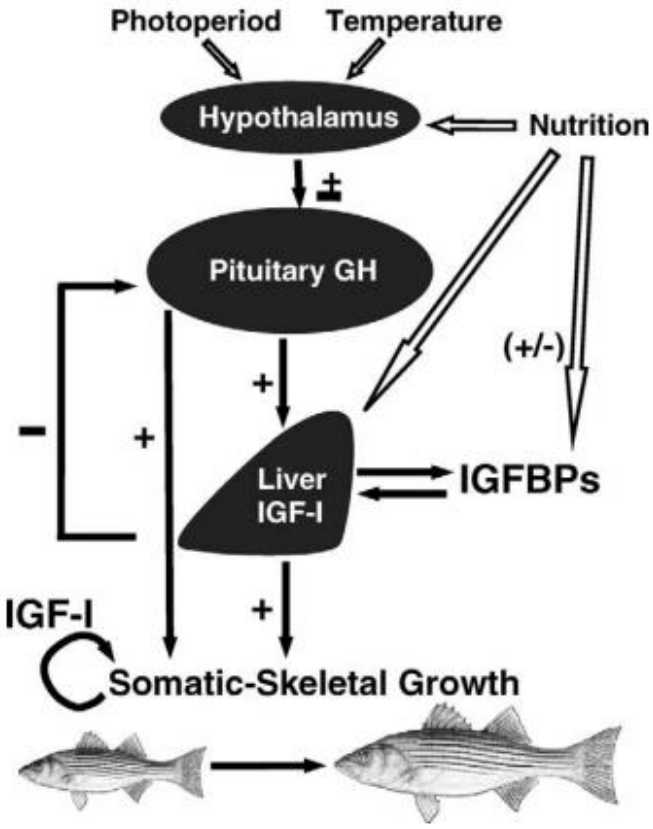


Figure 1.3. Diagram illustrating the growth hormone (GH) - insulin-like growth factor (IGF) system in fish (from Picha et al., 2008). Double arrowheads indicate interactions. (+) and (-) indicate stimulation and inhibition, respectively.

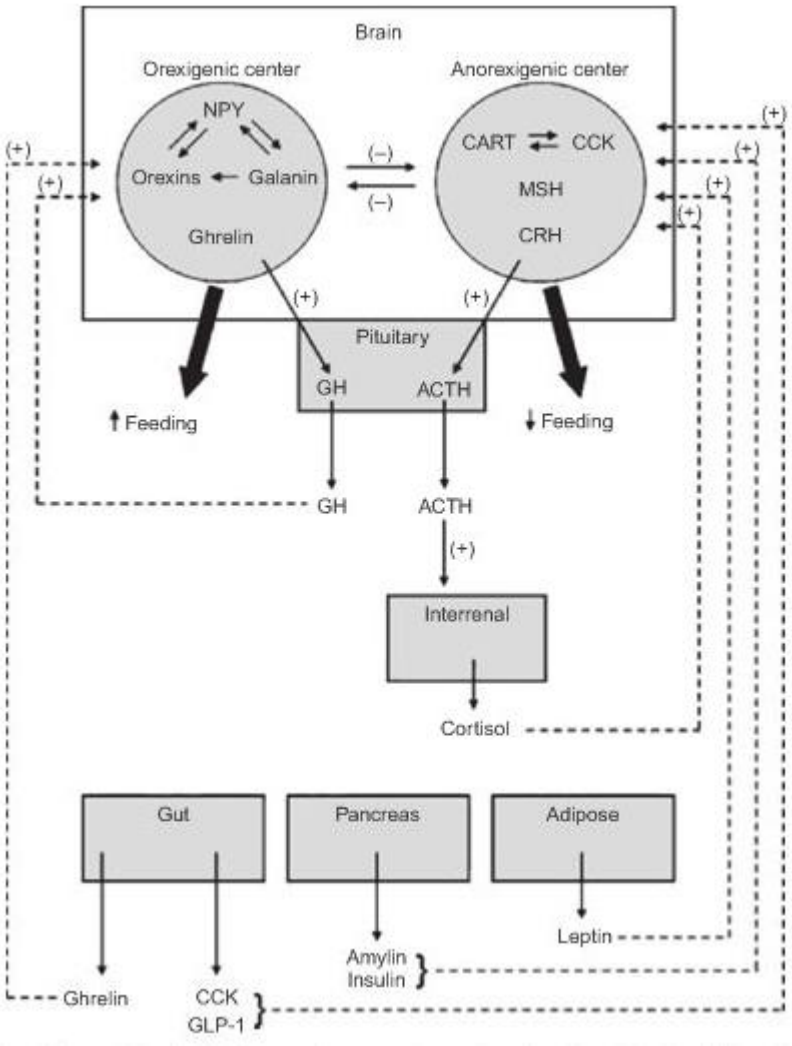


Figure 1.4. Diagram illustrating the current model of appetite regulation in fish (from Sheridan, 2011). The solid lines indicate direct actions; dashed lines indicate feedback mechanisms; and double arrowheads indicate interactions. NPY, Neuropeptide Y; CART, cocaine- and amphetamine- regulated transcript; CCK, cholecystokinin; MSH, melanocyte-stimulating hormone; CRH, corticotropin-releasing hormone; GH, growth hormone; ACTH, adrenocorticotropic hormone; GLP-1, glucagon-like peptide-1.

1.1.8. Fish energy metabolism

Fish, as is the case for other animals, require chemical energy to fuel numerous metabolic functions such as swimming, growth and reproduction. The overall use of chemical energy that occurs in living organisms is typically referred to as energy metabolism. Adenosine triphosphate (ATP) is the main carrier and source of metabolic energy, and is produced by most animals through the oxidation of energy substrates. Therefore, energy metabolism can be estimated by monitoring the rate of oxygen consumption (Cho et al., 1982; Steffensen, 2005), and using the equation;

$$C = (M_r + M_a + SDA) + (F+U) + (G_s + G_r)$$

(Metabolism) (Waste) (Growth)

C = rate of energy consumption

M_r = standard metabolic rate

M_a = metabolic rate increase (above the standard rate) due to activity

SDA = metabolic rate increase due to specific dynamic action, which is the cost of digestion, absorption and processing

F+U = waste losses due to egestion (feces) and excretion (urine) rates

G_s = somatic growth rate due to protein synthesis and lipid deposition

G_r = growth rate due to gonad (reproductive) development

Metabolic rate increases with body mass, and the relationship between metabolic rate and mass has been described by the allometric equation: $y = ax^b$; where y is metabolic rate, x is body weight, a is a constant, and b is the scaling exponent or slope of

the linearized relationship. The interspecific mass exponent (b) has been found to be in the range of 0.66-0.75 in endothermic vertebrates, and 0.80-1.0 in ectotherms, and depends on ambient temperature, lifestyle (Bokma, 2004; Glazier, 2005; 2008; Killen et al., 2010; Ohlberger et al., 2012), as well as level of metabolic rate [i.e. it has been shown that maximum metabolic rate scales with a higher b exponent than standard metabolic rate (Killen et al., 2007; Killen et al., 2010)]. For cod, the interspecific mass exponent (b) has been found to be 0.8 (Saunders, 1963; Edwards et al., 1972; Reidy et al., 1995).

Metabolic rate can be divided into three categories:

Basal/standard metabolic rate is defined as the minimum energy expenditure required to fuel essential life processes, and is measured in a post-absorptive, resting (inactive) fish under minimal stress.

Routine metabolism is measured in fish showing normal / spontaneous activity, and not necessarily in a post-absorptive state.

Active metabolism is the highest rate of energy expenditure possible, and usually occurs during high-speed sustained swimming. It is often measured in fish forced to swim at a maximum sustained (i.e. aerobic) speed using a swim-tunnel respirometer.

However, one of the most important measures of energy expenditure may be aerobic scope, and this parameter is calculated in two different ways:

Absolute metabolic scope is defined as the difference between active metabolic rate (AMR) and the fish's standard metabolic rate (SMR) (Fry, 1971; Brett, 1972; Brett and Groves, 1979; Steffensen, 2005), and is the energy available above that needed to perform essential life processes (Priede, 1985). Metabolic scope differs according to species, size, temperature and dissolved oxygen level, and limits the capacity of fish to preform vital functions such as growth / protein synthesis (Priede, 1985; Wieser et al., 1988; Wieser and Medgyesy, 1990) and digestion (Lucas and Priede, 1992; Blaikie and Kerr, 1996), or to deal with less than optimal environmental conditions [e.g., high temperature and hypoxia (Claireaux et al., 2000; Chabot and Claireaux, 2008; Petersen and Gamperl, 2010; Yang et al., 2013].

Factorial aerobic scope is defined as the ratio between active and standard metabolic rate. It is usually within the range of 3-7, with the majority of species lying towards the bottom end of this range (Jobling, 1994).

1.1.8.1. Swimming activity in fish

Measurements of oxygen consumption do not fully reflect the energetic costs of swimming activity since anaerobic metabolism (i.e. production of lactate) can provide a significant proportion of the energy consumed at high swimming speeds (Bennett, 1978; Priede, 1985). In salmonids and actively foraging species, the muscle begins to utilize anaerobic metabolism, and lactate begins to accumulate in the muscle, at swimming speeds of 2.0-5.0 bl s^{-1} depending on body size and species (Jobling, 1994). With regards

to Atlantic cod, it has been shown that the muscle begins using anaerobic metabolism at a swimming speed of 1.2 bl s^{-1} (Dutil et al., 2007).

Swimming activity has been classified into three categories – sustained, prolonged and burst swimming. These categories define the duration over which the fish can perform at a given level, and also give an indication of the metabolic pathways utilized. Sustained swimming is a term applied to swimming speeds, which can be maintained for long periods without resulting in muscular fatigue (usually 200 min or longer). Metabolism during sustained swimming is purely aerobic. Prolonged swimming is of a shorter duration (20 s – 200 min) than sustained swimming, and results in fatigue. The energy supply for prolonged swimming may be provided by both aerobic and anaerobic metabolism (Goolish, 1991). A special category of prolonged swimming is the critical swimming speed (U_{crit}), which indicates the maximum swimming velocity fish can maintain over an exact time period, and is used for comparing the swimming abilities of different sizes or species of fish (Brett, 1964; Plaut, 2001). By subjecting the fish to stepwise increases in swimming speed, and recording the maximum speed achieved prior to fatigue, the critical swimming speed can be calculated. The highest swimming speed of which fish are capable is termed burst swimming and can be maintained for only a very short period of time (less than 20 sec). The energy for burst swimming is provided, predominantly, by anaerobic metabolism (Blaxter, 1969; Beamish, 1978; Jobling, 1994; Hammer, 1995).

1.1.8.2. Dietary effects on metabolism and swimming performance

Diet quality, particularly the level of certain fatty acids, has been shown to affect the aerobic exercise performance of fishes (McKenzie et al., 1998; McKenzie, 2001; McKenzie et al., 2008). Fish take up fatty acids from their diet, store them as neutral lipids (triacylglycerols) and insert them into membranes as polar phosphoglycerides (Sargent et al., 1999; Tocher, 2003). Thus, the fatty acid composition of an animal's tissues appears to reflect that of the diet (McKenzie, 2001; Chatelier et al., 2006). Specific importance has been given to the ratio between highly unsaturated fatty acids (n-3 HUFAs) and saturated fatty acids (SFAs). Earlier studies showed that some HUFA's, specifically EPA and DHA, have beneficial effects on aerobic exercise and metabolic capacity. For example Atlantic salmon (Salmo salar) that were fed with higher levels of HUFA's, demonstrated higher U_{crit} values (Wagner et al., 2004). In addition, Adriatic sturgeon (Acipenser naccarii) and European eels (Anguilla anguilla) had significantly lower standard and routine metabolic rates when fed a diet rich in n-3 HUFA compared to a diet rich in saturated fatty acids (SFA) (McKenzie, 2001). In contrast, higher levels of SFA compared to n-3 HUFA have been reported to have beneficial effects upon the exercise performance and metabolic capacity of fishes (McKenzie et al., 1998; Chatelier et al., 2006).

With regards to nutritional differences in dietary live prey, it has been shown that copepods have higher levels of HUFAs (mainly DHA and EPA) compared to enriched rotifers /Artemia (Bell et al., 2003; Van der Meeren et al., 2008; Busch et al., 2010). However, very few studies have examined what are the effects of the type of live feed

used for larval diets on the aerobic and swimming performance of fishes. These measurements are valuable, as metabolic scope has been shown to be an important mediator of growth in several fish species including cod (Chabot and Dutil, 1999; Claireaux et al., 2000), and therefore, could explain whether the enhanced growth of copepod-fed cod was mediated by metabolic capacity. Finally, although one publication (Koedijk et al., 2010b) suggests that the enhanced growth of juvenile cod resulting from feeding larvae with zooplankton, was not associated with changes in metabolic capacity, this conclusion was based solely on measurements of metabolic enzyme activity and myosin mRNA expression.

1.1.9. Stress and the stress response in aquaculture

In intensive aquaculture operations, activities and conditions such as handling, sorting, grading, transport and poor water quality, as well as sudden changes in temperature, salinity and oxygen levels, can all impose stress on fish (Schreck, 1982; Barton and Iwama, 1991). The stress response is considered to be an adaptive mechanism that allows an organism to maintain its homeostatic state when exposed to stressors. However, if the stress is severe or long lasting, it may become maladaptive and have negative effects on fish growth, condition, resistance to disease, metabolic scope for activity, and eventually survival (Selye, 1974; Barton and Iwama, 1991). The physiological effects of stress in fish are categorized as primary [i.e. release of the stress hormones; catecholamines (epinephrine and norepinephrine) and corticosteroids (cortisol)], secondary (metabolic, cardiovascular, hydromineral balance, respiratory and immune functions) and tertiary [(whole animal responses that effect growth, disease

resistance and survival) (Barton and Iwama, 1991; Iwama, 1998; Barton, 2002; Davis, 2006; Pottinger, 2008)]. Cortisol is a primary stress hormone that is produced de novo from cholesterol in the interrenal cells of the head kidney in response to a hormonal cascade (i.e. throughout the hypothalamus-pituitary-interrenal axis) and released into the bloodstream (Donaldson, 1981; Mazeaud and Mazeaud, 1981; Sumpter, 1997). Due to many advantages [see review at Ellis at al. (2012)], cortisol has been established as a stress indicator and is commonly used in aquaculture (Barton and Iwama, 1991; Pottinger, 2008) to evaluate the fish's response to events such as handling (Ellis et al., 2007b), transfer (Weber et al., 2002), confinement (Pottinger, 2010), transportation (Barton and Peter, 1982), temperature shock (Donaldson, 1981; Barton and Peter, 1982), disease (Ellis et al., 2007a) and others (Flos et al., 1988; Tort et al., 2002).

Major efforts have been made to improve the ability of fish to tolerate stress by selecting for broodstock with low responsiveness to stressors in aquaculture (Pottinger and Carrick, 1999; Fevolden et al., 2002; Pottinger, 2003; Hori et al., 2012). In addition, diet appears to play an important part in stress sensitivity. For example, several studies have shown that adding highly unsaturated fatty acids [e.g., DHA (docosahexaenoic acid), EPA (eicosapentaenoic acid) and AA (arachidonic acid)] to larval diets can improve the larvae's ability to tolerate stress (Koven et al., 2001; Vagelli, 2004). Furthermore, improved stress resistance has been reported in larval mahimahi (*Euterpina acutifrons*) fed a copepod diet, compared to enriched *Artemia* (Kraul et al., 1993).

1.2. Rationale for study

In Newfoundland, at present, it is taking approx. 40 – 42 months to grow cod to the preferred market size of 3-4 kg, which is significantly longer than the 30-36 months that the industry has set as a target to ensure profitability (Clift Report, 2005; Gardner Pinfold Report, 2010). Many in the cod aquaculture industry believe that this slow growth is related to less than optimal diets, and have identified diet development as a research priority.

Recent studies show that feeding Atlantic cod (Gadus morhua) wild zooplankton, as opposed to enriched rotifers for even a short period (e.g., 2 weeks) can provide a better scope for growth and general development (i.e. a significantly lower incidence of skeletal deformities) during the larval period. Further, this improved larval growth has been shown to translate into significantly larger juveniles by 20-25% (Imsland et al., 2006; Busch et al., 2010; Koedijk et al., 2010a). While the window for feeding zooplankton appears to have been identified (Koedijk et al., 2010a), it is still not known whether feeding small amounts of zooplankton will achieve similar results (with regards to larval growth, skeletal deformities and survival) to those seen in earlier studies (i.e. Imsland et al., 2006; Busch et al., 2010; Koedijk et al., 2010a), or how / why feeding zooplankton vs. rotifers / Artemia, or adding protein hydrolysates to larval diets, improves cod growth performance. This information is required before current commercial enrichments can be effectively modified to achieve the growth rates required by the industry. To address this issue, a large multi-disciplinary project titled "Diet and the Early Development of Atlantic Cod" was funded. My M.Sc. thesis research is a component of this project, and

focuses on the physiology and production traits of cod as affected by three diets: Enriched rotifers / Artemia (RA); RA + protein hydrolysate (RA-PH); and RA supplemented with wild caught zooplankton (RA-Zoo).

1.3. Research objectives

1- **Examine whether partial dietary supplementation with zooplankton (5 – 10% of total prey items) or protein hydrolysate (three days per week) improves Atlantic cod production traits such as survival, growth, and the degree / types of deformities.**

Trials were conducted on a Newfoundland cod population, and the growth and production traits of fish fed the three diets were monitored for 18 months post-hatch. These results were then compared with those previously obtained in Norway / Iceland.

2- **Determine whether changes in growth associated with feeding zooplankton / protein hydrolysate are related to alterations in the expression of growth and appetite-regulating genes.**

The mRNA expression of the main growth-regulating hormones [insulin-like growth factors (IGF) I and II and growth hormone (GH)], GH receptors I and II and the growth inhibiting / suppressing hormone myostatin were measured in the three dietary groups in order to identify biomarkers for growth in cod larvae. In addition, in order to determine whether the growth enhancement seen in the

zooplankton fed larvae may be related to changes in food intake, we measured the mRNA expression of the appetite regulating genes NPY and CART.

3- **Examine whether feeding zooplankton / protein hydrolysate affects the basal metabolism, metabolic scope and swimming performance of juvenile cod.**

These measurements are valuable as they might explain whether any growth enhancement observed in the zooplankton group was associated with changes in basal energy expenditure or metabolic capacity.

4- **Examine whether these diets influence the stress (cortisol) response of juvenile cod.**

Early juvenile cod were exposed to a handling stress plus confinement, and the pre-and post-stress cortisol levels in fish from the three experimental groups were compared for 12 h post-stress.

2. Materials and Methods

Atlantic cod (Gadus morhua) broodstock, eggs and larvae were housed at the Dr. Joe Brown Aquatic Research Building (JBARB) at the Ocean Sciences Centre of Memorial University. The experiment was conducted until the cod reached 1.5 years of age (October 2011 to April 2013) and in accordance with the guidelines of the Canadian Council on Animal Care, and approved by the Institutional Animal Care Committee of Memorial University of Newfoundland (protocol # 11-30-KG).

2.1. Fish and experimental design

Broodstock were wild-caught fish from Smith Sound (Newfoundland) and held in a 37.7 m^3 flow through tank for several years prior to this study. The tank was supplied with aerated/oxygenated, UV-treated, and filtered seawater at 6.5-7 °C, and kept on a six month advanced photoperiod. The broodstock were fed mackerel and herring with a vitamin supplement twice a week. Eggs for this study were collected from these communally spawning broodstock at 9:00 AM on October 4, 2011, disinfected with ozone, and placed into two 0.3 m^3 cone shaped incubators with 32 ppt seawater at 6-7 °C until they reached 100% hatch. These eggs had an average diameter of 1.6 mm, a 95% fertilization rate and 91% of them had symmetrical cleavage; based on these metrics these eggs were considered to be high quality. At 93.4 degree days (October 19th, 2011) the larvae were transferred to 16, 400 L flow-through tanks at a density of 50 larvae l^{-1}. These tanks were then divided randomly into three different treatments based on feeding regime / diet (see Figure 2.1).

Treatment 1 (6 replicate tanks):

Rotifers/*Artemia* with Ori-Green enrichment (**RA**) three feedings per day (9 am, 3 pm and 9 pm). This is the standard larval feeding regime used in the JBARB and at many other commercial cod rearing facilities. Initial rotifer and *Artemia* prey densities during feeding ranged from 800 - 9000 and 1200-5400 l^{-1} (depending on larval age), respectively.

Treatment 2 (4 replicate tanks):

Rotifers/*Artemia* with Ori-Green enrichment, supplemented with 5-10% wild caught zooplankton (**RA-Zoo**) (see Section 2.3.3). This treatment had four replicates because the quantities of zooplankton that could be collected at the time of the study were limited. The larval diet was supplemented with zooplankton until 30 days post-hatch (dph) (see Figure 2.1). The numbers of rotifers/Artemia fed to each tank was reduced according to the amount of zooplankton that was added (~ 250,000 per feeding). . This ensured that the number of prey items was consistent between tanks.

Treatment 3 (6 replicate tanks):

Rotifers/*Artemia* with Ori-Green enrichment four days per week (Tuesday, Thursday, Saturday and Sunday) and Rotifers/*Artemia* with Protein Hydrolysate (**RA-PH**) enrichment three days per week (Monday, Wednesday and Friday).This feeding protocol was based on that used in previous experiments conducted by our NORA partners (Bjornsdottir et al., 2013). The fish (Pollock; *Pollachius virens*) protein hydrolysate was purchased from IceProtein Ltd. (Iceland) and fed to the rotifers or *Artemia* at a concentration of 0.1 g $liter^{-1}$ for two hours prior to these live feeds being offered to the cod larvae.

All groups were fed rotifers from 2 days post-hatch (dph) until they reached 9 mm in length, Artemia from 9 mm in length to 13 mm long (length was based on measurements obtained for each tank), and weaned onto a commercial micro-diet (Gemma micro W 0.2, Skretting, Vervins, France) with a co-feeding period of 10 days, thereafter (Figure 2.1.). When the larvae were fully weaned, each tank was fed with the micro-diet at a ration of approx. 10-12% body weight day^{-1}.

2.2. Larval rearing and water quality

Potters clay (400 ml) was added to all the tanks, twice a day, to increase tank turbidity and reduce bacterial numbers within the tanks (Prickett et al., 2010). Rearing temperature was increased from 6-7 °C (incubation temperature) to 10.5 °C over a period of 10 days, and water flow rate was gradually increased from 0.8 l min^{-1} (at 0 dph) to 4.5 l min^{-1} at 35 dph. The seawater supplied to the tanks was filtered through sand filters down to 30 µm, and went through ultraviolet sterilization, degassing, foam fractionation, and oxygenation prior to delivery to the larval tanks. This ensured high water quality and a pathogen free environment. Dissolved oxygen levels in the tanks were measured daily (YSI, ProODO, OH, USA) and kept at an average value of 117% saturation to alleviate any possible issues with nitrogen super-saturation. To ensure optimal growing conditions, regular maintenance was carried out on the tanks and included: vacuuming the tank bottom, and cleaning of the surface skimmers and center drains.

