EFFECT OF DIFFERENTLY LIPID-ENRICHED LIVE FEED ON GROWTH, SURVIVAL, AND LIPID COMPOSITION OF TWO LARVAL GADOIDS: ATLANTIC COD (GADUS MORHUA) AND HADDOCK (MELANOGRAMMUS AEGLEFINUS)

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Effect of differently lipid-enriched live feed on growth, survival, and lipid composition of two larval gadoids: Atlantic cod (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*)

by

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Abstract

Dietary lipids are recognized as one of the most important nutritional factors that affect fish larval growth and survival. Rotifers and *Artemia* sp. are widely used as live-feed during the larviculture of marine fish species. However, these organisms are naturally poor in essential fatty acids, and provide only sub-optimum nutrition to the larvae. The present thesis evaluated the use of commercial products for the enrichment of rotifers and *Artemia*, and the effects of these enriched live-feed on the early growth, survival and lipid composition of Atlantic cod and haddock larvae. For both species the enrichments tested during the rotifer phase were: 1) AlgaMac 2000®, 2) AquaGrow® Advantage, and 3) a combination of *Pavlova* sp. paste and AlgaMac 2000®. During the *Artemia* phase of cod larviculture, the same three treatments were tested in addition to a combination of DC DHA Selco® + AlgaMac 2000® as a fourth treatment. Treatments two, three, and four were tested during the *Artemia* phase of haddock larviculture. Larvae from both species performed better when fed with rotifers enriched with a combination of *Pavlova* sp. and AlgaMac 2000. At the end of the rotifer phase, larval dry weight ranged from 1.03 to 1.50 mg for cod, and from 1.03 to 1.10 mg for haddock. The larval lipid composition of both species was affected differently by the treatments. During the rotifer phase, larvae from both species maintained high levels of DHA (22:6ω3) while levels of EPA (20:5ω3) decreased significantly. During the *Artemia* phase, cod larvae fed with AlgaMac 2000-enriched *Artemia* and haddock larvae fed with DC DHA Selco + AlgaMac 2000-enriched *Artemia* showed the

best overall performance. The cod larvae final dry weight ranged from 5.31 to 12.06 mg, and haddock larvae final dry weight ranged from 4.47 to 6.40 mg. Larvae from both species had their lipid composition affected differently by the treatments. However, in contrast to the rotifer phase, during the *Artemia* phase, larvae from both species accumulated EPA and reduced DHA levels. Results from the present study indicate that two closely related gadoids raised under similar conditions have different growth, survival and lipid composition responses to lipid-enriched live-feed.

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List of Abbreviations and Symbols

α	Alpha
β	Beta
Δ	Delta
ω	Omega
%	Percentage
Ø	Diameter
Σ	Sum
µm	Micrometre
®	Registered
AA	arachidonic acid
ai	ante-iso
a.m.	ante meridiem
AMPL	acetone mobile polar lipids
ANCOVA	analysis of co-variance
ANOVA	analysis of variance
ARDF	Aquaculture Research Development Facility
BAME	bacterial acid methyl ester
BF_3	boron trifluoride
C	Carbon
°C	degree centigrade
ca	Circa

CA	California
cal	Calories
cm^2	centimetres square
Corp.	Corporation
COX-2	Cyclooxygenase
d	Days
dd	degree-days
DFO	Department of Fisheries and Oceans
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid
dph	days post-hatch
dw	dry weight
e.g.	exempli gratia
EPA	eicosapentaenoic acid
F	"F" statistic
FAME	fatty acid methyl ester
FAO	Food and Agriculture Organization of the United Nations
FID	flame ionization detection
FRCC	Fisheries Resource Conservation Council
g	Grams
GC	gas chromatograph
h	Hour
HUFA	highly unsaturated fatty acids

i	Iso
i.d.	internal diameter
Inc.	Incorporated
J	Joules
KET	Ketones
L	Litre
ln	natural logarithm
m	Metre
mg	Milligram
min	Minutes
mL	Millilitre
mm	Millimetre
MD	Maryland
MUFA	monounsaturated fatty acids
n	number of samples
N.b.	nota bene
NC	North Carolina
ON	Ontario
OSC	Ocean Sciences Centre
p	probability level
PA	Pennsylvania
PLA_2	phospholipase A_2
PL	Phospholipids

p.m.	post meridiem
psu	practical salinity unit
PUFA	polyunsaturated fatty acids
r^2	coefficient of determination
SE	standard error
SFA	saturated fatty acids
SGR	specific growth rate
sp.	Species
spp.	species plural
ST	Sterols
TAG	Triacylglycerols
TLC	thin layer chromatography
TN	Tennessee
USA	United States of America
UT	Utah

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CHAPTER I

General Introduction

I.1. Overview

The Gadidae family comprises medium to large sized bottom fish, living in cool seas, with most species inhabiting inshore waters and continental shelves, some to depths of 1300 m. Two subfamilies are recognized: Lotinae and Gadinae, the latter including cods and haddock (Scott and Scott, 1988). The fish from the Gadinae subfamily are of great economic importance and support extensive commercial fisheries in the North Atlantic. Atlantic cod, *Gadus morhua*, is one of the most famous fish species in the world and has been fished on the banks of the North Atlantic for centuries. Haddock, *Melanogrammus aeglefinus*, is one of the most highly prized fish caught for the Northeastern North American market.

The cod fisheries of the past provided a foundation for the social and economic structure in Atlantic Canada, especially in Newfoundland and Labrador, where this natural resource began to be exploited in the 1500s. In the 1800s, annual Atlantic cod landings in Canada ranged between 150,000 and 400,000 tones. The evolution of fishery gears and the introduction of new fishing technologies, such as automated floating fish factories in the 20th century, led to increasingly larger annual landings of Atlantic cod, reaching a peak during the 1960s of almost two million tones. From 1981 to 1990, the Atlantic cod fisheries were responsible for 59% of the northwest coast landings by value (DFO, 2006, http://www.dfo-mpo.gc.ca/kids-enfants/map-carte/map_e.htm). Between 1984

and 1992, total landings of northern cod stocks and northern gulf stocks declined from 38,648 to 11,828 tones, triggering concerns regarding the abundance of Atlantic cod stocks (Palmer and Sinclair, 1997). On July 2nd 1992, the Minister of Fisheries and Oceans announced a two-year moratorium on Atlantic cod fishing. The moratorium was subsequently extended to the "recreational" or "food" fishery. This moratorium was maintained indefinitely beyond its May 15th 1994 schedule termination and additional conservation measures—such as harvest planning, gear selectivity, seasonal closures, etc.—were imposed. In 2002, an independent advisory body, the Fisheries Resource Conservation Council (FRCC), presented to the Fisheries Minister an assessment report, which revealed that the Atlantic cod stocks had not shown any real signs of recovery. Finally, in March 24th 2003, the FRCC reported that stocks were at historically low levels and were not showing signs of imminent recovery, and the Atlantic cod fishery was maintained closed indeterminably. A wide range of social, economic, and environmental factors have been implicated in the collapse of the Atlantic cod stocks, including domestic and foreign overfishing, government mismanagement, lower annual water temperatures, and greater predation of Atlantic cod by seals (Schrank, 2005).

Interest in the culture of Atlantic cod in Canada began in the late 1800s, when a Norwegian expert was hired to establish and manage an Atlantic cod hatchery in Newfoundland with the purpose of enhancement of natural stocks (Baker et al., 1992). The rearing of Atlantic cod met with mixed success up to the

mid-1980s. After this period, there was a significant improvement in the production of Atlantic cod, arising from the use of large enclosures (Brown et al., 1995). In the early 1990s, the collapse of the Atlantic cod stocks, and consequently high market prices, renewed interest in Atlantic cod culture. In 2003, aquaculture operations in Europe produced 2,561 tones of Atlantic cod (FAO, 2006, Fisheries Global Information System-FIGIS, http://www.fao.org/figis/servlet/TabSelector).

Like many other Canadian groundfish stocks, haddock has also decreased in recent years, although high stock concentrations are sometimes found in the Georges Bank area. There are five haddock fishing areas in Canada, but fishing is only permitted in two of these areas at present. Haddock stocks were greatly overexploited by domestic and foreign fleets in the mid-1960's and the stocks are still recovering. Over the past decade, haddock landings in Canada have declined by almost one-third (DFO, 2006, http://www.dfo-po.gc.ca/media/infocus/2003/20031205/haddock_e.htm).

Research into haddock aquaculture is underway at several institutions in the United States, including the University of Maine, the University of New Hampshire, and the Massachusetts Institute of Technology. Outside of North America, researchers at the Ardtoe Aquaculture Unit in Scotland and at the Austevoll Aquaculture Research Station in Norway are trying to develop early rearing technology for haddock (Harmon, 2003). Here in Canada, two government hatcheries were producing juveniles of haddock for grow-out

evaluation at a private salmon culture facility, located at Deer Island, New Brunswick. After producing 180 tones in 2002, the project was discontinued when the private partner lost interest.

The Canadian aquaculture industry is almost entirely focused on salmon culture and has long recognized the need to diversify into alternative species. Both haddock and Atlantic cod are regarded as suitable alternate species for cold-water aquaculture in Atlantic Canada. Despite having good biological potential for culture, consistent low survival rates during the larval rearing still represent the major bottleneck in the mass production of both species. As with other marine species, problems related to nutrition of larvae are considered responsible for the majority of the mortality observed during the larviculture (Brown et al., 1995).

I.2. Characteristics of Marine Finfish Larvae Nutrition

The morphological and physiological transformations that fish larvae undergo during their development make the characteristics of larvae nutrition quite distinct from the nutrition during other life stages. In general, fish larvae have reduced size, impaired swimming ability, and incomplete or immature digestive systems. Most larval fish start feeding before the digestive system has developed into its adult form. The gut is usually just a simple transparent tube lacking in differentiation. Pyloric ceca have often not yet been formed. In most

cases, gastric glands do not exist and support organs such as the pancreas, gall bladder, and liver are also immature (Rust, 2002). In Atlantic cod for example, at the onset of exogenous feeding (around day four after hatching), the straight gut of the larvae consists of three segments (fore, mid and hindgut), and thus the Atlantic cod gut resembles that of fish larvae without functional stomachs, which will develop into functional organs at the end of metamorphosis (Kjørsvik et al., 1991b). Such larvae are known to have immature digestive mechanisms. These are characterized by lipid absorption and temporary lipid storage in the anterior part of the gut, and food protein ingestion and intracellular digestion by the rectal epithelial cells (Govoni et al., 1986). In addition, the digestive capacity of the larvae is less efficient than that of juveniles and adults. According to Perez-Casanova et al. (2006), in haddock and Atlantic cod larvae, general protease, trypsin-like enzymes, and pepsin-like enzymes are all present in the digestive system as early as at hatch, but a significant increase in these enzymes' activity was observed later in the development. In contrast, general lipase and alkaline phosphatase were present at hatch but the activity levels of these enzymes remain relatively constant through development. Based on these findings, the authors concluded that larvae of both species are capable of digesting protein and lipids at the time of mouth opening. Despite the fact that α-amylase has been reported in pyloric cecae of adult Atlantic cod (Overnell, 1973), Perez-Casanova et al. (2006) were unable to detect α-amylase activity in haddock and Atlantic cod larvae, except on occasions when large amounts of undigested rotifers were

present within the gut. The authors suggested that the prey was responsible for 100% of the carbohydrate digestion in larvae of both species.

Apart from the developmental status of the digestive system and the efficiency of the digestive capacity of first-feeding larvae, the mouth size usually mechanically restricts the size of the food particles which can be ingested. In general, mouth size is correlated with body size, which in turn is influenced by egg diameter and the period of endogenous feeding. In newly hatched Atlantic cod larvae (1-3 days, 4-5 mm), the cranial skeletal structures are simple, few in number, unarticulated and the mouth is formed but not open (Hunt von Herbing, 2001). Furthermore, the Atlantic cod larva's mouth is poorly developed during the first days post-hatch. The anterior part of the upper jaw is devoid of any skeletal elements during the first three days, and movements of the lower jaw were not observed until day four (Kjørsvik et al., 1991b). At first feeding (5-8 days, 5.6-6.3 mm), all feeding structures present at hatching increase in length and diameter. Three major articulations are formed; (1) between the Meckel's cartilage and the quadrate (jaw joint), (2) between the hyomandibula-symplecticum and the cranium, and (3) between the hyoid and hyomandibular cartilages. These joints permit simple movements of the jaw, primarily in the dorso-ventral direction, and a small degree of lateral expansion of the buccal cavity. These three joints and the linkage between the two major arches (buccal and branchial) result in mouth opening and closing and in the onset of first feeding (Hunt von Herbing, 2001). Prey size for first-feeding marine fish larvae is generally between 80 and 200 µm.

As the larvae grow, they will catch increasingly larger prey. The smallest prey eaten by Atlantic cod larvae in a basin was copepod nauplii of 230 μm, while the largest was amphipods of 1560 μm (Kjørsvik et al., 2004).

The natural diet of marine fish larvae is composed of a variety of plankton and zooplankton species. From the initiation of feeding, Atlantic cod larvae are opportunistic predators, which are able to ingest a variety of plankton groups, including algae, protozoans such as tintinnids and oligotrich ciliates, rotifers, polychaete larvae, and copepod eggs and nauplii (Olsen et al., 2004). Haddock larvae often co-occur with Atlantic cod larvae in the North Atlantic Ocean. Field studies indicate that these closely related species have similar feeding habits (Sherman et al., 1981). Atlantic cod are aggressive predators, which feed on large prey items shortly after yolk sac absorption. Haddock are more passive foragers depending largely on less motile prey such as copepod eggs, phytoplankton, and copepod nauplii. After the larvae reach approximately 6.0 mm, both species compete for the same food items (Kane, 1984). Atlantic cod and haddock larvae are generally selective feeders. For example, larvae from both species make a clear distinction between copepod nauplii and other prey categories of smaller size during the early larval development stage. The nauplii are among the largest catchable food items, and may be preferred owing to the higher energy gain compared with smaller zooplankton. Copepod nauplii are the main food item for newly hatched Atlantic cod larvae. Within only 2-3 weeks post-hatch, larger copepodites become more important than copepod nauplii as a food

source (Olsen et al., 2004). Kane (1984) studying the feeding habits of Atlantic cod and haddock larvae on Georges Bank, U.S. Continental Shelf, found that both species preyed heavily upon copepods, with the predominant components of their diet being the different life stages of *Pseudocalanus minutus*, *Oithona similis*, *Calanus finmarchicus*, and *Centropages typicus*. Copepod eggs were the most abundant food item consumed by both larvae (Kane, 1984). In another field study, McLaren and Avendaño (1995) evaluated the distribution of Atlantic cod larvae and plankton on an offshore nursery area on Western Bank, Scotian Shelf, and found that the densities of Atlantic cod larvae and copepods were positively correlated. Analysis of stomach content revealed that Atlantic cod larvae preyed upon at least eight different species of copepods, but copepodites and adults of *Pseudocalanus* spp. were the most abundant identified items. *Pseudocalunus* spp. copepodites were clearly eaten out of proportion to their relative abundance, since the three most abundant items in the plankton (*Microsetella norvegica*, *Oithona similis* and nauplii) were either never or seldom prey (McLaren and Avendaño, 1995). According to Shuvaev (1977) in the North Sea, newly hatched haddock larvae feed primarily on copepod eggs and nauplii below 0.35 mm in size (95-100% in terms of mass and numbers). However, on reaching a length of 6-10 mm, the larvae start feeding on copepodites and adult copepods, with the proportion of nauplii and eggs decreasing to 30%. The main species preyed upon were *Pseudocalanus elongates Oithona similis*, *Temora longicornis*, *Acartia clause*, and *Calanus finmarchicus* (Shuvaev, 1977).

A scarcity of energetically favorable food, such as copepod nauplii, induces non-selective feeding habit in Atlantic cod larvae (van der Meeren and Næss, 1993). Protozoans, such as tintinnids and oligotrich ciliates, are particularly frequent in the natural diet of Atlantic cod larvae. The minimum size of protozoans that Atlantic cod larvae can ingest seems to be 30-40 μm (Olsen et al., 2004).

Under aquaculture conditions, the food web structure in the larval tanks for the production of marine fish is among the simplest possible, normally having only four functional and interacting food web components present: microalgae, bacteria, rotifers or *Artemia*, and the fish larvae. Microalgae are not strictly necessarily for all species during larval feeding, but there is extensive evidence that the inclusion of algae in the tanks during the first days of larviculture enhance the survival, growth and food conversion index of more than 40 species of marine finfish (Muller-Feuga et al., 2003). The reasons for the apparently positive effects of microalgae on marine fish larvae are not fully understood. Several hypotheses have been proposed to explain this phenomenon, ranging from the stabilization or improvement of water quality and light contrast (Næs et al., 1992) to the role of direct or indirect nutrition (van der Meeren, 1991; Reitan et al., 1993) to the regulation of bacterial opportunistic populations by antibacterial or probiotic action (Skjermo and Vadstein, 1993). So far, only two zooplankton families have been produced regularly at a feasible cost for the production of marine finfish larvae: the rotifer *Brachionus* spp. and the small

branchiopod crustacean *Artemia* spp. Although first identified as a pest in the pond culture of eels in Japan during the fifties and sixties, rotifers have been used as live feed for cultured fish since the early seventies. Currently, there is a well-established technology for the mass production of rotifers applied worldwide. The availability of large quantities of rotifers has contributed to the successful production of more than 60 marine finfish species (Dhert, 1996). Major finfish species produced today using rotifers during the early developmental stages include yellowtail (*Seriola quinqueradiata*), red sea bream (*Pagrus major*), Asian sea bass (*Lates calcarifer*), gilthead sea bream (*Sparus aurata*), turbot (*Scopththalmus maximus*) and the European sea bass (*Dicentrarchus labrax*) (Lubzens and Zmora, 2003). The most commonly used species is *Brachionus plicatilis*, but the use of the smaller *Brachionus rotundiformis* in marine finfish culture has increased recently (Olsen, 2004). In the production protocol of Atlantic cod currently adopted at the Ocean Sciences Centre, Newfoundland, the rotifer *Brachionus plicatilis* is supplied to the larvae from day 4 up to day 48 post-hatch (Brown et al., 2003). Similarly, the feeding protocol adopted for the culture of haddock larvae also makes use of the rotifer *Brachionus plicatilis* between 2 and 25 days post-hatch (Harmon et al., 2002). Although not part of the natural diet of marine fish larvae, the use of nauplii of the brine shrimp *Artemia* is regarded as being responsible for the breakthrough in the culture of commercially important species (Sorgeloos, 1980). In its natural environment, when the conditions are not favorable, *Artemia* produces dormant embryos—so-called

"cysts"—that are available year-round in large quantities along shorelines of hypersaline lakes and coastal lagoons scattered throughout the tropical, subtropical and temperate climatic zones (Van Stappen, 1996). The cysts are metabolically inactive and do not develop further as long as they are kept dry. This unique characteristic of the life cycle of *Artemia* allows commercial production through harvesting and processing of the cysts, that are then made available in cans as a convenient "off the shelf" live food. As a result, nauplii of *Artemia* constitute the most widely used live food item in the larviculture of finfish and shellfish, representing approximately 40% of the total aquaculture demand for feeds for early stages (Sorgeloos et al., 2001). When rotifers become too small for the growing larvae to catch, *Artemia* nauplii are normally the choice of replacement. The larviculture of haddock and Atlantic cod are no exceptions, and *Artemia* nauplii are supplied to the larvae of both species from the end of the rotifer phase up to the weaning stage, when the larvae are introduced to artificial inert diets (Harmon, 2003; Brown et al., 2003).

I.3. Lipid Nutrition of Marine Finfish Larvae

The success of larval rearing is greatly influenced by first-feeding regimes and the nutritional quality of the diets used, with dietary lipids being recognized as one of the most important nutritional factors that affect larval growth and survival (Izquierdo et al., 2000). Lipids play major roles in the life history and

physiology of fish. Lipids and their constituent fatty acids are, along with protein, the main organic components of body tissues. Their importance is related to their multiple functions: 1) lipids are the preferred source of metabolic energy for fish, especially in the marine environment, where some species, such as capelin and herring, can accumulate more than 20% of their wet body weight as oil; 2) phosphoacylglycerols and their fatty acid composition have a major and very well-established role in maintaining the structure, permeability, and stability of cellular biomembranes; 3) lipids are a source of essential fatty acids; 4) lipids work as a vehicle for lipid-soluble nutrients such as vitamins; and 5) lipids are source of hormones, and some fatty acids are precursors of eicosanoids: bio-active molecules involved in a variety of biological functions (Takeuchi, 1997; Sargent et al., 1999a; Sargent et al., 1999b; Izquierdo et al., 2000; Sargent et al., 2002; Tocher, 2003).

The dietary requirement of highly unsaturated fatty acids (HUFA[1]) from the ω3 family— namely docosahexaenoic acid (DHA, 22:6ω3) and eicosapentaenoic acid (EPA, 20:5ω3) — for marine fish has long been known (Watanabe, 1993; Sargent et al. 1989; Sargent et al., 2002). More recently, arachidonic acid (AA, 20:4ω6) has also been recognized as an essential fatty acid for marine fish (Bell and Sargent, 1999; Izquierdo et al., 2000). This situation is the logical result of adaptations to a combination of the predominant polyunsaturated fatty acids

[1] The abbreviation "HUFA" will be used in this thesis to refer to fatty acids containing $\geq$ 20 carbon atoms with $\geq$ 4 double bonds in their chain.

(PUFA) in marine food webs and the carnivorous life style of the majority of marine fish. The major producers in marine ecosystems are unicellular algae. Actively growing algae can contain approximately 20% of their dry weight as lipid and 50% of this is present as ω3 PUFA (De Silva and Anderson, 1995). Diatoms tend to be rich in 20:5ω3 and dinoflagellates tend to be rich in 22:6ω3. The Rhodophyta (red algae) can be rich in arachidonic acid as well as ω3 PUFA. Therefore, the PUFA in marine food webs is dominated by ω3 HUFA, having 20:5ω3 and 22:6ω3 in abundance, with 18:3ω3 and 18:2ω6 being less abundant (Sargent and Henderson, 1995). The major consumers of phytoplankton are crustacean zooplankton. The ω3 PUFA are retained by the zooplankton, so they are transferred to planktivorous fish. Carnivorous marine fish consume smaller fish, that are rich in 20:5ω3 and 22:6ω3, derived from phytoplankton via zooplankton, and consequently, have no need to convert their very limited intake of 18:3ω3 to 20:5ω3 and 22:6ω3. Marine fish are considered to lack both the fatty acid Δ^5 and Δ^6 desaturases required for the production of C_{20} and C_{22} HUFA from C_{18} PUFA. According to Buzzi et al. (1996) the desaturases and elongases that act on the ω3 series also act on the ω6 series, therefore making it impossible for marine fish to convert 18:2ω6 to 20:4ω6 through the same pathway. For this reason, 20:5ω3, 22:6ω3 and 20:4ω6 are essential fatty acids for marine fish (Sargent et al., 1989). The marked chemical similarity of 22:6ω3, 20:5ω3 and 20:4ω6 lead to competitive interactions in the biochemical reactions they and their precursors and products undergo. Therefore, the requirement for

any individual PUFA cannot be considerate meaningfully in isolation. Rather, the ratios of all three long chain PUFA, 22:6ω3/20:5ω3/20:4ω6 must be considered, whether at a specific tissue or whole body level (Sargent et al., 1999a).

It was found that the 22:6ω3 contained in eggs of several finfish species, including Pacific cod (*Gadus macrocephalus*) decrease remarkably after hatching until around 10 to 20 days post-hatch. During the same period, the lipid quantity also decreased. The 22:6ω3 quantity in well fed cod larvae per gram wet weight was 6.1 mg on the first day and fell to 1.2 mg on day 15 (Takeuchi et al., 1994). However, in starved fish, 20:5ω3 reduced drastically while the 22:6ω3 quantity remained almost constant (Takeuchi, 1997). These results illustrate the importance of lipids, and more specifically 22:6ω3, during early development in cod. There is a considerable amount of literature concerning the effects of 22:6ω3, 20:5ω3 and 20:4ω6 in the early stages of development of marine fish. It has been shown that these essential fatty acids can affect survival, growth, neural development, stress resistance, pigmentation, schooling behavior, and the incidence of body deformities in marine fish larvae (Bell et al., 1995; Sargent et al. 1999b; Izquierdo et al., 2000; Ishizaki et al., 2001; Gapasin and Duray 2001; Koven et al., 2001; Copeman et al., 2002; Koven et al., 2003). The importance of 22:6ω3 in neural tissues of all vertebrates, including fish, is well established: It is known to be the major component of the brain, eyes, and nervous system of fish (Sargent et al., 2002). Dietary deficiency of 22:6ω3 resulted in larval herring, *Clupea harengus*, having low levels of this fatty acid in their retina, and

consequently, impaired ability to capture prey at natural light intensities (Bell et al., 1995). Ishizaki et al. (2001) demonstrated that 22:6ω3 is a factor that affects the ontogeny of schooling behavior and brain development in larval yellowtail. Yellowtail larvae fed with diets deficient in 22:6ω3 showed dispersion rather than schooling behavior, and had lower survival rates when compared with larvae fed 22:6ω3-rich diets. In addition, the whole brain volume, and the relative volume of regions of the brain associated with visual acuity (tectum opticus) and swimming performance (cerebellum) were significantly smaller in larvae fed with the diet deficient in 22:6ω3 (Ishizaki et al., 2001). Gapasin and Duray (2001) evaluated the effects of live-feed enriched with 22:6ω3 on growth, survival and incidence of opercular deformities in milkfish, *Chanos chanos*. During 25 days of larviculture, only survival was positively affected by 22:6ω3-enriched live food. However, after 60-day extensive culture in earthen nursery ponds, incidence of opercular deformities was significantly lower in milkfish fingerlings that had previously received 22:6ω3-enriched live-feed, when compared to those given an unenriched diet. Furthermore, total length was observed to be significantly longer in 85-day old milkfish juveniles fed 22:6ω3-enriched rotifers and *Artemia* during the larviculture. The elevation of dietary 20:5ω3 level from 0.3 to 1.1%, at constant 22:6ω3 (0.7%) and 20:4ω6 (0.06%) levels resulted in improvement in the growth of gilthead sea bream larvae. However, further elevation of 20:5ω3 to 1.7% at constant 22:6ω3 (1.1%) and 20:4ω6 (0.7%) reduced growth (Izquierdo et al., 2000). Koven et al. (2001, 2003) performed a series of studies with gilthead

sea bream larvae and showed that dietary 20:4ω6 promoted survival in fish exposed to handling stress. However, repetitive salinity change altered the nature of the stress response where dietary 20:4ω6 appeared to upregulate cortisol synthesis resulting in reduced growth and increased mortality (Koven et al., 2001; Koven et al., 2003). Abnormal pigmentation is a common problem in cultured marine flatfish larvae, which can be improved or eliminated with the enhancement of the level of 22:6ω3 in the diet (Sargent et al., 1999b). In larval yellowtail flounder, *Limanda ferruginea*, increased DHA/EPA ratio (8.2 with 28.2% 22:6ω3 and 3.5% 20:5ω3) resulted in improved growth and survival, while elevated 20:4ω6 (7.1%) exerted a negative effect on larval pigmentation (Copeman et al., 2002). According to Sargent et al. (1999b) given sufficient amounts of 22:6ω3 in turbot larval feeds, an excess of 20:5ω3 is not deleterious whereas an excess of 20:4ω6 results in impaired pigmentation and metamorphosis.