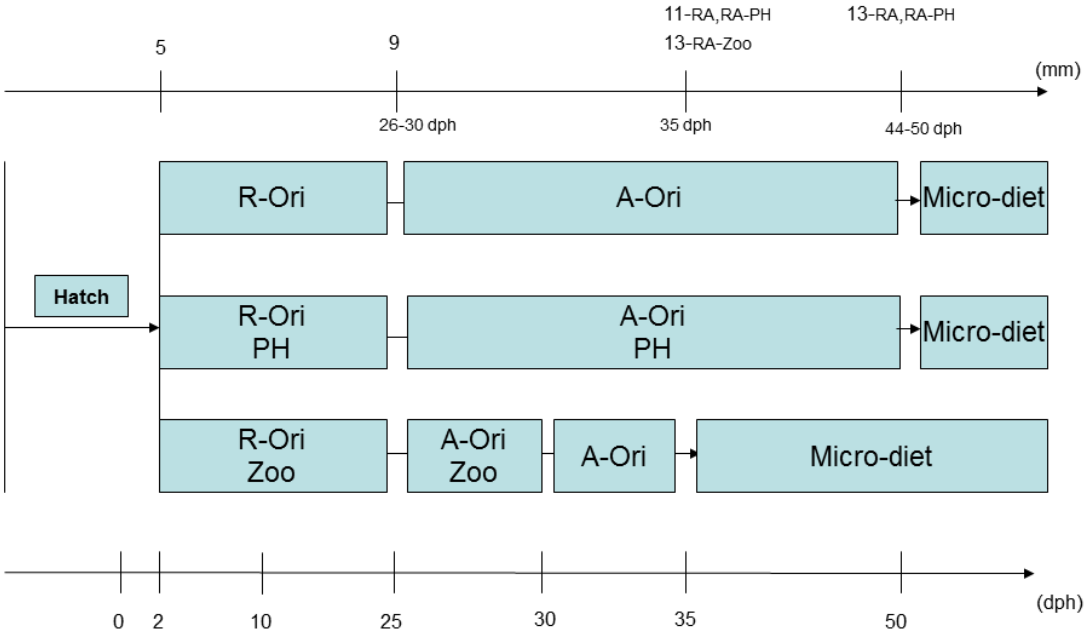


Figure 2.1. Schematic diagram of the feeding protocol used to investigate the effect of dietary supplementation with wild zooplankton or protein hydrolysate on cod production traits and physiology. The three diets fed to the cod larvae were, from top to bottom: RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers and Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. The top axis indicates developmental stage (length in mm) of the dietary groups [e.g., The three dietary groups were sampled at 35 days post-hatch (dph) or when the RA and RA-PH groups reached 11mm and RA-Zoo reached 13 mm in length].

2.3. Live feed

2.3.1. Cultivation, harvesting and enrichment of rotifers (Brachionus plicatilis)

The rotifers (*B. plicatilis)* were cultivated in four stagnant cone shaped culture tanks (1 x 300 l and 3 x 600 l) during October-November, 2011 (October 7 to Nov 25). Rotifer density was measured daily and kept between 500-2600 rotifers ml^{-1}. Rearing conditions were as follows: temperature, 25-28 °C; salinity, 30 ppt; oxygen saturation, 150-300%. Each culture tank was supplemented with Ori-culture (ORI-GO, Skretting, Vervins, France) (0.25-0.35 g million $rotifers^{-1}$) for four days, and on the fifth day the rotifers were concentrated, washed and stocked into two 300 l enrichment cones.

The rotifers were harvested from these cones using a 50 µm screen collector/concentrator and further enriched with Ori-green (ORI-GO, Skretting, Vervins, France) at a concentration of 0.15-0.25 g million $rotifers^{-1}$. The density during enrichment was 500-1000 rotifers ml^{-1} and the rotifers were enriched for approx. two hours before being washed and fed to the cod larvae.

2.3.2. Cultivation of Artemia sp.

The decapsulation of the Artemia cysts (from INVE, Great Salt Lake, UT, USA) was done according to Schumann (2000) in a 20 liter decapsulation cone, during November 2011. After decapsulation, the Artemia were collected in a 50 µm nitex bag and washed in 20 °C seawater to remove all chemicals from the decapped cysts. Then, they were placed in a 300 l cone-shaped hatching tank until 100% hatch was achieved. Eight hours after hatching, the Artemia were fed Ori-Culture for 8 h. Then, they were

enriched with Ori-green in a 300 l cone-shaped tank for 12 h, and washed again with seawater before being fed to the cod larvae. Seawater for the rotifers and Artemia was filtered to 0.35 µm before use.

2.3.3. Zooplankton collection, species identification and feeding

The zooplankton was collected from Conception Bay, Newfoundland, by towing a 100 µm mesh plankton net with a 1 m diameter mouth behind the Ocean Science Centre's (OSC) Boston Whaler. Zooplankton tows were conducted from early September to mid October 2011, to optimize collection methods/location in advance of the hatching of the cod larvae. To get the required numbers of zooplankton, 6 - 8 tows of 20 – 30 minutes in duration were required and continued until November 17, 2011; when the larvae were 30 dph. The number of copepods collected varied between days (see Table 2.1.). Once collected, the zooplankton were kept in an aerated cooler on the boat, and then transported to the OSC where they were passed through a 400 µm mesh filter and counted. The zooplankton were then kept in aerated, and chilled, containers for up 36-48 hours so that tows only had to be done every 2 - 3 days.

The zooplankton collected consisted primarily of copepods (Temora sp., Oithona sp. and Pseudocalanus sp.) (> 90%). However, a significant amount of the phytoplankton Ceratium was also collected (see Figure 2.2.), and could not be separated from the zooplankton. These organisms were fed to the cod larvae once per day for the first week (2 – 9 dph) and twice a day until 30 dph. Each feeding consisted of 250,000 zooplankton per tank, and this represented approx. 5-10% of the total prey items available. The feedings were at 9:00 PM during the first week and at 9:00 AM and 3:00 PM thereafter.

At all feedings, the larvae were allowed to feed exclusively on the zooplankton for one hour prior to adding the required number of enriched rotifers or Artemia. Gut squashes were conducted periodically, and these confirmed that the larvae were feeding on the zooplankton, but also ingesting some Ceratium.

2.4. Larval sampling and research methods

The larvae were randomly collected from each tank, anaesthetized in MS-222 (tricaine methane-sulphonate; 0.05 g liter^{-1}; Syndel Laboratories, B.C., Canada), and rinsed in UV sterilized seawater before being processed further.

Standard length (SL): 20 larvae were sampled at 0 dph, 24 larvae per treatment at 10, 20, 30 and 40 dph, and 40 larvae per treatment were collected at 60 dph. The larvae were individually photographed using a photomicroscope (Wild M420, ON, Canada) connected to a camera (Infinity 2-2c, ON, Canada), and measured from the tip of the snout to the end of the hypurals using a calibrated ocular micrometer.

Dry weight (DW): 8 samples of 30 larvae were collected at 0 dph and 12 samples per treatment were obtained at 10, 20, 30, 40 and 60 dph. The number of larvae per sample varied between 5 and 20 from 10 to 60 dph (as larvae size increased, fewer were needed per sample). Each sample was washed with isotonic ammonium formate three times, placed on Whatman filter paper that was under slight vacuum, and then rinsed three times with distilled water. The larvae and filter paper were then transferred to pre-weighed aluminum weigh boats and dried at 80 °C for a minimum 24 hours before being weighed on an analytical balance (Denver Instrument APX-60, Arvada, Co, USA).

Survival: Percent survival was calculated by subtracting the total number of sampled larvae from the initial number (i.e. at 0 dph) and dividing by the number of fish remaining at the time of grading.

Specific growth rate (SGR) was calculated according to the formula of Elliott (1975):

$G_w = 100\ [\ln (W_t / W_0)]/t]$, where (W) was weight at the beginning (W_0) and end (W_t) of a growth period of (t) days. Growth rate was calculated using dry weight during the larvae stages and wet weights during the juvenile stages.

Condition factor (K) was calculated according to the formula of Fulton (1904):

$K = W / (L^3)$, where (W) is dry weight and (L) is standard length.

Table 2.1. Number of zooplankton < 400 µm collected from Conception Bay, Newfoundland, and used to feed cod larvae from hatch to 30 dph.

Date	October					November							
	21	23	25	28	30	2	3	8	9	10	13	14	17
Vol. (L)	87	85	94	96	72	86	93	22	100	103	68	78	80
Count/ml	30.4	28.4	29.3	44.3	34.7	41.8	47.7	35.5	39.9	40.7	33.4	53.5	47.2
Total x 10^6	2.6	2.4	2.8	4.3	2.5	3.6	4.4	0.8	4.0	4.2	2.3	4.2	3.8

Figure 2.2. Pictures of the most prevalent zooplankton and phytoplankton collected in Conception Bay, Newfoundland during the plankton tows; A) copepods of the species Pseudocalanus, Oithona and Temora; and B) the phytoplankton Ceratium.

2.5. Larval bone deformities and skeletal malformation

To examine the incidence and type of deformities in the different treatments, 40 larvae per treatment were sampled at 60 dph, anaesthetized in MS-222 (tricaine methane-sulphonate; 0.05g liter^{-1}), stored in 4% formalin, and then stained with Alizarin Red according to Kjorsvik et al. (2009) (Figure 2.3). These fish were then placed in 40% glycerol for two days prior to being photographed using a photomicroscope (Wild M420, ON, Canada) connected to a camera (Infinity 2-2c, ON, Canada). The mean dry weights of the fish at 60 dph were: 14.30 ± 1.30 mg, 13.42 ± 1.07 mg and 57.95 ± 2.30 mg, respectively, for the RA, RA-PH and RA-Zoo groups, and their standard lengths were: 20.66 ± 0.23 mm, 21.12 ± 0.36 mm and 28.05 ± 0.60 mm, respectively.

The stained larvae from each of the three experimental groups were assessed for the following skeletal malformations (Lein et al., 2009): stargazer, lordosis, kyphosis, corkscrew, fused vertebrae and not fully ossified.

2.6. Juvenile rearing and sampling for growth and production traits

The experimental groups were hand graded according to size at 63 dph in order to minimize cannibalism and maximize survival in each group. RA were graded into groups of large, medium and small, and RA-Zoo were graded into groups of large and small. RA-PH were not graded due to their low survival (see Section 3.1.2), and to minimize stress to the remaining fish from that group. Automatic feeders were used after the fish were graded and they were fed with dry feed (Gemma Diamond, Skretting, Vervins, France) at

a ration of 8-3% body weight day^{-1} depending on size/age. The pellet size increased from 0.3 to 1.2 mm as the fish grew larger. All fish were kept in 400 l flow through tanks at 10 ^{o}C and 8 l min^{-1} water flow, and photoperiod was maintained at 12 hours light: 12 hours dark.

When the fish reached an average weight of 15 g (~ 192 dph) they were PIT-tagged [Passive Integrated Transponder (PIT) tags; Easy AV, Avid Identification Systems, Norco, CA, USA], and combined into two 3000 l tanks. The stocking density of each tank was 7 kg m^{-3}, and the fish were fed on 2 mm dry pellets (Gemma Diamond, Vervins, France) at a ration of 2% body weight day^{-1}. The feed ration from 6-18 months post-hatch was 1.5 - 1.0% body weight day^{-1} (depending on size/age) and pellet size was increased from 3 to 6 mm as the fish grew. Standard length (SL) and body wet-weight (VIC-6kg, Acculab, NY, U.S.A) were measured on all fish after they had been anaesthetized in MS-222 (tricaine methane-sulphonate; 0.1 g liter $^{-1}$). These measurements were carried out every 3 months on 6 - 18 month old fish (192-558 dph).

2.6.1. External deformities

Externally visible deformities (lordosis, scoliosis, stargazer and deformed lower jaw) were also measured on each fish (n=562 vs. n=444 vs. n=66 in the RA, RA-Zoo and RA-PH groups) when the juveniles were PIT-tagged (192 dph) and when they reached 18 months post-hatch (588 dph).

Figure 2.3. Photograph of 60 dph cod larvae stained with Alizarin Red. The scale bar is 3 mm.

2.7. Neuroendocrine regulation of growth and appetite

2.7.1. Animals and sampling regime

Larvae were collected from all tanks when they reached 9 mm (26-30 dph; i.e. prior to the start of feeding with Artemia), when the RA-Zoo group reached 13 mm (35 dph; prior to weaning onto microdiet) and when the RA-PH and RA groups reached 11 mm (35 dph) and 13 mm (44-50 dph) in length. At all these sampling points eight larvae per treatment were sampled. Fish dry weights at the 9, 11 and 13 mm sampling points were: 0.48 ± 0.04, 1.41 ± 0.26 and 4.31 ± 0.50 mg, respectively, in the RA group and 0.62 ± 0.04, 1.49 ± 0.19 and 3.75 ± 0.37 mg, respectively, in the RA-PH group. In the RA-Zoo group, fish dry weights at the 9 and 13 mm sampling points were 0.74 ± 0.04 and 2.75 ± 0.29 mg, respectively.

The sampled larvae were briefly anaesthetized in MS-222 (tricaine methane-sulphonate; 0.05g liter^{-1}), rinsed in UV sterilized seawater and transferred to RNase/DNase-free 1.5 ml Eppendorf tubes. They were then snap frozen in liquid nitrogen and immediately stored at -80 °C until homogenization and RNA extraction.

2.7.2. RNA extraction

Total RNA was extracted from frozen whole larvae using TRIzol reagent (Life Technologies, Burlington, ON). Samples were homogenized in 1.5 ml Eppendorf tubes with disposable plastic Kontes RNase-free Pellet pestles (Kimble Chase, Vineland, NJ), using a battery operated driver (VWR, Mississauga, ON) in 300 µl of TRIzol until no

visible solids remained. Then, an additional 450 µl of TRIzol was added, the samples were mixed by pipetting, and they were immediately stored at -80 °C. To finish the RNA extraction protocol the samples were thawed on ice and further disrupted using QIAshredder (QIAGEN, Mississauga, ON) spin columns following the manufacturer's instructions and refrozen. The TRIzol extracted RNA was then cleaned using column purification (RNeasy MinElute Column Cleanup kit, QIAGEN) and treated with DNAse-I according to the manufacturer's (QIAGEN) instructions. Briefly, TRIzol-extracted total RNA (45 µg) was incubated in a 100 µl reaction containing RDD DNA digest buffer (1x final concentration) and 6.8 Kunitz units of DNase-I (RNase-free DNase set, QIAGEN) at room temperature for 10 min. to degrade any residual genomic DNA. The entire reaction was then column cleaned according to the manufacturer's instructions.

For both the crude and column-purified RNA extracts, RNA quantity was assessed using A260 NanoDrop UV spectrophotometry. RNA purity was evaluated by A260/280 and A260/230 NanoDrop UV spectrophotometry and RNA integrity was verified using 1% agarose gel electrophoresis. The RNA in the gel was compared to a DNA size marker (1 kb plus ladder, Life Technologies) and the running buffer was 1x TAE; ethidium bromide (1 µg ml^{-1} final concentration) was added to the gel in order to visualize the RNA when exposed to UV light.

2.7.3. First strand cDNA synthesis

First strand cDNA was synthesized by reverse-transcribing 1 µg of DNAse-treated, column-purified total RNA using Moloney murine leukemia virus (M-MLV)

reverse transcriptase (Life Technologies) according to the manufacturer's instructions. Briefly, 1 µg of RNA, 1 µl of random hexamers (250 ng μl^{-1}), 1 µl of dNTPs (10 mM) and nuclease-free water (Life Technologies) (to a total reaction volume of 13 µl) were combined, mixed by pipetting and then heated at 65 °C for 5 min. The samples were subsequently placed on ice for 2 min and then, 4 µl of 5X manufacturer's first strand buffer, 2 µl of DTT (0.1 M), and 1 µl of M-MLV (200 U μl^{-1}) were added to a final reaction volume of 20 µl. All concentrations indicated are stock solution concentrations. Finally, samples were incubated at room temperature for 10 min at 37 °C for 50 min and then at 70 °C for 15 min.

2.7.4. Primer design for quantitative reverse transcription – polymerase chain reaction (QPCR)

Primers for QPCR were designed using Primer3 software (http://frodo.wi.mit.edu/primer3), and based on cDNA sequences for the genes of interest and the normalizer that were available for Gadus morhua in GenBank or ENSEMBL. Two primer sets were synthesized (by Integrated DNA Technologies, Coralville, Iowa) for each gene and tested for quality before use. In the case of gene isoforms (IGF-1 and IGF-2, and GHR-1 and GHR-2), cDNA sequences were aligned using AlignX (Vector NTI Advance 11, Life Technologies) to ensure that the primer sequences were isoform specific. The primer test included calculating amplification efficiencies (Pfaffl, 2001) for both a 9 mm and a 13 mm individual fish from the RA group. Briefly, a 5-point 1:3 dilution series starting with cDNA (corresponding to 10 ng of input total RNA) was

performed for each sample (i.e. 9 and 13 mm) and the reported efficiencies are an average of the two values. Amplification efficiencies were required to be between 80 and 110%. Melting curves were also analyzed in order to verify that the primers amplified a single product and that there were no primer-dimers or amplification in the no-template control. Lastly, the PCR products were electrophoresed on a 1.5% agarose gel with ethidium bromide staining alongside a DNA size marker (1 kb plus ladder, Life Technologies) to verify amplicon size. The primer set that met these parameters and had the best amplification efficiency, was chosen for each gene. Primer sequences, amplification efficiencies and amplicon sizes are shown in Table 2.2.

2.7.5. QPCR

QPCR reactions and analyses of transcript (mRNA) levels were performed using the 7500 Fast Real-Time PCR System (Life Technologies) with SYBR Green I dye chemistry. Transcript expression levels of the genes of interest were normalized to C3_RNA polymerase II elongation factor ELL2. This gene was chosen as the endogenous control (i.e. normalizer) gene based on its very stable expression profile in microarray studies (Jennifer Hall, personal communication) and in the following QPCR assessment. The florescence threshold cycle (C_T) values of the 24 samples from the different experimental groups [3 from each of the RA and RA-PH (9, 11 and 13 mm) groups and RA-Zoo (9 and 13 mm) groups] were stable; the average CT value was 28.2 and all 24 samples were within 0.3 cycles of this value.

PCR amplification was performed in a 13 µl reaction using 1 x (final concentration) Power SYBR Green PCR Master Mix (Life Technologies), 50 nM (final concentration) of each forward and reverse primer, and cDNA corresponding to 5 ng of input total RNA. The QPCR cycling parameters consisted of 1 cycle of 50 °C for 2 min, 1 cycle of 95 °C for 10 min, and 40 cycles of 95 °C for 15 sec and 60 °C for 1 min, and melt curve analysis was performed at the end of each reaction. On each 96-well plate, for every sample, target and normalizer genes were run in triplicate.

The C_T values were determined automatically using the 7500 Software Relative Quantification Study Application (Version 2.0) (Life Technologies). When a C_T value within a triplicate was greater than 0.7 cycles different from the other two technical replicate C_T values, it was considered to be an outlier, discarded and the average $C_T$value was calculated using the remaining two C_T values. The relative quantity (RQ) of each transcript was then calculated with this software using the $2^{-\Delta\Delta CT}$ relative quantification method (Livak and Schmittgen, 2001) and the amplification efficiencies for the gene of interest and normalizer. For each target gene, the individual with the lowest normalized expression (mRNA level) was set as the calibrator sample (assigned an RQ value = 1). Gene expression data are presented as means ($\pm$ 1 standard error).

Table 2.2. Primer sequences, amplification efficiencies and amplicon sizes for all the genes used in the QPCR studies

Gene name [1](Accession number)	[2]Primer name	Nucleotide sequence (5'-3')	Efficiency (%)	Amplicon size (bp)
CART (DQ167209)	CART_2 - F	CGTCCATGGAGCTGATCTTT	91.2	106
	CART_2 - R	CTGCCTCTCGTTGGTCAAGT		
NPY (AY822596)	NPY_2 - F	ACTCCGCATTGAGGCACTAT	92.3	108
	NPY_2 - R	TTTCCTTCAGCACCAGCTCT		
Myostatin (ENSGMOT00000013879)	MYO_1 - F	GACGGAGACGATCATGTTGA	102.9	112
	MYO_1 - R	GATGCGATTGGCTTGAATCT		
IGF-1 (HQ259081)	IGF-1_2 - F	ACACGCTGCAGTTTGTGTG	84.3	118
	IGF-1_2 - R	ATCTGGAAGCAGCACTCGTC		
IGF-2 (HQ263172)	IGF-2_2 - F	CCGAGAGGAGCATAATGACG	93.5	122
	IGF-2_2 - R	CTCTCCTCCGCACAGAGTTT		
GHR-1 (ENSGMOT00000008443)	GHR-1_1- F	GCTGGATGAGTCTGGCCTAC	92.1	110
	GHR-1_1 - R	GCGGATATGCACCTCGTACT		
GHR-2 (ENSGMOT00000011011)	GHR-2_1 - F	GTGGACCACGAGGTCAGAGT	97.0	124
	GHR-2_1 - R	TCAAGAGGATGGCAATAGGG		
GH (EU676171)	GH_2 - F	CCTACGGGGGCTACTACCAG	93.6	145
	GH_2 - R	CAGTTGTCCTCAGGGGAGAG		
C3_RNA polymerase II elongation factor ELL2 (FF416208)	ELL2_2 - F	GCTTCCGCATAAAGACAAGG	93.8	150
	ELL2_2 - R	GGATAACAGCGGCGTGTACT		

[1]Accession numbers starting with ENSGMOT are from ENSEMBL; the rest are from GenBank.

[2]F is forward and R is reverse direction.

2.8. Metabolic physiology

2.8.1. Animals and sampling regime

Measurements of oxygen consumption (MO_2; in mg O_2 $kg^{-0.8}$ hr^{-1}) and critical swimming speed (U_{crit}; in cm sec^{-1}) were performed on 5 months old juveniles from the RA (n = 13) and RA-Zoo groups (n = 14) within the size range of 5 to 11 g. This study was not performed on the RA-PH fish due to the low survival, and that these individuals were needed for the monitoring of long-term growth in this group.

2.8.2. Experimental protocol

Each juvenile cod was placed in a floating container (3.8 l volume) that was immersed in the holding tanks for 24 hours. Thereafter, they were lightly anaesthetized in seawater containing MS-222 (tricaine methane-sulphonate; 0.07 g $liter^{-1}$), and carefully placed into the swim-tunnel (see below) for a 24 hour acclimation period at a temperature of 10 – 10.5 °C. During this time, the water velocity in the swim tunnel was set to approx. 1 cm sec^{-1} and water was supplied to the tunnel using a peristaltic pump (Masterflex L/S Easy-load II, Cole-Parmer, IL, USA) at 15 – 20 ml min^{-1}. The floating container and the 24 hour recovery period were used to ensure that the juveniles had an empty gut (i.e. were in a post-absorptive state) at the time of MO_2 measurements.

After the acclimation period, two measurements of resting oxygen consumption were made over a 20 min period. The water velocity in the tunnel was then increased in approx. 3 cm sec^{-1} increments every 15 min and MO_2 measurements were made at each

swimming speed. Each 15 min cycle consisted of a 7 min measurement period (where water flow into the swim tunnel was stopped) followed by an 8 min flushing period. Swimming velocity was increased until the fish were exhausted. Exhaustion was determined as the point at which the fish rested on / could not free themselves from the grid at the back of the tunnel for 10 seconds. Using this protocol, there were no experiments in which water O_2 levels decreased below 90% of air saturation. After the fish were exhausted, they were removed from the swim tunnel, anaesthetized in MS-222 (tricaine methane-sulphonate; 0.2 g liter^{-1}), and several morphometric measurements were recorded: wet weight (g), total length (cm), depth (cm), width (cm), and condition factor (K). The liver and heart were also removed and used to calculate Hepatosomatic Index (HSI) and Relative Ventricular Mass (RVM) using the following formulas:

$$HSI = (\text{Liver weight (g)} / \text{Fish weight (g)}) \times 100,$$

$$RVM = (\text{Ventricular weight (g)} / \text{Fish weight (g)}) \times 100.$$

2.8.3. Oxygen consumption (MO_2) and Critical Swimming Speed (U_{crit}) measurements

Oxygen consumption and critical swimming speed (U_{crit}) were measured using a Blazka-type swim-tunnel (approx. 200 ml volume) (Loligo Systems, ApS; Tjele, Denmark), and a fiber-optic oxygen meter (model FIBOX 3 LCD, PreSens, Germany) with a pre-calibrated dipping probe at a temperature of 10 – 10.5 ˚C. The swim tunnel was covered with black foam in order to minimize disturbance to the fish while

swimming. Oxygen consumption (MO_2) was measured by turning off the inflow of water to the respirometer, and measuring the fall in water oxygen content (in mg O_2 l^{-1}) using the oxygen meter, which was connected to a computer that was running OxyView software (LCDPSTE V2.01). This data was then downloaded into Logger Pro (Version 3.4; Vernier Software and Technology; Beaverton, OR, USA) for the determination of MO_2 in mg O_2 l^{-1} min^{-1} by fitting a linear regression to the O_2-time data. Finally, MO_2 was converted into mass specific values using the fish's body weight and a weight exponent of 0.8 (Edwards et al., 1972), and was calculated using the formula:

$$MO_2 \text{ (mg } O_2 \text{ kg}^{-0.8} \text{ hr}^{-1}) = (\text{rate of decline in } [O_2] \times (V_t - V_w) \times 60 \text{ min}) / W^{0.8} \times h$$

Where rate of decline in O_2 was measured as the slope of the linear regression between time and water oxygen level; Vt is the tunnel volume in liters; Vw is the weight (volume) of the fish, assuming that 1 g is equal to 1 ml of sea water; W is the weight of the fish in kg; and h is hour.

Critical Swimming Speed (U_{crit}) was calculated using the formula:

$$U_{crit} = v + ((t_f / t_i) \times v_i)$$

Where v is the highest velocity at which the fish swam for the entire time increment (cm sec^{-1}); V_i is velocity increment (cm sec^{-1}); t_f is time elapsed from the last change in current velocity to fatigue (min); and t_i is the time between step increases in velocity (15 min).

The velocity at which the juveniles were swimming in the swim tunnel was corrected for the solid blocking effect using the equation of Bell and Terhune (1970):

$V_F = V_T [(1+ (\Gamma\lambda) (A_0/A_t)^{1.5}]$,

Where $V_{F=}$ corrected velocity, $V_{T=}$ test velocity, Γ=0.8, λ= 0.7 fish length/thickness, $A_{0 =}$ maximum cross-sectional area of fish, A_t= cross-sectional area of tunnel [Π (radius)^2], and thickness = [(fish width + fish depth)/4].

To eliminate / account for bacterial O_2 consumption the respirometer was cleaned daily with fresh water and 70% ethanol. In addition, weekly 'blank measurements' with an empty seawater-filled chamber were made. Under these conditions, oxygen consumption was always negligible (< 1%).

2.9. Stress response

2.9.1. Experimental design

To study the effect of the RA and RA-Zoo diets on the stress (cortisol) response, fish from these two treatments were sampled at 82 dph (i.e. when they were early juveniles) at rest (pre-stress) and at 1, 3, 6 and 12 hours post-stress, and whole body cortisol levels were measured. Again, this analysis was not performed on the RA-PH fish, given the limited survival in this group (see Section 3.1.2), and that these fish were needed for the monitoring of long-term growth.

The pre-stress samples were collected at 09:00 h, and all the fish were stressed before the fish in the tanks were fed. The fish were stressed by netting them, holding them

out of the water for 30 sec., and then confining them in small floating containers with mesh sides (250 ml volume) until sampling. At rest, and at each post-stress sampling point, nine and ten juveniles were quickly netted from the RA and RA-Zoo treatments, respectively, and immediately euthanized with an overdose of MS-222 (tricaine methane-sulphonate; 0.4 g liter^{-1}). Once the juveniles were immobilized, they were blotted dry on a mesh screen, quickly measured for wet weight and standard length, transferred to 2 ml Eppendorf tubes or sterile Whirl-Pak® bags (in cases when the juveniles were too big to fit the 2 ml tubes), and snap frozen in liquid nitrogen. This entire procedure took less than 3 minutes. Finally, the fish were then transferred to a - 80 °C freezer until homogenization and cortisol extraction.

2.9.2. Cortisol extraction and ELISA

Extraction of the samples was done by modifying the procedures of Hiroi et al. (1997). Briefly, ice-cold phosphate-buffered saline (0.1 M PBS + 140 nM NaCl, pH 7.6) was added to 15 ml or 50 ml conical tubes (depending on fish size) to a maximal initial volume of 4.5 or 20 ml, respectively. Frozen juvenile cod (weighing 0.2-2.7 g) were then quickly cut into a number of small pieces, transferred into the tubes, and immediately homogenized using a Polytron PT 10-35 homogenizer (KINEMATICA, Bohemia, NY, USA). The samples were homogenized for 1-1.5 min (depending on fish size) at a dial setting 5 until no visible tissue remained, and then placed on ice. Once all the samples were homogenized, they were sonicated (Branson Sonifier W-150; Branson Ultrasonics Corp. Danbury, CT, USA) on ice at 20 Watts for 30 sec. or until completely liquified, placed on ice for 30 sec., and sonicated for an additional 30 sec.

PBS was then added to each sonicated sample to obtain a final tissue concentration of 50 mg wet weight (ww) ml^{-1} of PBS, and 1.8 ml of each sonicated sample was centrifuged for 5 min at 3000x g. Finally, 1.5 ml of the sample was pipetted into a clean 1.5 ml microfuge tube, being careful not to transfer any of the pelleted insoluble material. These clear aliquots were then stored at - 80 °C.

After thawing the aliquots on ice, 250 µl of each sample were pipetted into a 12 x 75 mm glass culture tube and double extracted with ether as follows. Approximately 1.5 ml of ether was added to each glass tube and vortexed for 30 sec. The tube was then submerged in a dry ice- methanol bath for 60 sec, and immediately thereafter, the ether layer was poured into a second 12 x 75-glass culture tube and a second extraction performed. The two resulting ether extracted samples were pooled, and the aqueous layer from the second extraction was removed and again frozen in a dry ice-methanol bath. Any remaining ether from this final extraction was added to the pool as well. The ether was then evaporated under a gentle stream of nitrogen, and the dry extracts re-suspended in 300 µl of extraction buffer (prepared from the cortisol assay kit; NEOGEN Corp., Lexington, KY, USA). The extracts were then allowed to settle for a few minutes, and pipetted into fresh 1.5 ml microfuge tubes, which were stored at – 80 °C until the ELISA assay.

The ELISA assay was performed using a commercially available ELISA kit (NEOGEN Corp., Lexington, KY, USA), and according to the manufacturer's instructions. Briefly, serial dilutions of a 1 µg ml^{-1} of cortisol standard were made to create a standard curve of seven concentrations (0.04, 0.1, 0.2, 0.4, 1, 2 and 10 ng ml^{-1}).

Fifty µl of each of these standards and the extracted samples were added to the antibody (rabbit-anti cortisol) coated plate in duplicate. Fifty µl of enzyme conjugate (cortisol horseradish peroxidase) were then quickly added to each well using a repeater pipette, the plate was shaken for 20 sec., and the plate incubated in the dark for 1 h at room temperature. After the incubation period, the contents of the plate were discarded and each well washed three times using a wash buffer. One hundred and fifty µl of substrate (stabilized 3, 3', 5, 5' tetramethylbenzidine, TMB plus hydrogen peroxide, H_2O_2) were then added to each well using a multi-channel pipettor, and the plate was covered, mixed for 20 sec., and incubated in the dark for 30 min at room temperature. After the second incubation, the bottom of the plate was cleaned using a lint-free towel, the plate cover was removed, and the plate was shaken and read on a microplate reader (SpectraMax M5e, Molecular Devices, Sunnyvale, CA, USA) at an absorbance of 650 nm. Intra- and inter-assay variation did not exceed 10%.

2.10. Statistical and data analyses

2.10.1. Larval growth

The residuals of the data were tested for normality and homogeneity using the Shapiro-Wilk and Bartlett tests, respectively. Differences in growth between the groups were then examined using a Randomized Complete Block (RCB) one-way ANOVA [with treatments as the main effect and tank as the blocked factor] followed by Tukey's multiple comparisons of means test. A similar analysis was performed at each measurement point (10, 20, 30, 40 and 60 dph) for fish dry weight and standard length.

Due to uncertainly in pairing length (n=24) and weight (n=12) measurements, a randomization routine was employed to calculate 100 possible condition factors from randomly drawn length and weight pairs. From this distribution of condition factors, a mean and standard error were calculated, which reflected the uncertainty in the dataset.

2.10.2. Skeletal malformations

Significant differences in the frequency of each deformity type between groups were identified using Pearson's Chi-square test and Fisher's exact test for count data. These data are presented as a percentage of fish per treatment with a specific type of deformity.

2.10.3. Survival

Significant differences in total percent survival between the groups during the larval stage (i.e. up to grading), and during the juvenile stage from 6 (192 dph) to 18 (588 dph) months post-hatch, were identified using Pearson's Chi-square test and Fisher's exact test for count data.

2.10.4. Juvenile growth

The residuals of the data were tested for normality and homogeneity using the Shapiro-Wilk and Bartlett tests, respectively. When a data set was found to violate these assumptions, a non-parametric Kruskal-Wallis test was performed. Differences in growth (i.e. wet weight and standard length) between groups were examined using repeated

measures two-way ANOVAs with treatment and time as the main effects. However, a significant interaction between the main effects was found. Therefore, this analysis was followed by separate one-way ANOVAs and Tukey's post-hoc (HSD) tests at each time point to examine treatment effects.

2.10.5. Neuroendocrine regulation of growth and appetite

Prior to analyses, outliers were identified and removed using the ESD (extreme studentized deviate) method. Two-way ANOVAs were initially used to examine the effect of treatment and size on the RQ values of each gene of interest. This analysis was normally followed by separate one-way ANOVAs and Tukey's post-hoc (HSD) tests to examine the effects of: 1) age/developmental stage (9, 11 and 13 mm) within a treatment (RA, RA-PH, RA-Zoo); and 2) treatment within a particular age/developmental stage. However, in cases when there was significant interaction between the two main effects, only the latter analysis was performed. The residuals were tested for normality and homogeneity using the Kolmogorov Smirnov and Levene tests, respectively. When a data set was found to violate these assumptions, a non-parametric Kruskal-Wallis test was performed.

2.10.6. Metabolic physiology

Repeated measures two-way analysis of covariance (ANCOVA), with weight as the covariate, was first used to examine the effects of diet and swimming speed on MO_2. Then, separate one-way ANCOVAs were performed at each swimming speed to examine

the effect of diet on MO_2. Neither diet or weight significantly affected MO_2 at a particular swimming speed. Analysis of covariance (ANCOVA) was also used to examine the effect of diet on resting metabolic rate, maximum metabolic rate, absolute metabolic scope, factorial metabolic scope and U_{crit}. For all metabolic parameters, statistical analysis was performed before the data were weight corrected. The residuals in both analyses were also tested for normality and homogeneity using the Shapiro-Wilk and Bartlett tests, respectively. Morphometric parameters were compared between RA-Zoo and RA fish using un-paired t-tests.

2.10.7. Stress response

Initially, two-way analysis of covariance (ANCOVA) was used to examine the effects of diet and sampling point on the cortisol levels, with weight as the covariate. This analysis revealed a significant interaction between the two main effects, and therefore, we used: 1) One-way analysis of covariance (ANCOVA) followed by Dunnett's post-hoc tests to identify significant differences in cortisol levels between the control value (0 hr) and post-stress values (1, 3, 6 and 12 hr) within each diet group (RA, RA-Zoo); and 2) One-way analysis of covariance (ANCOVA) to examine the effect of diet at each time point (0, 1, 3, 6 and 12 hr), again with weight as the covariate. The residuals were tested for normality and homogeneity using the Shapiro-Wilk and Bartlett tests.

For all analyses, $P < 0.05$ was used as the level of statistical significance. Data in the text, in Figures and in Tables are presented as means ± 1 standard error of the mean (S.E.)

3. Results

3.1. Growth and production traits during the larval stage

3.1.1. Morphometric parameters and growth rate

Partially supplementing the larvae's diet with wild zooplankton (i.e. by 5-10%) dramatically enhanced growth (Figures 3.1 and 3.2), with the first appearance of a difference in larval dry weight and length occurring at 30 dph; the dry weights of the three groups at this time point were RA 0.6 ± $2*10^{-5}$ mg, RA-PH 0.7 ± $4*10^{-5}$ mg, and RA-Zoo 1.3 ± $1*10^{-4}$ mg. By the end of the larval stage (60 dph), the cod from the RA-Zoo group were approximately four fold heavier (57.9 ± 2.3 mg vs. 14.3 ± 1.3 mg and 13.4 ± 1.0 mg in the RA and RA-PH groups, respectively) and 30% longer (28 ± 0.6 mm vs. 20.7 ± 0.2 mm and 21.1 ± 0.3 mm in the RA and RA-PH groups, respectively). Condition factor (K) showed a similar trend, with the first appearance of a difference occurring at 30 dph; K of RA-Zoo being 62% and 84% greater as compared to the RA and RA-PH groups, respectively. Specific growth rate between 0 and 60 dph was approx. 2.5% day^{-1} higher in the zooplankton fed cod (10.4% body weight day^{-1}) as compared with the RA and RA-PH groups (approx. 8% body weight day^{-1}). Throughout this part of the experiment, there were no significant differences in morphometric parameters or growth between the RA and RA-PH fish, with the exception of K at 10 and 30 dph.

3.1.2. Survival

The number of fish remaining at the time of grading was quite low (see Table 3.1.) due to the large number of fish removed as part of the extensive sampling program, and likely, the stress induced by sampling. However, it was clear that the RA-PH treatment resulted in the poorest survival. Two of the 6 RA-PH tanks crashed (i.e. survival was 0) during the larval rearing period, and in total there were only 123 fish left at the time of grading. In contrast, there were 1267 fish left in the 6 RA tanks, and 587 fish left in the 4 RA-Zoo tanks. This latter difference was statistically significant (χ^2 = 955, $P < 0.05$).

3.1.3. Skeletal malformations

The incidence of the skeletal malformations of stargazer, lordosis, kyphosis, corkscrew, fused vertebrae and not fully ossified were recorded in alizarin stained 60 dph cod in each of the three dietary groups (RA, RA-PH and RA-Zoo). The deformities of stargazer, kyphosis and corkscrew were not encountered in any of the groups at 60 dph. The RA-Zoo group also had no occurrence of lordosis (0%) (Figure 3.3), but this deformity was found in 2.8 and 8.1% of the RA and RA-PH fish, respectively (Figure 3.4). However, this difference in occurrence between the RA and RA-PH groups was not statistically significant ($\chi^2 = 3.7$, $P > 0.05$).

The occurrence of skeletal elements that were not fully ossified (see Figure 3.3) was significantly lower in the RA-Zoo fish (2.5%) than in the RA and RA-PH (31.4% and

40.5%, respectively) groups at 60 dph (χ^2 = 16.7, P< 0.05) (Figure 3.4). In contrast, the degree of ossification between the RA and RA-PH groups was not statistically different.

3.2. Growth and production traits during the juvenile stage

3.2.1. Morphometric parameters and growth rate

The zooplankton fed fish were significantly longer (by 4-8%) as compared to the other two groups at 192 dph, and this difference was maintained until 588 dph (Figure 3.5). In contrast, while the zooplankton fish were always heavier than the other two groups during the juvenile period, this difference diminished with time (Figure 3.5). The RA-Zoo fish were significantly heavier at 192 dph by 30% (24.1 ± 0.4 g vs. 18.7 ± 0.2 g vs. 18.6 ± 0.9 g in the RA and RA-PH, respectively), but only by 11-14% from 368-558 dph. This decreasing treatment effect on body weight was also reflected in the growth rates for the three groups. The growth rates of the RA-Zoo and RA-PH were significantly lower (1.5% and 1.4% wet weight day^{-1}, respectively) than the RA group (1.57% wet weight day^{-1}) from 192-278 dph, and the growth rate of the RA-Zoo fish was significantly lower at 278-368 dph and 368-467 dph (0.99 and 0.59% wet weight day^{-1}, respectively) than measured in the RA (1.04 and 0.62% wet weight day^{-1}) and RA-PH (1.1% and 0.64% wet weight day^{-1}, respectively) groups (Figure 3.6). Furthermore, the overall growth rate of the zooplankton fed fish (0.89% wet weight day^{-1}) from 192-558 dph was significantly lower than the RA group (0.93% wet weight day^{-1}). No statistical differences were observed in length and weight between the RA and RA-PH groups at any of the sampling points.

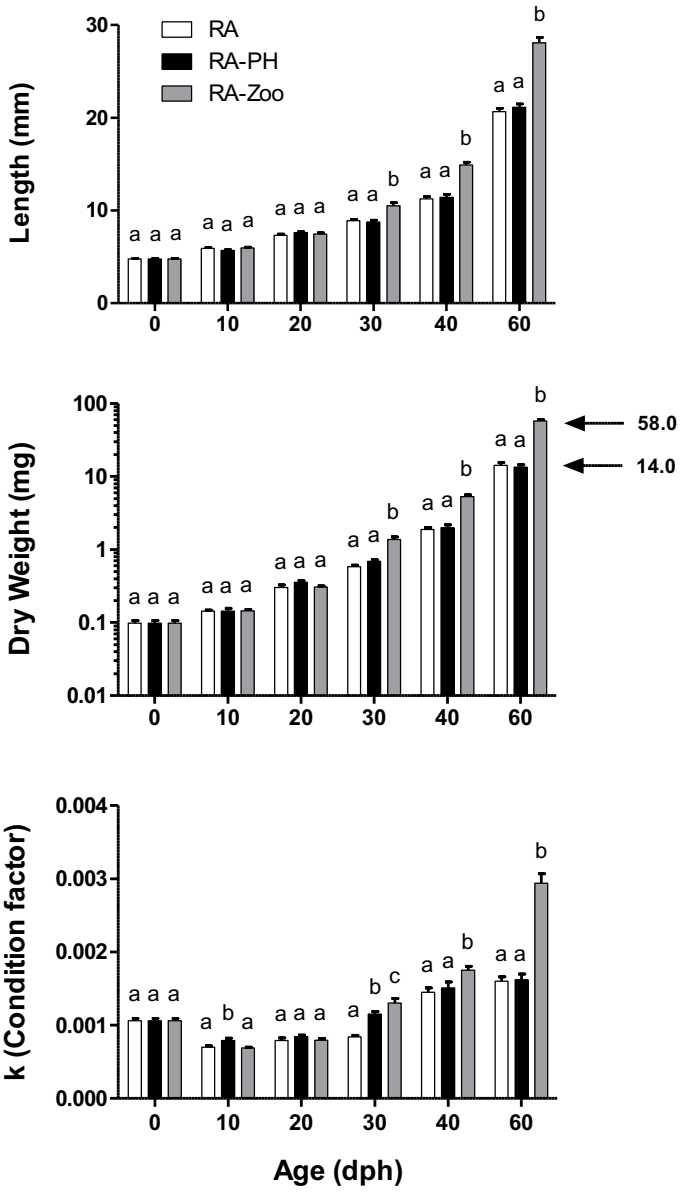


Figure 3.1. Changes in Atlantic cod morphometric parameters during the larval phase of rearing. The three diets fed to the cod were: RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers/Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. Bars represent means ± 1 S.E. (n= 24-40 and n=12 for the length and weight data, respectively). Dissimilar letters within a sampling point indicate a significant difference between the three groups (RCB ANOVA followed by Tukey's tests, $P < 0.05$).

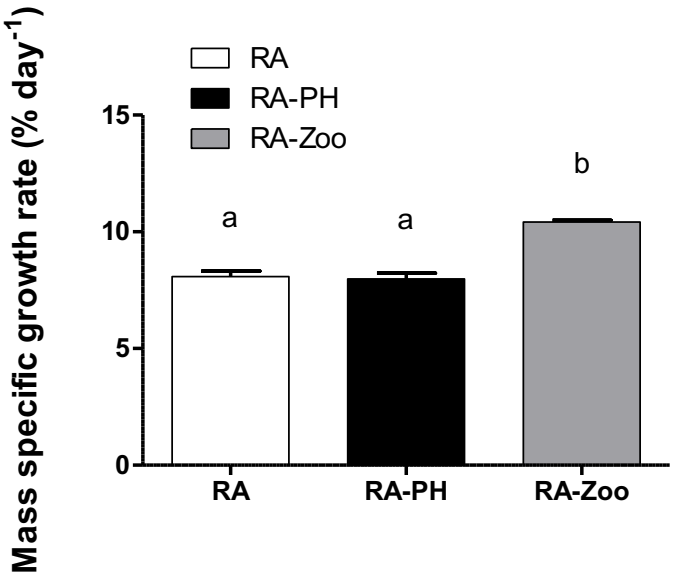


Figure 3.2. Growth rate (% body weight day^{-1}) of Atlantic cod fed three different diets from 0-60 dph. RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers/Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. Bars represent means $\pm$ 1 S.E. (n= 60-240). Dissimilar letters indicate a significant difference between the three groups (RCB ANOVA, $P < 0.05$).

Table 3.1. Number of larvae sampled and survival (%) throughout early development and at the time of grading. The number of larvae at the start of the experiment was different, as the RA-Zoo fish were only stocked into four tanks as compared to six tanks for the other two groups.

Treatment	Starting # of Larvae	JBARB Sampled	Sampled for Experiments	Total # Sampled	Total # of Fish Graded	% Survival
RA	123500	309	4636	4945	1267	1.07
RA-Zoo	82600	186	2673	2859	587	0.74
RA-PH	123500	243	3742	3985	123	0.10

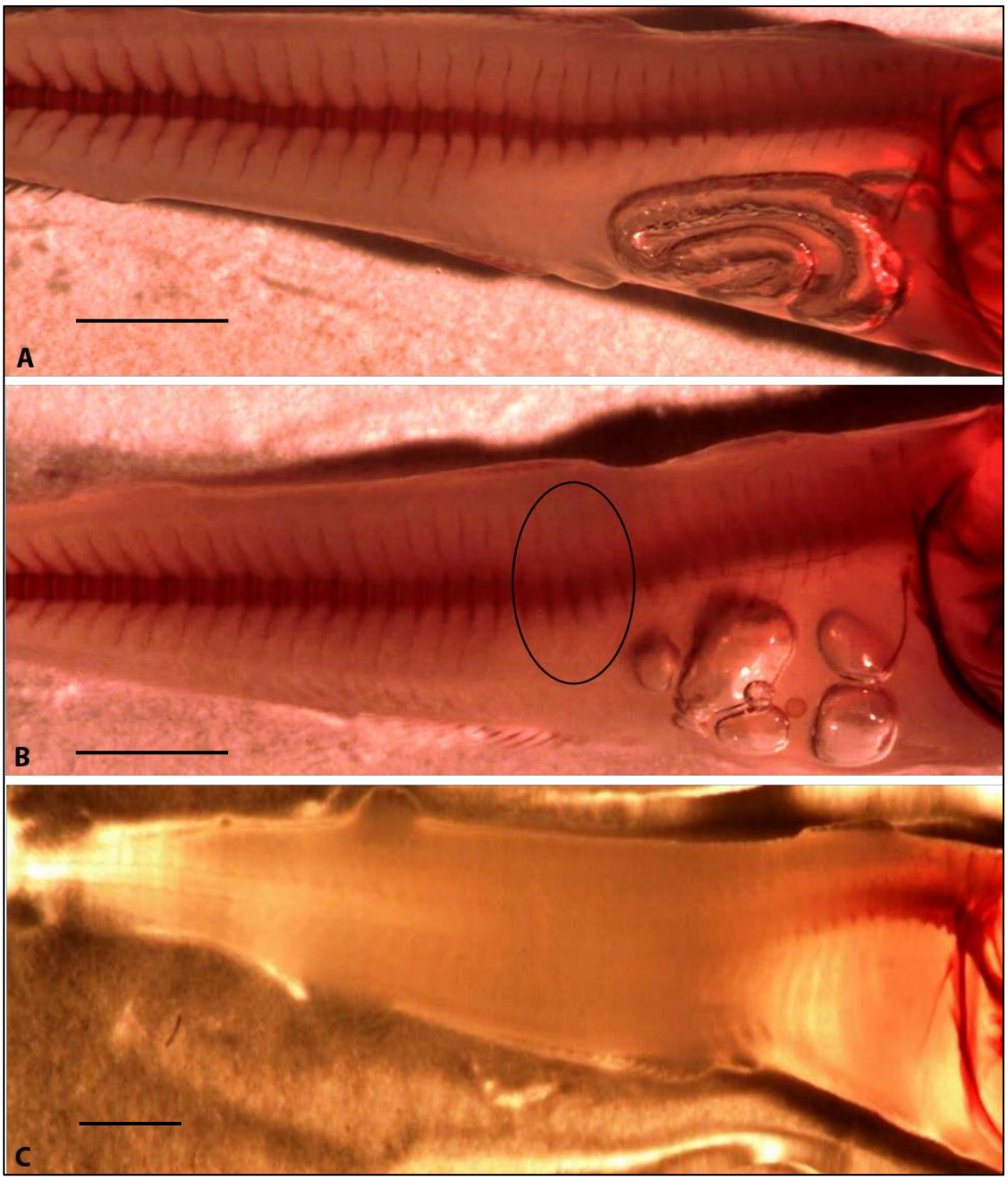


Figure 3.3. Pictures of cleared / stained cod larvae. A) Cod larva with no skeletal deformities. B) The encircled part shows a larva with mild lordosis of the pre-haemal vertebrae. C) Incompletely stained vertebral column indicating that the ossification process was not complete. Bars are 2 mm.