The results above exemplify the effects that diets containing inappropriate levels of essential fatty acids can have on larvae of several marine species; but there are still many aspects of fish lipid nutrition that are not well-understood (Sargent et al., 2002). For example, it is not clear which enzyme system is responsible for marine fish not being able to synthesize 22:6ω3, 20:5ω3 and 20:4ω6. Studies with radio-labeled substrates have shown that cultured turbot cells have low C_{18} to C_{20} fatty acid enlogase activity, whereas their Δ^5 fatty acid desaturase activity is functional. In contrast, cells line from gilthead sea bream

had active C_{18} to C_{20} and C_{20} to C_{22} fatty acid enlogase, but low Δ^5 fatty acid desaturase activity (Tocher and Ghioni, 1999). However, it remains unclear if other marine species are unable to convert C_{18} PUFA to C_{20} and C_{22} HUFA or if they have only limited ability to do so. In addition, it is not known if the findings observed in cultured cells will be confirmed *in vivo*, because there is no certainty that enzymes in the PUFA elongation/desaturation pathway are expressed in culture cells exactly as they are in living organisms (Tocher, 2003). Recently, results from studies with scallops and Atlantic cod strongly suggest that ω6 docosapentaenoic acid (ω6DPA, 22:5ω6) acts like an essential fatty acid at least in the early life stages of these two very different species (Milke et al., 2004; Pernet et al., 2005; Parrish, personal communication).

I.4. The Nutritional Value of Copepods, Rotifers and *Artemia* to Marine Finfish Larvae

The nutritional quality of copepods is generally accepted to be superior to rotifers and *Artemia* and believed to be ideal for marine finfish larvae. In general marine pelagic copepods are rich in protein (up to 82% dry weight) and have a well balanced amino acid profile, with the exception of methionine (1.6-1.8% crude protein) and histidine (1.3-1.9% crude protein) (Delbare et al., 1996). The energy content ranges from 9 to 31 J mg^{-1} dry weight, being generally higher in species from high latitudes (Støttrup, 2003). The lipid content of copepods is

affected by several factors such as latitude, food availability and season, with a range of 2-61% in low and medium-latitude species and 8-73% in high-latitude species (Båmstedt, 1986). High-latitude species need to store lipids to maintain fecundity in times of food scarcity, and therefore, these species are richer in energy. In addition, lipid storage also serves as a buoyancy aid (Sargent and Henderson, 1986). In general, lipid levels are higher in newly hatched nauplii than in later nauplii and stage I copepodites (Støttrup, 2003). The low lipid levels in the later stages imply a high proportion of phospholipids and consequently high levels of PUFA. Storage lipids in the late copepodite stages are accumulated in the form of wax ester or triacylglycerol (Hagen, 1988). Marine copepods contain very high levels of ω3 HUFA, in particular 20:5ω3 and 22:6ω3, which can be present in levels as high as 45-60% of total fatty acids (Olsen, 2004). These fatty acids are obtained mainly from their phytoplanktonic diet, although copepods are able to accumulate 20:5ω3 and 22:6ω3 despite low HUFA levels in the diet (Støttrup, 2003). A comparison between mixed species of copepods and Atlantic cod larvae revealed that copepods have levels of 22:6ω3, 20:5ω3 and 20:4ω6 (32.2, 12.1 and 1.0% total fatty acids, respectively) very close to those observed in the larvae (30.2, 15 and 1.8% total fatty acids, respectively) (Sargent et al., 1999b). Calanoid copepods, such as *Temora longicornis* and *Eurytemora* sp., have even higher levels of 22:6ω3 (40-45%) and 20:5ω3 (21-24%) in their fatty acid composition (Evjemo and Olsen, 1997). These findings reinforce the idea that copepods are the ideal live-feed for marine

fish larvae; however, their mass culture is very difficult. The application of copepods to larval rearing is largely restricted to extensive systems where wild zooplankton are collected using filter nets and fed either directly or allowed to proliferate in open outdoor ponds prior to being used as live-feed (Søttrup and Norsker, 1997). The intensive production of copepods in fish hatcheries is still in its infancy, and is normally characterized by small scale, usually in small volumes of one to a few litres, and limited duration (Støttrup, 2000).

According to Lubzens et al. (1989) the nutritional value of rotifers depends on their dry weight, caloric value and chemical composition. In general, the protein, lipid and carbohydrate contents range from 28 to 63%, 9 to 28%, and 10.5 to 27% dry weight, respectively (Lubzens and Zmora, 2003). The protein content of well-fed rotifers is significantly higher than starved rotifers, however, the amino acid content is very stable and is unaffected by either food ration or type of food provided (Olsen, 2004). The caloric value depends on the diet, and ranged from 1.3×10^{-3} cal per rotifer fed on baker's yeast, to 2.0×10^{-3} cal per rotifer enriched for 6 h with a formulated enrichment diet (Fernandez-Reiriz et al., 1993). The lipid classes of rotifers are constituted by 34-43% of phospholipids and 20-55% of triacylglycerols, with small amounts of sterols and free fatty acids (Lubzens and Zmora, 2003). The fatty acid profile of rotifers is highly dependent on their diet. Rotifers cultivated solely on baker's yeast, *Saccharomyces cerevisiae*, are nutritionally inadequate for marine fish larvae, as they lack appropriate amounts of 20:5ω3, 22:6ω3 and 20:4ω6 (Dhert, 1996; Sargent et al.,

1997; Lubzens and Zmora, 2003). Even rotifers fed on algae can be deficient in ω3 HUFA, depending on the algae of choice. For instance, high-density mass culture of marine rotifers using commercial condensed *Chlorella* is a common practice in marine finfish larvicultures in Japan. However, rotifers cultured with freshwater *Chlorella* contain almost no ω3 HUFA in their body composition (Yoshimatsu et al., 1997). The total lipid content and the fatty acid composition of rotifers vary depending on their growth rate (Olsen, 2004). The lipid content per dry weight of fast growing cultures was 40% lower than low growth cultures, whereas the individual content of lipids in the rotifers was constant for both cultures (Øie and Olsen, 1997). Although lipid per individual remains constant and does not depend on growth rate, the fatty acid composition shows great variation. The ω3 HUFA content decreases when growth rate increases, whereas the percentages of monounsaturated and saturated fatty acids become higher. In particular, 22:6ω3 and 20:5ω3 show a substantial reduction, with 22:6ω3 decreasing twice as fast as 20:5ω3 (Øie and Olsen, 1997; Olsen, 2004). The authors suggested that rotifers growth rate or nutritional state affect their metabolism of individual fatty acids differently.

The biochemical composition of newly hatched *Artemia* shows less variation than rotifers. Although strain, geographical location, and harvesting batch will account for some variation, these can be controlled by standardization of purchase practices and routine protocols (Olsen, 2004). Typically, the protein content of newly hatched *Artemia franciscana* nauplii is 45.5% dry weight,

whereas the lipid and ω3 HUFA contents are 20 and <0.5%, respectively (Evjemo and Olsen, 1999). The protein and amino acid profiles show less fluctuation between strain and life stages than do lipids (Dhont and Van Stappen, 2003). Nauplii contain lower levels of free amino acids when compared to wild copepods (Tonheim et al., 2000). Even so, the levels of the 10 amino acids that are considered essential for fish larvae are generally not a problem, but sulphur amino acids, like methionine, are the first limiting amino acids (Helland et al., 1999). *Artemia* nauplii have several proteolytic enzymes, are highly soluble and most of the protein is present as low molecular weight peptides and free amino acids, making their protein easy to digest by fish larvae (Merchie, 1996). The lipid content and fatty acid profile of *Artemia* adults and their offspring clearly reflects the composition of the parental diet, regardless of the strain (Lavens et al., 1989; Navarro and Amat, 1992). As with rotifers, *Artemia* nauplii contain markedly low levels of 22:6ω3, 20:5ω3, and 20:4ω6. According to Léger et al. (1986), oleic acid (18:1ω9) is often the most abundant fatty acid. Together with palmitic (16:0) and palmitoleic acids (16:1ω7), it accounts for 40-60% of the total fatty acids present in *Artemia*. Levels of linoleic acid (18:2ω6) show great variation, ranging from 0.4 to 33.6% (Léger et al. 1986). The levels of 20:5ω3 seem to be inversely proportional to linoleic acid, ranging from zero to 44.7% (Dhont and Van Stappen, 2003). *Artemia* tend to catabolise 22:6ω3 selectively at a far higher rate than other fatty acids, possibly retro-converting it to 20:5ω3 (Navarro et al.,

1999). *Artemia* nauplii typically have very low 22:6ω3 levels, with values ranging from zero to 0.4% of the total fatty acids (Dhont and Van Stappen, 2003).

The organisms that are part of the natural diet of marine fish larvae have feeding behaviour as either filter-feeders or micropredators, feeding on a wide diversity of bacteria, detritus, phytoplankton and/or smaller zooplankton (Omori and Ikeda, 1984). This high diversity of food organisms of different biochemical composition provides a good likelihood of meeting all nutritional requirements of the larvae. Although live-feed organisms cultivated in fish hatcheries only provide sub-optimum nutrition, these live feeds are preferred over natural plankton because of their availability, cost-efficiency, simplicity, as well as versatility in application (Sorgeloos and Léger, 1992; Lavens et al., 1995).

A range of techniques has been developed to manipulate and/or supplement live food organisms produced for aquaculture use. Taking advantage of the filter-feeding habit of rotifers and *Artemia*, a variety of algae and home-made or commercial products have been used to enhance their protein, vitamins, lipids and fatty acid composition in a process commonly known as enrichment (Coutteau and Sorgeloos, 1997). This improved nutrition has resulted in significant progress in larval culture performance for many cultured finfish species. Nevertheless, the technology for the intensive culture of Atlantic cod and haddock is still under development, and the practices adopted for the first feeding of these species are based on protocols developed for other marine species.

I.5. Objectives and Scope of the Study

The main objective of this thesis is to test differently lipid-enriched rotifers and *Artemia* during the first feeding of Atlantic cod and haddock, and evaluate their effects on early growth, survival and lipid composition of these two gadoids.

Chapter II examines the use of differently enriched rotifers during the early development of Atlantic cod larvae. Chapter III follows on from Chapter II, evaluating the effects of differently enriched *Artemia* as live-food for the late-stages of development of Atlantic cod larvae. These studies represent a "fine tunning" in the Atlantic cod production protocol adopted at the ARDF/OSC, and are one of the first attempts to evaluate products that are commercially available for the enrichment of live-feed during the larviculture of Atlantic cod. They are also innovative for testing the enriched live-food under mass production circumstances in the hatchery, rather than in laboratory conditions. In addition, these studies consider the importance of ω6DPA (22:5ω6) for the nutrition of Atlantic cod larvae, a fatty acid that so far, has been practically neglected in nutritional studies involving marine finfish larvae.

Chapter IV evaluates differently enriched rotifers during the early developmental stages of haddock larvae. Chapter V continues the experiment described in Chapter IV, examining the use of differently enriched *Artemia* during the larviculture of haddock. Similarly to the studies with Atlantic cod, these studies are the first to evaluate the use of enriched live-food during the rotifer and

Artemia phases of haddock larviculture, being performed in the hatchery under mass production circumstances.

In Chapter VI the general summary of the thesis, a comparison of the main results of each species, and some suggestions for future research are presented.

The specific objectives are as follows:

1. To evaluate the efficiency of different commercial products on the lipid enrichment of rotifers (Chapters II and IV).

2. To examine the effects of differently enriched rotifers on the early growth, survival and lipid composition of Atlantic cod larvae (Chapter II).

3. To evaluate the efficiency of different commercial products on the lipid enrichment of *Artemia* sp. (Chapters III and V).

4. To examine the effects of differently enriched *Artemia* on the growth, survival and lipid composition of Atlantic cod larvae (Chapter III).

5. To examine the effects of differently enriched rotifers on the early growth, survival and lipid composition of haddock larvae (Chapter IV).

6. To examine the effects of differently enriched *Artemia* on the growth, survival and lipid composition of haddock larvae (Chapter V).

CHAPTER II

A comparison among differently enriched rotifers (*Brachionus plicatilis*) and their effect on Atlantic cod (*Gadus morhua*) larvae early growth, survival and lipid composition.

II.1. Introduction

Interest in the intensive production of Atlantic cod (*Gadus morhua* L.) in Canada began in the early 1990s, driven by a reduced supply from the wild fisheries and consequently, high market prices. In addition, there was a perceived need to develop alternate species to salmon culture, and Atlantic cod has been shown to be a suitable candidate. Since the first attempts to rear newly hatched larvae at the Aquaculture Research Development Facility (ARDF) at the Ocean Sciences Centre (OSC) in St. John's, Newfoundland, considerably progress has been achieved, but low survival rates through larviculture are still a challenge for the successful production of this species. The current production protocol used in Newfoundland is based on use of rotifers (*Brachionus plicatilis*) as live-feed during first feeding. Rotifers are well known for being poor in polyunsaturated fatty acids (PUFA). Their content of eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA, 22:6ω3), and arachidonic acid (AA, 20:4ω6) — considered essential fatty acids for marine fish— are normally low when compared to the levels found in copepods, the main food item in the natural diet of marine larvae (Sargent et al., 1999b; Bell et al., 2003). The nutritional aspects of rotifers have received increased attention in the past 15 years (Rainuzzo et al., 1989; Fernadez-Reiriz et al., 1993; Rodríguez et al., 1996; Øie and Olsen, 1997; Lubzens et al., 1997; Dhert et al., 2001; Ando and Kobayashi, 2004; Palmtag et al., 2006) and several commercial products are now available to enhance lipid, protein, and vitamin contents in rotifers (Coutteau and Sorgeloos, 1997, Sorgeloos et al., 2001; Dhert et al., 2001;

Harel et al., 2002). Nevertheless, only a few recent studies compared and evaluated optimal rotifer enrichment procedures for Atlantic cod (O'Brien-MacDonald et al., 2006; Park et al., 2006). Results from these studies suggest that during the rotifer phase, Atlantic cod larvae require a diet that can deliver not only high energy levels, but also phospholipids (PL), and particularly, a high DHA/EPA ratio. Other studies have shown that the enrichment of live-feed can affect survival rates, growth, pigmentation and stress resistance in marine finfish larvae (Kanazawa, 1997; Rodríguez et al., 1997, Ishizaki et al., 2001, Copeman et al., 2002; Koven et al., 2003).

The objective of the present study was to evaluate the effects of commercial products used for the enrichment of rotifers on the growth, survival and lipid composition of Atlantic cod larvae.

II.2. Material and Methods

II.2.1. Rotifer culture

Rotifers were cultured in 3,000 litre (L) rectangular tanks on a combination of baker's yeast (0.5 g million^{-1}, four times a day), live *Nannochloropsis* sp. (20 litre at 2-3 x 10^6 cells mL^{-1}, once a day), and *Nannochloropsis* sp. paste (10 g at 68 x 10^9 cells mL^{-1}, once a day), on a 5 day cycle. From day 1 to day 3, Cultured Selco® (Inve Americas, Salt Lake City, UT, USA) was added to the culture tank (33.3 g day^{-1}) as supplemental food. The culture started with a volume of 900 L, a

salinity of 15 psu, and a density of 17 x 10^4 rotifers L^{-1}; and progressively increased in volume (final volume of 3,000 L) and salinity (final salinity 25 psu). The initial temperature was 25°C, which progressively decreased to 21°C over the five days of culture. The final density of the culture was 13 x 10^4 rotifers L^{-1}. Population and egg counts were performed daily on a number per millilitre basis. After five days, rotifers were removed from the tank, washed, concentrated, and placed in a 300 L conical tank for enrichment.

II.2.2. Larval rearing and rotifer enrichments

Newly hatched cod larvae were stocked in 3,000 L tanks at a density of 50 larvae L^{-1} (two replicates per treatment), and reared up to 37 days post hatch (dph), which is equivalent to 443.2 degree-days (dd), on rotifers enriched differently. Water management and feeding schemes followed previous protocols outlined in Brown et al. (2003). Water temperature ranged from 11 to 13°C over the course of the experiment. Three different treatments were tested using commercially available products as a single enrichment or in combination with algae paste. The products tested differed in their proximate and lipid composition (Tables II.1 and II.2). The treatments were: 1) AlgaMac 2000® (Aquafauna Bio-Marine Inc., Hawthorne, CA, USA) 2) AquaGrow® Advantage (Advanced

BioNutrition Corp., Columbia, MD, USA), and 3) Instant Algae®[2] (*Pavlova* sp. paste) (Reed Mariculture, Campbell, CA, USA) combined with AlgaMac 2000®.

Table II.1. Proximate composition of the enrichments tested.

	Enrichments		
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. paste
Proximate composition[1] (% dry weight basis)			
Protein	20.2	10.0	4.6
Lipids	38.1	23.0	1.76
Carbohydrate	17.1	9.0	1.9-2.1
Ash	20.4	44.0	*
Moisture	4.2	4.0	91.0
Vitamin A (IU/100 g)	<100	737.5	*
Vitamin C (mg/100 g)	4.8	*	*
Vitamin D (IU/100 g)	457.0	*	*
Vitamin E (IU/100 g)	12.0	*	*
Calcium (%)	*	0.3	*
Potassium (%)	*	0.6	*
Phosphorus (%)	*	0.2	*

[1]Values according to manufacturer's information. *N.b.* some of this information has changed over time.

* Information not available.

[2] The commercial name Instant Algae® refers to several algae species and/or a mixture of different species, and for this reason, will be replaced by *Pavlova* sp. in this thesis.

Rotifers were enriched in 300 L conical tanks at a density of $4x10^5$ rotifers L^{-1}, at 22°C for a period of 24 hours. Enrichments were added to the rotifer tanks twice in an eight-hour period, at 9:00 *a.m.* and 5:00 *p.m.*, with the amounts added each time as per manufacturer's directions. Larvae were fed twice a day, between 9:00 and 10:00 *a.m.*, and 4:00 and 5:00 *p.m.* Larvae from treatment 3 were fed with *Pavlova* sp.-enriched rotifers during the morning and AlgaMac 2000 + *Pavlova* sp.-enriched rotifers during the afternoon. For this treatment, rotifer batches were enriched with 8 mL of *Pavlova* sp. paste per $15x10^6$ rotifers (larvae morning feeding) and a combination of 8 mL of *Pavlova* sp. paste and 2.25 g of AlgaMac 2000 per $15x10^6$ rotifers (larvae afternoon feeding). Enriched rotifers were supplied to the larvae from 1 to 37 dph (11 to 443 dd) at a density of 4,000 L^{-1}. In order to maintain the desired prey concentration within each experimental tank, before each feeding, three 10 mL water aliquots were sampled from each tank. The number of prey in each sample was counted and the average number was used to adjust prey concentrations as needed.

Table II.2. Total lipid, lipid class and fatty acid composition of the enrichments tested.

	Enrichments		
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. paste
Total lipid (mg g^{-1} dw)	204.3 ± 3.4[a]	168.7 ± 8.1[b]	47.5 ± 3.0[c]
Lipid Class (% total lipids)			
Hydrocarbons	0.1 ± 0.0[a]	1.0 ± 0.1[b]	0.4 ± 0.0[c]
Steryl Esters/Wax Esters	3.6 ± 0.1[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]
Ketones	6.4 ± 0.5[a]	3.7 ± 0.3[b]	0.5 ± 0.1[c]
Triacylglycerols	70.2 ± 0.8[a]	64.5 ± 2.0[b]	34.4 ± 0.1[c]
Free fatty acids	1.2 ± 0.2[a]	2.9 ± 0.1[a]	13.2 ± 1.7[b]
Alcohols	3.5 ± 0.4[a]	1.1 ± 0.1[b]	1.9 ± 0.1[b]
Sterols	0.6 ± 0.1[a]	4.0 ± 0.2[b]	0.8 ± 0.1[a]
Acetone Mobile Polar Lipids	7.0 ± 0.8[a]	9.3 ± 0.3[b]	23.2 ± 0.5[c]
Phospholipids	5.4 ± 0.7[a]	13.5 ± 0.8[b]	25.5 ± 2.1[c]
Fatty acids (% total fatty acids)			
14:0	17.5 ± 0.1[a]	17.3 ± 0.1[a]	9.9 ± 1.1[b]
16:0	36.2 ± 0.0[a]	17.3 ± 0.3[b]	10.5 ± 1.5[c]
18:0	0.9 ± 0.0[a]	0.6 ± 0.0[b]	0.3 ± 0.1[c]
21:0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	2.0 ± 0.4[b]
ΣSFA[1]	56.0 ± 0.2[a]	27.3 ± 0.5[b]	25.5 ± 1.8[b]
16:1ω7	3.9 ± 0.1[a]	1.7 ± 0.0[b]	5.6 ± 0.2[c]
18:1ω9	0.0 ± 0.0[a]	8.7 ± 0.1[b]	13.6 ± 0.6[c]
18:1ω7	2.1 ± 0.0[a]	0.0 ± 0.0[b]	1.3 ± 0.1[c]
ΣMUFA[2]	6.4 ± 0.1[a]	2.0 ± 0.2[b]	24.4 ± 0.9[c]
18:2ω6	0.0 ± 0.0[a]	6.0 ± 0.1[b]	3.2 ± 0.1[c]
18:3ω3	0.0 ± 0.0[a]	0.0 ± 0.0[a]	1.9 ± 0.0[b]
18:4ω3	0.2 ± 0.5[a]	0.1 ± 0.0[a]	5.5 ± 0.1[b]
18:5ω3	0.0 ± 0.0[a]	0.0 ± 0.0[a]	6.7 ± 0.1[b]

20:3ω6	0.2 ± 0.0[a]	0.0 ± 0.0[a]	1.2 ± 0.2[b]
20:4ω6 (AA)	0.7 ± 0.0[a]	0.0 ± 0.0[b]	0.6 ± 0.3[a]
20:4ω3	0.5 ± 0.1[a]	0.0 ± 0.0[b]	0.3 ± 0.1[a]
20:5ω3 (EPA)	0.9 ± 0.0[a]	0.3 ± 0.0[b]	1.4 ± 0.1[c]
22:5ω6 (ω6DPA)	8.2 ± 0.1[a]	0.0 ± 0.0[b]	3.0 ± 0.2[c]
22:5ω3	0.2 ± 0.1[a]	0.6 ± 0.0[a]	0.5 ± 0.1[a]
22:6ω3 (DHA)	26.2 ± 0.4[a]	47.4 ± 0.2[b]	23.0 ± 1.0[c]
ΣPUFA[3]	37.7 ± 0.2[a]	51.2 ± 0.8[b]	50.0 ± 2.5[b]
Σω3	28.4 ± 0.1[a]	50.7 ± 0.5[b]	39.7 ± 1.7[c]
Σω6	9.2 ± 0.2[a]	0.6 ± 0.3[b]	9.2 ± 1.0[a]
ω3/ω6	3.1 ± 0.0[a]	87.5 ± 1.2[b]	4.5 ± 0.3[a]
DHA/EPA	30.0 ± 1.6[a]	156.7 ± 2.0[b]	16.8 ± 0.6[c]
EPA/AA	1.3 ± 0.1[a]	0.0 ± 0.0[b]	3.0 ± 0.8[a]

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids, which also includes: *i*15:0, 15:0, *ai*16:0, *i*17:0, *ai*17:0, 20:0, 22:0, and 23:0 at ≤ 0.68% each.

[2] Sum of monounsaturated fatty acids, which also includes: 14:1, 15:1, 16:1ω9, 16:1ω5, 17:1, 20:1ω11, 22:1ω11, 22:1ω9, and 24:1 at ≤ 1.98% each.

[3] Sum of polyunsaturated fatty acids, which also includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:4ω1, 20:2α, 20:2ω6, 20:3ω3, 21:5ω3, and 22:4ω6 at ≤ 1.16% each.

II.2.3. Sampling, growth and survival measurements

Pooled samples of larvae were collected at 1, 7, 15, 21, 30, and 37 dph (equivalent to 11, 79.8, 172.3, 243.2, 356.3, and 443.2 dd, respectively) for dry weight measurements (three replicates $tank^{-1}$). Between 30 (1 dph) and 10 (37 dph) larvae were collected on pre-weighed glass microfibre filters (24 mm diameter) and rinsed with 3% ammonium formate to remove salt. Each filter was placed on 1.5 cm^2 aluminum foil and dried at 60°C for 48 h. Filters were then

stored in a desiccator and re-weighed using a microbalance (UMT2, Mettler Toledo, Switzerland). Data for standard length were obtained by measuring 10 larvae per tank (20 per treatment) with a stereomicroscope and a calibrated eyepiece micrometer. Growth was then calculated as specific growth rate for dry weight (SGR; % d^{-1} = [ln (final dry weight) – ln (initial dry weight)/days] x 100) and for standard length (SGR; % d^{-1} = [ln (final standard length) – ln (initial standard length)/days] x 100). Unfortunately, standard length samples from 37 dph were not available and for this reason, SGR for standard length was calculated using data from 32 dph (382.4 degree-days).

Survival measurements were based on visual observations in order to reduce the stress that would be caused by handling and counting. Therefore, a nominal scale (0 to 5) was used to measure the weekly survival rates. The scale used considered 0 as total mortality (or 0% survival), 1 as poor survival, 2 as reasonable survival, 3 as good survival, 4 as very good survival, and 5 as total survival (or 100% survival). There was a consensus among the ARDF staff that under mass production circumstances in the hatchery, between 30 and 40% of survival at the end of the rotifer phase is considered a good survival rate for Atlantic cod larvae. Once a week, individual tanks were scored three times with a three-hour interval among recordings. Average weekly values were used for each tank.

II.2.4. Lipid analysis

Pooled samples of larvae were collected at 1, 15, 30, and 37 dph for lipid analysis (total lipids, lipid classes, and fatty acid composition). Samples of the experimental enrichments, algae paste and enriched live-feed were collected in triplicate for lipid analysis. Lipid samples were placed in 15 mL vials, covered with chloroform, and stored under nitrogen at -20°C until extraction. Lipids were extracted in chloroform/methanol according to Parrish (1998) using a modified Folch procedure (Folch et al., 1957). Lipid classes were determined using thin layer chromatography with flame ionization detection (TLC/FID) with a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel coated Chromarods and a three-stage development system was used to separate lipid classes. The first separation consisted of a 25-min and 20-min developments in 99:1:0.05 (v/v/v) hexane/diethyl ether/formic acid. The second separation consisted of a 40-min development in 80:20:1 (v/v/v) hexane/diethyl ether/formic acid. The last separation consisted of two 15-min developments in 100% acetone followed by two 10-min developments in 5:4:1 (v/v/v) chloroform/methanol/water. After each separation, the rods were scanned and three chromatograms were combined using T-data scan software (RSS, Bemis, TN, USA). The signal detected in millivolts was quantified using lipid standards (Sigma-Aldrich Corp., Oakville, ON, Canada).