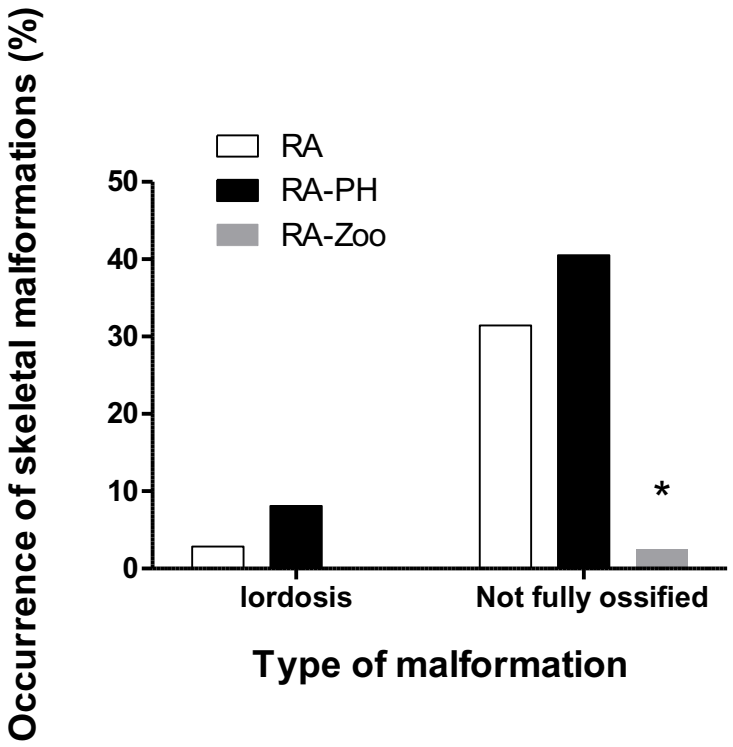


Figure 3.4. Percentage of larvae with the skeletal malformation of lordosis and whose skeleton was not fully ossified at 60 dph. RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers/Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. Bars represent the percentage of fish with a particular deformity in each treatment (n=40 per treatment). The asterisk represents a significant difference between the three diets for the same type of skeletal malformation (Pearson's Chi-square and Fisher tests, $\chi^2 = 16.7$, $P < 0.05$).

3.2.2. Survival

Percent survival, measured from PIT-tagging at 192 dph to 588 dph, was 92.9%, 78.8% and 91.7% in the RA, RA-PH and RA-Zoo groups, respectively. Survival of the RA-PH group was significantly lower as compared to the other two groups ($\chi^2 = 15.1$, $P < 0.05$).

3.2.3. External deformities

The incidence of externally visible deformities (lordosis, scoliosis, stargazer and deformed lower jaw) were recorded in each of the three dietary groups (RA, RA-PH and RA-Zoo) when the juveniles were PIT-tagged (192 dph) and when they reached 18 months post-hatch (588 dph). In almost all cases, the most common deformities were lordosis and scoliosis, a pattern that was seen at both measurement points (Table 3.2). The only deformity observed in the RA-PH group at 192 dph was lordosis, where overall, the RA-PH juveniles had a lower total percentage of deformities as compared to the RA and RA-Zoo fish (1.5% vs. 13.5% and 14.9%, respectively). However, at 558 dph large increases in the incidence of lordosis (by 35%) and scoliosis (by 23.1%) were evident in the RA-PH group, and as a result, the total percentage of deformities was higher in that group as compared to the RA and RA-Zoo fish (53.8% vs. 38.3% and 28.7%, respectively).

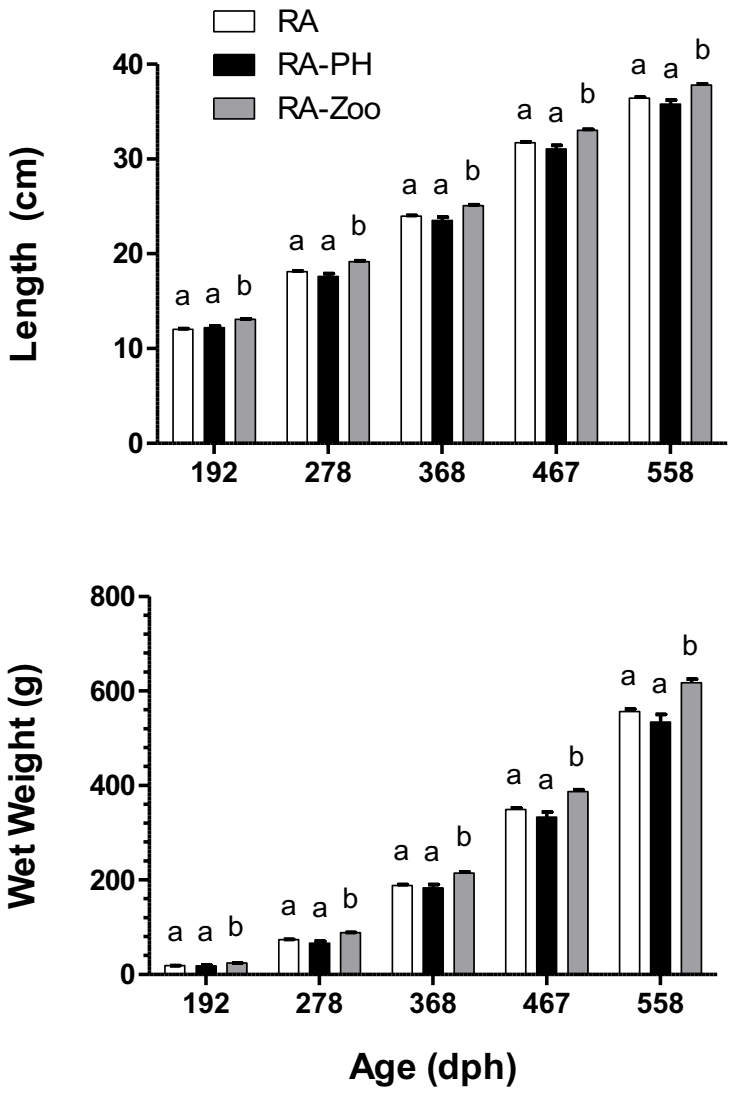


Figure 3.5. Changes in Atlantic cod length (SL) and wet weight during the juvenile phase of rearing. The three diets fed to the cod were: RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers/Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. Bars represent means $\pm$ 1 S.E. (n= 543, 57 and 430 in the RA, RA-PH and RA-Zoo groups, respectively). Dissimilar letters within a sampling point indicate a significant difference between the three groups (One-way ANOVA followed by Tukey's multiple comparison tests, $P < 0.05$).

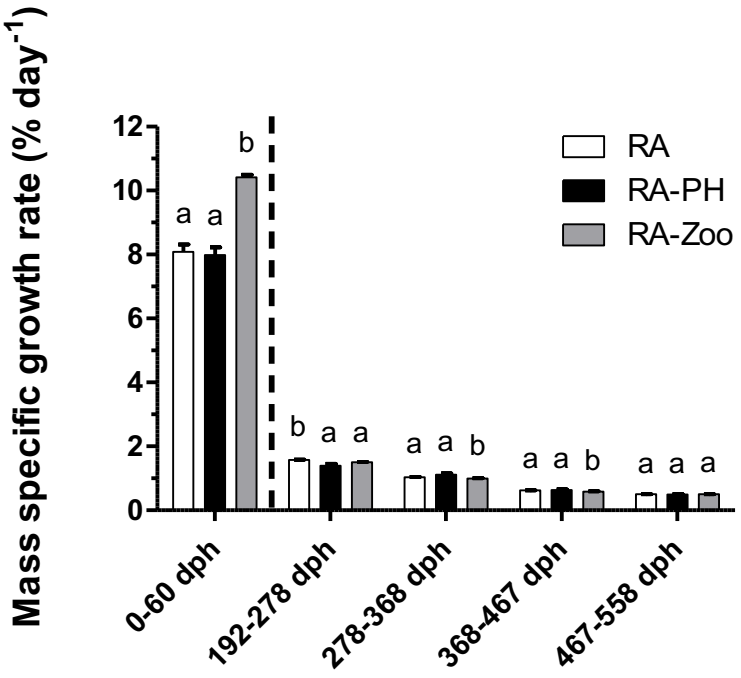


Figure 3.6. Growth rate of Atlantic cod during the larval (0-60 dph; % dry weight day^{-1}) and juvenile (192-558 dph; % wet weight day^{-1}) phases. The three diets fed to the cod were: RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers/Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. Bars represent means $\pm$ 1 S.E. (n = 60-240 and 57-543 for the larval and juvenile data, respectively). Dissimilar letters represent a significant difference between the three diets during the larval (RCB ANOVA) and juvenile phases (Kruskal-Wallis One-way analysis of variance). Both analyses were followed by Tukey's multiple comparison tests, $P < 0.05$.

Table 3.2. Deformity types and occurrence (% of fish) in all three diet groups at PIT-tagging (192 dph) and at the final sampling point (558 dph). *In some cases more than one type of deformity was observed per fish, therefore, the total represents individuals that had one or more deformity.

		Days Post-Hatch (dph)	
Deformity type (%)	**Group**	**192**	**558**
Lordosis	**RA**	8.7	21.1
	RA-Zoo	12.8	18.7
	RA-PH	1.5	36.5
Scoliosis	**RA**	5.0	15.1
	RA-Zoo	1.1	5.2
	RA-PH	0	23.1
Stargazer	**RA**	0	2.1
	RA-Zoo	0.2	2.5
	RA-PH	0	3.8
Deformed lower jaw	**RA**	0	4.8
	RA-Zoo	0.5	5.9
	RA-PH	0	3.8
***Total**	**RA**	13.5	38.3
	RA-Zoo	14.9	28.7
	RA-PH	1.5	53.8

3.3. Neuroendocrine regulation of growth and appetite

3.3.1. Dietary effects on the expression of the main growth-regulating genes

Insulin-like growth factor (IGF) 1 and 2 and growth hormone (GH) mRNA expression levels were not significantly different between diets at the 9 and 11 mm sizes or in the age matched (35 dph) comparison. However, at 13 mm, IGF-1 expression levels in the RA-PH group were significantly lower as compared to the other two groups, whereas, the expression levels of IGF-2 and GH showed the opposite trend (Figures 3.7 and 3.8). Myostatin mRNA expression was not significantly different between the three dietary groups at any fish size (i.e. 9, 11 or 13 mm), but was significantly higher in the RA-Zoo group as compared to the RA-PH group in the age-matched comparison (Figure 3.7). There were no significant differences in IGF-2 or GH mRNA expression as the fish grew. In contrast, IGF-1 mRNA levels increased in the RA-PH and RA groups from 9 to 11 mm, and then stabilized (RA) or returned to initial (9mm) levels. No significant changes in IGF-1 were evident with size in the RA-Zoo group (Figure 3.7).

3.3.2. Dietary effects on growth hormone receptor (GHR) gene expression

Diet also had significant effects on the mRNA expression of GHR-1 and GHR-2 (Figure 3.8). GHR-2 mRNA expression in the RA-PH group was consistently higher than measured in one or both of the other two diet treatments. For example, it was significantly higher in this group as compared to the RA-Zoo group at 9 mm, and higher than the RA group at 11 mm. Additionally, the RA-PH group had significantly higher GHR-2 mRNA

expression as compared to the other two groups at both 13 mm and in the age-matched comparison. In contrast, the effects of diet on GHR-1 mRNA expression were more variable. While GHR-1 expression was significantly higher in the RA-PH group as compared to the RA-Zoo group at 9 mm, it was significantly higher in the RA-Zoo group as compared to the RA group at 13 mm. Finally, there was no effect of diet on GHR-1 expression in the age-matched comparison.

3.3.3. Dietary effects on CART and NPY gene expression

While the expression levels of neuropeptide Y (NPY) were not significantly different between the three dietary groups at 9 mm, significant differences were evident in all of the other comparisons. NPY mRNA expression levels were highest in the RA group in the age-matched comparison and at 11 mm, whereas the RA-PH group showed the greatest expression of NPY mRNA at 13 mm (Figure 3.9). CART mRNA expression was significantly higher in the RA group as compared to the other two groups at 9 mm. However, this was the only significant difference found (Figure 3.9).

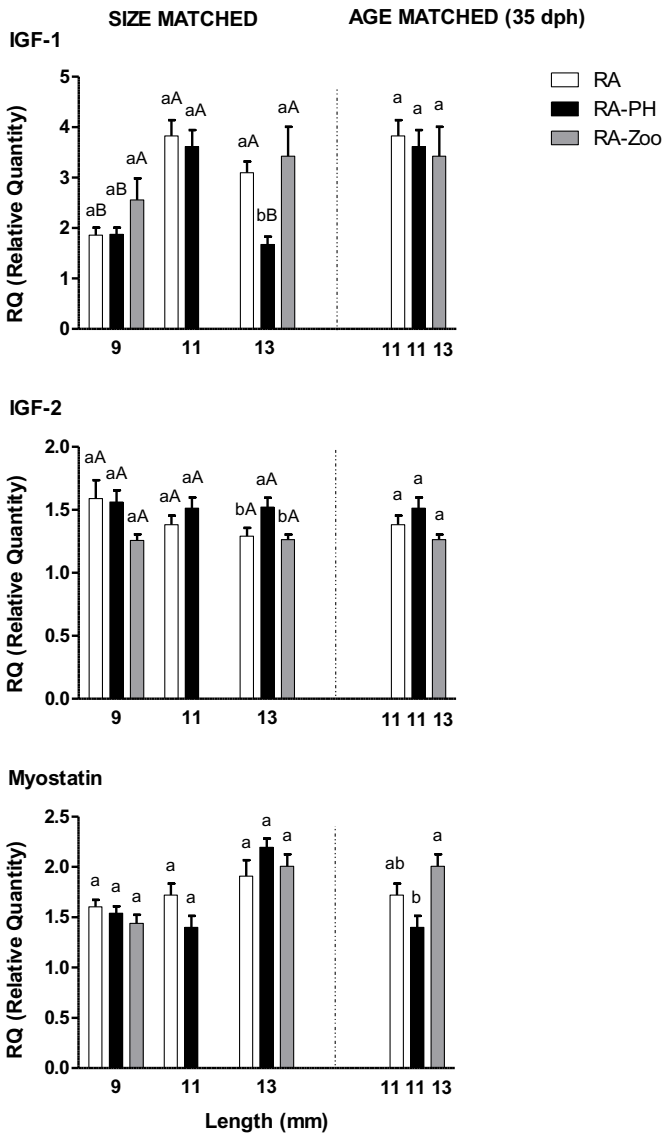


Figure 3.7. Insulin-like growth factors (IGF) 1 and 2, and myostatin mRNA expression levels in whole cod larvae fed the three diets; RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers/Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. RQ (relative quantity) values are normalized to C3_RNA polymerase II elongation factor expression, and calibrated to the individual with the lowest normalized expression for each gene of interest. Lower-case dissimilar letters within an age/developmental stage indicate a significant difference between the three groups, and upper-case dissimilar letters within a group indicate a significant difference between sizes (Two-way ANOVA, $P < 0.05$). All values are means $\pm$ 1 S.E. (n=8).

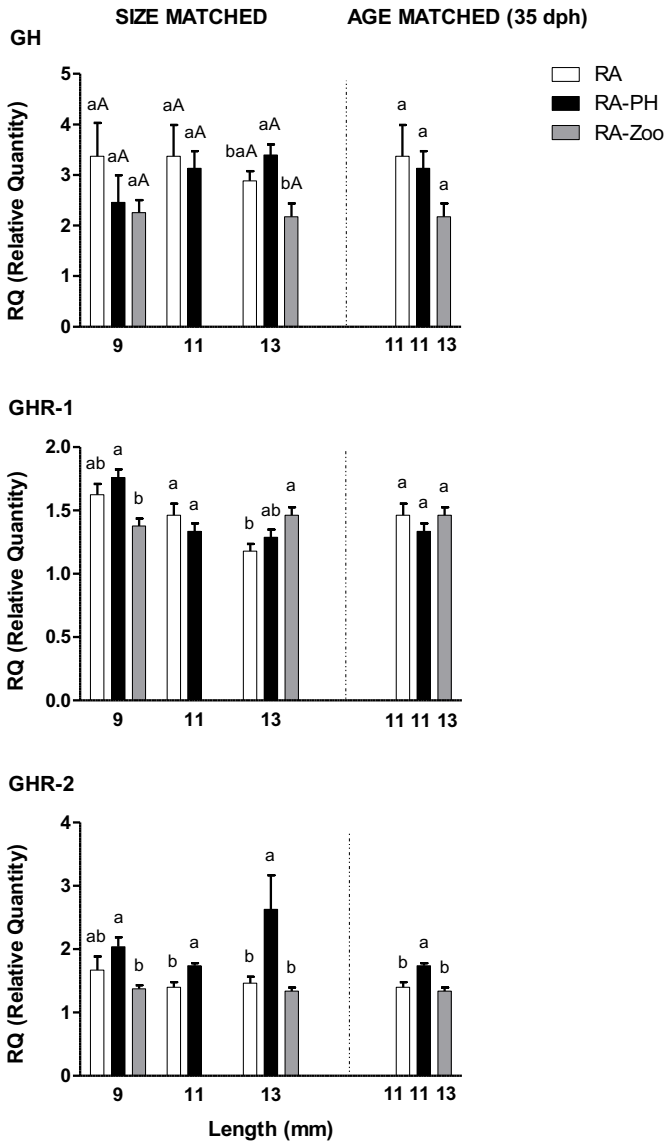


Figure 3.8. Growth hormone (GH) and growth hormone receptor (GHR) 1 and 2 mRNA expression levels in whole cod larvae fed the three diets; RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers/Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. RQ (relative quantity) values are normalized to C3_RNA polymerase II elongation factor expression and calibrated to the individual with the lowest normalized expression for each gene of interest. Lower-case dissimilar letters within an age/developmental stage indicate a significant difference between the three groups, and upper-case letters within a group indicate a significant difference between sizes (Two-way ANOVA, $P < 0.05$). All values are means $\pm$ 1 S.E. (n=8).

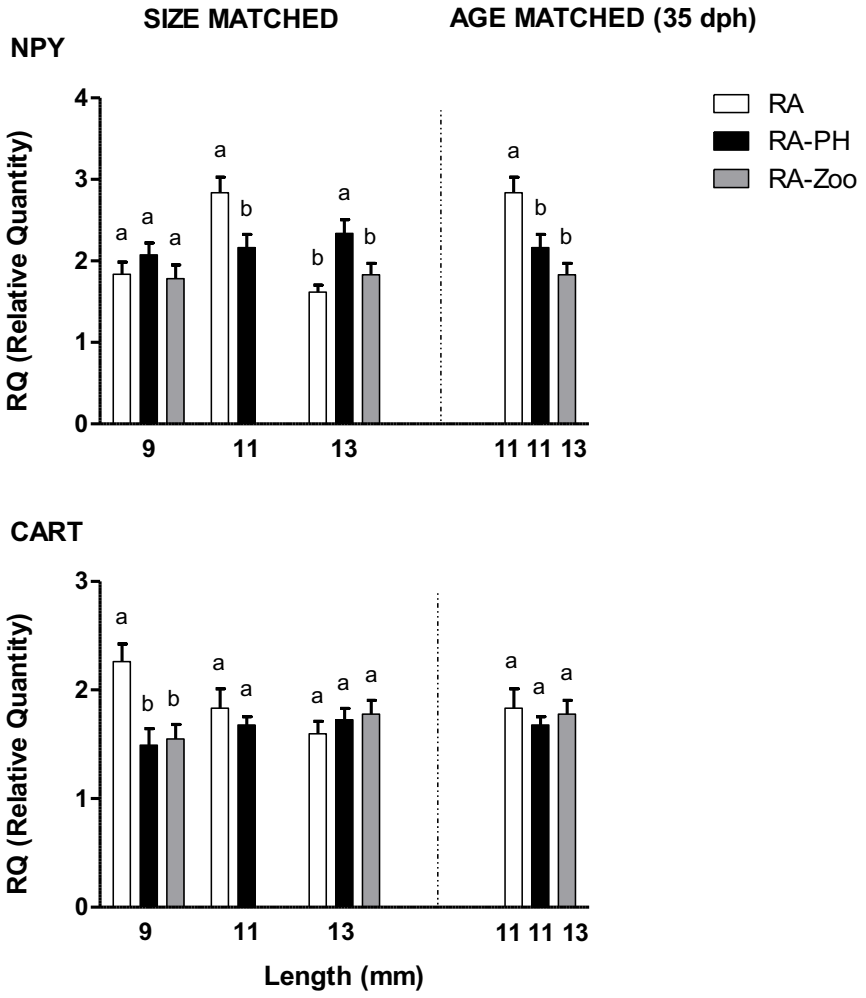


Figure 3.9. Neuropeptide Y (NPY) and cocaine and amphetamine regulated transcript (CART) mRNA expression in whole cod larvae fed the three diets; RA, rotifers and Artemia enriched with Ori-Green; RA-PH, rotifers/Artemia enriched with fish protein hydrolysate three days per week; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. RQ (relative quantity) values are normalized to C3_RNA polymerase II elongation factor expression, and calibrated to the individual with the lowest normalized expression for each gene of interest. RQ values are presented as means $\pm$ 1 S.E. (n=8). Lower-case dissimilar letters within age/developmental stage indicate a significant difference between the three groups (One way ANOVA, $P < 0.05$).

3.4. Metabolic physiology

3.4.1. Morphometric parameters

Fish weight, length and depth were not significantly different between groups, and averaged approx. 8.0 g, 10.3 cm, and 1.7 cm respectively. Ventricle weight and RVM were also not significantly different between the two groups (approx. 0.013 g and 0.16%, respectively) (see Table 3.3.). However, values of width (cm) (1.19 vs. 1.11 cm), condition factor (K) (0.76 vs. 0.70) and HSI (8.2 vs. 6.3 %) were significantly higher in the RA group ($P < 0.05$) (see Table 3.3).