Total lipids were analyzed for fatty acid composition. Fatty acid methyl esters (FAME) were prepared by transesterification with 10% BF_3 in methanol at

85°C for 1.5 h (Morrison and Smith, 1964; Budge and Parrish 2003). The efficiency of this method for esterification of the fatty acids present in acyl lipid classes was determined to be > 90%. A Varian model 3400 GC equipped with a Varian 8100 autosampler was used for fatty acid analysis (Varian, Palo Alto, CA, USA). The column was an Omegawax 320 column, 30 m, 0.32 mm i.d., 0.25 µm film thickness (Supelco, Bellefonte, PA, USA). Hydrogen was used as the carrier gas and the flow rate was 2 mL min^{-1}. The column temperature profile was: 65°C for 0.5 min, hold at 195°C for 15 min after increasing at 40°C min^{-1}, and hold at 220°C for 0.75 min after increasing at 2°C min^{-1}. The injector temperature increased from 150 to 250°C at 200°C min^{-1}. Peaks were detected by flame ionization with the detector held at 260°C. Fatty acid peaks were integrated using Varian Star Chromatography Software (version 5.50) and identification was made with reference to known standards (PUFA 1, PUFA 3, BAME and 37 Component FAME Mix, Supleco Canada, Oakville, ON).

II.2.5. Statistical analysis

Differences in growth between treatments were analyzed using an ANCOVA (analysis of covariance). The General Linear Model (Minitab Version 13.1) was employed and in all cases, the effect of tanks was not significant. Data from treatments were pooled. Lipid classes and fatty acid composition were analyzed using one-way ANOVAs with Tukey's multiple comparison test to compare differences (StatView®, SAS Institute, Cary, NC, USA). Residual versus fitted

values were examined for assumptions of normality, homogeneity and independence, and when necessary, randomization was employed to recompute new p-values. Linear regression was used to describe the relationship between larval dry weight and the concentration of selected fatty acids per individual larva. Significance was set at $\alpha = 0.05$ for all analyses.

II.3. Results

II.3.1. Total lipid, lipid class and fatty acid composition of rotifers

Only rotifers from the AlgaMac 2000 treatment had higher total lipid concentration than unenriched rotifers ($F_{4,9}=36.88$, $p<0.0001$). This treatment also resulted in the highest percentage of acetone mobile polar lipids (AMPL: 30.7 ± 1.5 % total lipids, $F_{4,10}=8.40$, $p=0.0031$) when compared to rotifers from other treatments. The ketone percentage (KET) decreased in all treatments after 24 h of enrichment. Rotifers enriched with AlgaMac 2000 and the combination of *Pavlova* sp. + AlgaMac 2000 showed a triacylglycerol (TAG) percentage significantly higher than unenriched rotifers or the other treatments ($F_{4,10}=7.35$, $p=0.005$). Rotifers enriched with AquaGrow Advantage had the sterol (ST) percentage (7.3 ± 1.3% total lipids), higher than AlgaMac 2000-enriched rotifers (1.6 ± 0.0 %) and *Pavlova* sp. + AlgaMac 2000-enriched rotifers (1.8 ± 0.5 %) ($F_{4,10}=5.36$, $p=0.014$). The phospholipid (PL) percentage decreased significantly in the AlgaMac 2000 and *Pavlova* sp. + AlgaMac 2000 treatments, when compared to unenriched rotifers

($F_{4,10}$=27.59, p<0.0001) (Table II.3). However, when considering the absolute values, there were no significant differences between unenriched rotifers and any of the enriched rotifers, with PL concentrations ranging from 8.3 ± 0.4 mg g^{-1} dry weight in *Pavlova* sp. + AlgaMac 2000-enriched rotifers to 9.4 ± 0.7 mg g^{-1} dry weight in *Pavlova* sp. enriched-rotifers.

Table II.3. Total lipid (mg g^{-1} dry weight) and percentage lipid class composition of rotifers before and after experimental enrichments.

	Treatments				
	Unenriched	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp.	*Pavlova* sp. + AlgaMac 2000®
Total lipid (mg g^{-1} dw)	17.4 ± 0.1^{a}	50.6 ± 6.4^{b}	19.7 ± 1.3^{a}	21.3 ± 1.0^{a}	25.5 ± 0.1^{a}
Lipid Class[1] (% total lipids)					
Hydrocarbons	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}	1.3 ± 0.2^{b}	1.0 ± 0.3^{ab}	1.1 ± 0.4^{b}
Steryl Esters/ Wax Esters	0.0 ± 0.0^{a}	6.3 ± 0.2^{b}	0.0 ± 0.0^{a}	1.2 ± 0.1^{c}	0.0 ± 0.0^{a}
Ketones	17.3 ± 0.5^{a}	4.0 ± 0.2^{b}	2.1 ± 0.4^{c}	11.0 ± 3.4^{c}	0.0 ± 0.0^{d}
Triacylglycerols	15.2 ± 2.5^{a}	35.4 ± 6.4^{b}	29.2 ± 0.1^{b}	10.5 ± 1.8^{a}	26.6 ± 4.6^{ab}
Free fatty acids	4.5 ± 0.2^{a}	3.9 ± 0.9^{a}	4.7 ± 1.3^{a}	7.8 ± 0.7^{ab}	11.2 ± 2.1^{b}
Sterols	5.6 ± 0.7^{ab}	1.6 ± 0.0^{a}	7.3 ± 1.3^{b}	4.9 ± 1.5^{ab}	1.8 ± 0.5^{a}
Acetone Mobile Polar Lipids	7.9 ± 1.7^{a}	30.7 ± 1.5^{c}	10.6 ± 3.3^{ab}	19.2 ± 2.2^{ab}	26.4 ± 6.2^{bc}
Phospholipids	50.2 ± 3.9^{a}	17.9 ± 0.9^{b}	44.1 ± 3.0^{a}	44.2 ± 2.0^{a}	32.8 ± 1.1^{c}

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different (p< 0.05).

In AlgaMac 2000-enriched rotifers and *Pavlova* sp. + AlgaMac 2000-enriched rotifers the percentage of palmitic acid (16:0) was higher ($F_{4,10}$=144.31, p<0.0001) than the other treatments and consequently, in these treatments the sum of saturated fatty acids (ΣSFA) was also higher ($F_{4,10}$=109.70, p<0.0001). AlgaMac 2000 was the only treatment that did not cause the percentage of AA in rotifers to decrease after enrichment (Table II.4). AlgaMac 2000-enriched rotifers also had the percentages of EPA (1.2 ± 0.1 % total fatty acids) and ω6DPA (4.9 ± 0.4 % total fatty acids) significantly higher than the other treatments ($F_{4,10}$=33.80, p<0.0001 and $F_{4,10}$=51.65, p<0.0001, respectively). In contrast, AquaGrow Advantage-enriched rotifers had DHA percentage (18.9 ± 0.8 % total fatty acids) higher than the other treatments ($F_{4,10}$=266.71, p<0.0001). This treatment resulted in rotifers with the highest ($F_{4,10}$=101.56, p<0.0001) sum of fatty acids from the ω3 family (Σω3=21.3 ± 1.6 % total fatty acids); and in the lowest amount of fatty acids from the ω6 family (but not significantly lower than in *Pavlova* sp. + AlgaMac 2000-rotifers). Consequently, rotifers enriched with AquaGrow Advantage had the highest ω3/ω6 ratio (7.4 ± 0.9) ($F_{4,10}$=46.58, p<0.0001). In addition, rotifers from this treatment had the highest DHA/EPA (25.0 ± 0.9) and EPA/AA (3.3 ± 0.2) ratios ($F_{4,10}$=342.32, p<0.0001 and $F_{4,10}$=28.71, p<0.0001, respectively). Rotifers enriched with AlgaMac 2000 and *Pavlova* sp. + AlgaMac 2000 had ω6DPA/AA ratio higher than the other treatments ($F_{4,10}$=13.72, p=0.0005)

Table II.4. Percentage fatty acid composition of rotifers before and after experimental enrichments.

	Treatments				
	Unenriched	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp.	*Pavlova* sp. + AlgaMac 2000®
Fatty acids (% total fatty acids)					
14:0	1.7 ± 0.1^{a}	10.5 ± 0.3^{b}	4.5 ± 0.5^{c}	3.0 ± 0.1^{a}	11.7 ± 0.6^{b}
16:0	6.8 ± 0.2^{a}	23.8 ± 0.6^{b}	11.8 ± 0.8^{c}	9.4 ± 0.3^{a}	23.9 ± 1.1^{b}
17:0	0.6 ± 0.0^{ac}	0.4 ± 0.0^{b}	0.5 ± 0.0^{ab}	0.8 ± 0.1^{c}	0.5 ± 0.1^{abc}
18:0	3.5 ± 0.2^{a}	3.3 ± 0.2^{a}	3.6 ± 0.4^{a}	4.4 ± 0.4^{a}	3.3 ± 0.3^{a}
ΣSFA[1]	17.3 ± 0.8^{a}	40.8 ± 0.7^{c}	24.5 ± 1.1^{b}	22.8 ± 0.4^{b}	43.2 ± 1.9^{c}
16:1ω7	22.4 ± 0.0^{a}	11.1 ± 0.2^{b}	13.2 ± 1.1^{b}	19.3 ± 0.3^{c}	15.7 ± 0.7^{d}
18:1ω11	3.0 ± 0.1^{ab}	1.8 ± 0.1^{a}	2.9 ± 0.3^{ab}	4.0 ± 0.3^{b}	2.4 ± 0.4^{a}
18:1ω9	23.2 ± 0.3^{a}	8.2 ± 0.0^{b}	6.1 ± 0.5^{c}	20.3 ± 0.7^{d}	13.8 ± 0.7^{e}
18:1ω7	7.4 ± 0.3^{bc}	6.3 ± 0.2^{ab}	3.1 ± 0.3^{ab}	9.1 ± 0.9^{c}	6.5 ± 0.2^{ab}
20:1ω9	3.8 ± 0.1^{bc}	2.0 ± 0.1^{a}	3.1 ± 0.3^{b}	4.0 ± 0.3^{c}	2.4 ± 0.2^{a}
ΣMUFA[2]	70.0 ± 1.0^{d}	33.7 ± 0.0^{a}	48.4 ± 1.8^{c}	66.0 ± 0.6^{d}	46.6 ± 1.4^{b}
18:2ω6	6.4 ± 0.9^{c}	1.4 ± 0.0^{a}	2.2 ± 0.2^{ab}	3.7 ± 0.4^{b}	1.4 ± 0.2^{a}
18:3ω3	0.3 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.6 ± 0.1^{b}	0.3 ± 0.1^{ab}
20:3ω6	0.7 ± 0.2^{c}	0.2 ± 0.1^{ab}	0.2 ± 0.0^{ab}	0.6 ± 0.1^{bc}	0.1 ± 0.1^{a}
20:4ω6 (AA)	0.8 ± 0.4^{a}	1.0 ± 0.0^{a}	0.2 ± 0.0^{b}	0.5 ± 0.0^{b}	0.3 ± 0.1^{b}
20:4ω3	0.0 ± 0.0^{a}	0.3 ± 0.0^{c}	0.0 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}
20:5ω3 (EPA)	0.3 ± 0.0^{a}	1.2 ± 0.1^{c}	0.8 ± 0.0^{b}	0.9 ± 0.0^{b}	0.4 ± 0.1^{a}
22:5ω6 (ω6DPA)	0.1 ± 0.0^{a}	4.9 ± 0.4^{c}	0.0 ± 0.0^{a}	0.2 ± 0.0^{a}	2.0 ± 0.5^{b}
22:5ω3	0.0 ± 0.0^{a}	0.4 ± 0.1^{b}	0.3 ± 0.0^{b}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
22:6ω3 (DHA)	0.2 ± 0.0^{a}	14.7 ± 0.8^{c}	18.9 ± 0.8^{d}	0.5 ± 0.0^{ab}	2.8 ± 0.4^{b}
ΣPUFA[3]	13.6 ± 0.3^{a}	25.3 ± 1.5^{b}	26.9 ± 2.8^{b}	10.9 ± 0.7^{a}	9.7 ± 1.4^{a}

Σω3	2.1 ± 0.1[a]	17.0 ± 0.9[b]	21.3 ± 1.6[c]	4.0 ± 0.3[a]	5.0 ± 0.5[a]
Σω6	9.0 ± 0.4[c]	7.6 ± 0.5[c]	3.0 ± 0.4[a]	5.3 ± 0.4[b]	3.0 ± 0.4[a]
ω3/ω6	0.2 ± 0.0[a]	2.2 ± 0.0[b]	7.4 ± 0.9[c]	0.8 ± 0.1[a]	1.7 ± 0.1[b]
DHA/EPA	0.6 ± 0.0[a]	12.4 ± 0.6[c]	25.0 ± 0.9[d]	0.6 ± 0.1[a]	8.9 ± 0.6[b]
EPA/AA	0.3 ± 0.0[a]	1.2 ± 0.1[ab]	3.3 ± 0.2[c]	1.6 ± 0.1[b]	1.8 ± 0.4[b]
DHA + ω6DPA	0.2 ± 0.0[a]	19.5 ± 0.7[b]	19.0 ± 0.8[b]	0.7 ± 0.0[a]	4.8± 0.7[b]
DHA/ω6DPA	2.0 ± 0.3[a]	2.9 ± 0.1[a]	-	2.2 ± 0.4[a]	1.6 ± 0.6[a]
ω6DPA/AA	0.06 ± 0.0[a]	5.1 ± 0.3[b]	0.0 ± 0.0[a]	0.4 ± 0.0[a]	9.5 ± 2.5[a]

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] Sum of saturated fatty acids, which includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 20:0, 22:0, and 23:0 at $\leq$ 1.38% each.

[2] Sum of monounsaturated fatty acids, which includes: 14:1, 15:1, 16:1ω9, 16:1ω5, 17:1, 20:1ω11, 22:1ω11, 22:1ω9, and 24:1 at $\leq$ 2.18% each.

[3] Sum of polyunsaturated fatty acids, which includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:4ω3, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 21:5ω3, and 22:4ω6 at $\leq$ 1.08% each.

II.3.2. Larval survival and growth

The combination of *Pavlova* sp. and AlgaMac 2000 resulted in the best survival during the experimental period. AquaGrow Advantage and AlgaMac 2000 treatments resulted in similar survival over the 37 days of larviculture (Figure II.1).

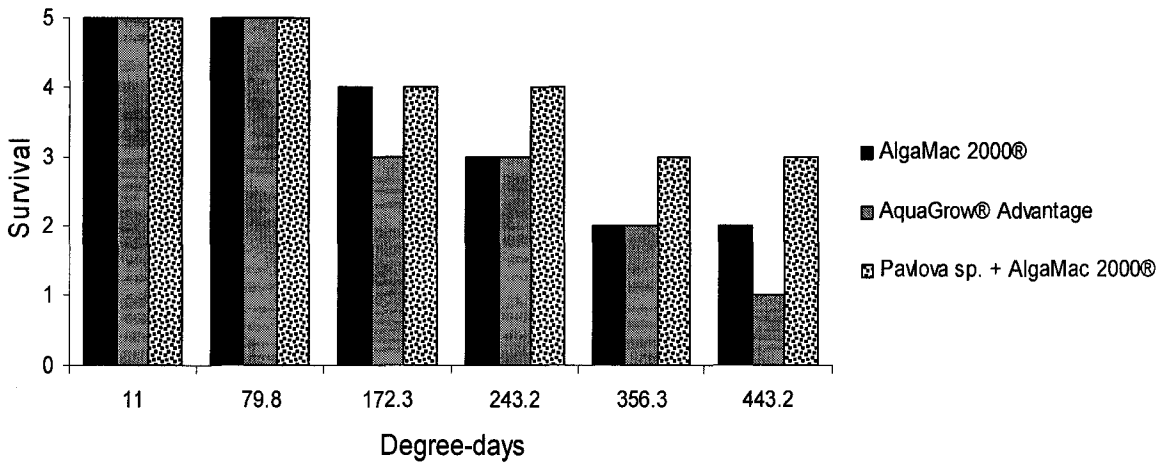

Figure II.1. Survival of Atlantic cod larvae fed differently enriched rotifers at 11, 79.8, 172.3, 243.2, 356.3, and 443.2 degree-days (equivalent to 1, 7, 15, 21, 30, and 37 days post hatch, respectively). Values are expressed as mean of twelve observations per treatment.

At the end of the experiment all treatments produced similar growth curves (Figure II.2A) however, larvae from the *Pavlova* sp. + AlgaMac 2000 treatment (1.50 ± 0.11 mg dry weight) were significantly heavier than larvae from the AquaGrow Advantage treatment (1.03 ± 0.04 dry weight) ($F_{1,1}$=8.32, p=0.006). At 32 dph (382.4 dd), larvae from the AquaGrow Advantage treatment were also shorter than the larvae from the other treatments ($F_{2,57}$=3.66, p=0.031) (Figure II.2B).

Table II.5. Growth of Atlantic cod larvae fed differently enriched rotifers. Dry weights of 10 larvae were determined at 37 dph (443.2 dd). Standard lengths of 20 larvae were determined at 32 dph (382.4 dd). Dry weight SGR were calculated between 1 and 37 dph (n=6). Standard length SGR were calculated between 1 and 32 dph (n=20).

	Treatments		
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®
Dry weight at 37 dph (mg)	1.21 ± 0.07^{ab}	1.03 ± 0.04^{a}	1.50 ± 0.11^{b}
Dry weight SGR (% d^{-1})	6.58 ± 0.20^{ab}	6.29 ± 0.04^{a}	7.10 ± 0.14^{b}
Standard length at 32 dph (mm)	10.23 ± 0.18^{a}	9.60 ± 0.22^{b}	10.23 ± 0.15^{a}
Standard length SGR (% d^{-1})	2.73 ± 0.05^{a}	2.53 ± 0.07^{b}	2.74 ± 0.04^{a}

Values (mean ± SE) with different superscript are significantly different ($p<0.05$).

Pavlova sp. + AlgaMac 2000-larvae showed a dry weight SGR significantly higher than AquaGrow Advantage-larvae ($F_{2,15}=7.79$, $p=0.004$). AquaGrow Advantage-larvae showed a standard length SGR lower than the larvae from other treatments ($F_{2,57}=3.79$, $p=0.02$) (Table II.5). During the three first weeks of larviculture the larval growth was not affected by the different treatments. After 21 dph (243.2 dd), differences in the growth curve produced by the different treatments became noticeable, but these differences were significant only at the end of the experiment (Figures II.2A and II.2B).

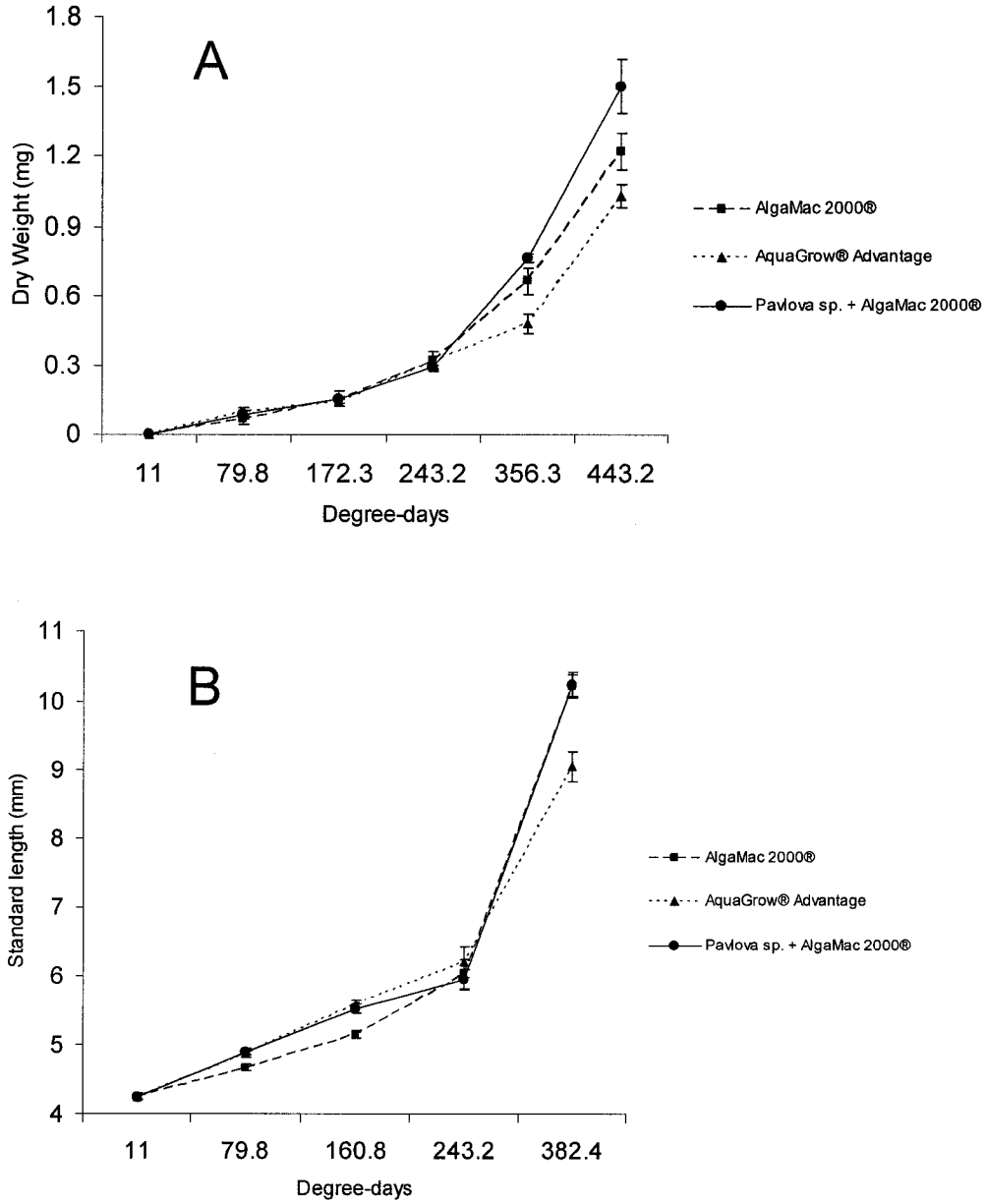

Figure II.2. Dry weight (A) and standard length (B) of Atlantic cod larvae fed differently enriched rotifers during 37 and 32 days of larviculture, respectively. Values expressed as mean ± SE of 6 samples for dry weight (except at 11 dd where n=3) and mean ± SE of 20 samples for standard length.

II.3.3. Total lipid, lipid class and fatty acid composition of larvae

In AquaGrow Advantage and AlgaMac 2000-larvae the total lipid concentration increased during the first two weeks of larviculture, and reached a peak at 15 dph (172.3 dd). After that, the total lipid concentration decreased progressively during the remaining experimental period. In contrast, *Pavlova* sp. + AlgaMac 2000-larvae experienced a constant increase in their total lipid concentration. The same trend was observed in the PL concentrations (Figures II.3A and II.3B). At the end of the experiment, larvae from all treatments significantly increased their total lipid concentration when compared to the levels observed in newly hatched larvae (Table II.6). However in *Pavlova* sp. + AlgaMac 2000-larvae (341.7 ± 6.4 mg g^{-1} dry weight) the total lipid concentration was higher than the other treatments ($F_{3,9}$=155.35, p<0.0001). Furthermore, these larvae also had the highest PL concentration (242.2 ± 6.6 mg g^{-1} dry weight) among all treatments ($F_{3,9}$=127.13, p<0.0001). After 37 days of larviculture, the percentages of hydrocarbons, TAG and AMPL decreased in larvae with all treatments ($F_{3,9}$=7.71, p=0.07; $F_{3,9}$=15.29, p=0.0007; and $F_{3,9}$=502.45, p<0.0001, respectively). The percentage of free fatty acids in AquaGrow Advantage-larvae was higher than in newly hatched larvae ($F_{3,9}$=7.77, p=0.007). AlgaMac 2000-larvae had higher percentage of ST than the larvae from the other treatments or newly hatched larvae ($F_{3,9}$=6.34, p=0.01).

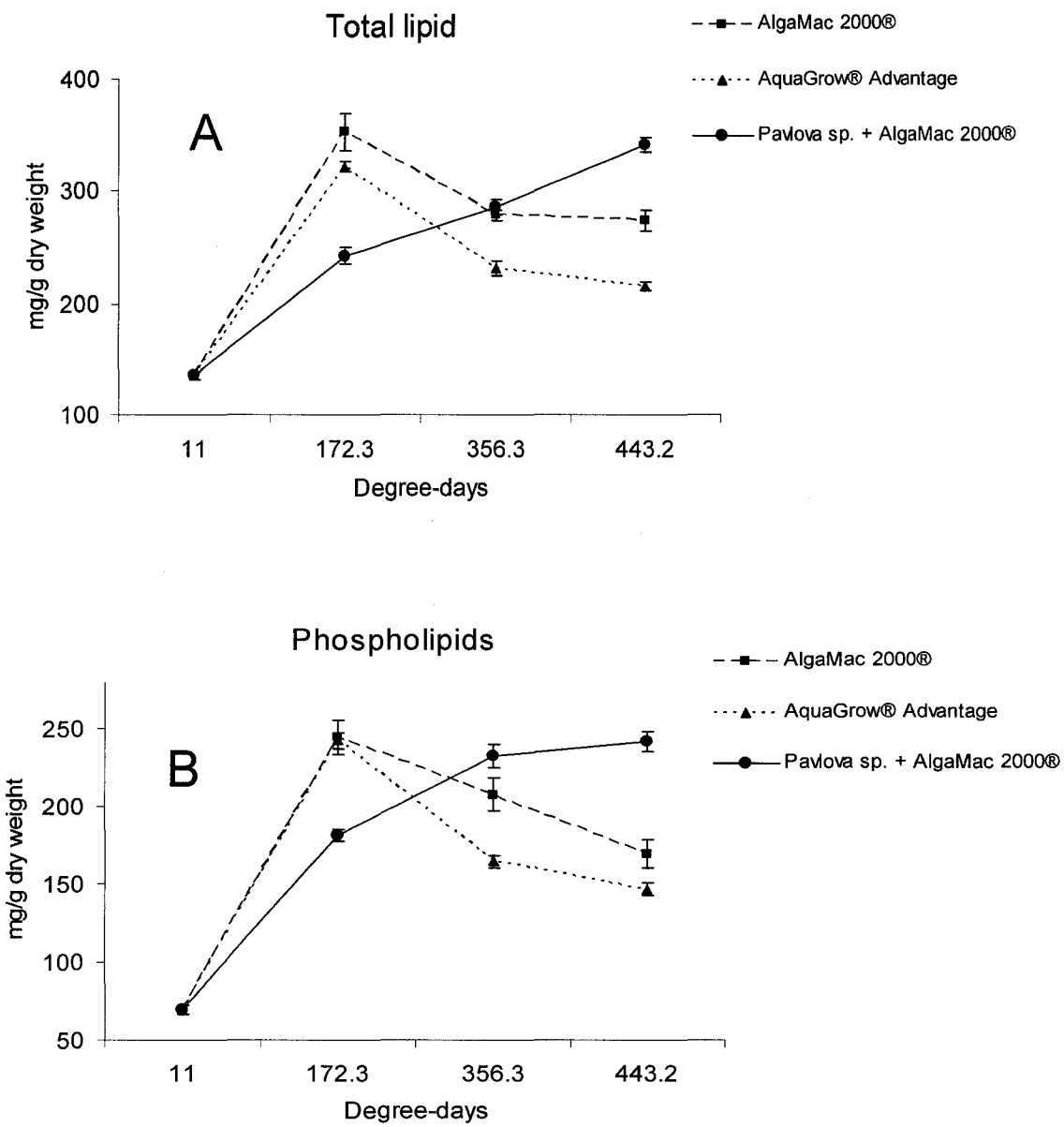

Figure II.3. Changes in total lipid (A) and phospholipids (B) concentrations in Atlantic cod larvae fed on differently enriched rotifers. Samples were collected at 11dd (1dph), 172.3 dd (15 dph), at 356.3 dd (30 dph), and 443.2 dd (37 dph). Values are expressed as mean ± SE of 4 samples, except at 11 dd (1dph) where n=3.