3.4.2. Metabolic parameters

Diet during the larval stage (RA vs. RA-Zoo) had no effect on the metabolic physiology of juvenile cod. Both groups had similar values for resting metabolic rate (67.4 ± 3.5 vs. 74.1 ± 4.5 mg O_2 $kg^{-0.8}$ h^{-1}) and maximum MO_2 (202.5 ± 6.1 vs. 221.5 ± 6.4 mg O_2 $kg^{-0.8}$ h^{-1}). Further, values of absolute and factorial metabolic scopes were comparable; these parameters approx. 140 mg O_2 $kg^{-0.8}$ h^{-1} and three-fold, respectively. Although more fish from the RA-Zoo group reached the highest swimming speeds (40-46 cm sec^{-1} depending on fish size) (see Figure 3.10), and the U_{crit} values were slightly higher for zooplankton fed cod (e.g., 3.76 ± 0.2 vs. 3.54 ± 0.1 bl s^{-1}), there was no significant ($P > 0.05$) difference in U_{crit} between the two groups (when measured in absolute terms or in bl s^{-1}) (Table 3.4.).

Table 3.3. Morphometric parameters for the juvenile RA and RA-Zoo (140-180 dph) cod used for the metabolic physiology study. Values are means ± 1 S.E. (n=13 and n=14 in the RA and RA-Zoo groups, respectively). Within each row, values without a letter in common are statistically different (t-test, $P < 0.05$).

	RA	RA-Zoo
Weight (g)	8.35 ± 0.60^{a}	7.93 ± 0.40^{a}
Total length (cm)	10.25 ± 0.22^{a}	10.34 ± 0.20^{a}
Depth (cm)	1.70 ± 0.05^{a}	1.65 ± 0.05^{a}
Width (cm)	1.19 ± 0.03^{a}	1.11 ± 0.02^{b}
Condition factor (K)	0.76 ± 0.01^{a}	0.70 ± 0.01^{b}
HSI (%)	8.20 ± 0.44^{a}	6.47 ± 0.27^{b}
Ventricle weight (g)	0.013 ± 0.0007^{a}	0.012 ± 0.0009^{a}
RVM (%)	0.165 ± 0.008^{a}	0.151 ± 0.004^{a}

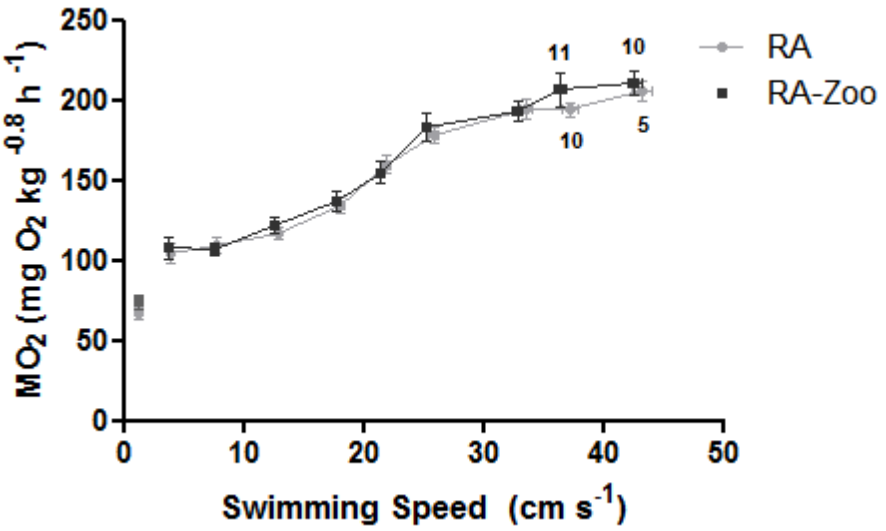


Figure 3.10. Oxygen consumption (MO_2) of 140-180 dph cod juveniles fed with RA or RA-Zoo during the larval period: RA, rotifers and Artemia enriched with Ori-Green; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. Values are means $\pm$ 1 S.E. (n=13 and n=14 in the RA and RA-Zoo groups, respectively). MO_2 was not significantly different at any swimming speed (two-way ANCOVA, $P > 0.05$). Values on the graph represent the number of fish remaining at the recorded swimming speed from each group.

Table 3.4. Metabolic parameters and critical swimming velocity (U_{crit}) of RA and RA-Zoo juveniles (140-180 dph). RA, rotifers and Artemia enriched with Ori-Green; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. Values are means $\pm$ 1 S.E. (n=13 and n=14 in the RA and RA-Zoo groups, respectively). There were no statistically significant differences between the treatment groups (One-way ANCOVA, $P > 0.05$).

	RA	RA-Zoo
Resting Metabolic Rate ($mg\ O_2\ kg^{-0.8}\ h^{-1}$)	67.4 $\pm$ 3.5	74.1 $\pm$ 4.5
Maximum Metabolic Rate ($mg\ O_2\ kg^{-0.8}\ h^{-1}$)	202.5 $\pm$ 6.1	221.5 $\pm$ 6.4
Absolute Scope ($mg\ O_2\ kg^{-0.8}\ h^{-1}$)	135.2 $\pm$ 7.5	147.3 $\pm$ 7.1
Factorial Scope	3.1 $\pm$ 0.2	3.1 $\pm$ 0.1
U_{crit} ($cm\ sec^{-1}$)	36.1 $\pm$ 1.4	38.6 $\pm$ 1.9
(body length sec^{-1})	3.54 $\pm$ 0.1	3.76 $\pm$ 0.2

3.5. Stress response

Resting (pre-stress; 8.0 ± 1.4 and 5.8 ± 0.9 ng g^{-1} wet weight, respectively) and maximum (41.1 ± 8.1 and 47.0 ± 10.8 ng g^{-1} wet weight, respectively) cortisol levels were not significantly different between the RA and RA-Zoo fish. However, there was a difference in the pattern of post-stress cortisol elevation between the two groups (Figure 3.11). Cortisol levels peaked in the RA cod juveniles at 3 hours post-stress before returning to pre-stress levels at 12 h. In contrast, whole body cortisol levels did not decrease in the RA-Zoo fish between 3 and 6 hours, and this resulted in significantly higher ($P < 0.05$) cortisol values in RA-Zoo as compared to RA fish at this latter time point.

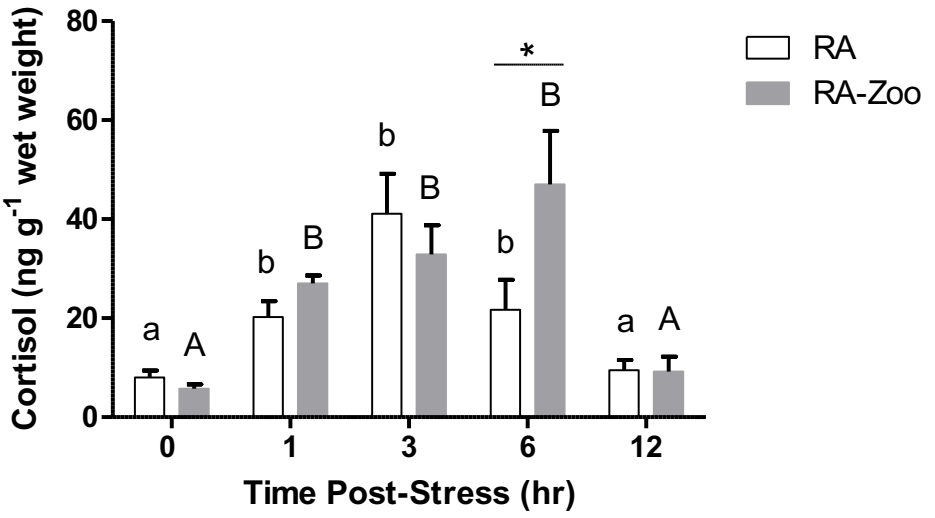


Figure 3.11. Pre- and post-stress whole body cortisol levels in 82 dph cod juveniles from the RA and RA-Zoo treatments: RA, rotifers and Artemia enriched with Ori-Green and; RA-Zoo, rotifers and Artemia supplemented with wild caught zooplankton. Values are means $\pm$ 1 S.E. (n = 9 and n = 10 in the RA and RA-Zoo groups, respectively). Lower and upper case letters indicate significant differences in cortisol levels between the control value (0 hr) and the post-stress values (1, 3, 6 and 12 hr) within each diet group (RA and RA-Zoo, respectively). The asterisk (with underlining) indicates a significant difference between the groups at 6 hours post-stress (One-way ANCOVA, $P < 0.05$).

4. Discussion

4.1. Effect of dietary supplementation on larval growth and production traits

4.1.1. Growth and survival

Zooplankton

Partial dietary supplementation (i.e. 5-10%) with wild zooplankton significantly enhanced the growth of Atlantic cod larvae, as compared to the RA and RA-PH treatments (Figure 3.1 and 3.2). This improved growth was detected as early as 30 dph, where dry weight (by 2.4 fold), length (by 1.2 fold) and condition factor (by 1.55 and 1.13 fold compared to the RA and RA-PH treatments, respectively) were all greater in the RA-Zoo group, and continued throughout the larval stage. By the end of the larval stage (i.e. 60 dph) the zooplankton fed cod were four-fold heavier and 30% longer as compared to the other two dietary treatments (Figure 3.1), and this was associated with a significantly greater mass specific growth rate between 0 and 60 dph [10.4% body weight day^{-1} in the zooplankton fed cod as compared with approx. 8% body weight day^{-1} in the RA and RA-PH treatments (Figure 3.2)]. The extent of growth enhancement observed with partial zooplankton supplementation is in the range of that observed in earlier experiments, where cod (Gadus morhua) larvae were exclusively fed zooplankton for varying periods and their growth compared with those given enriched rotifers. Koedijk et al. (2010a) fed wild zooplankton (i.e. mainly Temora sp.) until 36 dph and showed a six-fold greater mass at time of weaning (i.e. 50 dph), whereas Hansen (2011) reported a 1.9-

fold mass increase at 60 dph in cod that were fed cultivated copepods (Acartia tonsa) until 28 dph. Further, the mass specific growth rate in the latter experiment was found to be 10.2% day^{-1} in the zooplankton group vs. 9.1% day^{-1} in the enriched rotifer group. These growth rate values are very similar to those reported here, and thus, it appears that only a small amount of zooplankton is needed to significantly improve the growth of larval cod.

This enhanced growth was likely due to the nutritional benefits associated with feeding zooplankton. For example, the zooplankton-fed larvae had the highest levels of DHA, EPA and total ω3 fatty acids (reflecting levels in the zooplankton themselves), and the highest levels of triglycerides (TG) at the 11 mm sampling (i.e. at 35 dph) (Rocha et al., unpubl). However, there are several alternative explanations or other factors that need to be considered. It is also possible that part of the enhancement in growth and production traits was related to a difference in dietary micronutrient levels. Although we do not have data on the micronutrient composition of the various diets or larvae from the three experimental groups, natural zooplankton have higher levels of iodine, manganese and selenium as compared to rotifers, and these elements have beneficial effects on growth, survival and the incidence of skeletal deformities in fish larvae (Hamre, 2006; Hamre et al., 2008a; Nguyen et al., 2008). With regards to vitamins, vitamin C levels have also been found to be higher and less variable in zooplankton as compared to rotifers and Artemia (Hamre, 2006; Van der Meeren et al., 2008), and consistently higher levels of the pigment astaxanthin, which serves as precursor for Vitamin A (Moren et al., 2005; Palace and Werner, 2006), have been reported in copepods. Astaxanthin has profound

antioxidant properties, and appears to enhance survival (Okimasu et al., 1992) and play a critical role in salmonid growth (Christiansen and Torrissen, 1996). Likewise, we cannot eliminate the possibility that some of the growth enhancement was related to prey size and/or size variability, or the general appearance and behaviour of the zooplankton (e.g., prey movement and swimming speed) (Beck and Turingan, 2007). For example, earlier studies have shown that zooplankton swim faster than rotifers and that this has a positive effect on their capture by 15 dph fish larvae (Beck and Turingan, 2007), and Hansen et al. (2011) reported that zooplankton-fed cod were more active, swam less for each prey item caught, and caught significantly more Artemia sp. than fish fed a rotifer (control) diet. Finally, the enhanced growth of zooplankton-fed cod could be related to alterations in gut microflora (Tang, 2005; Brunvold et al., 2007; Tang et al., 2009; Nayak, 2010). While metagenomics analysis by our Icelandic collaborators (Jóhannsdóttir et al., unpubl.) did not reveal any overall differences in bacterial species richness (Chao estimate) or diversity (using Simpson and Shannon indices), particular bacterial groups (i.e. Mycoplasmatales and Vibrionales) were more prevalent in the guts of the zooplankton fed fish as compared to the other two dietary groups.

In earlier studies where wild zooplankton was used as live feed in intensive cod culture, Koedijk et al. (2010a) reported similar survival rates at 50 dph for larvae fed rotifers (26.4%) vs. those fed zooplankton (25.9%), whereas Busch et al. (2010) only achieved a survival rate of 7.1% in both first feeding groups at 41 dph, and Otterlei et al. (1999) had a survival rate of 5 to 45% in zooplankton-fed fish at 56 dph. These studies suggest two things. First, feeding zooplankton, does not improve survival during the larval stage. Second, survival, even within the same experiment, can vary widely. These

finding were generally supported by the current study, despite the low survival rates that were associated with repeated sampling of the larvae. Survival in the RA-Zoo group was 0.74% up to grading (63 dph) as compared to 1.07% in the RA group.

Protein Hydrolysate

The protein hydrolysate enrichment used in this study did not improve growth and had a negative effect on survival as compared to the other two treatments (Figures 3.1, 3.2 and Table 3.1). These findings are in contrast to other studies on marine finfish species which show improved larval performance when fed protein hydrolysates (Zambonino Infante et al., 1997; Cahu et al., 1999; Carvalho et al., 2004; Savoie et al., 2011), including Bjornsdottir et al. (2013) who also used pollock protein hydrolysate (100 ppm, three days per week) to enrich rotifers and reported significantly improved survival in cod larvae (18%) as compared to those provided with a standard rotifer diet (12.8%). However, they are consistent with Solberg (unpubl.) who showed that cod larvae fed with protein hydrolysate enriched rotifers for 5 weeks had a similar mass at 44 dph (approx. 15.5 mg) as compared to those fed a standard enriched rotifer diet (approx. 14.9 mg).

In the current study, the RA-PH larvae had triglyceride levels and triglyceride sterol (TG:SL) values (approx. 1:10; 0.1) that were only approx. 1/3 of those in the RA and RA-Zoo groups at 9 mm (Rocha et al., unpubl). Given that the ratio of TG:SL has been used as a condition index for marine larvae (Fraser, 1989), and Håkanson (1993) suggests that larvae with a TG:SL value less than 0.2 are in a poor nutritional condition, it appears that the poor survival of the RA-PH larvae was diet related. Why the RA-PH larvae were in poor nutritional condition is not clear, but could be related to the way in

which the protein hydrolysate was processed / prepared. For example, Bjornsdottir et al. (2013) used pollock protein hydrolysate that was prepared using freeze-drying, whereas the product we were provided with by IceProtein Inc. was heat dried. Heat drying can alter the quality of the protein and as a result reduce its digestibility (Lan and Pan, 1993; Garcia-Ortega et al., 2000), or have other effects on the protein including denaturation, crosslinking or the leaching of soluble nutrients into the water (Klostermeyer and Reimerdes, 1976; Boye et al., 1997; Mohammed et al., 2000; Kvåle et al., 2006; Nordgreen et al., 2009). The level of hydrolyzed protein (i.e. free amino acids) also appears to be an important determinant of how fish larvae respond to feeding with protein hydrolysates. However, it does not appear that the level of inclusion (% free amino acids) of protein hydrolysates in the current study (39.1%) was responsible for the lower survival. It is generally accepted that low to moderate levels of hydrolysed protein (i.e. < 40 – 50%) improve the survival and growth of marine fish larvae (Zambonino Infante et al., 1997; Cahu et al., 1999; Carvalho et al., 2004; Kotzamanis et al., 2007), and cod larvae fed protein hydrolysates with an inclusion level of 40% at weaning, had improved survival (18%) as compared to lower levels of hydrolysed protein (0-30%) at 41 dph (Kvåle et al., 2009).

4.1.2. Skeletal malformations

The only deformities recorded in the 60 dph cod were lordosis and the lack of a fully ossified skeleton. At this life-history stage, the zooplankton fed fish had no occurrence of lordosis (0%), whereas the incidence of this deformity in the RA and RA-PH groups was 2.8% and 8.1%, respectively (Figure 3.4). The occurrence of skeletal

elements that were not fully ossified was also lower in the RA-Zoo fish (2.5%) as compared to the RA and RA-PH groups (31.4% and 40.5%, respectively) (Figure 3.4).

The finding that feeding zooplankton reduces the incidence of skeletal malformations and improves ossification is consistent with several previous studies on fish larvae. Hansen (2011) reported that 60 dph copepod fed cod had a lower incidence of lordosis (12%) and not fully ossified skeletal elements (0%) as compared to fish fed enriched rotifers (18% and 22%, respectively). Kjørsvik et al. (2009) showed that cod larvae fed with a similar n-3 HUFA composition (i.e. mainly DHA and EPA) and lipid source as natural zooplankton had significantly faster ossification of the vertebral column as compared with those fed other diets. Hamre et al. (2002) reported a significantly lower incidence of developmental disorders (i.e. malpigmentation and impaired eye migration) in Atlantic halibut (Hippoglossus hippoglossus, L.) that were start-fed on wild zooplankton (32% and 12%) as opposed to enriched Artemia (93% and 90%). Finally, Zouiten et al. (2011) showed that the occurrence of skeletal malformations in 37 dph sea bass (Dicentrarchus labrax) larvae was lower when reared in natural mesocosms as compared with intensive culture systems. Zouiten et al. (2011) suggested that the nutritional contribution of wild zooplankton present in the mesocosms had major effects on larvae development, and this supports the prevailing view that the nutritional composition (mainly lipids, proteins, minerals and vitamins) of copepods is more suitable for fulfilling the needs of fish larvae and plays a major role in reducing the extent of skeletal malformations (Hamre et al., 2002; Cahu et al., 2003; Hamre, 2006; Imsland et al., 2006). Nonetheless, we cannot exclude the possibility that the improved skeletal

ossification of RA-Zoo cod at 60 dph was not at least, in part, due to their enhanced growth rate, and thus, greater size at the time of sampling.

The RA-PH larvae exhibited the highest rate of skeletal malformations, which is consistent with the poor survival of this group. It is very possible that the same characteristics of the protein hydrolysate (as discussed earlier) that resulted in poorer survival were also responsible for the higher incidence of skeletal malformations. Interestingly, Hermannsdottir et al. (2009) showed that feeding 300,000 prey liter^{-1} twice per day that were enriched with 0.02 g l^{-1} of pollock protein hydrolysate per liter, negatively influenced the percentage of halibut larvae that successfully metamorphosed (i.e. 72 vs. 92%). This feeding regimen is very similar to that used in this study (i.e. 0.1 g liter^{-1} protein hydrolysate with a prey density of 1,000,000 liter^{-1}), and thus, may have also contributed to the higher incidence of skeletal malformation in the RA-PH group.

4.2. Effect of dietary supplementation on juvenile growth and production traits

The RA-Zoo fish were longer by 4-8% throughout the period from 6 – 18 month's post-hatch, and heavier by 30% at 192 dph as compared to the other two dietary groups. However, this weight difference decreased to 20% by 278 dph and to 11-14% between 368 and 558 dph, and was concomitant with lower growth rates (Figure 3.6).

The reported weight differences at 192 – 278 dph are similar to those measured in previous experiments where cod larvae were initially fed wild zooplankton. Koedijk et al. (2010a) showed that zooplankton-fed fish were heavier than the enriched rotifer group by

23% at 253 dph, and Imsland et al. (2006) reported a 17% mass difference in favour of the zooplankton group at approx. 7.5 months (~ 225 days) post-hatch. However, the lower growth rate of the RA-Zoo cod as compared to the other two groups during the juvenile period in the current study, contrasts with these studies, which showed that the higher growth rates of zooplankton fed fish were maintained during the juvenile stages. Specifically, Koedijk et al. (2010a) and Imsland et al. (2006) showed that the zooplankton fed cod had a significantly higher growth rate (by approx. 0.2% day^{-1}) as compared to the rotifer-fed fish between 169-253 dph and 150-225 dph, respectively. However, the accelerated growth of the zooplankton fed cod in Koedijk et al. (2010a) may have been due to differences in rearing temperature between the groups during part of the juvenile period. We currently have no explanation as to why the zooplankton fed fish in this experiment failed to maintain their size difference. However, it is important to mention that these fish were fed at a ration of 1.5 - 1.0% body weight day^{-1} during this growth period (i.e. 6-18 month's post hatch), and a different response may have been observed if they had been fed to satiation.

Survival and the incidence of deformities were recorded separately during the juvenile stage (192-588 dph) in order to examine whether there were long-term treatment effects on these parameters. The RA-PH fish continued to have a sigificantly lower rate of survival (78.8%) than the other two groups (92.9 and 91.7% in the RA and RA-Zoo, respectively). Further, this was concommitant with a higher incidence of external deformities [lordosis (36.5%) and scoliosis (23.1%)] at 558 dph (see Table 3.2). Some studies have linked skeletal deformities such as lordosis in cod with swim bladder abnormalities. Swim bladder abnormalities are characterized by a slight upward tilt of the

head and an indented dorsal body contour at the transition between the head and the trunk. This is first evident when the cod reach the juvenile stage, and can result in impaired swimming and foraging behaviour, and thus, increased mortality (Grotmol et al., 2005; Hansen et al., 2011).

When comparing the level of deformities reported in the current study to the literature, it appears that values are in the same range. Kolstad et al. (2006) reported that the percentage of deformed cod at two years of age ranged from 28-74%, while Fjelldal et al. (2009) determined that the incidence of vertebral deformities was 75% in one and two year old cod based on radiological examination. These levels are significantly higher than that found in other fish species used for aquaculture (e.g., salmon and trout), and thus, represent a major bottleneck to the successful commercialisation of cod (Rosenlund and Halldorsson, 2007).

4.3. Neuroendocrine regulation of growth and appetite

4.3.1. Dietary effects on the expression of growth regulating genes

There were several statistical differences in the mRNA expression of growth regulating hormones (GH, IGF-1, IGF-2, myostatin) and GH receptors (GHR-1 and GHR-2) (Figures 3.7 – 3.8). However, there was no consistent pattern in mRNA expression between the groups (with the exception of GHR-2 in RA-PH fish), and the data did not reflect the enhanced growth displayed by the RA-Zoo group during the larval period. These fish had a 2.5% higher overall growth rate (during 0-60 dph) and a four-fold greater dry weight by the end of the larval period (Figures 3.1 and 3.2). This lack of a clear relationship between growth performance and growth regulating gene expression is

in contrast to some studies. Olivotto et al. (2008a) demonstrated that the improved growth of copepod (i.e. Centropages typicus) fed clownfish was concomitant with higher IGF-1 and IGF-2 mRNA expression and a reduction in the expression of myostatin, as compared to those fed an enriched rotifer diet. Further, previous studies have shown similar relationships between the expression of these genes (i.e. an increase in IGFs and decrease in myostatin) and growth in fish that were fed diets rich in HUFAs (Avella et al., 2007; Olivotto et al., 2010). In contrast, other studies have failed to see a relationship between somatic growth and growth regulating gene expression. For example, Lanes et al. (2012) were unable to demonstrate that the improved growth of cod larvae that were fed with nucleotide-enriched live feed was related to the expression levels of IGF-1, IGF-2, Fst, (activin binding protein that antagonizes the activity of several members of the TGF-β superfamily including myostatin) or other key myogenic genes, as compared to the unenriched dietary group. Similarly, differences in growth observed in cod and sea bass (Dicentrarchus labrax, L.) larvae that were fed different first-feeding diets were not related to the mRNA expression of either GH (Kortner et al., 2011a) or myostatin (Carnevali et al., 2006), respectively.

The hypothalamic-pituitary-growth axis (see section 1.1.6) is a very complex system that involves several hormones and other interacting components [e.g., GH and IGFs binding proteins (GHBPs and IGFBPs) and their specific proteases, IGF- receptors (IGFRs), GH receptors etc.], and is regulated by the integration of many environmental factors (e.g., food availability, temperature, salinity, season, photoperiod, osmotic pressure) (Duan, 1997; Wood et al., 2005; Kelley et al., 2006; Canosa et al., 2007; Chang and Wong, 2009; Reinecke, 2010). Therefore, differences in the fish species examined

and in experimental conditions could explain the contrasting results between studies. Furthermore, it has been shown that several components of the insulin-like growth factor (IGF) system such as IGF-1, IGF-2 (Duan, 1997; Zou et al., 2009), and GHRs (Calduch-Giner et al., 2001; Canosa et al., 2007) undergo post-translation modification in teleost fish. Thus, the mRNA expression of these genes may not be directly related to hormone levels or be a valuable biomarker of growth in fishes in all cases (Picha et al., 2008). It is unlikely that the use of homogenized whole larvae was responsible for the lack of a clear pattern in mRNA expression of growth-regulating genes in this study. This method was used in earlier experiments that showed a relationship between growth and the transcript levels of these genes (Avella et al., 2007; Olivotto et al., 2008a).

4.3.2. Ontogenetic effects on the expression of growth regulating genes

Only one significant difference in mRNA expression was evident with development (ontogeny) of the cod larvae. IGF- 1 expression was approx. two-fold higher at 11 mm (35 dph) as compared to 9 mm (26 – 30 dph) in the RA and RA-PH groups, whereas stable IGF-1 expression was observed in 9 mm (26 – 30 dph) vs. 13 mm (35 dph) RA-Zoo larvae (Fig. 3.7). These findings are very similar to Lanes et al. (2012) who showed that cod larvae with improved growth had a stable expression pattern for IGF-1 between 30 to 38 dph, while larvae that grew less demonstrated increasing expression levels of these gene over this period. These data may provide some insights into the important role played by IGF-1 during these specific developmental stages. However, the number of aged-matched samples was very limited in the current study (2 – 3) and this could partly explain the lack of significant differences with development in the other

growth regulating genes. For example, it has been shown that whole body GH mRNA expression levels in cod fluctuates considerably between hatch and 50 dph (Kortner et al., 2011b). Clearly, additional studies where larvae are frequently sampled are required before the temporal dynamics of growth-regulating gene expression can be thoroughly understood.

4.3.3. Dietary effects on CART and NPY gene expression

It is well established that NPY (Narnaware et al., 2000; Narnaware and Peter, 2002; MacDonald, 2008) and CART (Volkoff and Peter, 2001b; Kehoe and Volkoff, 2007) gene expression are influenced by nutrient and diet composition in fishes, including cod. However, very little is known regarding the underlying mechanism(s) by which dietary regimes cause an increase in somatic growth and the relationship to appetite (Volkoff, 2006; Panserat and Kaushik, 2010; Hoskins and Volkoff, 2012). In the current study, the dietary treatments resulted in few significant changes in mRNA expression of the appetite regulating genes NPY and CART (Figure 3.9). The mRNA expression of the anorexigenic neuropeptide CART was significantly lower in the RA-Zoo as compared to the RA group at 9 mm (Figure 3.9), and thus, may partially explain the enhanced growth of the zooplankton-fed fish. However, NPY mRNA expression levels were not significantly different between the RA-Zoo and the RA groups at 9 mm (26-30 dph) which is in agreement with Kortner et al. (2011a). Further, Kortner et al. (2011a) showed that there was a general increase in NPY transcript levels in zooplankton-fed cod throughout early ontogeny (from 8 to 29 dph), and suggested based on this work and that of Kortner et al. (2011b) that the endocrine regulation of appetite and digestion in cod are

closely linked and influenced by dietary regimes from these early developmental stages. Thus, the lack of clear relationships between growth, NPY expression and ontogeny in the current study could again be due to the fact that larvae were sampled only between 30 and 50 dph.

4.4. Metabolic physiology

In this study, I measured the metabolic physiology and swimming performance of juvenile cod from the RA and RA-Zoo dietary groups, and report that zooplankton supplementation during the larval stage had no significant effect on the resting metabolic rate (approx. 185 vs. 209 mg O_2 kg^{-1} h^{-1}, respectively), maximum metabolic rate (approx. 544 vs. 604 mg O_2 kg^{-1} h^{-1}, respectively), metabolic scope (approx. 372 vs. 409 mg O_2 kg^{-1} h^{-1}, respectively) or critical swimming velocity (U_{crit}; approx. 3.6 vs. 3.95 body length sec^{-1}) of juvenile fish.

The above values are comparable to some other studies that have investigated the metabolic physiology of early juvenile stage (< 10 g) cod. For example, Hansen and von Herbing (2009) measured the standard metabolic and active metabolic rates of 2-3 g (wet mass) fish at 10 °C, and reported values of 184.6 and 645.8 mg O_2 kg^{-1} h^{-1}, respectively, whereas Peck et al. (2003) reported a routine respiration rate of approximately 200 mg O_2 kg^{-1} h^{-1} for cod in the same size range. However, these findings are not comparable to all studies on juveniles of this species. For example, Hunt von Herbing and White (2002) reported much lower routine and active (maximum) metabolic rate values (76.8-115.2 and 96-153.6 mg O_2 kg^{-1} h^{-1}, respectively) for 1-3 g (wet mass) cod juveniles. This latter study used the 'chase' method to induce exhaustion, and while this might explain the difference

in maximum metabolic rate with my research (Reidy et al., 1995), it does not explain the discrepancy in resting (routine) metabolism. The U_{crit} values for cod are also comparable with previous studies on juvenile gadids. For example, Hansen and von Herbing (2009) reported values of 2-4 bl s^{-1} for 0.5-3 g Atlantic cod juveniles at 10 °C, and Ottmar and Hurst (2012) found that the U_{crit} for 10 cm Pacific cod juveniles was 3.6 bl s^{-1} at 9 °C.

Metabolic scope is considered to be an important mediator of growth in cod, as a direct positive relationship has been observed between metabolic scope (mg O_2 kg^{-1} h^{-1}) and specific growth rate (% body weight day^{-1}) (Chabot and Dutil, 1999; Claireaux et al., 2000). Thus, the similar metabolic scope values in the two dietary groups suggest that these fish (RA and RA-Zoo) had comparable growth rates during this life history stage, as they did from 192 – 278 dph (1.57 and 1.50% day^{-1}, respectively). These results complement those of Koedijk et al. (2010b) who found no significant differences in the metabolic enzyme [i.e. lactate dehydrogenase (LDH), malate dehydrogenase (MDH) and aspartate amino transferrase (AAT)] activity of 10-70 g juvenile cod that were start-fed with wild zooplankton as opposed to enriched rotifers. Collectively, these data suggest that feeding wild zooplankton during the early life history stages does not result in long-term changes in the metabolic capacity of Atlantic cod.

It is worth mentioning that we also attempted to measure the oxygen consumption of RA and RA-Zoo larvae between 15 and 30 dph. Measurements of resting metabolic rate were made on groups of 10 larvae using small glass respirometers (60 ml) over a 24-h period. Unfortunately, the larvae were quite 'fragile' during this period and only survived for approximately 6-8 hours once placed into the respirometers. This indicated that the larvae were under severe stress; i.e. measurements of oxygen consumption would

not be indicative of a resting animal and make the interpretation of differences in metabolism between the groups difficult / impossible. However, these data would be extremely valuable to collect, and likely reveal whether enhanced metabolic capacity during the larval stages contributed to the enhanced growth of the zooplankton-fed cod.

4.5. Stress response

Resting (pre-stress; 8.0 ± 1.4 vs. 5.8 ± 0.9 ng g^{-1} wet weight, respectively) and maximum (41.1 ± 8.1 vs. 47 ± 10.8 ng g^{-1} wet weight, respectively) cortisol levels were not significantly different between the RA and RA-Zoo early juveniles at 82 dph. However, there was a difference in the pattern of post-stress cortisol elevation between the two groups (see Figure 3.11). Cortisol levels peaked in the RA cod at 3 hours post-stress before returning to pre-stress levels at 12 h, whereas whole body cortisol levels in the RA-Zoo fish were comparable between 3 and 6 hours and then returned to pre-stress levels at 12 h.

Resting whole body cortisol levels for cod during the late larval and early juvenile stages have been reported to be in the range of 2-10 ng g^{-1} wet weight (King and Berlinsky, 2006; Westelmajer, 2008). In addition, Westelmajer (2008) showed that whole body immunoreactive corticosteroid (IRC) concentration reached a maximum level of ~ 25 ng g^{-1} wet weight (a 10 fold-increase) in 59 dph larvae at 1 hour after air exposure stress, while King and Berlinsky (2006) reported a whole body cortisol level of ~ 68 ng g^{-1} at 1 hour following a similar stressor, and that it returned to resting levels by 24 hours post-stress. These resting levels, the magnitude of the increase in cortisol levels, and the

temporal nature of the cortisol response were all quite similar to that observed for the cod fed enriched rotifers and Artemia in this study.

Very few studies have examined the effects of feeding zooplankton on the stress response of fishes. However, Kraul et al. (1993) showed that larval mahimahi (Coryphaena hippurus) that were fed with copepods had significantly higher post-stress survival after an acute 120 sec air exposure, as compared to those fed an enriched Artemia diet. Further, Piccinetti et al. (2012) reported that early common sole juveniles that were fed with 50% wild zooplankton supplementation during the larvae stages had lower resting (by ~ 1.9 fold) and higher maximum post-stress levels (by ~ 1.4) of whole body cortisol as compared to those fed a traditional rotifer / Artemia based diet. The difference in resting cortisol levels between the dietary treatments in the current study is somewhat similar to Piccinetti et al. (2012) (1.4-fold lower levels in the RA-Zoo group). However, if anything, the current results suggest that post-stress cortisol levels were similar, if not higher (e.g., levels may have still been increasing between 3 and 6 hours post-stress), and more prolonged in the RA-Zoo fish. While the reason for the discrepancy in findings between this study and Piccinetti et al. (2012) is unknown, it is consistent with Westelmajer (2008) who showed that cod fed rotifers and Artemia enriched with high levels of the HUFAs DHA, AA and w6DPA (docosapentaenoic acid: 22:5w6), and given a 15 sec air exposure stress, had a more prolonged elevation in cortisol as compared to the other commercially enriched rotifers and Artemia treatments. These studies, as well as others (Kanazawa, 1997; Koven et al., 2001; Vagelli, 2004; Ganga et al., 2006), suggest that the nutritional effects associated with feeding zooplankton, such as enhanced level of

HUFAs and phospholipids, are reflected in stress tolerance (survival) and the corticosteroid response to stress.

5. Summary and future perspectives

The present study has several principal findings. 1) Only small amounts of zooplankton (i.e. 5-10% of total prey items) are needed to significantly improve the growth and general development (i.e. incidence of skeletal malformations) of Atlantic cod larvae. This result strongly suggests that the feeding of cultured zooplankton may be a viable and cost-effective strategy for increasing the growth (and potentially health) of marine finfish produced through intensive culture practices. However, it is also apparent from my data that zooplankton feeding alone will not be sufficient to reach the target that the Newfoundland aquaculture industry has set to insure profitability (30-36 months to reach market size of 3-4 kg) (Clift report, 2005; Gardner Pinfold Report, 2010). This standard requires an increase in growth of approx. 20-30%, and at the end of my study (18 months), the zooplankton-reared cod were only approx. 11% larger than their RA fed counterparts. Clearly, additional improvements in husbandry practices / rearing protocols (e.g., the optimization of juvenile and adult diet formulations) or broodstock selection will be required to meet this goal.

2) The impetus to use pollock protein hydrolysate as a live feed enrichment in these experiments was based on its previously demonstrated beneficial effects on growth and incidence of deformities by our NORA (Nordic Atlantic Cooperation) partners (Bjornsdottir et al., 2013; Johannsdottir et al., 2013). However, the protein hydrolysate enrichment used in this study did not improve growth, and had a negative effect on

survival and skeletal deformities. These contrasting results may be due to the way the protein hydrolysate was processed, as these earlier studies used freeze-drying and our product was produced by heat-drying. Additional studies must be performed to: 1) assess how methods for preparing fish protein hydrolysates as an additive in aquaculture feeds influences their 'quality' and ability to positively influence production related traits, and 2) determine the optimal level of inclusion when used in cod live feed diets, given this parameters importance (Kvåle et al., 2009; Liu et al., 2010; Mamauag et al., 2011).

3) The growth enhancement achieved by feeding the cod larvae wild zooplankton was not related to alterations in the mRNA expression of the main growth regulating hormones (IGF I and II, GH, GH receptors I and II, and myostatin), and this suggests that the transcript levels of these hormones / receptors are not valuable biomarkers of growth in cod larvae (i.e. between 9 and 13 mm; 26 to 50 dph). This conclusion is supported by other studies on cod and various fish species (Carnevali et al., 2006; Kortner et al., 2011a; Lanes et al., 2012). mRNA expression of the appetite regulating neuropeptides (NPY and CART) was also not related to the growth of the RA-Zoo fish. This result suggests that the enhanced growth of this group was not due to higher food intake. However, this will have to be investigated further given the lack of a relationship between growth and the transcript levels of various growth mediators at this life history stage.

4) The zooplankton fed fish did not maintain their growth advantage and displayed significantly lower growth rates as compared to the RA treatment during the juvenile period (6-18 months post-hatch). It is not clear whether this reduced growth rate was related to slower growth of the RA-Zoo fish, or enhanced (catch up, compensatory) growth in the RA cod during the juvenile stage. Nonetheless, my results suggest that the

growth potential of fish is not necessarily determined (set) in the larval stage, and this is in contrast to conclusions by other authors (Steinarsson et al., 2012).

References

Avella, M.A., Olivotto, I., Gioacchini, G., Maradonna, F. and Carnevali, O., 2007. The role of fatty acids enrichments in the larviculture of false percula clownfish Amphiprion ocellaris. Aquaculture 273, 87-95.

Ayson, F.G., de Jesus-Ayson, Evelyn, G.T. and Takemura, A., 2007. mRNA expression patterns for GH, PRL, SL, IGF-I and IGF-II during altered feeding status in rabbitfish, Siganus guttatus. General and Comparative Endocrinology 150, 196-204.

Baeverfjord, G., Helland, S. and Hough, C., 2009. Control of malformations in fish aquaculture: Science and practice (Fine fish) Liege: Federation of European Aquaculture Producers, Belgum.

Bail, P.Y., Gentil, V., Noel, O., Gomez, J., Carre, F., Goff, P. and Weil, C., 1998. Structure, function, and regulation of insulin-like growth factors in fish. Annals of the New York Academy of Sciences 839, 157-161.

Bardon, A., Vandeputte, M., Dupont-Nivet, M., Chavanne, H., Haffray, P., Vergnet, A. and Chatain, B., 2009. What is the heritable component of spinal deformities in the European sea bass (Dicentrarchus labrax)? Aquaculture 294, 194-201.

Barton, B. and Peter, R., 1982. Plasma cortisol stress response in fingerling rainbow trout, Salmo gairdneri Richardson, to various transport conditions, anaesthesia, and cold shock. Journal of Fish Biology 20, 39-51.

Barton, B.A., 2002. Stress in fishes: a diversity of responses with particular reference to changes in circulating corticosteroids. Integrative and Comparative Biology 42, 517-525.

Barton, B.A. and Iwama, G.K., 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. Annual Review of Fish Diseases 1, 3-26.

Beamish, F.W.H., 1978. Swimming capacity. In: Hoar, W.S., Randall, D.J. (Eds.), Fish Physiology. Academic Press, New york, pp. 101-187.

Beck, J.L. and Turingan, R.G., 2007. The effects of zooplankton swimming behavior on prey-capture kinematics of red drum larvae, Sciaenops ocellatus. Marine Biology 151, 1463-1470.

Beckman, B.R., Shimizu, M., Gadberry, B.A. and Cooper, K.A., 2004. Response of the somatotropic axis of juvenile coho salmon to alterations in plane of nutrition with an analysis of the relationships among growth rate and circulating IGF-I and 41kDa IGFBP. General and Comparative Endocrinology 135, 334-344.

Bell, J.G., McEvoy, L.A., Estevez, A., Shields, R.J. and Sargent, J.R., 2003. Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture 227, 211-220.

Bell, W.H. and Terhune, L.D.B., 1970. Water tunnel design for fisheries research. Journal of the Fisheries Research Board of Canada. Technical Reports. 195, 1-69

Bendiksen, E.Å., Jobling, M. and Arnesen, A., 2002. Feed intake of Atlantic salmon parr Salmo salar L. in relation to temperature and feed composition. Aquaculture Research 33, 525-532.

Bennett, A.F., 1978. Activity metabolism of the lower vertebrates. Annual Review of Physiology 40, 447-469.

Berg, L., 1997. Commercial feasibility of semi-intensive larviculture of Atlantic halibut (Hippoglossus hippoglossus L.). Aquaculture 155, 333-340.

Bjornsdottir, R., Johannsdottir, J., Gamperl, A.K., Boyce, D., Jonassen, T.M., Solberg, C., Vargas, C., Steinarsson, A., Øie, G. and Hagemann, A., 2013. Novel approach for determining quality through comparative studies of intensively produced cod larvae and juveniles. Final report. Avaliable: http://www.nora.fo. Apr., 2014.

Blaikie, H.B. and Kerr, 1996. Effect of activity level on apparent heat increment in Atlantic cod, Gadus morhua. Canadian Journal of Fisheries and Aquatic Sciences/Journal Canadien des Sciences Halieutiques et Aquatiques. Ottawa 53, 2093-2099.

Blaxter, J.H., 1969. Swimming speeds of fish. Food and Agriculture Organisation, Fisheries Report.Vol. 2, pp. 69-100.

Blier, P.U., Pelletier, D. and Dutil, J.D., 1997. Does aerobic capacity set a limit on fish growth rate? Reviews in Fisheries Science 5, 323-340.

Boglione, C., Gagliardi, F., Scardi, M. and Cataudella, S., 2001. Skeletal descriptors and quality assessment in larvae and post-larvae of wild-caught and hatchery-reared gilthead sea bream (Sparus aurata L.) Aquaculture 192, 1-22.

Boglione, C., Costa, C., Di Dato, P., Ferzini, G., Scardi, M. and Cataudella, S., 2003. Skeletal quality assessment of reared and wild sharpsnout sea bream and pandora juveniles. Aquaculture 227, 373-394.

Bokma, F., 2004. Evidence against universal metabolic allometry. Functional Ecology 18, 184-187.

Bolliet, V., Aranda, A. and Boujard, T., 2001. Demand-feeding rhythm in rainbow trout and European catfish: Synchronisation by photoperiod and food availability. Physiology and Behavior 73, 625-633.

Booman, M., Borza, T., Feng, C.Y., Hori, T.S., Higgins, B., Culf, A., Léger, D., Chute, I.C., Belkaid, A. and Rise, M.L., 2011. Development and experimental validation of a 20K Atlantic cod (Gadus morhua) oligonucleotide microarray based on a collection of over 150,000 ESTs. Marine Biotechnology 13, 733-750.

Boujard, T., Burel, C., Médale, F., Haylor, G. and Moisan, A., 2000. Effect of past nutritional history and fasting on feed intake and growth in rainbow trout Oncorhynchus mykiss. Aquatic Living Resources 13, 129-137.

Boye, J.I., Ma, C.Y. and Hardwalker, V.R., 1997. Thermal denaturizing and coagulation of proteins. In: Damodaran, S., Paraf, A. (Eds.), Food Proteins and Their Applications. Marcel Dekker, New York, pp. 25-26.

Brett, J., 1964. The respiratory metabolism and swimming performance of young sockeye salmon. Journal of the Fisheries Board of Canada 21, 1183-1226.

Brett, J., 1972. The metabolic demand for oxygen in fish, particularly salmonids, and a comparison with other vertebrates. Respiration Physiology 14, 151-170.

Brett, J. and Groves, T.D.D., 1979. Physiological Energetics. Fish Physiology 8, 279-352.

Bristow, G., 1990. Parasitter i zooplankton: Doedelighet hos kveitelarver og yngel i startforingsfasen. Norsk Fiskeoppdrett 15.

Brown, J.A., Minkoff, G. and Puvanendran, V., 2003. Larviculture of Atlantic cod (Gadus morhua): progress, protocols and problems. Aquaculture 227, 357-372.

Brunvold, L., Sandaa, R.A., Mikkelsen, H., Welde, E., Bleie, H. and Bergh, Ø., 2007. Characterisation of bacterial communities associated with early stages of intensively reared cod (Gadus morhua) using Denaturing Gradient Gel Electrophoresis (DGGE). Aquaculture 272, 319-327.

Busch, K.E.T., Falk-Petersen, I.-B., Peruzzi, S., Rist, N.A. and Hamre, K., 2010. Natural zooplankton as larval feed in intensive rearing systems for juvenile production of Atlantic cod (Gadus morhua L.). Aquaculture Research 41, 1727-1740.

Cahu, C., Zambonino Infante, J. and Takeuchi, T., 2003. Nutritional components affecting skeletal development in fish larvae. Aquaculture 227, 245-258.

Cahu, C.L., Zambonino Infante, J.L., Quazuguel, P. and Le Gall, M.M., 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass Dicentrarchus labrax larvae. Aquaculture 171, 109-119.

Calduch-Giner, J.-À., Duval, H., Chesnel, F., Boeuf, G., Pérez-Sánchez, J. and Boujard, D., 2001. Fish growth hormone receptor: molecular characterization of two membrane-anchored forms. Endocrinology 142, 3269-3273.

Canosa, L.F., Chang, J.P. and Peter, R.E., 2007. Neuroendocrine control of growth hormone in fish. General and Comparative Endocrinology 151, 1-26.

Carnevali, O., de Vivo, L., Sulpizio, R., Gioacchini, G., Olivotto, I., Silvi, S. and Cresci, A., 2006. Growth improvement by probiotic in European sea bass juveniles (Dicentrarchus labrax, L.), with particular attention to IGF-1, myostatin and cortisol gene expression. Aquaculture 258, 430-438.

Carvalho, A.P., Sá, R., Oliva-Teles, A. and Bergot, P., 2004. Solubility and peptide profile affect the utilization of dietary protein by common carp (Cyprinus carpio) during early larval stages. Aquaculture 234, 319-333.

Chabot, D. and Dutil, J.D., 1999. Reduced growth of Atlantic cod in non-lethal hypoxic conditions. Journal of Fish Biology 55, 472-491.

Chabot, D. and Claireaux, G., 2008. Environmental hypoxia as a metabolic constraint on fish: The case of Atlantic cod, Gadus morhua. Marine Pollution Bulletin 57, 287-294.

Chang, J.P. and Wong, A.O.L., 2009. Chapter 4 Growth hormone regulation in fish: a multifactorial model with hypothalamic, peripheral and local autocrine/paracrine signals. Fish Physiology 28, 151-195.

Chatelier, A., McKenzie, D.J., Prinet, A., Galois, R., Robin, J., Zambonino, J. and Claireaux, G., 2006. Associations between tissue fatty acid composition and physiological traits of performance and metabolism in the seabass (Dicentrarchus labrax). Journal of Experimental Biology 209, 3429-3439.

Cho, C., Slinger, S. and Bayley, H., 1982. Bioenergetics of salmonid fishes: energy intake, expenditure and productivity. Comparative Biochemistry and Physiology Part B: Comparative Biochemistry 73, 25-41.

Christiansen, R. and Torrissen, O., 1996. Growth and survival of Atlantic salmon, Salmo salar L. fed different dietary levels of astaxanthin. Juveniles. Aquaculture Nutrition 2, 55-62.