Table II.6. Total lipid (mg g^{-1} dry weight) and percentage lipid class composition of Atlantic cod larvae at the start (newly hatched larvae) and the end of the experiment (37 days post hatch).

		Treatments		
	Newly hatched larvae (1dph)	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®
Total lipids[1] (mg g^{-1} dw)	135.2 ± 3.8[a]	274.4 ± 9.9[b]	215.3 ± 3.5[c]	341.8 ± 6.4[d]
Lipid classes (% total lipids)				
Hydrocarbons	0.6 ± 0.1[a]	0.3 ± 0.0[b]	0.3 ± 0.0[b]	0.2 ± 0.1[b]
Triacylglycerols	4.7 ± 0.7[a]	1.8 ± 0.4[b]	1.3 ± 0.2[b]	2.1 ± 0.3[b]
Free fatty acids	0.7 ± 0.1[a]	1.3 ± 0.2[ab]	1.7 ± 0.1[b]	1.3 ± 0.4[ab]
Sterols	24.6 ± 2.6[a]	33.1 ± 1.5[b]	27.6 ± 1.4[a]	24.3 ± 0.4[a]
Acetone Mobile Polar Lipids	13.5 ± 0.6[a]	1.4 ± 0.2[b]	0.3 ± 0.0[b]	0.7 ± 0.1[b]
Phospholipids	52.5 ± 4.7[a]	61.7 ± 2.0[ab]	68.1 ± 1.3[b]	70.8 ± 0.6[b]

Values (mean ± SE of three replicates, except for AquaGrow® Advantage where n=4) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] May also contain steryl esters, ketones, and alcohols at ≤ 2.47% each.

In AlgaMac 2000-larvae the palmitic acid percentage was comparable to values observed in newly hatched larvae and higher than in the other treatments ($F_{3,11}$=180.39, $p<0.0001$). This treatment resulted in larvae with the highest ΣSFA ($F_{3,11}$=218.28, $p<0.0001$) (Table II.7). *Pavlova* sp. + AlgaMac 2000-larvae showed the highest percentage of 16:1ω7 and the highest sum of monounsaturated fatty acids (ΣMUFA, $F_{3,11}$=52.56, $p<0.0001$ for both analyses). AlgaMac 2000-larvae had the highest percentage of AA and the lowest percentage of EPA ($F_{3,11}$=126.56, $p<0.0001$ and $F_{3,11}$=3733.82, $p<0.000$, respectively). Consequently, larvae from

this treatment showed the lowest EPA/AA ratio ($F_{3,11}$=462.08, p<0.0001). AquaGrow Advantage-larvae had the highest DHA percentage and *Pavlova* sp. + AlgaMac 2000-larvae the highest ω6DPA percentage ($F_{3,11}$=114.07, p<0.0001 and $F_{3,11}$=137.63, p<0.0001, respectively).

Table II.7. Percentage fatty acid composition of Atlantic cod larvae at the start (newly hatched larvae) and end of the experiment (37 days post hatch).

		Treatments		
	Newly hatched larvae (1dph)	AlgaMac 2000®	AquaGrow Advantage®	AlgaMac 2000® + *Pavlova* sp.
Fatty acids (%)				
14:0	1.6 ± 0.1^{a}	1.3 ± 0.1^{ab}	0.9 ± 0.0^{b}	1.4 ± 0.1^{a}
16:0	19.8 ± 0.1^{a}	19.7 ± 0.2^{a}	14.5 ± 0.2^{b}	12.7 ± 0.3^{c}
18:0	2.1 ± 0.1^{a}	8.0 ± 0.2^{c}	7.4 ± 0.3^{bc}	6.7 ± 0.3^{b}
22:0	0.4 ± 0.0^{a}	0.1 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{b}
Σ SFA[1]	25.3 ± 0.2^{a}	31.5 ± 0.0^{b}	25.2 ± 0.3^{a}	23.9 ± 0.2^{c}
16:1ω7	2.3 ± 0.1^{a}	3.8 ± 0.1^{b}	3.4 ± 0.1^{b}	5.3 ± 0.2^{c}
18:1ω9	8.1 ± 0.1^{a}	8.2 ± 0.3^{a}	11.3 ± 0.2^{b}	11.2 ± 0.4^{b}
18:1ω7	3.1 ± 0.0^{a}	5.7 ± 0.2^{b}	4.3 ± 0.1^{c}	5.1 ± 0.1^{d}
20:1ω9	3.4 ± 0.1^{a}	0.7 ± 0.1^{b}	1.2 ± 0.1^{bc}	1.4 ± 0.2^{c}
Σ MUFA[2]	18.7 ± 0.2^{a}	25.0 ± 0.0^{b}	24.7 ± 0.3^{b}	29.3 ± 1.0^{c}
18:2ω6	0.6 ± 0.1^{a}	1.1 ± 0.1^{b}	2.2 ± 0.0^{c}	3.2 ± 0.1^{d}
18:3ω3	0.3 ± 0.0^{a}	0.1 ± 0.0^{b}	0.1 ± 0.0^{b}	0.2 ± 0.0^{a}
18:4ω3	0.4 ± 0.1^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.2 ± 0.1^{b}
20:3ω6	0.0 ± 0.0^{a}	0.6 ± 0.0^{b}	0.7 ± 0.0^{b}	1.0 ± 0.1^{c}
20:4ω6 (AA)	1.0 ± 0.0^{a}	4.2 ± 0.1^{b}	2.2 ± 0.1^{c}	3.5 ± 0.2^{d}
20:4ω3	1.5 ± 0.1^{a}	0.3 ± 0.0^{a}	1.0 ± 0.8^{a}	0.5 ± 0.1^{a}
20:5ω3 (EPA)	16.0 ± 0.2^{a}	2.3 ± 0.0^{b}	3.4 ± 0.1^{c}	3.0 ± 0.0^{c}

22:5ω6 (ω6DPA)	0.2 ± 0.0[a]	4.2 ± 0.3[b]	0.7 ± 0.1[a]	5.7 ± 0.2[c]
22:5ω3	1.3 ± 0.0[a]	0.8 ± 0.0[b]	1.4 ± 0.1[a]	0.6 ± 0.1[b]
22:6ω3 (DHA)	32.1 ± 0.1[a]	28.3 ± 0.6[b]	35.8 ± 0.3[c]	26.0 ± 0.2[b]
Σ PUFA[3]	56.0 ± 0.3[a]	43.2 ± 0.1[b]	50.0 ± 0.6[c]	47.5 ± 0.7[d]
Σ ω3	53.2 ± 0.3[a]	32.0 ± 0.6[b]	42.0 ± 0.6[c]	30.7 ± 0.3[b]
Σ ω6	2.5 ± 0.0[a]	10.5 ± 0.5[b]	6.8 ± 0.1[c]	14.1 ± 0.6[d]
ω3/ω6	21.4 ± 0.3[a]	3.2 ± 0.2[b]	6.3 ± 0.1[c]	2.1 ± 0.0[c]
DHA/EPA	2.0 ± 0.0[a]	12.1 ± 0.0[b]	10.6 ± 0.4[c]	8.6 ± 0.1[d]
EPA/AA	16.6 ± 0.7[a]	0.6 ± 0.0[b]	1.6 ± 0.0[c]	0.9 ± 0.0[c]
DHA + ω6DPA	32.3 ± 0.1[a]	32.6 ± 0.3[a]	36.5 ± 0.3[b]	32.1 ± 0.4[a]
DHA/ω6DPA	179.7 ± 23.3[a]	6.7 ± 0.8[b]	53.3 ± 3.4[c]	4.3 ± 0.1[b]
ω6DPA/AA	0.2 ± 0.0[a]	1.0 ± 0.1[b]	0.3 ± 0.0[a]	1.6 ± 0.0[c]

Values (mean ± SE of four replicates, except for newly hatched larvae where n=3) in the same row not sharing a superscript are significantly different ($p<0.05$).

[1] Sum of saturated fatty acids, which includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 22:0, and 23:0 at $\leq$ 0.68% each .

[2] Sum of monounsaturated fatty acids, which includes: 16:1ω9, 16:1ω5, 17:1, 18:1ω11, 18:1ω5, 20:1ω11, 22:1ω11, and 24:1 at $\leq$ 1.27% each.

[3] Sum of polyunsaturated fatty acids, which includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 20:2α, 20:2β, 20:2ω6, 20:3ω3, and 21:5ω3 at $\leq$ 1.07% each.

The larval ω3/ω6 ratio decreased in all treatments after 37 days of larviculture, but in *Pavlova* sp. + AlgaMac 2000-larvae, this ratio was significantly lower than in the larvae from the other treatments ($F_{3,11}$=415.03, $p<0.0001$). On the other hand, the larval DHA/EPA ratio increased in larvae from all treatments, but *Pavlova* sp. + AlgaMac 2000-larvae showed the lowest increment ($F_{3,11}$=249, $p<0.0001$). In larvae from all treatments, the sum of DHA and ω6DPA remained constant around 32% from hatch up to the end of the experiment, with the exception of AquaGrow Advantage-larvae (Figure II.4), in which this value was

higher than the other treatments at 37 dph (443.2 dd; 36.5 ± 0.3%) ($F_{3,11}$=42.53, p<0.0001). The larval DHA/ω6DPA ratio decreased, whereas the larval ω6DPA/AA ratio increased in all treatments during the experimental period. At 37 dph (443.2 dd), AquaGrow Advantage-larvae had the highest DHA/ω6DPA ratio (53.3 ± 3.4) and the lowest ω6DPA/AA ratio (0.3 ± 0.0) when compared to the larvae from the other treatments ($F_{3,11}$=70.85, p<0.0001 and $F_{3,11}$=299.61, p<0.0001, respectively).

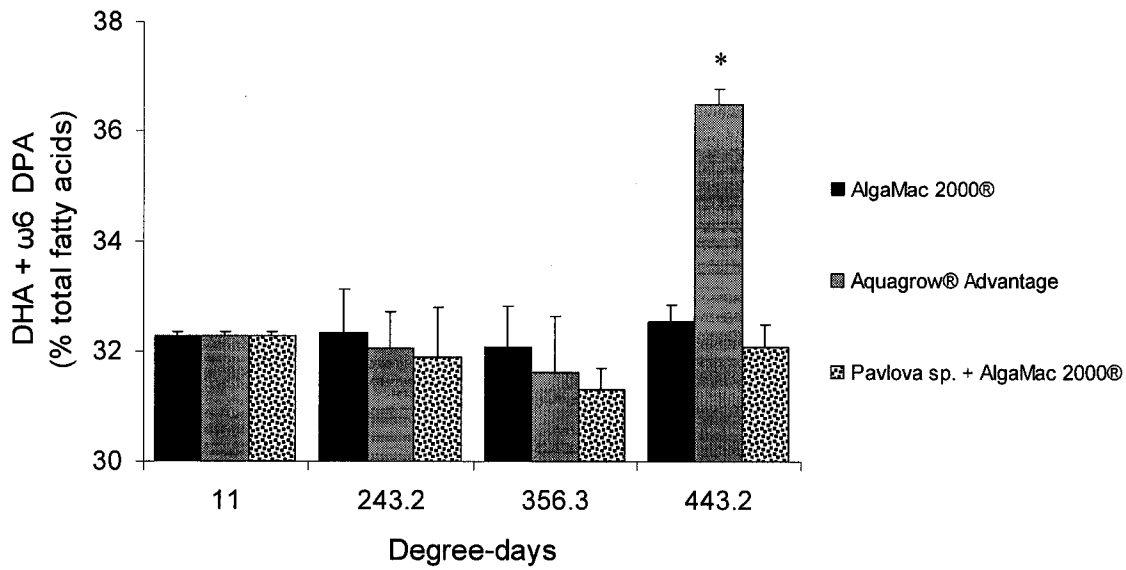

Figure II.4. Levels of 22:6ω3 + 22:5ω6 in Atlantic cod larvae fed differently enriched rotifers at 11, 243.2, 356.3, and 443.2 degree-days (equivalent to 1, 15, 30, and 37 days post hatch, respectively). Values are expressed as mean + SE of 4 samples, except at 11 dd (1dph) where n=3. * Denotes differences at p<0.05.

The larval dry weight correlated positively with larval contents of DHA, AA, and EPA (r^2=88.8%, p<0.001, r^2=77%, p<0.001, and r^2=76%, p<0.001, respectively) over the experimental period. There was also a positive correlation between larval dry weight and the larval content of ω6DPA (r^2=60%, p=0.001); however, this correlation was not as strong as with DHA, AA, and EPA (Figure II.5). Other major variables were tested but did not correlate with larval dry weight.

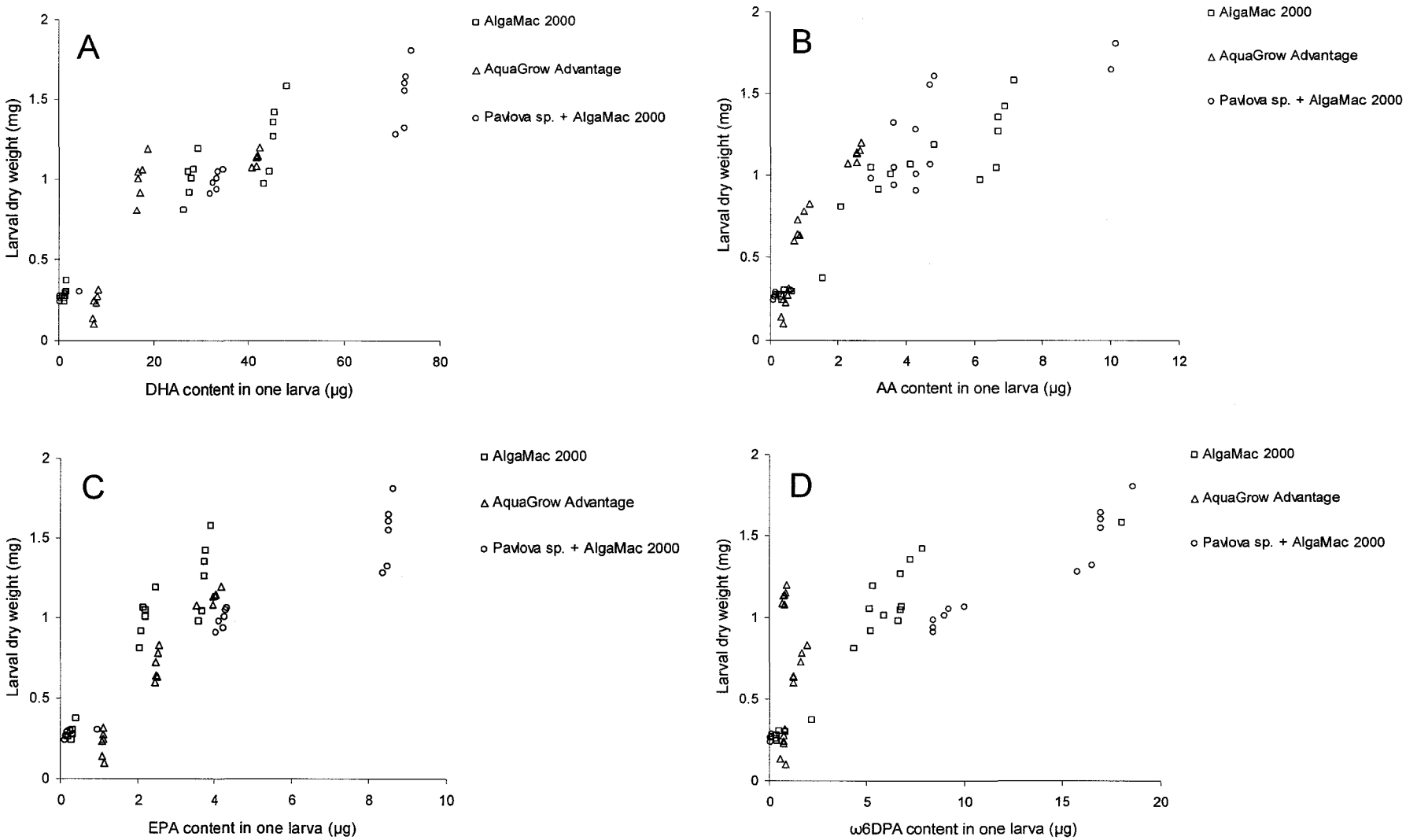

Figure II.5. The relationship between larval dry weight and (A) DHA content of cod larvae, (B) AA content of cod larvae, (C) EPA content of cod larvae, and (D) ω6DPA content of cod larvae. Values of fatty acids expressed as content in one larva (µg).

The larval concentrations of DHA, EPA, AA, and ω6DPA did not reflect the relative composition of enriched rotifers and their diets. Although *Pavlova* sp. and *Pavlova* sp. + AlgaMac 2000-enriched rotifers contained the lowest concentrations of these fatty acids, *Pavlova* sp. + AlgaMac 2000-larvae showed the highest concentrations of DHA, EPA, AA, and ω6DPA (Figures II.6 and II.7).

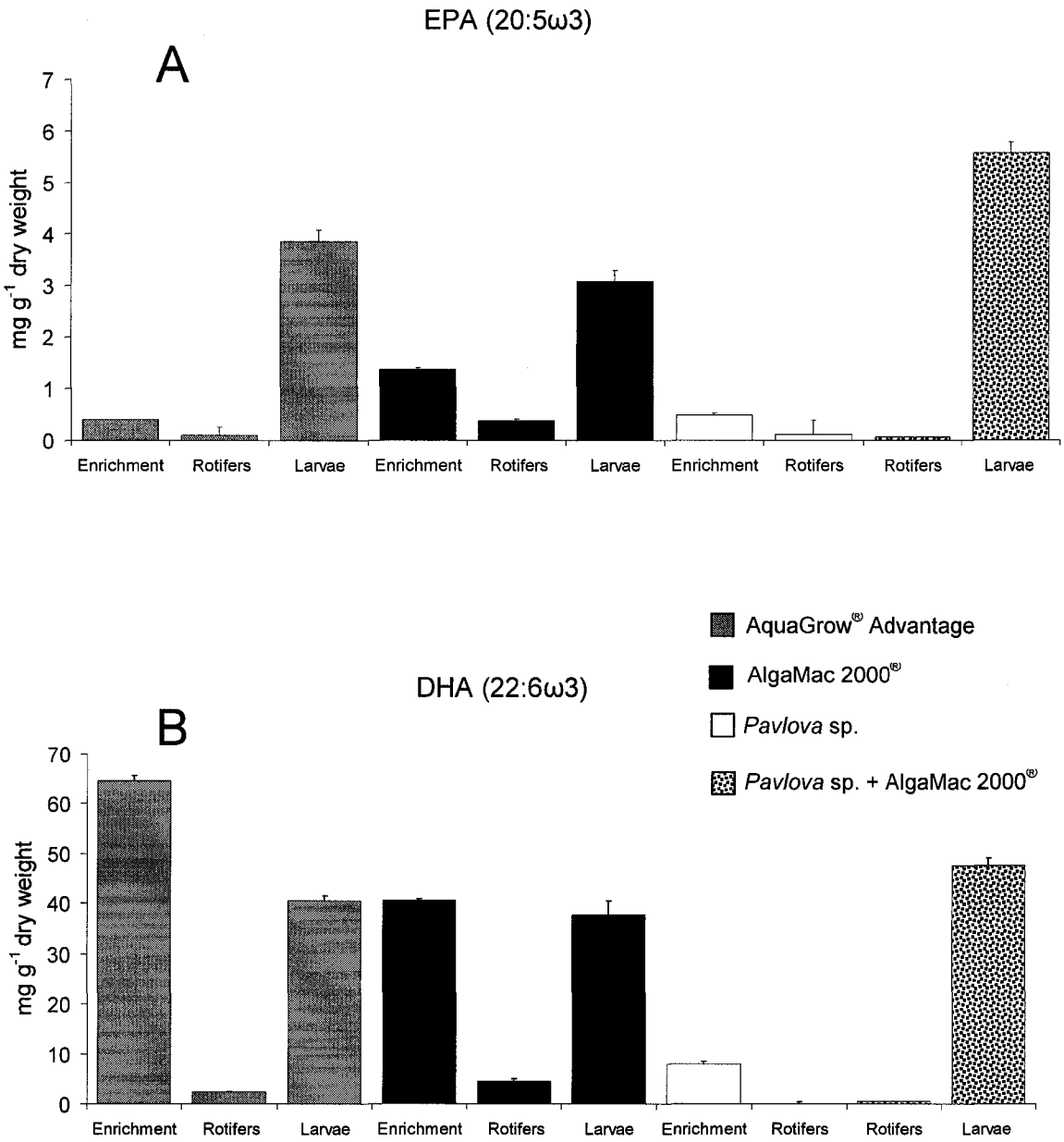

Figure II.6. Concentrations of two fatty acids from the ω3 family, EPA (A) and DHA (B), in commercial enrichments, enriched rotifers, and cod larvae at 37 days post hatch (443.2 dd) fed on them. Values are expressed as mean + SE of 3 samples, except for larvae where n=4.

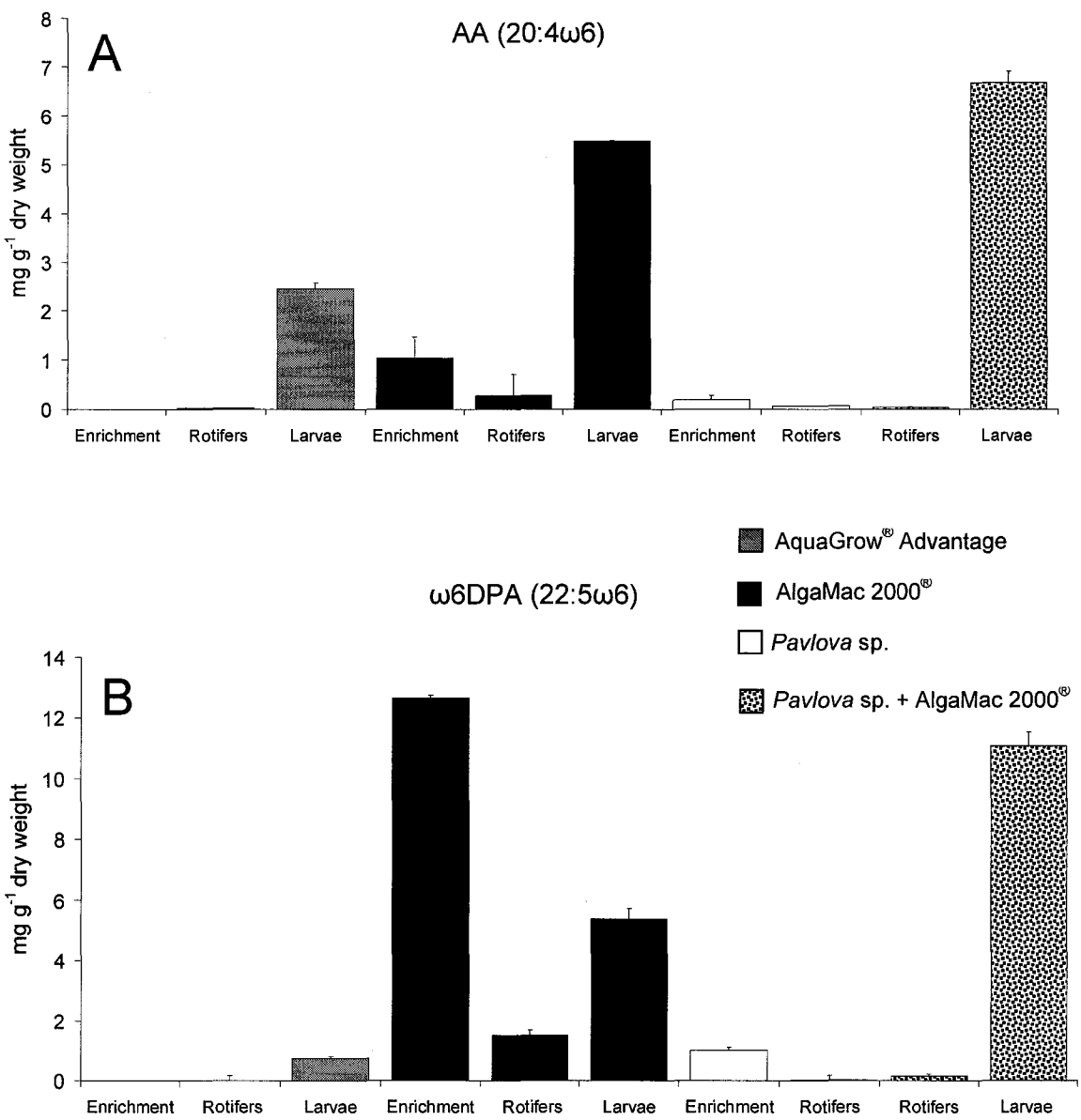

Figure II.7. Concentrations of two fatty acids from the ω6 family, AA (A) and ω6DPA (B), in commercial enrichments, enriched rotifers, and cod larvae at 37 days post hatch (443.2 dd) fed on them. Values are expressed as mean + SE of 3 samples, except for larvae where n=4.

II.4. Discussion

The lipid class composition of enriched rotifers showed a trend similar to the composition of their diet. Although in AlgaMac 2000-enriched rotifers the PL proportion was significantly lower than in the other treatments, in absolute terms, different treatments delivered similar concentrations of PL to the larvae. The fact that one of the treatments resulted in rotifers with the PL percentage significantly lower than others is somewhat surprising. The polar lipid class composition of live organisms tends to be conservative (Tackaert et al., 1991), and rotifers in particular, tend to conserve a constant PL level independent of the lipid class composition of the diets (Rainuzzo et al. 1994). However, rotifers rapidly break down the PL fraction of their diet (Dhert et al., 2001), and since other nutritional parameters of the enriched rotifers were different (such as total lipid content, TAG percentage, and fatty acid composition), it is possible that the metabolism of PL in the AlgaMac 2000 rotifers was faster than in the other treatments.

In the present study, the *Pavlova* sp. + AlgaMac 2000 treatment resulted in the best larval performance overall. Larvae from this treatment had not only better growth rates but also superior survival during the 37 days of larviculture. The growth rates observed in this study at 37 dph (443.2 dd) are comparable to those obtained with cod larvae at 36 dph by Park et al. (2006). These authors tested four commercial preparations with three different DHA/EPA ratios— 0, 33.6, and 95.7— for the enrichment of rotifers. After 43 days of larviculture, the growth produced by rotifers enriched with a DHA/EPA ratio of 33.6 (giving rotifers with a DHA/EPA ratio of 4.0)

was significantly lower than the other treatments. On the other hand, rotifers with DHA/EPA ratio of 9.2 (40% TAG and 39% PL) resulted in superior survival, and growth comparable to the growth produced by rotifers with DHA/EPA ratio of 9.7 (32% TAG and 61% PL). In a different study with cod larvae, O'Brien-MacDonald et al. (2006) found that larvae fed with rotifers with DHA/EPA ratio of 3.7 (78.3 ± 8.6 mg g^{-1} of total lipids and ~21% TAG and 22% PL) grew better, had higher survival rates, and were more efficient at catching prey than larvae fed with rotifers with a DHA/EPA ratio of 0.3 (44.4 ± 4.8 mg g^{-1} of total lipids and ~15% TAG and 55% PL). In our study, larvae from the *Pavlova* sp. + AlgaMac 2000 treatment were fed rotifers with DHA/EPA ratio of 0.59 and 8.89 (11 to 27% TAG and 33 to 44% PL). This feeding regime supplied to the larvae high proportions of EPA and DHA, in the morning and afternoon feeding, respectively. The *Pavlova* sp. + AlgaMac 2000 treatment resulted in larval DHA/EPA ratio of 8.57. These results indicate that the PL content of the diet is not as influential as the DHA/EPA ratio is on Atlantic cod larvae growth and survival during the rotifer phase. Park et al. (2006) also speculated that PL levels in the diet do not have a dominant influence on the growth of Atlantic cod larvae. Rather, the DHA proportions were positively correlated with larval growth and survival, and the authors suggested that larval Atlantic cod require a high DHA/EPA ratio (Park et al., 2006). According to Sargent et al. (1999a), the requirement of marine fish larvae for DHA is species and life stage-specific. Although a DHA/EPA ratio of 2 is normally found in many marine fish eggs and larvae (Tocher and Sargent, 1984), DHA/EPA ratios different from 2 are often associated with good larval growth and survival. For example, yellowtail flounder (*Limanda ferruginea*) larvae fed rotifers with a DHA/EPA

ratio of 8.2 grew faster and with better survival rates than larvae fed rotifers with DHA/EPA ratio of 1.9 (Copeman et al., 2002). Although growth and survival rates were not affected by different DHA/EPA ratios, the percentage of turbot (*Scophthalmus maximus*) larvae with normal pigmentation was higher in a group fed rotifers with DHA/EPA ratio of 0.5 (83%) than in a group fed rotifers with DHA/EPA ratio of 2 (~33%) (Estévez et al., 1999). There were no differences in growth and survival rates of haddock (*Melanogrammus aeglefinus*) larvae fed rotifers with DHA/EPA ratio of 4.5 when compared to larvae fed rotifers with DHA/EPA ratio of 1.6 (Castell et al., 2003).

The essential fatty acids from the ω3 family, DHA and EPA, are well known for the role they play in larval growth, survival and resistance to stress (Sargent et al., 1999a; Izquierdo et al., 2000) but other nutritional factors should be considered when evaluating the quality of diets for marine fish larvae. The importance of fatty acids from the ω6 family, especially AA, has received increased attention (Bell and Sargent, 1999; Izquierdo et al., 2000; Koven et al., 2003). There is a competitive interaction between EPA and AA for the synthesis of eicosanoids. Eicosanoids are a range of highly bioactive compounds with twenty carbon atoms formed in small amounts by every tissue in the body, and involved in a great variety of physiological functions, including immune response and stress resistance (Sargent et al., 2002; Tocher, 2003). The major precursor of eicosanoids in fish is AA, with eicosanoids formed from EPA being less biologically active than those formed from AA. Furthermore, EPA competitively inhibits the formation of eicosanoids from AA (Sargent et al., 1999b). Consequently, EPA works as eicosanoid modulators in the body, with high tissue ratios of EPA/AA resulting in decreased eicosanoid action. The physiological function

that EPA exerts in eicosanoid action may help to explain why in the present study, the diet with the highest DHA/EPA ratio (AquaGrow Advantage, DHA/EPA of ~ 25) resulted in poor larval growth and survival. Rotifers enriched with AquaGrow Advantage had the lowest AA percentage and an EPA/AA ratio significantly higher than the other treatments. It is worthwhile to note that the same trend was observed in the larval ratios, with AquaGrow Advantage-larvae having the highest EPA/AA ratio (1.55). It is possible that larvae from this treatment produced higher proportions of EPA-derived eicosanoids, making them less successful at overcoming stressful situations, and having consequently, reduced survival and growth. These results are in agreement with the Park et al. (2006) study, were the diet with the lowest EPA/AA ratio resulted in the highest survival rates. The EPA/AA ratio also explains the results exemplified above, where DHA/EPA ratios different from 2 resulted in good larval performance.

The question of which DHA/EPA ratio is optimal for a given species of fish cannot be answered without considering the EPA/AA ratio, since elevating the level of DHA in the diet will simultaneously reduce the level of EPA, therefore altering the EPA/AA ratio. Consequently, the interactions in eicosanoid production between EPA and AA are affected, which in turn affect larval growth, survival and stress resistance. Sargent et al. (1999a) suggested that an optimum DHA/EPA/AA ratio for marine fish would probably be around 10/5/1. However, these authors cautiously pointed that this ratio may not apply universally to all marine fish, since both the optimum concentration and ratio of DHA, EPA and AA are species specific. In the present study, a combination of two rotifers enriched differently—*Pavova* sp.-enriched and *Pavlova* sp.

+ AlgaMac 2000-enriched rotifers—resulted in the best larval performance. The DHA/EPA/AA ratios in these preys were approximately 1/1.6/1 and 11/1.5/1, respectively. The daily food requirement of Atlantic cod larvae is related to the larvae's specific growth rate. It has been estimated to range from a few prey items per larva at the onset of the exogenous feeding to up to >100 prey items per larva later in the development (Olsen et al., 2004). Likewise, the proportions of prey items ingested during different feeding periods (morning and afternoon) also show variation during the larviculture (ARDF-staff, personal communication). Because the feeding ratio was not measured in the present study, it is not possible to estimate the contribution of each type of rotifer as a source of essential fatty acids to the larvae. Nevertheless, it is unlikely that the DHA/EPA/AA ratio observed in *Pavlova* sp.-enriched rotifers is the best ratio to be supplied to Atlantic cod larvae. In the study performed by Park et al. (2006) rotifers containing DHA/EPA/AA ratio of approximately 10/1/1 resulted in the best larval performance (growth and survival). Considering this value, and the ratio proposed by Sargent et al. (1999a), the DHA/EPA/AA ratio observed in *Pavlova* sp. + AlgaMac 2000-enriched rotifers (11/1.5/1) is probably closer to an optimum DHA/EPA/AA ratio for early developmental stages of Atlantic cod larvae.

To date, ω6DPA (22:5ω6) has not been considered an essential fatty acid for marine fish larvae. This fatty acid can act as a substitute for DHA in the developing brain of mammals under DHA-deficient diets (Moriguchi et al., 2000). According to Moriguchi et al. (2000) it is possible that ω6DPA supplementation would avoid an inadequate amount of 22-carbon polyunsaturated fatty acids during a critical stage in early nervous system development. In mammals, this supplementation would prevent

losses in behavioral performance associated with ω3 deficiency. Eldho et al. (2003) investigated the effects of the replacement of DHA by ω6DPA in the physical properties of membranes. These authors found that parameters traditionally used to compared bilayers, such as main phase transition temperature, overall bilayer geometry, bilayer thickness, and area per molecule, were identical in the control group and in the bilayer in which DHA was replaced by ω6DPA. However, there was an important difference between DHA- and ω6DPA-containing membranes: a difference in the packing of hydrocarbon chains. The loss of a single double bond from DHA to ω6DPA resulted in a more even distribution of chain densities along the bilayer normal, with the less flexible tail section of ω6DPA favoring more extended chain formations (Eldho et al., 2003). This characteristic of ω6DPA-containing membranes may serve to maintain membrane fluidity or structural integrity during shifts in ambient temperature, which would be important in species such as Atlantic cod. According to Sundby (2000), during the period from the egg stage to pelagic juvenile stage, Atlantic cod undergoes the largest temperature changes through lifetime, with larvae from the Northern stock off Labrador/Newfoundland being found even at temperatures below 0°C. Results with cold-water bivalves support this hypothesis. Marked accumulation of ω6DPA was observed in sea scallop larvae irrespective of diet and life stage. Accumulation of ω6DPA was reported in polar lipids of larval *Placopecten maximus* (Delaunay et al., 1993), *Placopecten magellenicus* (Feindel, 2000) and *Argopecten purpuratus* (Farias et al., 2003). Moreover, high mortalities and poor growth of *P. maximus* larvae were related to low ω6DPA in the diet (Pernet et al., 2005). Apparently, ω6DPA plays a crucial role in the development of bivalve larvae and it is

possible that sea scallops require this fatty acid either as a substitute for DHA or as an essential fatty acid *per se* (Milke et al., 2004; Pernet et al., 2005). In the present study, the two groups of larvae with top performances showed a substantial accumulation of ω6DPA through the experimental period. At 37 days post hatch, the level of ω6DPA in AlgaMac 2000-larvae was 22 times higher than in newly hatched larvae, while in *Pavlova sp.* + AlgaMac 2000-larvae the increment was approximately thirty-fold. In contrast, in AquaGrow Advantage larvae, the increment of ω6DPA was less than four-fold. In addition the sum of DHA and ω6DPA was maintained around 32% in larvae from all treatments from hatch up to the end of the experiment, with the only exception being the AquaGrow Advantage larvae. These results indicate that despite replacement of DHA by ω6DPA, a constant level of around 32% of 22-carbon fatty acids is needed during the first 4 weeks of development of Atlantic cod larvae. In a study performed to determine the molecular and stable isotopic composition of long-chain fatty acids in a short food chain leading to Atlantic cod larvae, ω6DPA showed the smallest isotopic change among 17 fatty acids for which stable isotope data were available. This indicates a lack of kinetic fractionation that would occur during enzyme-catalyzed reaction steps. In addition, this fatty acid showed a thirty-fold enrichment in Atlantic cod larvae between day 1 and day 11, compared to <three-fold enrichment of AA and DHA (Parrish et al., 2007).

Besides its possible structural role in membranes, ω6DPA may be precursor of bioactive docosanoids (22-carbon atom compounds with functions similar to eicosanoids). Recently, Hong et al. (2005) shown that rainbow trout (*Oncorhynchus mykiss*) brain cells biosynthesize a range of bioactive docasanoids—including

neuroprotectin D1, resolvin D5, resolvin D1 and resolvin D2—from endogenous sources of DHA when challenged *in vitro*. These compounds are known for their potent anti-inflammatory and immunoregulatory actions (Hong et al., 2003). The enzymes involved in the production of docosanoids from DHA, such as phospholipase A_2 (PLA_2) and cyclooxygenase 2 (COX-2), could work on ω6DPA to form a parallel series of competitive products as found with the C_{20} EPA and AA (Simopoulos, 2002). Elevated concentrations of ω6DPA could compete for the active sites of both PLA_2 and COX-2, thus helping to retain DHA (DeMar Jr. et al., 2004). In the present study, the larvae with the poorest performance had the highest DHA/ω6DPA ratio. However, if the poor performance of this group is to be related to the DHA/ω6DPA ratio, the relationship should be established on the basis of docosanoid production and not DHA retention, since larvae from this treatment had DHA levels significantly higher than the larvae from other treatments. The results above suggest that ω6DPA could act as an essential fatty acid during early development of Atlantic cod larvae. If so, it would not be surprising, considering that up to the mid-1990s AA was not regarded as an essential fatty acid in marine fish nutrition.

Sargent et al. (1999b) pointed out that the role of saturated and monounsaturated fatty acids as a major energy-yielding nutrient in fish is often neglected in nutritional studies. There is a strong indication that 16:0, 18:1ω9, 20:1ω9, and 22:1ω11 are heavily catabolized to generate metabolic energy in fish because they are all consumed in large amounts during the growth of farmed fish species (Sargent et al., 2002). In our study, rotifers from the treatment that resulted in the best larval performance had the highest levels of 18:1ω9, 20:1ω9 and ΣMUFA (*Pavlova*

sp.-rotifer), and of 16:0 and ΣSFA (*Pavlova* sp. + AlgaMac 2000-rotifer). Although HUFA (including EPA and DHA) are catabolized to generate energy in fish (Sargent et al., 2002; Tocher, 2003), diets containing high levels of saturated and monounsaturated fatty acids give a clear advantage to the larvae in terms of energy supply, permitting more valuable essential fatty acids to be spared for membrane composition and/or eicosanoid production.

Our results indicate that the combination of *Pavlova* sp. and AlgaMac 2000 provided rotifers with an appropriate fatty acid profile for the initial feeding of Atlantic cod larvae. Among the treatments tested, this combination resulted in rotifers with the best balance between DHA and EPA and between EPA and AA, high levels of ω6DPA, and high levels of fatty acids that can be used as an energy source. More experiments involving stress resistance and variation in ω6DPA levels are needed to better understand the effects of essential fatty acids and ω6DPA in the early development of Atlantic cod larvae.

CHAPTER III

Growth, survival and lipid composition of Atlantic cod (*Gadus morhua*) larvae in response to differently enriched *Artemia franciscana*.

III.1. Introduction

Feeding fish larvae is the most challenging step during the culture of marine fish species. In general, marine fish larvae have reduced size, a small mouth, and incomplete or immature digestive systems. Their natural diet is composed of motile prey organisms, and young larvae do not accept well inert/dry diets. Attempts at replacement of live-feed by formulated inert diets during the first feeding of altricial larvae (those that, when the yolk sac is exhausted, remain in a relatively undeveloped state) has resulted in poor growth performance and high mortality, making the use of live food organisms obligatory for successful culture of early life stages of most marine larvae (Bengtson, 2003). During the mass-rearing of Atlantic cod (*Gadus morhua*), rotifers, *Brachionus plicatilis*, and brine shrimp, *Artemia* spp. are offered to the larvae as live-feed (Brown et al., 2003; Svåsand et al., 2004). In the standard protocol used in Newfoundland, Canada, the rotifer feeding stage normally lasts between 20 and 40 days from hatch (Brown et al., 2003). The point at which *Artemia* should replace rotifers as diet for the larvae has not yet been adequately defined for Atlantic cod. The replacement occurs gradually and normally starts around 20 days post hatch (dph) when the larvae have obtained a total length between 8 and 9 mm (Rosenlund et al., 1993; Puvanendran and Brown, 1999). However, the introduction of *Artemia* has been reported to start as early as 6.5 mm total length (Brown et al., 2003). Baskerville-Bridges and Kling (2000a) succeeded at culturing Atlantic cod larvae through metamorphosis without using *Artemia*; however, the absence of *Artemia* severely affected the larval growth rate. Larvae raised without *Artemia* attained a dry weight of

13 mg by 71 days post hatch, while the larvae that received *Artemia* reached the same age weighing 20 mg (Baskerville-Bridges and Kling, 2000a).

Although *Artemia* are not part of the natural diet of marine fish, they are widely used in fish hatcheries because they are readily available as "off the shelf" live-feed, are simple to prepare, have suitable size and swimming behavior, and are credited as the least labor-intensive live-feed available for aquaculture (Van Stappen, 1996). The biochemical composition and nutritional value of *Artemia* reflect a variety of influences such as strain, season of harvesting, geographical source, and life-stage (Dhont and Van Stappen, 2003), but, in general, *Artemia* nauplii have markedly low levels of eicosapentaenoic acid (EPA, 20:5ω3) and arachidonic acid (AA, 20:4ω6), and docosahexaenoic acid (DHA, 22:6ω3) is practically absent. These fatty acids are considered to be essential for marine fish (Sargent et al., 2002) and their influence on larval growth, survival, pigmentation, and stress resistance has been demonstrated for several fish species (Watanabe, 1993; Furuita et al., 1998; Estévez et al., 1999; Gapasin and Duray, 2001; Koven et al., 2001; Copeman and Parrish, 2002; Van Anholt et al., 2004; Villalta et al., 2005; Hamre, et al., 2005).

After about 8 hours post-hatch, *Artemia* nauplii start feeding on particles smaller than 25 μm irrespective of their nature (Van Stappen, 1996). Taking advantage of this non-selective filter feeding, simple methods were developed to enhance their nutritional profile before feeding them to predatory larvae. Live microalgae, spray-dried algae, yeast-based diets, microcapsules, and marine oil emulsions have been used in commercial hatcheries to improve the fatty acid composition of *Artemia* nauplii in a process known as enrichment or bioencapsulation

(Coutteau and Mourente, 1997; Coutteau and Sorgeloos, 1997, Dhont and Van Stappen, 2003). However, the enrichment process is poorly understood in terms of the mechanisms of lipid assimilation and efficiency, and it is now known that the *Artemia* nauplii do not act as a mere "capsule", but rather can actively change the lipid composition of the diet (Navarro et al., 1999). To date, we only know about a few studies that tested different live-feed enrichments for Atlantic cod larvae during the *Artemia* phase (Zhang et al., 1996; Galloway et al., 1998; Cutts et al. 2006). In the study performed by Cutts et al. (2006), the three enrichments tested differed in their proximate composition, however, there were no differences in survival, growth, and larval fatty acid content of lipids during the *Artemia* feeding stage.

The objectives of the present study were to evaluate the effects of different commercial products for the enrichment of *Artemia* metanauplii, and their effect on growth, survival and lipid composition of Atlantic cod larvae.

III.2 Material and Methods

III.2.1. Larval rearing and *Artemia* enrichments

Atlantic cod larvae (37 days post hatch) were stocked in 3,000 litre (L) circular tanks at a density of 4 larvae L^{-1} (two replicates per treatment) and reared up to 59 days post hatch (dph), which is equivalent to 709.7 degree-days (dd), on *Artemia* enriched differently. Water management and feeding schemes followed previous protocols outlined in Brown et al. (2003). Water temperature ranged from 11 to 13°C

over the course of the experiment. Four different treatments were tested using commercially available products as a single enrichment or in combination with algae paste. The products tested differed in their proximate and lipid composition (Tables III.1 and III.2). The treatments were: 1) AlgaMac 2000® (Aquafauna Bio-Marine Inc., Hawthorne, CA, USA) 2) AquaGrow® Advantage (Advanced BioNutrition Corp., Columbia, MD, USA), 3) *Pavlova* sp. paste (Reed Mariculture, Campbell, CA, USA) combined with AlgaMac 2000®, and 4) DC DHA Selco (Inve Americas, Salt Lake City, UT, USA) combined with AlgaMac 2000®. Prior to the start of the experiment, larvae were reared from hatch up to 37 dph, in the same tanks, and fed rotifers enriched with the same treatments, except larvae from treatment 4. Feeding schedules and rotifers enrichments used during the rotifer phase were as described in Chapter II. At the end of the rotifer phase (37 dph; 443 dd) larvae fed with *Pavlova* sp. + AlgaMac 2000-enriched rotifers had better survival rates than the larvae from the other treatments. Excess larvae from this treatment were stocked at density of 4 larvae L^{-1} in 500 L tanks (two replicates) and used for treatment 4.

Table III.1. Proximate composition of the enrichments tested.

	Enrichments			
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. paste	DC DHA Selco®
Proximate composition[1] (%)				
Protein	20.2	10.0	4.6	*
Lipids	38.1	23.0	1.76	65.0
Carbohydrate	17.1	9.0	1.9-2.1	*
Ash	20.4	44.0	*	3.0
Moisture	4.2	4.0	91.0	30.0
Vitamin A (IU/100 g)	<100	737.5	*	150
Vitamin C (mg/100 g)	4.8	*	*	0.8
Vitamin D (IU/100 g)	457.0	*	*	150
Vitamin E (IU/100 g)	12.0	*	*	3.6
Calcium (%)	*	0.3	*	*
Potassium (%)	*	0.6	*	*
Phosphorus (%)	*	0.2	*	0.2

[1] Values according to manufacturer's information. *N.b.* some of this information has changed over time.

* Information not available.

Artemia cysts were hydrated, decapsulated, and hatched according to standard practices and subsequently enriched for 24 hours. *Artemia* metanauplii were enriched in 300 L conical tanks at a density of $8x10^4$ metanauplii L^{-1}, at 22°C with protocols similar to those described in Chapter II for rotifers. Larvae were fed twice a day, between 9:00 and 10:00 *a.m.*, and 4:00 and 5:00 *p.m.* The enrichment of metanauplii

for treatments 1 and 2 followed the manufacturer's directions. Larvae in treatment 3 were fed with *Pavlova* sp.-enriched metanauplii during the morning and *Pavlova* sp. + AlgaMac 2000-enriched metanauplii during the afternoon. For this treatment, *Artemia* metanauplii batches were enriched with 8 ml of *Pavlova* sp. paste per $15x10^6$ metanauplii (larvae morning feeding) and a combination of 8 ml of *Pavlova* sp. paste and 22.5 g AlgaMac 2000 per $15x10^6$ metanauplii (larvae afternoon feeding). Larvae from treatment 4 were fed with DC DHA Selco-enriched metanauplii during the morning and with DC DHA Selco + AlgaMac 2000-enriched metanauplii during the afternoon. Enrichments for treatment 4 were carried out using 2 g DC DHA Selco per $1x10^6$ metanauplii (larvae morning feeding) and a combination of 1 g DC DHA Selco and 0.5 g AlgaMac 2000 per $1x10^6$ metanauplii (larvae afternoon feeding). The enriched *Artemia* were harvested, concentrated in a sieve and thoroughly rinsed with 20°C water to remove all residual enrichment. Before being supplied to the larvae, enriched *Artemia* were put in 10 L buckets with 6 L seawater and kept in the same room as the experimental tanks. Air stones were added to the buckets to provide constant aeration, and 4 L of seawater from the larval tanks were added to further dilute the *Artemia* and acclimate the prey to the tank temperature. Enriched *Artemia* were supplied to the larvae from 37 to 59 dph (443.3 to 709.7 dd) twice a day as necessary to sustain a prey density of 2000 L^{-1}. In order to maintain the desired prey concentration within each experimental tank, before each feeding, three 10 ml water aliquots were sampled from each tank. The number of prey in each sample was counted and the average number was used to adjust prey concentrations as needed.

Table III.2. Total lipid, lipid class and fatty acid composition of the enrichments tested.

	Enrichments			
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. paste	DC DHA Selco®
Total lipid (mg g^{-1} dw)	204.3 ± 3.4[a]	168.7 ± 8.1[b]	47.5 ± 3.0[c]	421.0 ± 5.0[d]
Lipid Class (% total lipids)				
Hydrocarbons	0.1 ± 0.0[a]	1.1 ± 0.1[b]	0.4 ± 0.0[c]	0.4 ± 0.0[c]
Steryl Esters/Wax Esters	3.6 ± 0.1[a]	0.0 ± 0.0[b]	0.0 ± 0.0[b]	2.3 ± 0.0[c]
Ketones	6.4 ± 0.5[a]	3.7 ± 0.3[b]	0.5 ± 0.0[c]	3.9 ± 0.0[b]
Triacylglycerols	70.2 ± 0.8[a]	64.4 ± 2.0[a]	34.4 ± 0.1[b]	63.7 ± 1.9[a]
Free fatty acids	1.2 ± 0.2[a]	2.9 ± 0.1[a]	13.2 ± 1.7[b]	0.8 ± 0.1[a]
Alcohols	3.5 ± 0.4[a]	1.1 ± 0.1[b]	1.9 ± 0.1[ab]	3.9 ± 1.0[a]
Sterols	0.6 ± 0.1[a]	4.1 ± 0.2[b]	0.8 ± 0.1[a]	0.4 ± 0.1[a]
Acetone Mobile Polar Lipids	7.0 ± 0.8[a]	9.3 ± 0.3[a]	23.2 ± 0.5[b]	17.8 ± 1.3[c]
Phospholipids	5.4 ± 0.7[a]	13.5 ± 0.8[b]	25.5 ± 2.1[c]	6.8 ± 0.9[a]
Fatty acids (% total fatty acids)				
14:0	17.5 ± 0.1[a]	17.3 ± 0.1[a]	9.9 ± 1.1[b]	3.6 ± 0.2[c]
16:0	36.2 ± 0.0[a]	17.3 ± 0.3[b]	10.5 ± 1.5[c]	20.3 ± 0.1[b]
18:0	0.9 ± 0.0[a]	0.6 ± 0.0[a]	0.3 ± 0.1[a]	5.4 ± 0.3[b]
21:0	0.0 ± 0.0[a]	0.0 ± 0.0[a]	2.0 ± 0.4[b]	0.3 ± 0.0[a]
ΣSFA[1]	56.0 ± 0.2[a]	27.3 ± 0.5[b]	25.5 ± 1.8[b]	33.5 ± 0.6[c]
16:1ω7	3.9 ± 0.1[a]	1.7 ± 0.0[b]	5.6 ± 0.2[c]	6.1 ± 0.3[c]
18:1ω9	0.0 ± 0.0[a]	8.7 ± 0.1[b]	13.6 ± 0.6[c]	0.0 ± 0.0[a]
18:1ω7	2.1 ± 0.0[a]	0.0 ± 0.0[b]	1.3 ± 0.1[c]	18.7 ± 0.3[d]
ΣMUFA[2]	6.4 ± 0.1[a]	2.0 ± 0.2[b]	24.4 ± 0.9[c]	31.1 ± 0.1[d]
18:2ω6	0.0 ± 0.0[a]	6.0 ± 0.1[b]	3.2 ± 0.1[c]	0.2 ± 0.0[a]
18:3ω3	0.0 ± 0.0[a]	0.0 ± 0.0[a]	1.9 ± 0.0[b]	0.0 ± 0.2[a]
18:5ω3	0.0 ± 0.0[a]	0.0 ± 0.0[a]	6.7 ± 0.1[b]	0.0 ± 0.0[a]
20:3ω6	0.2 ± 0.0[a]	0.0 ± 0.0[a]	1.2 ± 0.1[b]	0.1 ± 0.1[a]
20:4ω6 (AA)	0.7 ± 0.0[a]	0.0 ± 0.0[b]	0.6 ± 0.3[a]	0.7 ± 0.0[a]
20:4ω3	0.5 ± 0.0[ac]	0.0 ± 0.0[b]	0.3 ± 0.1[a]	0.7 ± 0.0[c]

20:5ω3 (EPA)	0.9 ± 0.0^{a}	0.3 ± 0.0^{b}	1.4 ± 0.1^{c}	6.3 ± 0.3^{d}
22:5ω6 (ω6DPA)	8.2 ± 0.1^{a}	0.0 ± 0.0^{b}	3.0 ± 0.2^{c}	1.4 ± 0.0^{d}
22:5ω3	0.2 ± 0.1^{a}	0.6 ± 0.0^{a}	0.5 ± 0.1^{a}	1.6 ± 0.1^{b}
22:6ω3 (DHA)	26.2 ± 0.4^{a}	47.4 ± 0.2^{b}	23.0 ± 1.0^{c}	20.0 ± 0.6^{d}
ΣPUFA[3]	37.7 ± 0.2^{a}	51.2 ± 0.8^{b}	50.0 ± 2.5^{b}	34.1 ± 0.6^{a}
Σω3	28.4 ± 0.1^{a}	50.7 ± 0.5^{b}	39.7 ± 1.7^{c}	29.8 ± 0.7^{a}
Σω6	9.2 ± 0.2^{a}	0.6 ± 0.3^{b}	9.2 ± 1.0^{a}	3.7 ± 0.1^{c}
ω3/ω6	3.1 ± 0.0^{a}	87.5 ± 1.2^{b}	4.5 ± 0.3^{a}	8.1 ± 0.3^{c}
DHA/EPA	30.0 ± 1.6^{a}	156.7 ± 2.0^{b}	16.8 ± 0.6^{c}	3.2 ± 0.3^{d}
EPA/AA	1.3 ± 0.1^{ab}	0.0 ± 0.0^{b}	3.0 ± 0.8^{ac}	4.4 ± 0.0^{c}

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids, which also includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 20:0, 21:0, 22:0, and 23:0 at ≤ 0.68% each.