Claireaux, G., Webber, D., Lagardère, J.-P. and Kerr, S., 2000. Influence of water temperature and oxygenation on the aerobic metabolic scope of Atlantic cod Gadus morhua. Journal of Sea Research 44, 257-265.

Clift, T., 2005. Strategic analysis of the Newfoundland cod aquaculture sector. Available: http://www.ccfi.ca/Projects/ProjectDisplay. Sept., 2011.

Cobcroft, J.M., Pankhurst, P.M., Sadler, J. and Hart, P.R., 2001. Jaw development and malformation in cultured striped trumpeter Latris lineata. Aquaculture 199, 267-282.

Davie, A., Porter, M.J., Bromage, N.R. and Migaud, H., 2007. The role of seasonally altering photoperiod in regulating physiology in Atlantic cod (Gadus morhua). Part II. Somatic growth. Canadian Journal of Fisheries and Aquatic Sciences 64, 98-112.

Davis, K.B., 2006. Management of physiological stress in finfish aquaculture. North American Journal of Aquaculture 68, 116-121.

De Pedro, N., López-Patiño, M.A., Guijarro, A.I., Pinillos, M.L., Delgado, M.J. and Alonso-Bedate, M., 2000. NPY receptors and opioidergic system are involved in NPY-induced feeding in goldfish. Peptides 21, 1495-1502.

Devresse, B., Leger, P., Sorgeloos, P., Murata, O., Nasu, T., Ikeda, S., Rainuzzo, J.R., Reitan, K.I., Kjorsvik, E. and Olsen, Y., 1994. Improvement of flat fish pigmentation through the use of DHA-enriched rotifers and Artemia. Aquaculture 124, 287-288.

Dhert, P., Rombaut, G., Suantika, G. and Sorgeloos, P., 2001. Advancement of rotifer culture and manipulation techniques in Europe. Aquaculture 200, 129-146.

Donaldson, E.M., 1981. The pituitary-interrenal axis as an indicator of stress in fish. In: Pickering, A. D. (Eds.), Stress and Fish. Academic Press, New York, pp. 1-98.

Drillet, G., Frouël, S., Sichlau, M.H., Jepsen, P.M., Højgaard, J.K., Joarder, A.K. and Hansen, B.W., 2011. Status and recommendations on marine copepod cultivation for use as live feed. Aquaculture 315, 155-166.

Duan, C., 1997. The insulin-like growth factor system and its biological actions in fish. American Zoologist 37, 491-503.

Duan, C. and Xu, Q., 2005. Roles of insulin-like growth factor (IGF) binding proteins in regulating IGF actions. General and Comparative Endocrinology 142, 44-52.

Dutil, J.D., Sylvestre, E.L., Gamache, L., Larocque, R. and Guderley, H., 2007. Burst and coast use, swimming performance and metabolism of Atlantic cod Gadus morhua in sub-lethal hypoxic conditions. Journal of Fish Biology 71, 363-375.

Edwards, R.R.C., Finlayson, D.M. and Steele, J.H., 1972. An experimental study of the oxygen consumption, growth, and metabolism of the cod (Gadus Morhua L.). Journal of Experimental Marine Biology and Ecology 8, 299-309.

Elliott, J.M., 1975. The growth rate of brown trout (Salmo trutta L.) fed on maximum rations. Journal of Animal Ecology 44, 805-821.

Ellis, T., Bagwell, N., Pond, M., Baynes, S. and Scott, A.P., 2007a. Acute viral and bacterial infections elevate water cortisol concentrations in fish tanks. Aquaculture 272, 707-716.

Ellis, T., James, J.D., Sundh, H., Fridell, F., Sundell, K. and Scott, A.P., 2007b. Non-invasive measurement of cortisol and melatonin in tanks stocked with seawater Atlantic salmon. Aquaculture 272, 698-706.

Ellis, T., Yildiz, H.Y., López-Olmeda, J., Spedicato, M.T., Tort, L., Øverli, Ø. and Martins, C.I., 2012. Cortisol and finfish welfare. Fish Physiology and Biochemistry 38, 163-188.

Evjemo, J.O., Reitan, K.I. and Olsen, Y., 2003. Copepods as live food organisms in the larval rearing of halibut larvae (Hippoglossus hippoglossus L.) with special emphasis on the nutritional value. Aquaculture 227, 191-210.

Faulk, C.K., Holt, G.J. and Davis, D.A., 2005. Evaluation of fatty acid enrichment of live food for yellowtail snapper Ocyurus chrysurus larvae. Journal of the World Aquaculture Society 36, 271-281.

Feng, C.Y., Johnson, S.C., Hori, T.S., Rise, M., Hall, J.R., Gamperl, A.K., Hubert, S., Kimball, J., Bowman, S. and Rise, M.L., 2009. Identification and analysis of differentially expressed genes in immune tissues of Atlantic cod stimulated with formalin-killed, atypical Aeromonas salmonicida. Physiological Genomics 37, 149-163.

Fevolden, S.E., Røed, K.H. and Fjalestad, K.T., 2002. Selection response of cortisol and lysozyme in rainbow trout and correlation to growth. Aquaculture 205, 61-75.

Finn, R.N., Rønnestad, I., Van Der Meeren, T. and Fyhn, H.J., 2002. Fuel and metabolic scaling during the early life stages of Atlantic cod Gadus morhua. Marine Ecology Progress Series 243, 217-234.

Fjelldal, P.G., van der Meeren, T., Jørstad, K.E. and Hansen, T.J., 2009. A radiological study on vertebral deformities in cultured and wild Atlantic cod (Gadus morhua, L.). Aquaculture 289, 6-12.

Fletcher, R., Roy, W., Davie, A., Taylor, J., Robertson, D. and Migaud, H., 2007. Evaluation of new microparticulate diets for early weaning of Atlantic cod (Gadus morhua): Implications on larval performances and tank hygiene. Aquaculture 263, 35-51.

Flos, R., Reig, L., Torres, P. and Tort, L., 1988. Primary and secondary stress responses to grading and hauling in rainbow trout, Salmo gairdneri. Aquaculture 71, 99-106.

Folkvord, A., 1991. Growth, survival and cannibalism of cod juveniles (Gadus morhua): effects of feed type, starvation and fish size. Aquaculture 97, 41-59.

Fox, B.K., Breves, J.P., Hirano, T. and Grau, E.G., 2009. Effects of short-and long-term fasting on plasma and stomach ghrelin, and the growth hormone / insulin-like growth factor I axis in the tilapia, Oreochromis mossambicus. Domestic Animal Endocrinology 37, 1-11.

Fraser, A.J., 1989. Triacylglycerol content as a condition index for fish, bivalve, and crustacean larvae. Canadian Journal of Fisheries and Aquatic Sciences 46, 1868-1873.

Fruchtman, S., Jackson, L. and Borski, R., 2000. Insulin-like growth factor I disparately regulates prolactin and growth hormone synthesis and secretion: studies using the teleost pituitary model. Endocrinology 141, 2886-2894.

Fry, F.E.J., 1971. The effect of environmental factors on the physiology of fish. In: Hoar, W.S. and Randall, D.J. (Eds.), Fish Physiology. Academic Press, pp. 1-98.

Fulton, T.W., 1904. The rate of growth of fishes. 22nd Annual Report of the Fishery Board of Scotland 3, 141-241.

Gahete, M.D., Durán-Prado, M., Luque, R.M., Martínez-Fuentes, A.J., Quintero, A., Gutiérrez-Pascual, E., Córdoba-Chacón, J., Malagón, M.M., Gracia-Navarro, F. and Castaño, J.P., 2009. Understanding the multifactorial control of growth hormone release by somatotropes. Annals of the New York Academy of Sciences 1163, 137-153.

Ganga, R., Tort, L., Acerete, L., Montero, D. and Izquierdo, M.S., 2006. Modulation of ACTH-induced cortisol release by polyunsaturated fatty acids in interrenal cells from gilthead seabream, Sparus aurata. Journal of Endocrinology 190, 39-45.

Garcia-Ortega, A., Verreth, J., Van Hoornyck, A. and Segner, H., 2000. Heat treatment affects protein quality and protease activity in decapsulated cysts of Artemia when used as starter food for larvae of African catfish Clarias gariepinus (Burchell). Aquaculture Nutrition 6, 25-31.

Gardner Pinfold Report, 2010. Factors affecting the viability of cod aquaculture in Canada. Available: http://www.gardnerpinfold.ca. March, 2012.

Gavaia, P.J., Dinis, M.T. and Cancela, M., 2002. Osteological development and abnormalities of the vertebral column and caudal skeleton in larval and juvenile stages of hatchery-reared Senegal sole (Solea senegalensis). Aquaculture 211, 305-323.

Georgakopoulou, E., Katharios, P., Divanach, P. and Koumoundouros, G., 2010. Effect of temperature on the development of skeletal deformities in Gilthead seabream (Sparus aurata Linnaeus, 1758). Aquaculture 308, 13-19.

Gjerde, B., Pante, J.R. and Baeverfjord, G., 2005. Genetic variation for a vertebral deformity in Atlantic salmon (Salmo salar). Aquaculture 244, 77-87.

Glazier, D.S., 2005. Beyond the '3/4-power law': variation in the intra-and interspecific scaling of metabolic rate in animals. Biological Reviews 80, 611-662.

Glazier, D.S., 2008. Effects of metabolic level on the body size scaling of metabolic rate in birds and mammals. Proceedings of the Royal Society B: Biological Sciences 275, 1405-1410.

Goolish, E.M., 1991. Aerobic and anaerobic scaling in fish. Biological Reviews 66, 33-56.

Grotmol, S., Kryvi, H. and Totland, G.K., 2005. Deformation of the notochord by pressure from the swim bladder may cause malformation of the vertebral column in cultured Atlantic cod Gadus morhua larvae: a case study. Diseases of Aquatic Organisms 65, 121-128.

Gutiérrez, J., Navarro, I., Planas, J.V., Montserrat, N., Rojas, P., Castillo, J., Chystiakova, O.V., Gabillard, J.C., Smith, A., Chan, S.J. and Leibush, N.B., 2006. Insulin and IGF receptors in fish. In: Reinecke, M., Zaccone, G. and Kapoor, B.G. (Eds.), Fish Endocrinology. Science Publishers, New Hampshire, pp. 131-165.

Håkanson, J., 1993. Nutritional condition and growth rate of anchovy larvae (Engraulis mordax) in the California Current: two contrasting years. Marine Biology 115, 309-316.

Hammer, C., 1995. Fatigue and exercise tests with fish. Comparative Biochemistry and Physiology Part A: Physiology 112, 1-20.

Hamre, K., 2006. Nutrition in cod (Gadus morhua) larvae and juveniles. ICES Journal of Marine Science 63, 267-274.

Hamre, K., Mollan, T.A., Sæle, Ø. and Erstad, B., 2008a. Rotifers enriched with iodine and selenium increase survival in Atlantic cod (Gadus morhua) larvae. Aquaculture 284, 190-195.

Hamre, K., Srivastava, A., Rønnestad, I., Mangor-Jensen, A. and Stoss, J., 2008b. Several micronutrients in the rotifer Brachionus sp. may not fulfil the nutritional requirements of marine fish larvae. Aquaculture Nutrition 14, 51-60.

Hamre, K., Opstad, I., Espe, M., Solbakken, J., Hemre, G.I. and Pittman, K., 2002. Nutrient composition and metamorphosis success of Atlantic halibut (Hippoglossus hippoglossus, L.) larvae fed natural zooplankton or Artemia. Aquaculture Nutrition 8, 139-148.

Hansen, M.H., 2011. Effects of feeding with copepod nauplii (Acartia tonsa) compared to rotifers (Brachionus ibericus, Cayman) on quality parameters in Atlantic cod (Gadus morhua) larvae. Master Thesis. Norwegian University of Science and Technology. Trondheim, Norway. 91 pp.

Hansen, Ø.J., Puvanendran, V., Jøstensen, J.P. and Ous, C., 2011. Effects of dietary levels and ratio of phosphatidylcholine and phosphatidylinositol on the growth, survival and deformity levels of Atlantic cod larvae and early juveniles. Aquaculture Research 42, 1026-1033.

Hansen, S.L. and von Herbing, I.H., 2009. Aerobic scope for activity in age 0 year Atlantic cod Gadus morhua. Journal of Fish Biology 74, 1355-1370.

Hansen, T., Karlsen, O., Taranger, G.L., Hemre, G.I., Holm, J.C. and Kjesbu, O.S., 2001. Growth, gonadal development and spawning time of Atlantic cod (Gadus morhua) reared under different photoperiods. Aquaculture 203, 51-67.

Helland, S., Lein, I., Hjelde, K. and Bæverfjord, G., 2009. Effects of water speed on lordosis and heart ventricle weight in cod. Control of malformations in fish aquaculter: Sence and practice (Fine fish). Liege: Federation of European Aquaculture Producers, Luxenbourg, 53-58.

Hermannsdottir, R., Johannsdottir, J., Smaradottir, H., Sigurgisladottir, S., Gudmundsdottir, B. and Bjornsdottir, R., 2009. Analysis of effects induced by a pollock protein hydrolysate on early development, innate immunity and the bacterial community structure of first feeding of Atlantic halibut (Hippoglossus hippoglossus L.) larvae. Fish and Shellfish Immunology 27, 595-602.

Hiroi, J., Sakakura, Y., Tagawa, M., Seikai, T. and Tanaka, M., 1997. Developmental changes in low-salinity tolerance and responses of prolactin, cortisol and thyroid hormones to low-salinity environment in larvae and juveniles of Japanese flounder, Paralichthys olivaceus. Zoological Science 14, 987-992.

Hori, T.S., Rise, M.L., Johnson, S.C., Afonso, L.O.B. and Gamperl, A., 2012. The mRNA expression of cortisol axis related genes differs in Atlantic cod (Gadus morhua) categorized as high or low responders. General and Comparative Endocrinology 175, 311-320.

Hoskins, L.J. and Volkoff, H., 2012. The comparative endocrinology of feeding in fish: Insights and challenges. General and Comparative Endocrinology 176, 327-335.

Howell, B.R., 1984. The intensive rearing of juvenile cod, Gadus morhua L. In: Dahl, E., Danielssen, D.S., Moksness, E., Solemdal, P. (Eds.), Flødevigen Rapportser, pp. 657-675.

Hunt von Herbing, I. and White, L., 2002. The effects of body mass and feeding on metabolic rate in small juvenile Atlantic cod. Journal of Fish Biology 61, 945-958.

Hutchings, J.A. and Myers, R.A., 1994. What can be learned from the collapse of a renewable resource? Atlantic cod, Gadus morhua, of Newfoundland and Labrador. Canadian Journal of Fisheries and Aquatic Sciences/Journal Canadien des Sciences Halieutiques et Aquatiques. Ottawa ON 51, 2126-2146.

Imsland, A.K., Foss, A., Koedijk, R., Folkvord, A., Stefansson, S.O. and Jonassen, T.M., 2006. Short-and long-term differences in growth, feed conversion efficiency and deformities in juvenile Atlantic cod (Gadus morhua) startfed on rotifers or zooplankton. Aquaculture Research 37, 1015-1027.

Imsland, A.K., Foss, A., Gunnarsson, S., Berntssen, M.H.G., FitzGerald, R., Bonga, S.W., Ham, E.v., Nævdal, G. and Stefansson, S.O., 2001. The interaction of temperature and salinity on growth and food conversion in juvenile turbot (Scophthalmus maximus). Aquaculture 198, 353-367.

Iwama, G.K., 1998. Stress in fish. Annals of the New York Academy of Sciences 851, 304-310.

Jakobsen, T., 1993. Management of northeast Arctic cod: Past, present -- and future? In: Kruse, G., Eggers, D.M., Marasco, R.J., Pautzke, C., Quinn, T.J., II (Eds.). Alaska Sea Grant College Program, University of Alaska Fairbanks, Fairbanks, Alaska, pp. 18-338.

Jobling, M., 1994. Respiration and metabolism. Fish bioenergetics, 121-145.

Johannsdottir, J., Heimisdottir, H.L., Hakonardottir, K., Hrolfsdottir, L., Steinarsson, A., Imsland, A.K., Thorarensen, H., Bergsson, A.B. and Bjornsdottir, R., 2013. Improved performance of Atlantic cod (Gadus morhua L.) larvae following enhancement of live feed using a fish protein hydrolysate. Aquaculture Nutrition, 10.1111/anu.12080.

Kanazawa, A., 1997. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. Aquaculture 155, 129-134.

Karlsen, O., Holm, J.C. and Kjesbu, O.S., 1995. Effects of periodic starvation on reproductive investment in first-time spawning Atlantic cod (Gadus morhua L.). Aquaculture 133, 159-170.

Kehoe, A.S. and Volkoff, H., 2007. Cloning and characterization of neuropeptide Y (NPY) and cocaine and amphetamine regulated transcript (CART) in Atlantic cod (Gadus morhua). Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 146, 451-461.

Kelley, K.M., Price, T.D., Galima, M.M., Sak, K., Reyes, J.A., Zepeda, O., Hagstrom, R., Tuan, A., Truong, T. and Lowe, C., 2006. Insulin-like growth factor binding proteins (IGFBPs) in fish: beacons for (disrupted) growth endocrine physiology. Fish Endocrinology, 167-195.

Killen, S.S., Atkinson, D. and Glazier, D.S., 2010. The intraspecific scaling of metabolic rate with body mass in fishes depends on lifestyle and temperature. Ecology Letters 13, 184-193.

Killen, S.S., Costa, I., Brown, J.A. and Gamperl, A.K., 2007. Little left in the tank: metabolic scaling in marine teleosts and its implications for aerobic scope. Proceedings of the Royal Society B: Biological Sciences 274, 431-438.

King, W. and Berlinsky, D.L., 2006. Whole-body corticosteroid and plasma cortisol concentrations in larval and juvenile Atlantic cod Gadus morhua L. following acute stress. Aquaculture Research 37, 1282-1289.

Kjørsvik, E., Olsen, C., Wold, P.A., Hoehne-Reitan, K., Cahu, C.L., Rainuzzo, J., Olsen, A.I., Øie, G. and Olsen, Y., 2009. Comparison of dietary phospholipids and neutral lipids on skeletal development and fatty acid composition in Atlantic cod (Gadus morhua). Aquaculture 294, 246-255.

Klein, S.E. and Sheridan, M.A., 2008. Somatostatin signaling and the regulation of growth and metabolism in fish. Molecular and Cellular Endocrinology 286, 148-154.

Klostermeyer, H. and Reimerdes, E., 1976. Heat induced crosslinks in milk proteins and consequences for the milk system. Advances in Experimental Medicine and Biology 86, 263-275.

Koedijk, R., Folkvord, A., Foss, A., Pittman, K., Stefansson, S., Handeland, S. and Imsland, A., 2010a. The influence of first-feeding diet on the Atlantic cod Gadus morhua phenotype: survival, development and long-term consequences for growth. Journal of Fish Biology 77, 1-19.

Koedijk, R.M., Le François, N.R., Blier, P.U., Foss, A., Folkvord, A., Ditlecadet, D., Lamarre, S.G., Stefansson, S.O. and Imsland, A.K., 2010b. Ontogenetic effects of diet during early development on growth performance, myosin mRNA expression and metabolic enzyme activity in Atlantic cod juveniles reared at different salinities. Comparative Biochemistry and Physiology-Part A: Molecular & Integrative Physiology 156, 102-109.

Kolstad, K., Thorland, I., Refstie, T. and Gjerde, B., 2006. Genetic variation and genotype by location interaction in body weight, spinal deformity and sexual maturity in Atlantic cod (Gadus morhua) reared at different locations off Norway. Aquaculture 259, 66-73.

Konturek, S., Pepera, J., Zabielski, K., Konturek, P., Pawlik, T., Szlachcic, A. and Hahn, E., 2003. Brain-gut axis in pancreatic secretion and appetite control. Journal of Physiology and Pharmacology: An Official Journal of the Polish Physiological Society 54, 293-317.

Kortner, T.M., Overrein, I., Øie, G., Kjørsvik, E. and Arukwe, A., 2011a. The influence of dietary constituents on the molecular ontogeny of digestive capability and effects on growth and appetite in Atlantic cod larvae (Gadus morhua). Aquaculture 315, 114-120.

Kortner, T.M., Overrein, I., Øie, G., Kjørsvik, E., Bardal, T., Wold, P.A. and Arukwe, A., 2011b. Molecular ontogenesis of digestive capability and associated endocrine control in Atlantic cod (Gadus morhua) larvae. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 160, 190-199.

Kotzamanis, Y., Gisbert, E., Gatesoupe, F., Zambonino Infante, J. and Cahu, C., 2007. Effects of different dietary levels of fish protein hydrolysates on growth, digestive enzymes, gut microbiota, and resistance to Vibrio anguillarum in European sea bass (Dicentrarchus labrax) larvae. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 147, 205-214.

Koumoundouros, G., 2010. Morpho-anatomical abnormalities in Mediterranean marine aquaculture. In: Koumoundouros, G. (Eds.), Recent Advances in Aquaculture Research. Transworld Research Network, Kerala, pp. 125-148.

Koven, W., Barr, Y., Lutzky, S., Ben-Atia, I., Weiss, R., Harel, M., Behrens, P. and Tandler, A., 2001. The effect of dietary arachidonic acid (20: 4n-6) on growth, survival and resistance to handling stress in gilthead seabream (Sparus aurata) larvae. Aquaculture 193, 107-122.

Kraul, S., Brittain, K., Cantrell, R., Nagao, T., Ogasawara, A., Ako, H. and Kitagawa, H., 1993. Nutritional factors affecting stress resistance in the larval mahimahi Coryphaena hippurus. Journal of the World Aquaculture Society 24, 186-193.

Kvåle, A., Harboe, T., Mangor-Jensen, A. and Hamre, K., 2009. Effects of protein hydrolysate in weaning diets for Atlantic cod (Gadus morhua L.) and Atlantic halibut (Hippoglossus hippoglossus L.). Aquaculture Nutrition 15, 218-227.

Kvåle, A., Yúfera, M., Nygård, E., Aursland, K., Harboe, T. and Hamre, K., 2006. Leaching properties of three different micropaticulate diets and preference of the diets in cod (Gadus morhua L.) larvae. Aquaculture 251, 402-415.

Lall, S.P. and Lewis-McCrea, L.M., 2007. Role of nutrients in skeletal metabolism and pathology in fish—an overview. Aquaculture 267, 3-19.

Lan, C.C. and Pan, B.S., 1993. Invitro digestibility simulating the proteolysis of feed protein in the midgut gland of grass shrimp (Penaeus-monodon). Aquaculture 109, 59-70.

Lanes, C.F.C., Bolla, S., Fernandes, J.M., Nicolaisen, O., Kiron, V. and Babiak, I., 2012. Nucleotide enrichment of live feed: a promising protocol for rearing of Atlantic cod Gadus morhua larvae. Marine Biotechnology 14, 544-558.

Lein, I., Hjelde, K., Helland, S. and Bæverfjord, G., 2006. Assessment of deformities in cod. Akvaforsk Rapport No. 1/06, 13 (In Norwegian).

Lein, I., Hjelde, K., Helland, S. and Baeverfjord, G., 2009. Survey of skeletal deformities in farmed cod. FineFish. Available: http://www.finefish.info/finefish. March 2011.

Lewis, L.M., Lall, S.P. and Eckhard Witten, P., 2004. Morphological descriptions of the early stages of spine and vertebral development in hatchery-reared larval and juvenile Atlantic halibut (Hippoglossus hippoglossus). Aquaculture 241, 47-59.

Liu, X.D., Liang, M.Q., Zhang, L.M., Wang, J.Y., Chang, Q., Wang, J.L. and Yannis, K., 2010. Effect of fish protein hydrolysate levels on growth performance and biological and physiological parameters in tongue sole (Cynoglossus semilaevis gunther, 1873) post- larvae. Acta Hydrobiologica Sinica 34, 242-249.

Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using Real-Time Quantitative PCR and the 2 (- ΔΔCT) method. Methods 25, 402-408.

Lucas, M. and Priede, I., 1992. Utilization of metabolic scope in relation to feeding and activity by individual and grouped zebrafish, Brachydanio rerio (Hamilton-Buchanan). Journal of Fish Biology 41, 175-190.

MacDonald, E.E., 2008. NPY, CART and CCK identification in winter flounder (Pleuronectes americanus) and winter skate (Raja ocellata): molecular cloning, tissue distribution and response to season and fasting. General and Comparative Endocrinology 161, 252-261.

MacKenzie, D.S., VanPutte, C.M. and Leiner, K.A., 1998. Nutrient regulation of endocrine function in fish. Aquaculture 161, 3-25.

Mamauag, R.E.P., Koshio, S., Ishikawa, M., Yokoyama, S., Gao, J., Nguyen, B.T. and Ragaza, J.A., 2011. Soy peptide inclusion levels influence the growth performance, proteolytic enzyme activities, blood biochemical parameters and body composition of Japanese flounder, Paralichthys olivaceus. Aquaculture 321, 252-258.

Mazeaud, M. and Mazeaud, F., 1981. Adrenergic responses to stress in fish. In: Pickering, A. (Eds.), Stress and Fish. Academic Press, London, pp. 49-75.

McEvoy, L.A., Naess, T., Bell, J.G. and Lie, Ø., 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (Hippoglossus hippoglossus) fed enriched Artemia: a comparison with fry fed wild copepods. Aquaculture 163, 237-250.

McKenzie, D.J., Lund, I. and Pedersen, P.B., 2008. Essential fatty acids influence metabolic rate and tolerance of hypoxia in Dover sole (Solea solea) larvae and juveniles. Marine Biology 154, 1041-1051.

McKenzie, D.J., 2001. Effects of dietary fatty acids on the respiratory and cardiovascular physiology of fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 128, 605-619.

McKenzie, D.J., Higgs, D.A., Dosanjh, B.S., Deacon, G. and Randall, D.J., 1998. Dietary fatty acid composition influences swimming performance in Atlantic salmon (Salmo salar) in seawater. Fish Physiology and Biochemistry 19, 111-122.

Milione, M. and Zeng, C., 2008. The effects of temperature and salinity on population growth and egg hatching success of the tropical calanoid copepod, Acartia sinjiensis. Aquaculture 275, 116-123.

Mohammed, Z.H., Hill, S.E. and Mitchell, J.R., 2000. Covalent crosslinking in heated protein systems. Journal of Food Science 65, 221-226.

Moren, M., Gundersen, T.E. and Hamre, K., 2005. Quantitative and qualitative analysis of retinoids in Artemia and copepods by HPLC and diode array detection. Aquaculture 246, 359-365.

Myers, R.A., Barrowman, N.J., Hoenig, J.M. and Qu, Z., 1996. The collapse of cod in eastern Canada: the evidence from tagging data. ICES Journal of Marine Science 53, 629-640.

Narnaware, Y.K. and Peter, R.E., 2001. Effects of food deprivation and refeeding on neuropeptide Y (NPY) mRNA levels in goldfish. Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology 129, 633-637.

Narnaware, Y.K. and Peter, R.E., 2002. Influence of diet composition on food intake and neuropeptide Y (NPY) gene expression in goldfish brain. Regulatory Peptides 103, 75-83.

Narnaware, Y.K., Peyon, P.P., Lin, X. and Peter, R.E., 2000. Regulation of food intake by neuropeptide Y in goldfish. American Journal of Physiology 279, R1025-R1034.

Nayak, S.K., 2010. Role of gastrointestinal microbiota in fish. Aquaculture Research 41, 1553-1573.

Nguyen, V.T., Satoh, S., Haga, Y., Fushimi, H. and Kotani, T., 2008. Effect of zinc and manganese supplementation in Artemia on growth and vertebral deformity in red sea bream (Pagrus major) larvae. Aquaculture 285, 184-192.

Nordgreen, A., Tonheim, S. and Hamre, K., 2009. Protein quality of larval feed with increased concentration of hydrolysed protein: effects of heat treatment and leaching. Aquaculture Nutrition 15, 525-536.

Ohlberger, J., Mehner, T., Staaks, G. and Hölker, F., 2012. Intraspecific temperature dependence of the scaling of metabolic rate with body mass in fishes and its ecological implications. Oikos 121, 245-251.

Øiestad, V., Kvenseth, P. and Folkvord, A., 1985. Mass production of Atlantic cod juveniles Gadus morhua in a Norwegian saltwater pond. Transactions of the American Fisheries Society 114, 590-595.

Okimasu, E., Matsumoto, M., Yoshida, Y. and Amemura, A., 1992. The effect of pigments of Rhodobacter capsulatus on free radicals and application of the bacterium as feed to fish larvae. Nippon Suisan Gakkaishi 58, 1487-1491.

Olivotto, I., Tokle, N.E., Nozzi, V., Cossignani, L. and Carnevali, O., 2010. Preserved copepods as a new technology for the marine ornamental fish aquaculture: A feeding study. Aquaculture 308, 124-131.

Olivotto, I., Buttino, I., Borroni, M., Piccinetti, C., Malzone, M. and Carnevali, O., 2008a. The use of the Mediterranean calanoid copepod Centropages typicus in yellowtail clownfish (Amphiprion clarkii) larviculture. Aquaculture 284, 211-216.

Olivotto, I., Capriotti, F., Buttino, I., Avella, A.M., Vitiello, V., Maradonna, F. and Carnevali, O., 2008b. The use of harpacticoid copepods as live prey for Amphiprion clarkii larviculture: Effects on larval survival and growth. Aquaculture 274, 347-352.

Olsen, Y., 1997. Larval-rearing technology of marine species in Norway. Hydrobiologia 358, 27-36.

Otterlei, E., Nyhammer, G., Folkvord, A. and Stefansson, S.O., 1999. Temperature- and size-dependent growth of larval and early juvenile Atlantic cod (Gadus morhua): a comparative study of Norwegian coastal cod and northeast Arctic cod. Canadian Journal of Fisheries and Aquatic Sciences 56, 2099-2111.

Ottmar, M. and Hurst, T., 2012. Thermal effects on swimming activity and habitat choice in juvenile Pacific cod (Gadus macrocephalus). Marine Biology 159, 2185-2194.

Palace, V.P. and Werner, J., 2006. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: a review. Scientia Marina 70, 41-57.

Pankhurst, N.W., Ludke, S.L., King, H.R. and Peter, R.E., 2008. The relationship between acute stress, food intake, endocrine status and life history stage in juvenile farmed Atlantic salmon, Salmo salar. Aquaculture 275, 311-318.

Panserat, S. and Kaushik, S.J., 2010. Regulation of gene expression by nutritional factors in fish. Aquaculture Research 41, 751-762.

Peck, M.A. and Holste, L., 2006. Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in Acartia tonsa (Calanoida: Copepoda): Optimizing intensive cultures. Aquaculture 255, 341-350.

Peck, M.A., Buckley, L.J. and Bengtson, D.A., 2003. Energy losses due to routine and feeding metabolism in young-of-the-year juvenile Atlantic cod (Gadus morhua). Canadian Journal of Fisheries and Aquatic Sciences 60, 929-937.

Pérez-Casanova, J.C., Rise, M.L., Dixon, B., Afonso, L.O.B., Hall, J.R., Johnson, S.C. and Gamperl, A.K., 2008. The immune and stress responses of Atlantic cod to long-term increases in water temperature. Fish and Shellfish Immunology 24, 600-609.

Petersen, L. and Gamperl, A., 2010. Effect of acute and chronic hypoxia on the swimming performance, metabolic capacity and cardiac function of Atlantic cod (Gadus morhua). The Journal of Experimental Biology 213, 808-819.

Pfaffl, M.W., 2001. A new mathematical model for relative quantification in real-time RT–PCR. Nucleic Acids Research 29, e45-e45.

Piccinetti, C.C., Ricci, L.A., Tokle, N., Radaelli, G., Pascoli, F., Cossignani, L., Palermo, F., Mosconi, G., Nozzi, V., Raccanello, F. and Olivotto, I., 2012. Malnutrition may affect common sole (Solea solea L.) growth, pigmentation and stress response: molecular, biochemical and histological implications. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 161, 361-371.

Picha, M.E., Turano, M.J., Beckman, B.R. and Borski, R.J., 2008. Endocrine biomarkers of growth and applications to aquaculture: a minireview of growth hormone, insulin-like growth factor (IGF)-I, and IGF-binding proteins as potential growth indicators in fish. North American Journal of Aquaculture 70, 196-211.

Plaut, I., 2001. Critical swimming speed: its ecological relevance. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 131, 41-50.

Pottinger, T., 2003. The selection of trout for high and low responsiveness to stress: progress and prospects. Trout News 36, 14-16.

Pottinger, T., 2010. A multivariate comparison of the stress response in three salmonid and three cyprinid species: evidence for inter-family differences. Journal of Fish Biology 76, 601-621.

Pottinger, T. and Carrick, T., 1999. Modification of the plasma cortisol response to stress in rainbow trout by selective breeding. General and Comparative Endocrinology 116, 122-132.

Pottinger, T.G., 2008. The stress response in fish-mechanisms, effects and measurement. In: Branson, E. (Eds.), Fish Welfare. Blackwell Publishing Ltd, UK, pp. 32-48.

Prickett, R., Boyce, D. and Monk, J., 2010. Marine finfish hatchery in Canada uses clay to rear cod. The Cold Harvester, Newfoundland Aquaculture Spring Edition, 18-19.

Priede, I.G., 1985. Metabolic scope in fishes. In: Peter, T., Peter, C. (Eds.), Fish Energetics. Springer, Rotterdam, pp. 33-64.

Puvanendran, V., Burt, A.L. and Brown, J.A., 2006. Can Atlantic cod (Gadus morhua) larvae be weaned faster onto dry feed at higher temperatures? Aquaculture 255, 334-340.

Puvanendran, V., Laurel, B.J. and Brown, J.A., 2008. Cannibalism of Atlantic cod Gadus morhua larvae and juveniles on first-week larvae. Aquatic Biology 2, 113-118.

Reidy, S., Nelson, J., Tang, Y. and Kerr, S., 1995. Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. Journal of Fish Biology 47, 377-386.

Reinecke, M., 2010. Influences of the environment on the endocrine and paracrine fish growth hormone–insulin-like growth factor-I system. Journal of Fish Biology 76, 1233-1254.

Rognerud, C., 1887. Hatching cod in Norway. Bulletin of the United States Fish Commission 8, 113-119.

Rosenlund, G. and Skretting, M., 2006. Worldwide status and perspective on gadoid culture. ICES Journal of Marine Science: Journal du Conseil 63, 194-197.

Rosenlund, G. and Halldorsson, O., 2007. Cod juvenile production: research and commercial developments. Aquaculture 268, 188-194.

Rosenlund, G., Meslo, I., Rødsjø, R. and Torp, H., 1993. Large scale production of cod. In: Reinertsen, H., Dahle, H., Jorgensen, L. (Eds.), Fish Farming Technology. Balkema, Rotterdam, pp. 141-146

Samuelsen, O.B., Nerland, A.H., Joergensen, T., Schroeder, M.B., Svaasand, T. and Bergh, O., 2006. Viral and bacterial diseases of Atlantic cod Gadus morhua, their prophylaxis and treatment: a review. Diseases of Aquatic Organisms 71, 239-254.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J. and Tocher, D., 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture 179, 217-229.

Saunders, R.L., 1963. Respiration of the Atlantic cod. Journal of the Fisheries Board of Canada 20, 373-386.

Savoie, A., Le François, N.R., Lamarre, S.G., Blier, P.U., Beaulieu, L. and Cahu, C., 2011. Dietary protein hydrolysate and trypsin inhibitor effects on digestive capacities and performances during early-stages of spotted wolffish: Suggested mechanisms. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 158, 525-530.

Schmid, A.C., Lutz, I., Kloas, W. and Reinecke, M., 2003. Thyroid hormone stimulates hepatic IGF-I mRNA expression in a bony fish, the tilapia Oreochromis mossambicus, in vitro and in vivo. General and Comparative Endocrinology 130, 129-134.

Schreck, C.B., 1982. Stress and rearing of salmonids. Aquaculture 28, 241-249.

Schumann, K., 2000. Artemia FAQ 2.0. Available: http://www.aqualink.com/marine/z-artemia. Sep., 2013.

Schwartz, M.W., 2001. Brain pathways controlling food intake and body weight. Experimental Biology and Medicine 226, 978-981.

Segner, H., Storch, V., Reinecke, M., Kloas, W. and Hanke, W., 1994. The development of functional digestive and metabolic organs in turbot, Scophthalmus maximus. Marine Biology 119, 471-486.

Selye, H., 1974. In: Weisman, A.D. (Eds.), Stress without Distress. McClelland Stewart, Toronto, pp. 165-171.

Shelbourne, J.E., 1964. The artificial propagation of marine fish. In: Russel, F.S. (Eds.), Advances in Marine Biology. Vol. 2, Academic Press, London, pp. 1-83.

Sheridan, M.A., 2011. Hormonal control of reproduction and growth. In: Farrell, A.P. (Eds.), Encyclopedia of Fish Physiology. Academic Press, San Diego, pp. 1483-1489.

Shields, R.J., 2001. Larviculture of marine finfish in Europe. Aquaculture 200, 55-88.

Shved, N., Berishvili, G., Baroiller, J.F., Segner, H. and Reinecke, M., 2008. Environmentally relevant concentrations of 17α-ethinylestradiol (EE2) interfere with the growth hormone (GH)/insulin-like growth factor (IGF)-I system in developing bony fish. Toxicological Sciences 106, 93-102.

Silverstein, J.T. and Plisetskaya, E.M., 2000. The effects of NPY and insulin on food intake regulation in fish. American Zoologist 40, 296-308.

Silverstein, J.T., Shearer, K.D., Dickhoff, W.W. and Plisetskaya, E.M., 1999. Regulation of nutrient intake and energy balance in salmon. Aquaculture 177, 161-169.

Simpson, A., Metcalfe, N., Huntingford, F. and Thorpe, J., 1996. Pronounced seasonal differences in appetite of Atlantic salmon parr, Salmo salar: effects of nutritional state and life-history strategy. Functional Ecology, 760-767.

Solemdal, P., Dahl, E., Danielssen, D.S. and Moksness, E., 1984. The cod hatchery in Flodevigen - background and realities. Flodevigen Rapportserie 1, 17-45.

Star, B., Nederbragt, A.J., Jentoft, S., Grimholt, U., Malmstrom, M., Gregers, T.F., Rounge, T.B., Paulsen, J., Solbakken, M.H., Sharma, A., Wetten, O.F., Lanzen, A., Winer, R., Knight, J., Vogel, J.H., Aken, B., Andersen, O., Lagesen, K., Tooming-Klunderud, A., Edvardsen, R.B., Tina, K.G., Espelund, M., Nepal, C., Previti, C., Karlsen, B.O., Moum, T., Skage, M., Berg, P.R., Gjoen, T., Kuhl, H., Thorsen, J., Malde, K., Reinhardt, R., Du, L., Johansen, S.D., Searle, S., Lien, S., Nilsen, F., Jonassen, I., Omholt, S.W., Stenseth, N.C. and Jakobsen, K.S., 2011. The genome sequence of Atlantic cod reveals a unique immune system. Nature 477, 207-210.

Steffensen, F.J., 2005. Respiratory systems and metabolic rates. In: Farrell, A.P., Steffensen J.F., (Eds.), The Physiology of Polar Fishes. Academic Press, San Diego, pp. 203-238.

Steinarsson, A., Kristjánsson, T. and Ingimarsson, K., 2012. Growth potential of farmed cod. Final activity report – Lokaskýrsla.Vol. 1, pp. 1-47.

Støttrup, J., 2000. The elusive copepods: their production and suitability in marine aquaculture. Aquaculture Research 31, 703-711.

Støttrup, J.G., 2003. Production and nutritional value of copepods. In: Støttrup, J.P. and McEvoy, L.A. (Eds.), Live Feeds in Marine Aquaculture. Blackwell Publishing, Oxford, pp. 145-205.

Støttrup, J.G., 2006. Review on the status and progress in rearing copepods for marine laviculture. Advantages and disadvantages. Among calanoid, harpacticoid and cyclopoid copepods. In: Suárez, L.E., Salazar, M.T., López, M.G.N., Cavazos, D.A.V., Cruz, A.C.P. and Ortega, A.G. (Eds.), Advances in Aquaculture Nutrition. VIII International Symposium, Universidad Autónoma de Nuevo León, Monterrey, Nuevo León, México, pp. 62-83.

Strader, A.D. and Woods, S.C., 2005. Gastrointestinal hormones and food intake. Gastroenterology 128, 175-191.

Sumpter, J., 1997. The endocrinology of stress. Fish Stress and Health in Aquaculture 819, 95-118.

Sunuma, T., Amano, M., Yamanome, T., Furukawa, K. and Yamamori, K., 2007. Self-feeding activity of a pleuronectiform fish, the barfin flounder. Aquaculture 270, 566-569.

Svåsand, T., 1998. Cod enhancement studies in Norway - background and results with emphasis on releases in the period 1983-1990. Bulletin of Marine Science 62, 313-324.

Svåsand, T., Otterå, H., Taranger, G., Litvak, M., Skiftesvik, A., Bjelland, R., Pavlov, D., Holm, J.C., Harboe, T. and Mangor-Jensen, A., 2004. The status and perspectives for the species. Culture of Cold-Water Marine Fish, 433-474.

Tang, K., Dziallas, C., Hutalle-Schmelzer, K. and Grossart, H.P., 2009. Effects of food on bacterial community composition associated with the copepod Acartia tonsa Dana. Biology Letters 5, 549-553.

Tang, K.W., 2005. Copepods as microbial hotspots in the ocean: effects of host feeding activities on attached bacteria. Aquatic Microbial Ecology 38, 31-40.

Tilseth, S., 1990. New marine fish species for cold-water farming. Aquaculture 85, 235-245.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Reviews in Fisheries Science 11, 107-184.

Tonheim, S.K., Espe, M., Hamre, K. and Rønnestad, I., 2005. Pre-hydrolysis improves utilisation of dietary protein in the larval teleost Atlantic halibut (Hippoglossus hippoglossus L.). Journal of Experimental Marine Biology and Ecology 321, 19-34.

Tonheim, S.K., Espe, M., Raae, A.J., Darias, M.J. and Rønnestad, I., 2004. In vivo incorporation of [U]-14C-amino acids: an alternative protein labelling procedure for use in examining larval digestive physiology. Aquaculture 235, 553-567.

Tort, L., Puigcerver, M., Crespo, S. and Padros, F., 2002. Cortisol and haematological response in sea bream and trout subjected to the anaesthetics clove oil and 2-phenoxyethanol. Aquaculture Research 33, 907-910.

Vagelli, A.A., 2004. Significant increase in survival of captive-bred juvenile banggai cardinalfish Pterapogon kauderni with an essential fatty acid-enriched diet. Journal of the World Aquaculture Society 35, 61-69.

Van der Meeren, T. and Næss, T., 1993. How does cod (Gadus morhua) cope with variability in feeding conditions during early larval stages? Marine Biology 116, 637-647.

Van der Meeren, T. and Naas, K.E., 1997. Development of rearing techniques using large enclosed ecosystems in the mass production of marine fish fry. Reviews in Fisheries Science 5, 367-390.

Van der Meeren, T., Olsen, R.E., Hamre, K. and Fyhn, H.J., 2008. Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. Aquaculture 274, 375-397.

Very, N.M. and Sheridan, M.A., 2002. The role of somatostatins in the regulation of growth in fish. Fish Physiology and Biochemistry 27, 217-226.

Volkoff, H., 2006. The role of neuropeptide Y, orexins, cocaine and amphetamine-related transcript, cholecystokinin, amylin and leptin in the regulation of feeding in fish. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 144, 325-331.

Volkoff, H. and Peter, R.E., 2001a. Interactions between orexin A, NPY and galanin in the control of food intake of the goldfish, Carassius auratus. Regulatory Peptides 101, 59-72.

Volkoff, H. and Peter, R.E., 2001b. Characterization of two forms of cocaine-and amphetamine-regulated transcript (CART) peptide precursors in goldfish: molecular cloning and distribution, modulation of expression by nutritional status, and interactions with leptin. Endocrinology 142, 5076-5088.

Volkoff, H., Xu, M., MacDonald, E. and Hoskins, L., 2009. Aspects of the hormonal regulation of appetite in fish with emphasis on goldfish, Atlantic cod and winter flounder: notes on actions and responses to nutritional, environmental and reproductive changes. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 153, 8-12.

Volkoff, H., Canosa, L., Unniappan, S., Cerda-Reverter, J., Bernier, N., Kelly, S. and Peter, R., 2005. Neuropeptides and the control of food intake in fish. General and Comparative Endocrinology 142, 3-19.

Wagner, G.N., Balfry, S.K., Higgs, D.A., Lall, S.P. and Farrell, A.P., 2004. Dietary fatty acid composition affects the repeat swimming performance of Atlantic salmon in seawater. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology 137, 567-576.

Weber, E.D., Borthwick, S.M. and Helfrich, L.A., 2002. Plasma cortisol stress response of juvenile Chinook salmon to passage through Archimedes lifts and a Hidrostal pump. North American Journal of Fisheries Management 22, 563-570.

Westelmajer, S.K.M., 2008. Ontogeny of the corticosteroid stress response and effect of differentially enriched live feed on growth, lipid composition and acute stress tolerance of larval Atlantic cod, Gadus morhua. Master Thesis. Memorial University of Newfoundland. St. John's, NL. 148 pp.

Wieser, W. and Medgyesy, N., 1990. Aerobic maximum for growth in the larvae and juveniles of a cyprinid fish, Rutilus rutilus (L.): implications for energy budgeting in small poikilotherms. Functional Ecology, 233-242.

Wieser, W., Forstner, H., Medgyesy, N. and Hinterleitner, S., 1988. To switch or not to switch: partitioning of energy between growth and activity in larval cyprinids (Cyprinidae: Teleostei). Functional Ecology, 499-507.

Wold, P.A., Hoehne-Reitan, K., Cahu, C.L., Infante, J.Z., Rainuzzo, J. and Kjørsvik, E., 2009. Comparison of dietary phospholipids and neutral lipids: effects on gut, liver and pancreas histology in Atlantic cod (Gadus morha L.) larvae. Aquaculture Nutrition 15, 73-84.

Wood, A.W., Duan, C. and Bern, H.A., 2005. Insulin-like growth factor signaling in fish. International Review of Cytology 243, 215-285.

Wullimann, M.F. and Mueller, T., 2004. Teleostean and mammalian forebrains contrasted: evidence from genes to behavior. Journal of Comparative Neurology 475, 143-162.

Yang, Y., Cao, Z.d. and Fu, S.-j., 2013. Effects of water temperature on the critical swimming speed and metabolic scope of juvenile Parabramis pekinensis. Shengtaixue Zazhi 32, 1260-1264.

Zambonino Infante, J.L., Cahu, C.L. and Peres, A., 1997. Partial substitution of di-and tripeptides for native proteins in sea bass diet improves Dicentrarchus labrax larval development. The Journal of Nutrition 127, 608-614.

Zou, S., Kamei, H., Modi, Z. and Duan, C., 2009. Zebrafish IGF genes: gene duplication, conservation and divergence, and novel roles in midline and notochord development. PLoS One 4, e7026.

Zouiten, D., Ben Khemis, I., Slaheddin Masmoudi, A., Huelvan, C. and Cahu, C., 2011. Comparison of growth, digestive system maturation and skeletal development in sea bass larvae reared in an intensive or a mesocosm system. Aquaculture Research 42, 1723-1736.