[2]Sum of monounsaturated fatty acids, which also includes: 14:1, 15:1, 16:1ω9, 16:1ω5, 17:1, 18:1ω11, 20:1ω11, 22:1ω11, 22:1ω9, and 24:1 at ≤ 2.0% each.

[3]Sum of polyunsaturated fatty acids, which also includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:4ω3, 18:4ω1, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 21:5ω3, and 22:4ω6 at ≤ 1.16% each.

III.2.2. Sampling, growth and survival measurements

Pooled samples of larvae were collected at 37, 44, 52, and 59 dph (equivalent to 443.2, 534.1, 630, and 709.7 dd) for dry weight measurements (three replicates tank^{-1}). Growth measurements were performed as described in Chapter II. Briefly, between 10 (37 dph) and 5 (59 dph) larvae were collected in pre-weighed glass microfibre filters and dried at 60°C for 48 h. Filters were then re-weighed using a microbalance (UMT2, Mettler Toledo, Switzerland). Data for standard length were obtained by measuring 10 larvae per tank (20 per treatment, except for treatment 4 where n=10) with a stereomicroscope. Growth was then calculated as specific growth

rate for dry weight (SGR; % d^{-1} = [ln (final dry weight) – ln (initial dry weight)/days] x 100) and for standard length (SGR; % d^{-1} = [ln (final standard length) – ln (initial standard length)/days] x 100). Unfortunately, standard length samples from 37 and 59 dph were not available. SGR for standard length was calculated using data between 32 and 50 dph (382.4 and 607 degree-days).

Survival measurements were based on visual observations and performed as described in Chapter II. A nominal scale (0 to 5) was used to measure the weekly survival rates. The scale used considered 0 as total mortality (or 0% survival), 1 as poor survival, 2 as reasonable survival, 3 as good survival, 4 as very good survival, and 5 as total survival (or 100% survival). There was a consensus among the ARDF staff that under mass production circumstances in the hatchery, between 20 and 30% survival at the end of the *Artemia* phase is considered a good survival rate for Atlantic cod larvae.

III.2.3. Lipid analysis

Pooled samples of larvae were collected at 37, 52, and 59 dph for lipid analysis (total lipids, lipid classes, and fatty acid composition). Samples of the experimental enrichments, algae paste and enriched live-feed were collected in triplicate for lipid analysis. Lipid extraction, and lipid class and fatty acid analyses were performed as described in Chapter II. Briefly, lipids were extracted in chloroform/methanol according to Parrish (1998) using a modified Folch procedure (Folch et al., 1957). Lipid classes were determined using thin layer chromatography with flame ionization detection

(TLC/FID) with a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel coated Chromarods and a three-stage development system was used to separate lipid classes. Fatty acid methyl esters (FAME) were prepared by transesterification as described by Budge and Parrish (2003). A Varian model 3400 GC equipped with a Varian 8100 autosampler was used for fatty acid analysis (Varian, Palo Alto, CA, USA). Peaks were detected by flame ionization. Fatty acid peaks were integrated using Varian Star Chromatography Software (version 5.50) and identification was made with reference to known standards (PUFA 1, PUFA 3, BAME and 37 Component FAME Mix, Supleco Canada, ON).

III.2.4. Statistical analysis

Growth and lipid composition data were analyzed as described in Chapter II. The General Linear Model (Minitab Version 13.1) was employed and in all cases, the effect of tanks was not significant. Data from treatments were pooled. Linear regression was used to describe the relationship between larval dry weight and the concentration of selected fatty acids per individual larva. Significance was set at α = 0.05 for all analyses.

III.3. Results

III.3.1. Total lipid, lipid class and fatty acid composition of *Artemia*

Total lipid content of *Artemia* after 24 h enrichment ranged from 107.7 ± 4.3 mg g^{-1} in *Pavlova* sp.-enriched *Artemia* to 232.0 ± 1.1 mg g^{-1} in DC DHA Selco-enriched *Artemia* (Table III.3). In AquaGrow Advantage and *Pavlova* sp.-enriched *Artemia* the total lipid concentration after the enrichment process was similar to the concentration observed in unenriched *Artemia* ($F_{2,6}$=1.40, p=0.31). The ketone (KET) percentage showed great variation among treatments after enrichment, with AlgaMac 2000, DC DHA Selco, and DC DHA Selco + AlgaMac 2000-enriched *Artemia* having lower proportions than unenriched *Artemia* ($F_{6,14}$=75.78, p<0.0001). The triacylglycerol (TAG) percentage decreased significantly in all treatments after enrichment ($F_{6,14}$=39.82, p<0.0001), however the smallest reduction was observed in DC DHA Selco + AlgaMac 2000-enriched *Artemia* (15.7%), and the biggest in *Pavlova* sp.-enriched *Artemia* (27.6%). The enrichment process caused a significant increase in the sterol (ST) percentage of *Pavlova* sp.-enriched *Artemia* ($F_{6,14}$=11.34, p=0.0001). Like the KET percentage, the acetone mobile polar lipids (AMPL) percentage also showed great variation, with values ranging from 3.3 ± 0.7 to 20.2 ± 1.2% total lipid, in DC DHA Selco and AlgaMac 2000-enriched *Artemia*, respectively. The AMPL percentage was significantly higher in AlgaMac 2000-enriched *Artemia* when compared to unenriched *Artemia* or AquaGrow Advantage, *Pavlova* sp., and DC DHA Selco-enriched *Artemia* ($F_{6,14}$=26.62, p=<0.0001). The phospholipid (PL) percentage

was significantly higher in AlgaMac 2000, AquaGrow Advantage, and DHA Selco-enriched *Artemia* when compared to unenriched *Artemia* ($F_{6,14}$=47.77, p<0.0001).

The *Artemia* strain used in this experiment had approximately 65% of its total fatty acids composed by only four fatty acids (16:0, 18:1ω9, 18:1ω7, and 18:3ω3) (Table III.4). After the enrichment process, AlgaMac 2000-enriched *Artemia*, had higher percentages of myristic acid (14:0) and palmitic acid (16:0) than the other treatments ($F_{6,14}$=30.64, p<0.0001 and $F_{6,14}$=43.22, p<0.0001, respectively).

Table III.3. Total lipid (mg g^{-1} dry weight) and percentage lipid class composition of *Artemia* before and after experimental enrichments.

	Treatments						
	Unenriched	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp.	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco®	DC DHA Selco® + AlgaMac 2000®
Total lipid (mg g^{-1} dw)	89.5 ± 7.1^{a}	174.6 ± 8.9^{b}	110.2 ± 5.6^{a}	107.7 ± 4.3^{a}	137.5 ± 5.7^{b}	232.0 ± 1.1^{c}	175.7 ± 0.5^{b}
Lipid Class (% total lipid)							
Hydrocarbons	0.6 ± 0.1^{a}	0.3 ± 0.0^{a}	1.0 ± 0.3^{a}	0.9 ± 0.1^{a}	1.0 ± 0.5^{a}	0.4 ± 0.0^{a}	0.6 ± 0.0^{a}
Ketones	2.1 ± 0.3^{a}	0.4 ± 0.0^{b}	2.5 ± 0.2^{a}	6.0 ± 1.7^{c}	7.4 ± 1.8^{d}	0.0 ± 0.0^{b}	0.4 ± 0.0^{b}
Triacylglycerols	77.0 ± 0.4^{a}	51.4 ± 1.7^{bc}	49.6 ± 2.3^{c}	49.4 ± 0.9^{c}	52.8 ± 2.0^{bc}	58.7 ± 1.9^{cd}	61.3 ± 0.2^{d}
Free fatty acids	4.0 ± 0.6^{a}	7.8 ± 1.2^{b}	3.3 ± 0.3^{ad}	16.9 ± 0.6^{c}	7.8 ± 0.0^{b}	0.7 ± 0.2^{d}	5.4 ± 0.7^{ab}
Sterols	2.4 ± 0.1^{a}	2.9 ± 0.3^{a}	4.8 ± 0.4^{a}	8.2 ± 1.7^{b}	5.9 ± 0.8^{a}	3.5 ± 0.7^{a}	2.6 ± 0.4^{a}
Diacylglycerol	0.0 ± 0.0^{a}	0.9 ± 0.1^{b}	4.1 ± 0.6^{c}	0.0 ± 0.0^{a}	0.7 ± 0.1^{b}	0.0 ± 0.0^{a}	0.4 ± 0.0^{ab}
Acetone Mobile Polar Lipids	10.8 ± 0.8^{a}	20.2 ± 1.2^{b}	14.9 ± 1.5^{ac}	10.0 ± 1.8^{a}	16.5 ± 1.1^{cb}	3.3 ± 0.6^{d}	19.2 ± 0.1^{cb}
Phospholipids	3.2 ± 0.5^{a}	16.2 ± 1.9^{bc}	19.9 ± 1.1^{c}	8.7 ± 1.7^{a}	8.1 ± 0.5^{a}	33.5 ± 2.7^{d}	10.1 ± 0.0^{ab}

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

With the exception of the AquaGrow Advantage treatment, this treatment also resulted in a significantly higher sum of saturated fatty acids (ΣSFA) ($F_{6,14}$= 14.12, p<0.0001). The percentage of 18:1ω7 in AquaGrow Advantage and *Pavlova* sp.-enriched *Artemia* was higher than the other treatments ($F_{6,14}$=10.12, p=0.0002). In AlgaMac 2000 and *Pavlova* sp. + AlgaMac 2000-enriched *Artemia*, the percentage of 18:1ω9 decreased significantly when compared to unenriched *Artemia* ($F_{6,14}$=49.96, p<0.0001). Consequently, these treatments resulted in the lowest sum of monounsaturated fatty acids (ΣMUFA) ($F_{6,14}$=28.44, p<0.0001). With the exception of the *Pavlova* sp. treatment, all treatments caused the percentage of linolenic acid (18:3ω3) to significantly decrease when compared to unenriched *Artemia* ($F_{6,14}$=47.39, p<0.0001). AlgaMac 2000-enriched *Artemia* had the highest levels of AA and ω6DPA, with the latter being higher than the other treatments ($F_{6,14}$=82.41, p<0.0001). With the exception of the *Pavlova* sp. treatment, all treatments resulted in an increase in DHA proportion of *Artemia*, with AlgaMac 2000 and *Pavlova* sp. + AlgaMac 2000-enriched *Artemia* having significantly higher levels when compared to unenriched *Artemia* or the *Artemia* from the other treatments ($F_{6,14}$=123.77, p<0.0001). These treatments also resulted in significantly higher DHA/EPA and ω6DPA/AA ratios ($F_{6,14}$=141.14, p<0.0001, and $F_{6,14}$=147.18, p<0.0001, respectively) and in significantly higher sums of DHA and ω6DPA when compared to unenriched *Artemia* or the *Artemia* from the other treatments ($F_{6,14}$=124.46, p<0.0001). The highest EPA/AA and DHA/ω6DPA ratios were observed in AquaGrow Advantage-enriched *Artemia* ($F_{6,14}$=26.68, p<0.0001, and $F_{6,14}$=8.91, p=0.0004, respectively).

Table III.4. Percentage fatty acid composition of *Artemia* before and after experimental enrichments.

	Treatments						
	Unenriched	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp.	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco®	DC DHA Selco® + AlgaMac 2000®
Fatty acids (% total fatty acids)							
14:0	0.8 ± 0.1^{a}	2.9 ± 0.1^{b}	1.8 ± 0.1^{c}	0.6 ± 0.0^{a}	1.9 ± 0.3^{c}	0.9 ± 0.0^{a}	1.8 ± 0.0^{c}
16:0	11.7 ± 0.1^{bc}	19.2 ± 0.6^{d}	13.5 ± 0.1^{c}	10.6 ± 0.2^{ab}	13.3 ± 0.8^{c}	10.3 ± 0.3^{ab}	13.2 ± 0.5^{c}
17:0	0.6 ± 0.2^{a}	0.6 ± 0.1^{a}	0.6 ± 0.1^{a}	0.7 ± 0.0^{a}	0.5 ± 0.0^{a}	0.5 ± 0.2^{a}	0.6 ± 0.0^{a}
18:0	6.0 ± 0.2^{a}	5.7 ± 0.5^{a}	7.3 ± 0.3^{b}	8.6 ± 0.1^{b}	5.5 ± 0.2^{a}	5.9 ± 0.3^{a}	5.2 ± 0.1^{a}
ΣSFA[1]	24.2 ± 0.1^{ab}	32.7 ± 1.6^{c}	28.3 ± 0.3^{bc}	25.1 ± 0.4^{ab}	24.7 ± 1.6^{ab}	21.6 ± 0.7^{a}	25.0 ± 0.6^{ab}
16:1ω7	3.2 ± 0.1^{bcd}	2.2 ± 0.0^{ab}	2.7 ± 0.1^{abc}	2.3 ± 0.0^{ab}	1.9 ± 0.1^{a}	3.4 ± 0.0^{cd}	4.0 ± 0.5^{d}
18:1ω9	18.3 ± 0.2^{a}	11.4 ± 0.3^{b}	17.9 ± 0.1^{a}	17.5 ± 0.3^{a}	12.4 ± 0.3^{b}	18.7 ± 0.1^{a}	16.8 ± 1.0^{a}
18:1ω7	8.9 ± 0.3^{bc}	6.8 ± 0.3^{a}	9.8 ± 0.4^{c}	10.3 ± 0.7^{c}	7.8 ± 0.3^{ab}	7.5 ± 0.5^{ab}	7.3 ± 0.2^{ab}
18:1ω5	0.3 ± 0.2^{a}	0.1 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.1^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}
ΣMUFA[2]	33.9 ± 0.4^{a}	22.7 ± 0.3^{b}	34.2 ± 0.8^{a}	33.4 ± 0.6^{a}	23.4 ± 0.7^{b}	32.1 ± 0.8^{a}	30.8 ± 1.9^{a}
18:2ω6	6.0 ± 0.0^{cd}	2.9 ± 0.0^{a}	4.2 ± 0.1^{b}	5.4 ± 0.3^{c}	3.9 ± 0.0^{b}	6.6 ± 0.1^{d}	5.2 ± 0.3^{c}
18:3ω3	25.9 ± 0.7^{d}	13.3 ± 0.4^{a}	19.5 ± 0.6^{c}	23.4 ± 0.6^{d}	16.9 ± 0.5^{bc}	17.3 ± 0.5^{bc}	14.6 ± 1.1^{ab}
18:4ω3	4.7 ± 0.0^{d}	1.7 ± 0.1^{a}	2.4 ± 0.0^{ab}	3.5 ± 0.2^{c}	2.8 ± 0.1^{bc}	2.7 ± 0.1^{b}	2.3 ± 0.2^{ab}
20:3ω6	0.1 ± 0.1^{ab}	0.2 ± 0.4^{ab}	0.1 ± 0.0^{a}	0.1 ± 0.0^{ab}	0.2 ± 0.0^{b}	0.1 ± 0.4^{ab}	0.1 ± 0.0^{ab}
20:4ω6 (AA)	0.6 ± 0.2^{ab}	1.9 ± 0.1^{d}	0.4 ± 0.1^{a}	0.9 ± 0.1^{ab}	1.9 ± 0.1^{cd}	1.35± 0.1^{bcd}	1.1 ± 0.2^{bc}
20:4ω3	0.6 ± 0.0^{ab}	0.6 ± 0.0^{ab}	0.4 ± 0.0^{a}	0.8 ± 0.2^{b}	0.8 ± 0.0^{b}	0.7 ± 0.0^{ab}	0.8 ± 0.1^{ab}
20:5ω3 (EPA)	1.7 ± 0.1^{a}	3.7 ± 0.3^{b}	3.7 ± 0.2^{b}	3.4 ± 0.3^{b}	4.5 ± 0.2^{b}	6.4 ± 0.3^{c}	3.8 ± 0.2^{b}
22:5ω6 (ω6DPA)	0.0 ± 0.0^{a}	5.3 ± 0.3^{c}	0.2 ± 0.1^{a}	0.0 ± 0.0^{a}	4.8 ± 0.3^{c}	0.5 ± 0.0^{ab}	2.2 ± 0.5^{b}
22:5ω3	0.0 ± 0.4^{a}	0.4 ± 0.0^{bc}	0.2 ± 0.1^{ab}	0.0 ± 0.0^{a}	0.2 ± 0.3^{ab}	0.7 ± 0.0^{d}	0.5 ± 0.1^{cd}

22:6ω3 (DHA)	0.3 ± 0.1^{a}	13.2 ± 0.7^{c}	4.4 ± 0.2^{b}	0.3 ± 0.1^{a}	13.3 ± 1.0^{c}	6.8 ± 0.4^{b}	10.7 ± 0.3^{b}
ΣPUFA[3]	41.8 ± 0.4^{ab}	44.6 ± 1.8^{bc}	37.6 ± 0.7^{a}	41.1 ± 0.8^{ab}	51.9 ± 2.3^{c}	46.3 ± 1.4^{bc}	44.1 ± 1.3^{ab}
ΣOBFA[4]	6.7 ± 0.2	4.8 ± 0.7	5.4 ± 0.2	3.3 ± 0.2	4.9 ± 0.2	3.5 ± 0.1	4.2 ± 0.3
Σω3	34.2 ± 0.5^{bc}	33.4 ± 1.4^{ab}	31.6 ± 0.6^{ab}	33.2 ± 0.4^{ab}	39.4 ± 1.9^{c}	35.5 ± 1.3^{bc}	33.3 ± 1.5^{ab}
Σω6	7.4 ± 0.2^{b}	10.7 ± 0.6^{cd}	5.4 ± 0.0^{a}	7.2 ± 0.5^{b}	11.5 ± 0.5^{d}	9.4 ± 0.1^{c}	9.3 ± 0.3^{c}
ω3/ω6	4.6 ± 0.2^{a}	3.1 ± 0.1^{b}	5.9 ± 0.1^{c}	4.6 ± 0.3^{a}	3.4 ± 0.0^{d}	3.8 ± 0.1^{d}	3.6 ± 0.0^{d}
DHA/EPA	0.2 ± 0.0^{a}	3.6 ± 0.4^{c}	1.2 ± 0.1^{b}	0.1 ± 0.0^{a}	3.0 ± 0.1^{c}	1.1 ± 0.0^{b}	2.8 ± 0.2^{c}
EPA/AA	3.4 ± 0.8^{ab}	1.9 ± 0.1^{a}	9.9 ± 0.6^{c}	3.8 ± 0.5^{ab}	2.4 ± 0.1^{a}	4.7 ± 0.1^{b}	3.6 ± 0.5^{ab}
DHA + ω6DPA	0.3 ± 0.1^{a}	18.4 ± 1.0^{b}	4.5 ± 0.2^{c}	0.3 ± 0.0^{a}	18.1 ± 1.3^{b}	7.4 ± 0.4^{c}	12.9 ± 0.7^{d}
DHA/ω6DPA	7.6 ± 3.9^{a}	2.5 ± 0.1^{a}	26.9 ± 4.1^{b}	12.1 ± 4.8^{a}	2.8 ± 0.0^{a}	13.4 ± 1.0^{ab}	5.1 ± 0.9^{a}
ω6DPA/AA	0.1 ± 0.0^{a}	2.7 ± 0.1^{b}	0.4 ± 0.2^{a}	0.0 ± 0.0^{a}	2.5 ± 0.0^{b}	0.4 ± 0.0^{a}	2.0 ± 0.2^{c}

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] Sum of saturated fatty acids, which also includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 20:0, 22:0, and 23:0 at ≤ 1.54% each.

[2] Sum of monounsaturated fatty acids, which also includes: 14:1, 15:1, 16:1ω9, 16:1ω5, 17:1, 20:1ω11, 20:1ω9, 20:1ω7, 22:1ω11, and 24:1 at ≤ 1.43% each.

[3] Sum of polyunsaturated fatty acids, which also includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:3ω4, 18:4ω1, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 20:4ω3, 21:5ω3, 22:4ω6, and 22:4ω3 at ≤ 0.98% each.

[4] Sum of odd and/or branched fatty acids. Includes: *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, and 17:1.

III.3.2. Larval survival and growth

Larvae fed *Pavlova* sp. + AlgaMac 2000-enriched *Artemia* had superior survival during the trial. However, at 59 dph (709.7 dd), larvae from this treatment had survival similar to DC DHA Selco + AlgaMac 2000-larvae. Larvae fed with AquaGrow Advantage or AlgaMac 2000-enriched *Artemia* had similar survival during the 22 days of larviculture (Figure III.1).

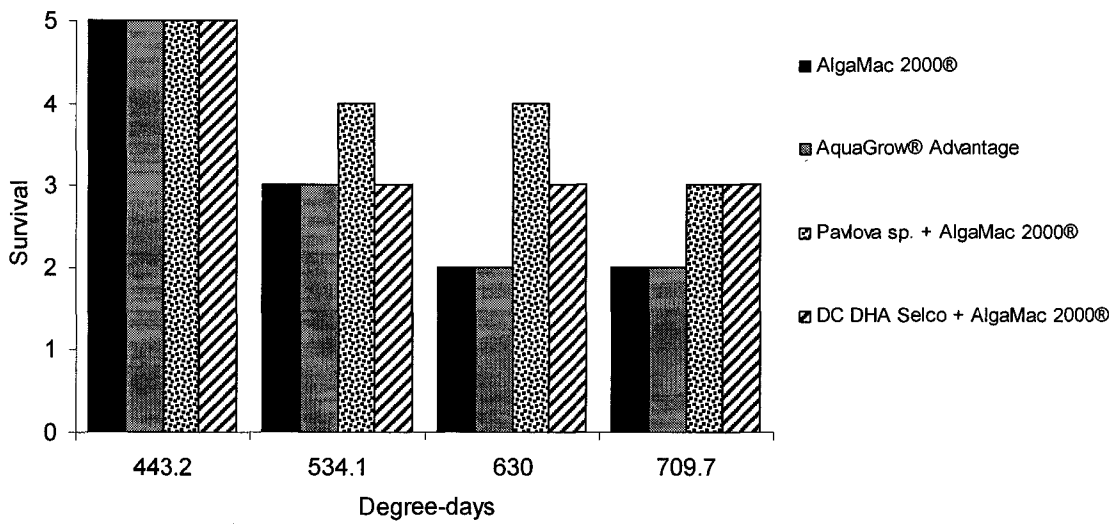

Figure III.1. Survival of Atlantic cod larvae fed differently enriched *Artemia* at 443.2, 534.1, 630, and 709.7 degree-days (equivalent to 38, 44, 52, and 59 days post hatch, respectively). Values are expressed as mean of twelve observations per treatment.

Between 52 and 59 dph (630 and 709.7 dd), the fastest growth rate for all treatments were observed (Figure III.2). At the end of the experiment, the AlgaMac 2000 treatment resulted in the highest final weight. Larvae from this treatment (12.06 ± 1.13 mg dry weight) were significantly heavier than *Pavlova* sp. + AlgaMac 2000-larvae (6.49 ± 0.32 mg dw, $F_{1,31}$=20.79, p<0.001) or DC DHA Selco + AlgaMac 2000-larvae (5.31 ± 0.20 mg dw, $F_{1,31}$=44.73, p<0.001). There was no statistical difference in the final weight between AlgaMac 2000-larvae and AquaGrow Advantage-larvae ($F_{1,31}$=2.73, p=0.108) nor between *Pavlova* sp. + AlgaMac 2000-larvae and DC DHA Selco + AlgaMac 2000-larvae ($F_{1,20}$=1.13, p=0.30). It is important to note that although *Pavlova* sp. + AlgaMac 2000-larvae were heavier than AquaGrow Advantage-larvae at the start of the experiment, the latter group grew faster during the *Artemia* phase than *Pavlova* sp. + AlgaMac 2000-larvae, showing that the growth results were not simply a reflection of the initial condition.

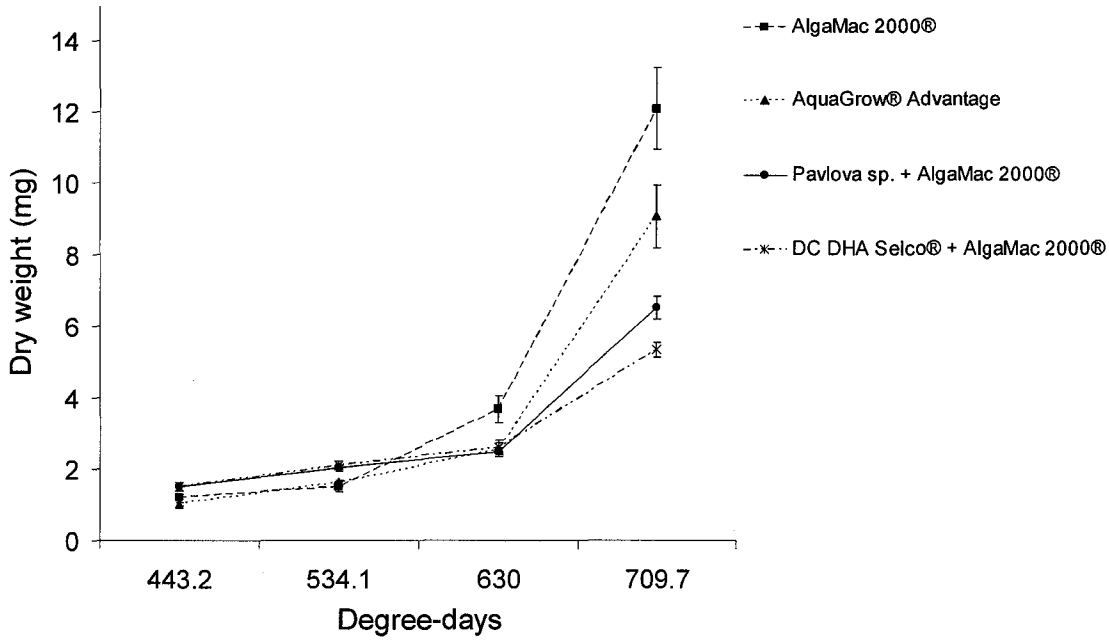

Figure III.2. Dry weight of Atlantic cod larvae fed differently enriched *Artemia* at 443.2, 534.1, 630, and 709.7 degree-days (equivalent to 37, 44, 52 and 59 days post hatch, respectively). Values expressed as mean ± SE of 6 samples.

At 50 dph (607 dd) there were no significant differences in standard lengths of larvae from different treatment groups ($F_{3,66}$=2.29, p=0.085) (Figure III.3). In addition, there was no significant difference in standard length SGR among larvae from the different treatment groups ($F_{3,66}$=2.48, p=0.07) (Table III.5).

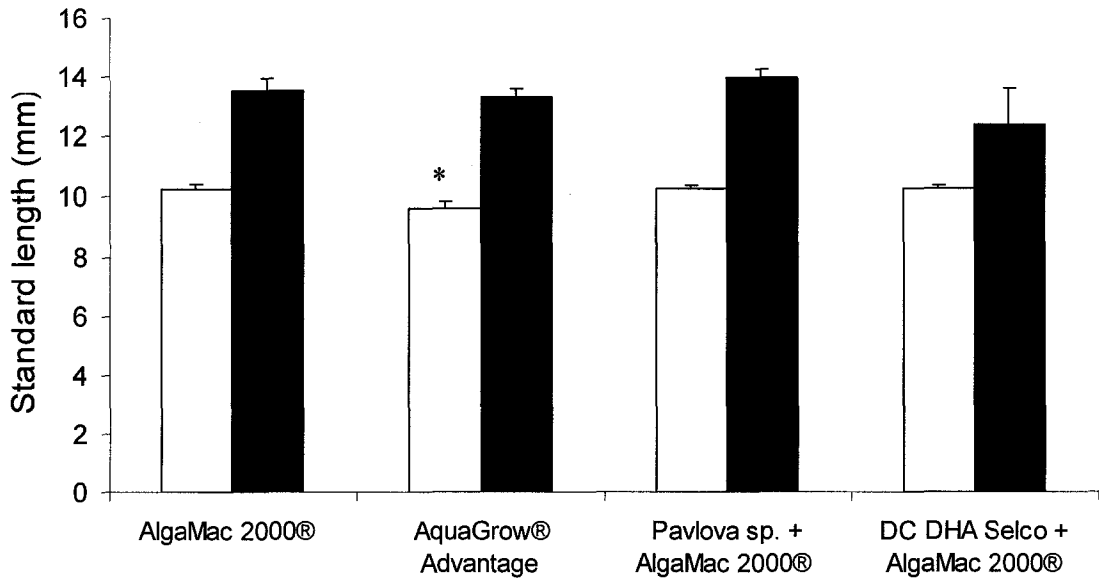

Figure III.3. Standard length of Atlantic cod larvae at 382.4 (□) and 607 (■) degree-days (equivalent to 32 and 50 days post hatch, respectively) fed differently enriched *Artemia*. Values are expressed as mean + SE of 20 samples, except for DC DHA Selco + AlgaMac 2000-larvae at 607 days post hatch where n=10. * Denotes differences in bars with the same fill at $p<0.05$.

AlgaMac 2000-larvae showed a dry weight SGR significantly higher than *Pavlova* sp. + AlgaMac 2000-larvae or DC DHA Selco-larvae ($F_{3,20}=24.91$, $p<0.0001$). There were no differences between the dry weight SGR of AlgaMac 2000-larvae and AquaGrow Advantage-larvae, or between *Pavlova* sp. + AlgaMac 2000-larvae and DC DHA Selco + AlgaMac 2000-larvae (Table III.5).

Table III.5. Growth of Atlantic cod larvae fed differently enriched *Artemia*. Dry weights of 5 larvae were determined at 59 dph (709.7 dd). Standard lengths of 20 larvae were determined at 50 dph (607 dd), except for the DC DHA Selco + AlgaMac 2000 treatment where n=10.

	Treatments			
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco® + AlgaMac 2000®
Dry weight at 59 dph (mg)	12.06 ± 1.13^{a}	9.05 ± 0.86^{ab}	6.49 ± 0.32^{bc}	5.31 ± 0.20^{c}
Dry weight SGR (% d^{-1})[1]	10.43 ± 0.43^{a}	9.44 ± 0.80^{a}	6.87 ± 0.21^{b}	4.86 ± 0.39^{b}
Standard length at 50 dph (mm)	13.53 ± 0.44^{a}	13.33 ± 0.27^{a}	13.93 ± 0.30^{a}	12.37 ± 1.25^{a}
Standard length SGR (% d^{-1}) [2]	1.49 ± 0.18^{a}	1.80 ± 0.11^{a}	1.69 ± 0.12^{a}	1.17 ± 0.16^{a}

Values (mean ± SE) with different superscript are significantly different ($p<0.05$).

[1] Dry weight SGR were calculated between 37 and 59 dph (n=6).

[2] Standard length SGR were calculated between 32 and 50 dph (n=20, except for the DC DHA Selco + AlgaMac 2000 treatment where n=10).

III.4.3. Total lipid, lipid class and fatty acid composition of larvae

All treatments resulted in a reduction in the larval total lipid content during the *Artemia* phase (Figure III.4A). At the end of the experiment (59 dph; 607 dd) larvae from all treatments had similar total lipid contents, with the exception of DC DHA Selco + AlgaMac 2000-larvae, which had a significantly higher total lipid content than the other larvae ($F_{6,19}=336.95$, $p<0.0001$) (Table III.6). Similarly, the ST concentration in larvae from all treatments decreased during the experimental period (Figure III.4B). At the end of the experiment, larvae from all treatments had lower ST percentages than

they had at the start ($F_{6,19}$=19.20, p<0.0001). There were no significant differences in the ST percentage of larvae from the different treatments at 59 dph. In contrast, the TAG, AMPL and PL percentages in larvae from all treatments increased over the experimental period ($F_{6,19}$=12.85, p<0.0001, $F_{6,19}$=11.58, p<0.0001, and $F_{6,19}$=14.28, p<0.0001, respectively). While there was no difference in the TAG percentage among treatments, in AquaGrow Advantage-larvae the AMPL percentage was lower than in the larvae from the other treatments. Furthermore, in larvae from this treatment the PL percentage was significantly higher than in AlgaMac 2000-larvae and DC DHA Selco + AlgaMac 2000-larvae (Table III.6). In *Pavlova* sp. + AlgaMac 2000-larvae, the hydrocarbon and free fatty acid percentages increased significantly during the experimental period when compared to the values observed at 37 dph ($F_{6,19}$=9.19, p<0.0001 and $F_{6,19}$=5.91, p=0.001, respectively).

Larvae form all treatments reached 59 dph with lower levels of saturated fatty acids (14:0, 16:0, and 18:0) than they had at the beginning of the experiment ($F_{6,21}$=21.69, p<0.0001, $F_{6,21}$=98.96, p<0.0001, and $F_{6,21}$=11.39, p<0.0001, respectively). Although there were no statistical differences when larval levels of saturated fatty acids were compared among treatments, AlgaMac 2000-larvae had slightly higher levels of palmitic acid (16:0) at 59 dph, resulting in a significantly higher ΣSFA when compared to the levels observed at 59 dph in the larvae from the other treatments ($F_{6,21}$=105.23, p<0.0001). Larval levels of AA and EPA increased over the experimental period (except AA for AquaGrow Advantage-larvae; Figures III.5A and III.5B), and at 59 dph, AlgaMac 2000-larvae had significantly higher levels of AA ($F_{6,21}$=121.94, p<0.0001), while AquaGrow Advantage-larvae had significantly higher

levels of EPA ($F_{6,21}$=180.21, $p<0.0001$). On the other hand, DHA levels decreased in all treatments over the experiment (Figure 6B), with AquaGrow Advantage-larvae experiencing the highest reduction (24%) and having the lowest DHA level at 59 dph ($F_{6,21}$=199.03, $p<0.0001$).

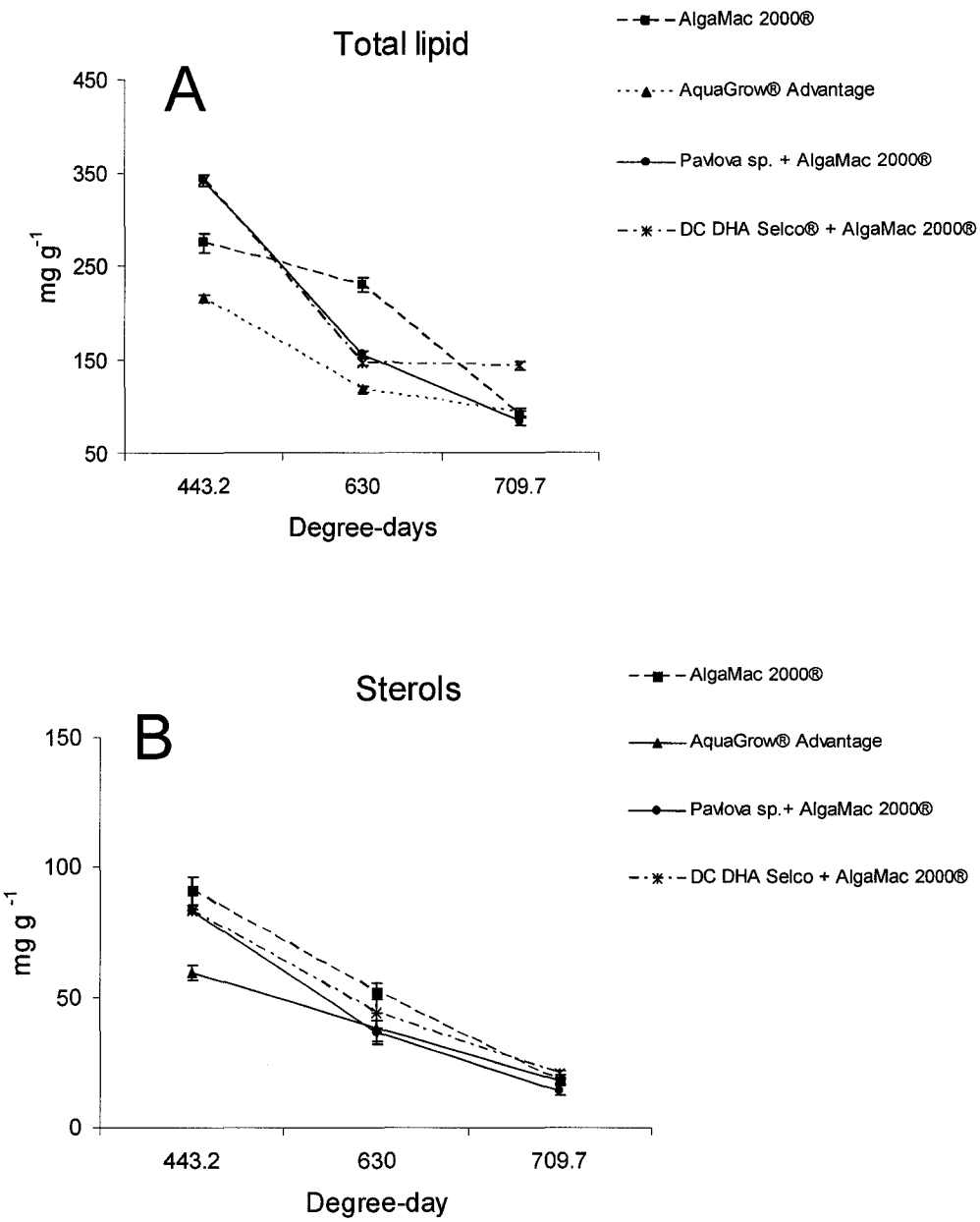

Figure III.4. Changes in total lipid (A) and sterols (B) concentrations in Atlantic cod larvae fed differently enriched *Artemia*. Samples were collected at 443.2 dd (37 dph), 630 dd (52 dph), and 709.7 dd (59 dph). Values are expressed as mean ± SE of 4 samples.

Table III.6. Total lipid (mg g^{-1} dry weight) and percentage lipid class composition of Atlantic cod larvae fed differently enriched *Artemia* at the start (37 dph; 443.2 dd) and the end of the experiment (59 dph; 709.7 dd).

	Treatments						
	Start of the experiment (37 days post hatch)			End of the experiment (59 days post hatch)			
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco® + AlgaMac 2000®
Total lipids[1] (mg g^{-1} dw)	274.4 ± 9.9^{a}	215.3 ± 3.5^{b}	341.8 ± 6.4^{c}	90.8 ± 4.2^{d}	93.0 ± 5.4^{d}	84.5 ± 4.6^{d}	142.4 ± 5.3^{e}
Lipid classes (% total lipids)							
Hydrocarbons	0.3 ± 0.0^{bc}	0.3 ± 0.0^{bc}	0.2 ± 0.1^{ab}	0.2 ± 0.0^{ab}	0.1 ± 0.0^{ab}	0.4 ± 0.1^{c}	0.1 ± 0.0^{ab}
Triacylglycerols	1.8 ± 0.4^{ab}	1.3 ± 0.2^{a}	2.1 ± 0.3^{ab}	22.8 ± 3.8^{c}	12.5 ± 1.6^{bc}	14.2 ± 1.9^{c}	21.0 ± 3.9^{c}
Free fatty acids	1.3 ± 0.2^{ab}	1.7 ± 0.1^{ab}	1.3 ± 0.4^{ab}	4.2 ± 0.4^{bc}	1.8 ± 0.3^{ab}	5.3 ± 1.1^{c}	3.9 ± 1.5^{bc}
Sterols	33.1 ± 1.5^{d}	27.6 ± 1.4^{cd}	24.3 ± 0.4^{bc}	19.9 ± 1.3^{ab}	19.4 ± 0.4^{ab}	17.1 ± 2.5^{a}	16.0 ± 1.1^{a}
Acetone Mobile Polar Lipids	1.4 ± 0.2^{ab}	0.3 ± 0.0^{a}	0.7 ± 0.1^{a}	3.5 ± 0.5^{c}	1.9 ± 0.3^{ab}	2.6 ± 0.4^{bc}	2.4 ± 1.1^{bc}
Phospholipids	61.7 ± 2.0^{cd}	68.1 ± 1.3^{cd}	70.8 ± 0.6^{d}	47.1 ± 2.6^{a}	63.5 ± 1.7^{cd}	59.5 ± 3.0^{bc}	54.7 ± 3.0^{ab}

Values (mean ± SE of four replicates, except for AlgaMac 2000® 37 dph, and *Pavlova* sp. + AlgaMac 2000® 37 dph where n=3) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] May also contain steryl esters and/or ketones at ≤ 1.76% each.

Over the experiment levels of ω6DPA increased in AlgaMac 2000 and AquaGrow Advantage-larvae, but decreased in *Pavlova* sp. + AlgaMac 2000 and DC DHA Selco + AlgaMac 2000-larvae (Figure III.6A). AlgaMac 2000-larvae had the highest level of this fatty acid, while AquaGrow Advantage-larvae had the lowest ($F_{6,21}$=92.44, p<0.0001). The DHA/EPA ratio ranged from 1.2 ± 0.0 in AquaGrow Advantage-larvae to 3.3 ± 0.4 in DC DHA Selco + AlgaMac 2000-larvae, with the ratio observed in AquaGrow Advantage-larvae being significantly lower than in larvae from other treatments ($F_{6,21}$=528.15, p<0.0001). Conversely, AquaGrow Advantage-larvae had a significantly higher EPA/AA ratio (5.2 ± 0.1) when compared to the ratios observed in the larvae from the other treatments ($F_{6,21}$=551.30, p<0.0001). Furthermore, this treatment was the only one in which larval levels of DHA + ω6DPA were below 21% (Table 7). The DHA/ω6DPA ratio ranged from 3.4 ± 0.1 in AlgaMac 2000-larvae to 7.3 ± 0.7 in AquaGrow Advantage-larvae, although the differences were significant only when compared to the values observed at the start of the experiment ($F_{6,21}$=183.30, p<0.0001). *Pavlova* sp. + AlgaMac 2000-larvae had the highest ω6DPA/AA ratio (1.4 ± 0.1) and AquaGrow Advantage-larvae the lowest (0.8 ± 0.1) ($F_{6,21}$=49.07, p<0.0001).

Larval dry weight correlated positively with the larval content of DHA, EPA, AA, and ω6DPA (Figure III.7) over all time points. However, the correlation was stronger with EPA (r^2= 75.7%, p<0.0001) than with AA, DHA or ω6DPA (r^2= 51.9%, p<0.001, r^2=45.5%, p<0.001, and r^2=39.7%, p<0.001, respectively). Other major variables were tested but did not correlate with larval dry weight.

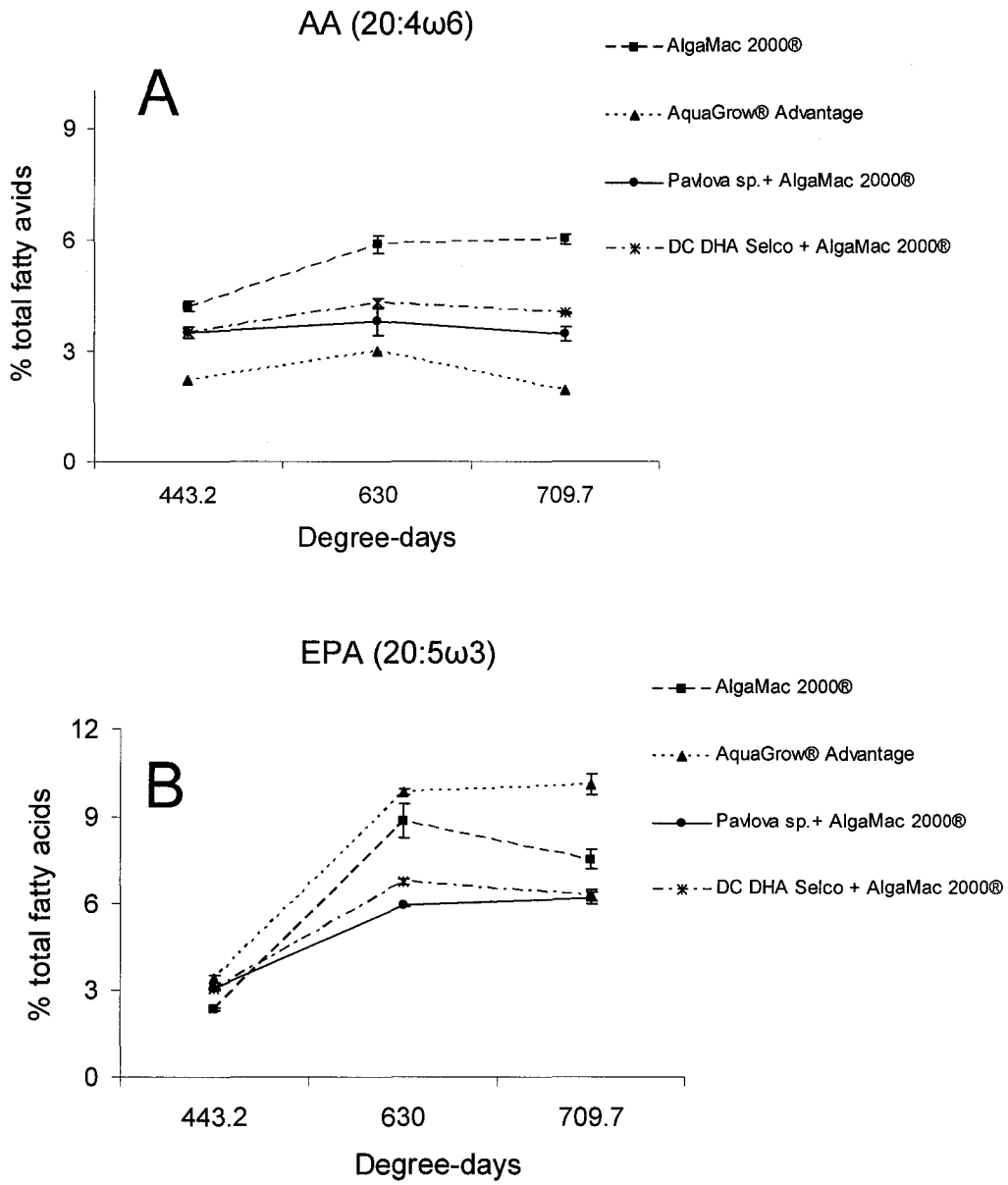

Figure III.5. Changes in the levels of AA (A) and EPA (B) in Atlantic cod larvae fed differently enriched *Artemia.* Samples were collected at 443.2 dd (37 dph), 630 dd (52 dph), and 709.7 dd (59 dph). Values are expressed as mean ± SE of 4 samples.

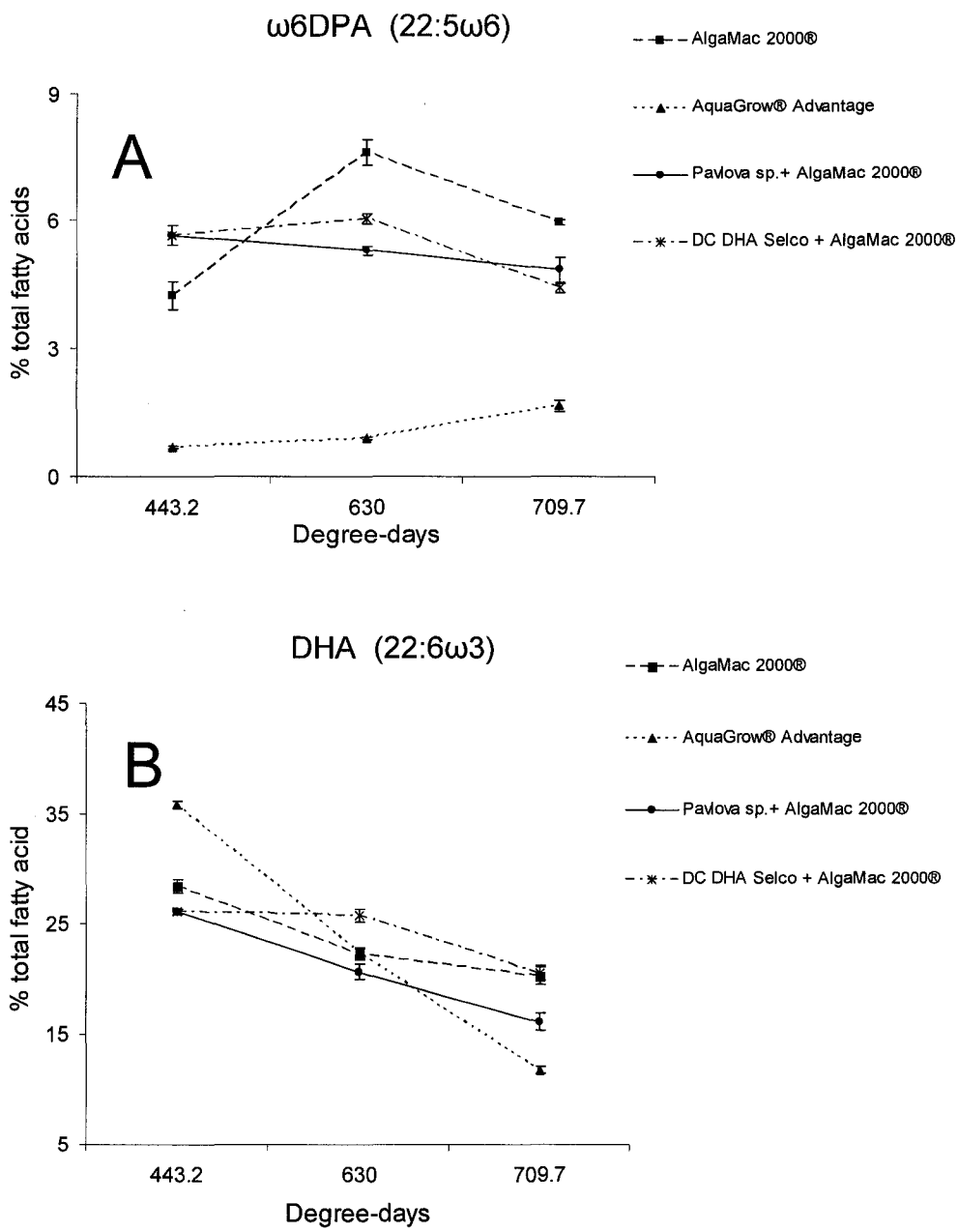

Figure III.6. Changes in the levels of ω6DPA (A) and DHA (B) in Atlantic cod larvae fed differently enriched *Artemia*. Samples were collected at 443.2 dd (37 dph), 630 dd (52 dph), and 709.7 dd (59 dph). Values are expressed as mean ± SE of 4 samples.

Table III.7. Fatty acid composition of Atlantic cod larvae fed differently enriched *Artemia* at the start (37 dph; 443.2 dd) and the end of the experiment (59 dph; 709.7 dd).

	Treatments						
	Start of the experiment (37 days post hatch)			End of the experiment (59 days post hatch)			
Fatty acids (% total fatty acids)	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco® + AlgaMac 2000®
14:0	1.3 ± 0.1^{a}	0.9 ± 0.0^{b}	1.4 ± 0.1^{a}	0.6 ± 0.0^{b}	0.6 ± 0.0^{b}	0.8 ± 0.1^{b}	0.7 ± 0.0^{b}
16:0	19.7 ± 0.2^{d}	14.5 ± 0.2^{c}	12.7 ± 0.3^{ab}	13.7 ± 0.3^{bc}	12.9 ± 0.0^{ab}	11.8 ± 0.4^{a}	12.7 ± 0.3^{ab}
18:0	8.0 ± 0.2^{b}	7.4 ± 0.3^{b}	6.7 ± 0.3^{ab}	6.1 ± 0.2^{a}	5.8 ± 0.1^{a}	6.2 ± 0.3^{a}	5.8 ± 0.2^{a}
22:0	0.1 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.3 ± 0.1^{b}	0.3 ± 0.1^{b}	0.4 ± 0.1^{b}	0.2 ± 0.0^{ab}
ΣSFA[1]	31.5 ± 0.0^{c}	25.2 ± 0.3^{b}	23.9 ± 0.2^{b}	23.7 ± 0.4^{b}	23.1 ± 0.2^{a}	22.9 ± 0.7^{a}	22.5 ± 0.5^{a}
16:1ω7	3.8 ± 0.1^{b}	3.4 ± 0.1^{b}	5.3 ± 0.2^{c}	1.3 ± 0.1^{a}	1.6 ± 0.0^{a}	1.7 ± 0.1^{a}	1.6 ± 0.0^{a}
18:1ω9	8.2 ± 0.3^{a}	11.3 ± 0.2^{c}	11.2 ± 0.4^{bc}	10.8 ± 0.3^{b}	13.1 ± 0.2^{d}	13.1 ± 0.2^{d}	11.0 ± 0.3^{bc}
18:1ω7	5.7 ± 0.2^{b}	4.3 ± 0.1^{a}	5.1 ± 0.1^{ab}	5.5 ± 0.1^{b}	7.3 ± 0.0^{c}	6.9 ± 0.5^{c}	5.2 ± 0.1^{ab}
20:1ω9	0.7 ± 0.1^{a}	1.2 ± 0.1^{b}	1.4 ± 0.2^{b}	0.4 ± 0.1^{a}	0.8 ± 0.0^{a}	0.7 ± 0.0^{a}	0.5 ± 0.0^{a}
ΣMUFA[2]	25.0 ± 0.0^{c}	24.7 ± 0.3^{c}	29.3 ± 1.0^{d}	19.7 ± 0.4^{a}	26.8 ± 0.2^{c}	24.7 ± 0.6^{c}	21.9 ± 0.1^{b}
18:2ω6	1.1 ± 0.1^{a}	2.2 ± 0.0^{b}	3.2 ± 0.1^{c}	2.8 ± 0.1^{bc}	4.2 ± 0.0^{d}	3.4 ± 0.3^{c}	3.3 ± 0.2^{c}
18:3ω3	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	8.9 ± 0.6^{b}	13.0 ± 0.2^{c}	11.3 ± 0.7^{d}	7.9 ± 0.4^{b}

18:4ω3	0.2 ± 0.0^{a}	0.2 ± 0.0^{a}	0.2 ± 0.1^{a}	0.9 ± 0.1^{b}	1.3 ± 0.0^{cd}	1.6 ± 0.1^{d}	1.1 ± 0.0^{bc}
20:3ω6	0.6 ± 0.0^{bc}	0.7 ± 0.0^{c}	1.0 ± 0.1^{d}	0.3 ± 0.0^{a}	0.3 ± 0.5^{a}	0.3 ± 0.0^{ab}	0.2 ± 0.1^{a}
20:4ω6 (AA)	4.2 ± 0.1^{c}	2.2 ± 0.1^{a}	3.5 ± 0.2^{b}	6.0 ± 0.1^{d}	1.9 ± 0.0^{a}	3.5 ± 0.2^{b}	4.0 ± 0.0^{bc}
20:4ω3	0.3 ± 0.0^{a}	1.0 ± 0.8^{a}	0.5 ± 0.1^{a}	0.6 ± 0.0^{a}	0.8 ± 0.0^{a}	0.9 ± 0.0^{a}	0.7 ± 0.0^{a}
20:5ω3 (EPA)	2.3 ± 0.0^{a}	3.4 ± 0.1^{b}	3.0 ± 0.0^{ab}	7.6 ± 0.3^{d}	10.1 ± 0.4^{e}	6.2 ± 0.1^{c}	6.3 ± 0.2^{c}
22:5ω6 (ω6DPA)	4.2 ± 0.3^{b}	0.7 ± 0.0^{a}	5.7 ± 0.2^{bc}	6.0 ± 0.1^{c}	1.6 ± 0.1^{a}	4.9 ± 0.3^{b}	4.4 ± 0.1^{b}
22:5ω3	0.8 ± 0.0^{b}	1.4 ± 0.1^{d}	0.6 ± 0.1^{a}	0.6 ± 0.0^{ab}	0.7 ± 0.0^{ab}	1.1 ± 0.0^{c}	0.7 ± 0.0^{ab}
22:6ω3 (DHA)	28.3 ± 0.6^{d}	35.8 ± 0.3^{e}	26.0 ± 0.2^{d}	20.3 ± 0.8^{c}	11.8 ± 0.3^{a}	16.2 ± 0.8^{b}	20.5 ± 0.6^{c}
ΣPUFA[3]	43.2 ± 0.1^{a}	50.0 ± 0.6^{b}	47.5 ± 0.7^{c}	56.8 ± 0.3^{d}	50.3 ± 0.2^{be}	52.4 ± 0.9^{e}	55.5 ± 0.5^{d}
ΣOBFA[4]	2.1 ± 0.1^{ab}	2.1 ± 0.2^{ab}	2.4 ± 0.3^{bc}	2.8 ± 0.2^{bcd}	2.9 ± 0.2^{bcd}	3.4 ± 0.4^{cd}	3.7 ± 0.4^{d}
Σω3	32.0 ± 0.6^{a}	42.0 ± 0.6^{d}	30.7 ± 0.3^{a}	39.7 ± 0.5^{d}	39.2 ± 0.5^{c}	38.2 ± 0.3^{b}	38.5 ± 1.2^{b}
Σω6	10.5 ± 0.5^{b}	6.8 ± 0.1^{a}	14.1 ± 0.6^{c}	9.8 ± 0.1^{b}	9.0 ± 0.2^{b}	8.3 ± 0.6^{a}	13.0 ± 0.3^{c}
ω3/ω6	3.2 ± 0.2^{b}	6.3 ± 0.1^{e}	2.1 ± 0.0^{a}	4.0 ± 0.0^{c}	4.4 ± 0.1^{cd}	5.0 ± 0.4^{d}	2.9 ± 0.0^{b}
DHA/EPA	12.1 ± 0.0^{e}	10.6 ± 0.4^{d}	8.6 ± 0.1^{c}	2.7 ± 0.2^{b}	1.2 ± 0.0^{a}	2.6 ± 0.1^{b}	3.3 ± 0.4^{b}
EPA/AA	0.6 ± 0.0^{a}	1.6 ± 0.0^{cd}	0.9 ± 0.0^{b}	1.3 ± 0.1^{c}	5.2 ± 0.1^{e}	1.8 ± 0.1^{d}	1.6 ± 0.0^{cd}
DHA + ω6DPA	32.6 ± 0.3^{d}	36.5 ± 0.3^{e}	32.1 ± 0.4^{d}	26.2 ± 0.8^{c}	13.5 ± 0.4^{a}	21.0 ± 0.8^{b}	25.0 ± 0.8^{c}
DHA/ω6DPA	6.9 ± 0.8^{a}	53.3 ± 3.4^{b}	4.3 ± 0.1^{a}	3.4 ± 0.1^{a}	7.3 ± 0.7^{a}	3.4 ± 0.2^{a}	4.6 ± 0.0^{a}
ω6DPA/AA	1.0 ± 0.1^{b}	0.3 ± 0.0^{a}	1.6 ± 0.0^{c}	1.0 ± 0.0^{b}	0.8 ± 0.1^{b}	1.4 ± 0.1^{c}	1.1 ± 0.0^{b}

Values (mean ± SE of four replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids, which also includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 20:0, 21:0, and 23:0 at ≤ 0.98% each.

[2]Sum of monounsaturated fatty acids, which also includes: 15:1, 16:1ω9, 16:1ω5, 17:1, 18:1ω11, 18:1ω5, 20:1ω11, 20:1ω7, 22:1ω11, 22:1ω9, and 24:1 at ≤ 1.26% each.

[3]Sum of polyunsaturated fatty acids, which also includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:3ω4, 18:4ω1, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 22:1ω7, 22:4ω6, and 22:4ω3 at ≤ 1.08% each.

[4]Sum of odd and/or branched fatty acids. Includes: *i*15:0, *ai*15:0, 15:0, 15:1, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, and 17:1.

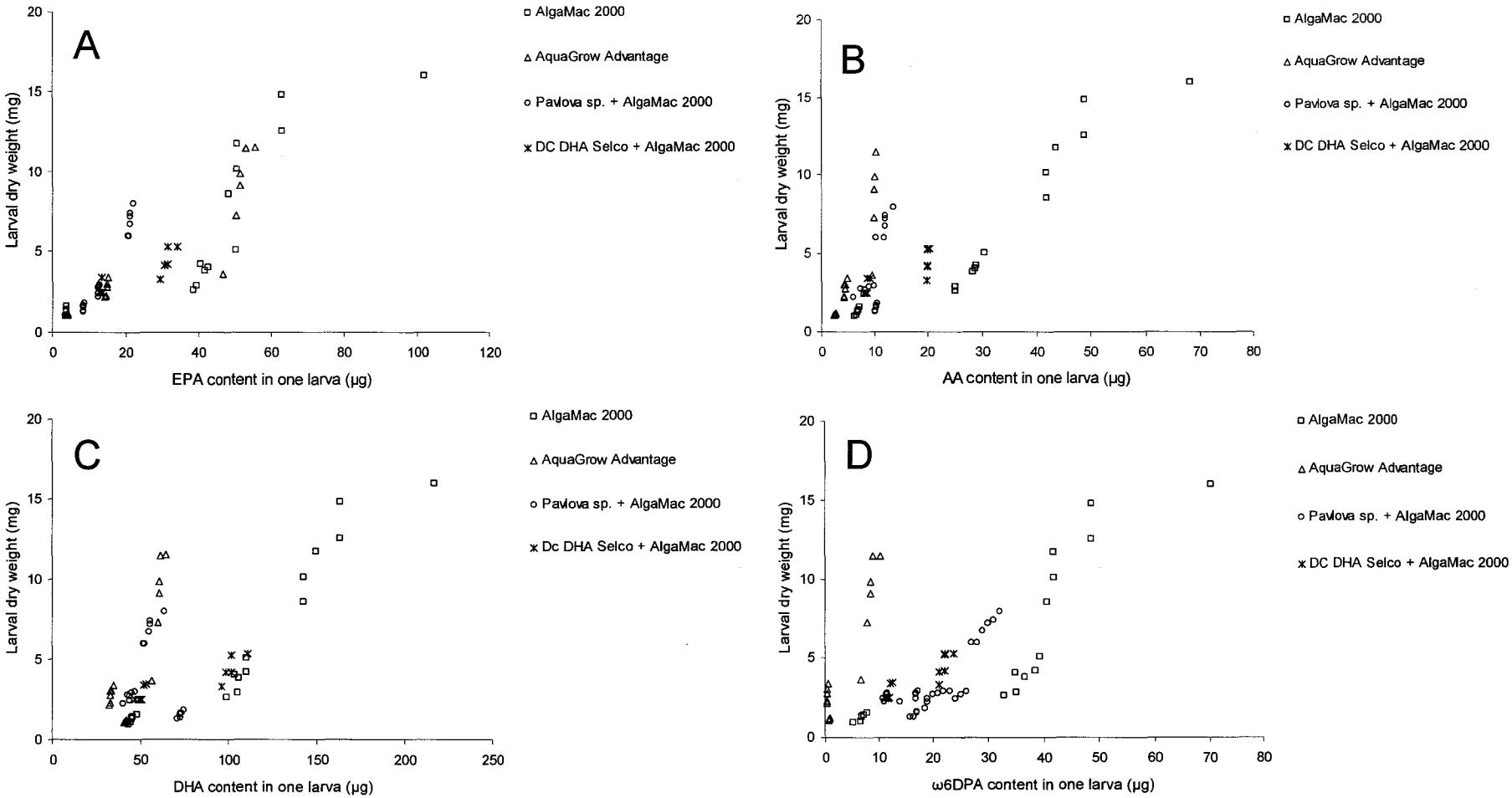

Figure III.7. The relationship between larval dry weight and (A) EPA content of Atlantic cod larvae, (B) AA content of Atlantic cod larvae, (C) DHA content of Atlantic cod larvae, (D) ω6DPA content of Atlantic cod larvae. Values of fatty acids expressed as content in one larva (µg).

III.5. Discussion

The lipid class composition of enriched *Artemia* did not mirror the composition of the enrichments. For example, DC DHA Selco had low PL and relatively high AMPL percentages; however, *Artemia* metanauplii enriched with this emulsion showed the highest PL and lowest AMPL percentages. Similarly, in AquaGrow Advantage the TAG percentage was almost twice as much as in *Pavlova* sp. paste, but *Pavlova* sp.-enriched *Artemia* had virtually the same TAG percentage as AquaGrow Advantage-enriched *Artemia*. In addition to the lipid classes, *Artemia* also modified the fatty acid composition of the diet during the enrichment process. The level of AA was higher in DC DHA Selco than in AlgaMac 2000, but both treatments resulted in *Artemia* with similar levels of this fatty acid. In AquaGrow Advantage the DHA concentration was approximately 59% higher than in AlgaMac 2000; however, AlgaMac 2000-enriched *Artemia* had more than four-fold the concentration of DHA in AquaGrow Advantage-enriched *Artemia*. Previous studies have demonstrated that *Artemia* can actively change the lipid class and fatty acid composition of their diets during the enrichment process (McEvoy et al., 1996; Coutteau and Mourente, 1997; Navarro et al., 1999). Coutteau and Mourente (1997) observed an increase of the total lipid content of *Artemia* (from 20 to ~28% dry matter) after 12 h of enrichment. The increment of total lipid was due to a significant accumulation of neutral lipid, mainly as TAG, regardless of the use of either pure TAG or a mixture of TAG (56%) and ethyl esters (44%) as a lipid source in the enrichment diet. Similarly,

Navarro et al. (1999) reported that fatty acids given to *Artemia* as ethyl esters rapidly appeared in other lipid classes, especially TAG and PL. In addition, using radiolabelled substrates, Navarro et al. (1999) demonstrated that *Artemia* metanauplii have the ability to retroconvert incorporated DHA to EPA during enrichment and subsequent starvation. In the present study, the increase in the total lipid content observed in *Artemia* after enrichment (between 20 and 159% of increase) was due mainly to increases in the PL concentration (between 3 and 27-fold increase), rather than in the TAG reserves. Published data on the total lipid content, lipid class and fatty acid composition of *Artemia* after enrichment show high variability due to differences in *Artemia* populations and harvests, enrichment diets tested as well as analytical methodology. Furthermore, the mechanisms of lipid assimilation by the live-feed during the enrichment process are not completely understood. Navarro et al. (1999) pointed out that differences in the enrichment conditions, *e.g.*, amounts of dissolved oxygen, hydrodynamics in the enrichments tanks, and size of micelles in the emulsion, may influence the *Artemia* enrichment process. These factors could affect the efficiency of the enrichment or, secondarily, the developmental rates of the nauplii and thus their metabolic rates. Nevertheless, at present, there is enough evidence that *Artemia* metanauplii are not mere passive carries of fatty acids; and therefore, as noted earlier by Navarro et al. (1999), the use of the term "bioencapsulation" should be avoided, since it mistakenly suggests that *Artemia* simply serve as a capsule to deliver unaltered nutrients to their predators.

The best larval growth and survival were not produced by the same treatment. AlgaMac 2000-enriched *Artemia* resulted in the heaviest larval dry weight at 59 dph (709.7 dd), but *Pavlova* sp. + AlgaMac 2000-larvae had the best survival overall. It is worth mentioning that the *Pavlova* sp. + AlgaMac 2000 treatment resulted in the best larval performance (growth and survival) during the rotifer phase (Chapter II). Additionally, DC DHA Selco + AlgaMac 2000-larvae showed survival comparable to *Pavlova* sp. + AlgaMac 2000-larvae. Both treatments were tested with larvae that fed on *Pavlova* sp. and *Pavlova* sp. + AlgaMac 2000-enriched rotifers during the rotifer phase. Moreover, it was observed that larvae from the *Pavlova* sp. + AlgaMac 2000 treatment had better resistance to stress caused by handling, since during the sampling procedure these larvae showed a more active swimming behavior, less abnormal swimming orientation, and less mortality than any other larvae.

It is important to note that the larvae from different treatments were not fed the same diet during the rotifer phase, and although they had similar percentages of TAG and PL at the start of the experiment, other major lipid variables such as total lipid concentration, ΣSFA, ΣPUFA, and AA percentage were significantly different among larvae from different treatments. Therefore, the different biochemical composition of the larvae at the start of the experiment should also be considered whem interpreting the results from the present study. Larvae from the two treatments that resulted in good growth performance had higher sum of saturated fatty acids, sum of ω3 fatty acids, EPA and sterol levels, and a lower ω6DPA/AA ratio than the larvae from the two treatments that resulted in good

survival. Fatty acid oxidation is the favored source of metabolic energy in several tissues in fish, with all saturated and monounsaturated fatty acids being readily catabolized by mitochondrial β-oxidation (Sargent et al., 1989; Sargent et al., 2002). During periods of rapid growth, there is a high demand for energy, and the higher tissue deposition of saturated fatty acids observed in the larvae from AlgaMac 2000 and AquaGrow Advantage treatments suggests that these larvae had more energy available for growth than the larvae from the other two treatments. Consistent with this, AlgaMac 2000 and AquaGrow Advantage-enriched *Artemia* had high levels of 14:0, 16:0, and 18:1ω7, and high sum of saturated fatty acids. There is some evidence that medium-chain fatty acids may be potentially useful as energy source in fish, and may also affect the deposition of fat in the tissues (Tocher, 2003).

Sterols are important for the physiology of eukaryotic organisms. They are structural components of cellular membranes, where they modulate their fluidity and function, and participate as secondary messengers in developmental signaling. Cholesterol, the main sterol in animal membranes, affects membrane fluidity by preventing the tight packing of the long hydrocarbon tails. As a result, cholesterol tends to abolish sharp transition temperatures and may also increase the stability and decrease the permeability of the membrane. Like sterols, EPA is also a structural component of membranes, and although it can be oxidized to generate energy, EPA has a clearly established role in membrane structure and function, and in eicosanoid production. Therefore, higher supply of energy and

higher levels of structural components in the tissues are both compatible with faster growth rates.

The reduction in the larval lipid content during the *Artemia* phase (between 57 and 75%) is likely the result of the rapid larval growth observed. As the larva grows, proteins become the major organic material, and their proportion reaches levels as high as 65 to 75% of the dry weight in adult fish (Wilson, 2002). Percentage levels of larval DHA also dropped markedly, from 26-35.8% to 11-20.5%. In contrast, larvae from all treatments accumulated EPA during the *Artemia* phase, reaching 59 dph with higher levels of this fatty acids than at the end of the rotifer phase (increment of 100 to 224%). Larval AA levels seemed to be more conservative, with only AlgaMac 2000®-larvae having higher levels of AA at 59 dph than at the start of the experiment. Cutts et al. (2006) observed a similar trend in Atlantic cod larvae fed rotifers and *Artemia* enriched differently. Despite the different enrichments, Atlantic cod DHA levels were approximately six times lower at 50 dph in all treatments, when compared to the period where the larvae were still feeding on rotifers (at 10 or 22 dph). Atlantic cod AA levels were more stable, with larvae from all treatments exhibiting similar levels throughout the entire trial (Cutts et al., 2006). A preferential accumulation of AA and reduction of DHA levels have also been observed in Atlantic halibut, *Hippoglossus hippoglossus* (Hamre et al., 2002), and Senegal sole, *Solea senegalensis,* larvae (Villalta et al., 2005) fed *Artemia*. According to Sargent et al. (2002), in marine fish the larval requirements for highly unsaturated fatty acids from the ω3 family (ω3 HUFA) is higher than those of juveniles and pre-adults.

The importance of ω3 HUFA for the early development of vertebrates is strongly related to growth and tissue differentiation. DHA is a major component of eyes, brain and nervous tissues in vertebrates (Sargent et al., 2002), and for this reason, is particularly important for very young and fast-growing stages of fish larvae. Atlantic cod larvae for example, will double their biomass during the first week or two (Olsen, 2004). The weaning of pelagic marine fish larvae traditionally starts once the fish larva has metamorphosed or while it is going through metamorphosis (Stoss et al., 2004), which normally coincides with the end of the *Artemia* period. Once weaned, an adequate supply of ω3 HUFA and, particularly DHA is generally not a major problem (Sargent et al., 2002). Therefore, it is not surprising that in the present study, at the end of the *Artemia* phase Atlantic cod larvae had lower DHA levels than at the end of the rotifer phase. On the other hand, larvae from all treatments had higher AA levels at 59 dph than at the start of the experiment. The onset of metamorphosis for Atlantic cod is considered to be at 12 mm larval length (Otterlei et al., 1999) and during this period, the major obstacle for the larvae is to overcome the stress posed by the morphological and physiological changes that will take place during the metamorphosis process. Both AA and EPA are precursors of eicosanoids in fish tissues (Tocher, 2003) with EPA-derived eicosanoids being less biologically active than those formed from AA. These highly active molecules are involved in a variety of physiological actions, and as stated by Sargent et al. (1999b), in broad terms, they are produced in response to stressful situations, both at a cellular and whole body level. The effects of dietary AA in survival and/or stress resistance have been

demonstrated for some marine fish, including turbot (Castell et al., 1994), summer flounder (Willey et al., 2003), and gilthead seabream (Bessonart et al., 1999; Koven et al., 2001; 2003).

The ω6 docosapentaenoic acid (ω6DPA) has been neglected in nutritional studies with marine fish larvae. In mammalian tissues, DHA are precursors of novel bioactive lipid mediators, collectively termed docosanoids. These 22-carbon molecules are involved in immune and/or neuroprotective response, and have function similar to eicosanoids (Hong et al., 2003). It is possible that the same enzymes involved in the production of docosanoids from DHA would work on ω6DPA to form a parallel series of lipid mediators, as observed with EPA and AA in the production of eicosanoids. The possible role of ω6DPA as an essential fatty acid for Atlantic cod larvae has been discussed earlier in Chapter II. In the present study, the larvae with best growth and survival performances had both relatively high ω6DPA levels, with levels in AlgaMac 2000-larvae being significantly higher than in larvae from other treatments. It is noteworthy that two (AlgaMac 2000-enriched *Artemia* and *Pavlova* sp. + AlgaMac 2000-enriched *Artemia*) out of the three enriched *Artemia* types that resulted in the best larval growth and survival, had ω6DPA significantly higher than the other treatments.

Larvae fed *Artemia* with a DHA/EPA/AA ratio of 6.8/1.9/1 had the heaviest final dry weight, while feeding a combination of *Pavlova* sp.-enriched *Artemia* (DHA/EPA/AA ratio of 0.3/3.7/1) and *Pavlova* sp. + AlgaMac 2000-enriched *Artemia* (7/2.4/1) resulted in the highest survival rate. The number of prey consumed by the larvae during different feeding periods (morning or afternoon)

were not measured, and therefore is difficult to estimate the contribution of *Pavlova* sp.-enriched *Artemia* to the overall supply of essential fatty acids. Nevertheless, considering both growth and survival results together, and the fact that two out of the three prey types used to produce such results had very similar DHA/EPA/AA ratios, it is unlikely that the DHA/EPA/AA ratio in *Pavlova* sp.-enriched *Artemia* was responsible for the higher survival rates observed in the *Pavova* sp. + AlgaMac 2000 treatment. A possible explanation for the positive effect of *Pavlova*-sp.-enriched *Artemia* on larval survival could be a change in the bacterial composition of *Artemia* caused by the enrichment with algae. During the hatching and enrichment process, the numbers of bacteria in *Artemia* increase exponentially (Ritar et al., 2004). *Artemia* is a filter-feeding bacteriovore capable of concentrating large amounts of bacteria. Once the larvae ingest the live-feed, the bacteria associated with the prey proliferate exponentially in the fish gut, making live-feed the major influx of bacteria to the gastrointestinal tract of fish (Makridis et al., 2000, Makridis et al., 2001). High mortalities in intensive rearing of marine fish larvae—including Atlantic cod—could be partly explained by the increased number of opportunistic bacteria in the fish gut during early development (Olafsen, 2001; Vadstein et al., 2004). It has been demonstrated that in *Artemia* fed with microalgae, new bacterial species from the algal culture are introduced, resulting in a more stable and diverse flora associated with the animals (Olsen et al., 2000; Makridis et al., 2006). In addition, microalgae promote a reduction in the bacterial numbers in *Artemia*, by expelling the gut contents, which act as a substrate for bacterial proliferation (Olsen et al., 2000).

This trend was observed in the present study, with *Artemia* enriched with *Pavlova* sp. having lower levels of bacterial fatty acids (odd and/or branched fatty acids-OBFA). Algae can possibly also affect the bacterial community in the live feed by production of antibacterial substances, as reported for *Tetraselmis suecica* (Austin and Day, 1990; Austin et al., 1992). Although we did not study the bacteria associated with the live-feed and/or the larvae, Korsnes et al. (2006) reported that different enrichment media affected not only the quantity but also the quality of the microbiota composition in the gut of Atlantic cod larvae. It has been demonstrated that the presence of certain bacterial species in sufficient amounts may induce mortality, whereas other bacteria may even increase survival (Hjelm et al., 2004). In the present study, the larval sum of OBFA was not different among treatments; however, there was a significant difference in the levels of *ai*17:0 ($F_{2,9}$=11.76, p=0.003), indicating a difference in the composition of the bacterial population. Therefore, more research is necessary to confirm the hypothesis that the use of microalgae for the enrichment of live-food affects the microbiota composition of prey and predator in a beneficial way, resulting in improved Atlantic cod larval survival.

On the other hand, considering the treatments that resulted in poor growth and survival performance, the DHA/EPA/AA ratios were 11/9/1, 5/5/1, and 10/3.5/1 in AquaGrow Advantage-enriched *Artemia*, DC DHA Selco-enriched *Artemia,* and DC DHA Selco + AlgaMac 2000-enriched *Artemia*, respectively. Two out of three of these *Artemia* have low DHA/EPA ratio and high EPA/AA

ratio, suggesting that Atlantic cod larvae require diets with relatively high proportions of DHA and AA.

While our results can be partially explained by our lipid data, other factors should also be considered for the interpretation of the overall larval performance. The two treatments that resulted in top growth performance, AlgaMac 2000 and AquaGrow Advantage, had lower survival rates when compared to *Pavlova* sp. + AlgaMac 2000 and DC DHA Selco + AlgaMac 2000. High stocking density has been demonstrated to influence the social behavior, survival, and growth of several species of fish, including Arctic charr, *Salvelinus alpinus* (Brown et al., 1992), Atlantic cod (Baskerville-Bridges and Kling, 2000b), and spotted wolffish, *Anarhichas minor* (Jonassen, 2002). At high stocking densities, there is normally a reduction in social dominance and antagonistic behavior among individuals, leading to an increase in survival, but generally paralleled with a decrease in growth. Baskerville-Bridges and Kling (2000b) cultured Atlantic cod larvae at two different densities (150 or 300 larvae L^{-1}) during the rotifer and *Artemia* phases. Although there were no significant differences in larval length and dry weight during the rotifer phase, two weeks after introducing *Artemia*, it was observed that at higher density, larval survival was 12% greater, but the specific growth rate was 1% less per day. The authors suggested that food became limiting in the higher density tanks, resulting in lower growth rates. Further experiments where Atlantic cod larvae were cultured at different densities (50, 100, 200, and 300 larvae L^{-1}) but with food fed in excess in all tanks, showed no differences in larval length, dry weight or specific growth rates among different densities (Baskerville-

Bridges and Kling, 2000b). These results confirmed their first hypothesis that food was a limiting factor at high densities. The results reported by Baskerville-Bridges and Kling (2000b) cannot be directly compared to the findings in the present study because there are major differences in the experimental design of both experiments. Baskerville-Bridges and Kling (2000b) used small tanks (22 L) and worked with unusually high larval densities (150 and 300 larvae L^{-1}). Although it would be precipitated to use the results reported by these authors to conclude that in the two treatments with higher survival the larval growth was limited by food availability, recent experiments at the Ocean Sciences Centre have shown that at higher prey densities (>2,000 *Artemia* L^{-1}) larval growth is improved. As a result, in the current production protocol, *Artemia* is supplied to the larvae at a density of approximately 2,700 L^{-1}. The larval lipid composition data show that growth was not limited by the lipid composition of the diet. In DC DHA Selco-larvae the total lipid content, and the PL and TAG concentrations were all higher than in the other larvae, and in *Pavlova* sp. + AlgaMac 2000-larvae these parameters were not different from AlgaMac 2000 and AquaGrow Advantage-larvae. Other factors such as social behavior and water quality are known to affect fish growth, and could also be related to the arrested growth observed in the two treatments with higher larval densities. More research is necessary to better understand the effects of larval density on Atlantic cod larvae growth.

Based on the above, our results suggest that during the *Artemia* phase of Atlantic cod larviculture, prey items containing a DHA/EPA/AA ratio of 7/2/1 result in good larval performance; that prey items should contain proportionally high

DHA and AA levels; and that the use of microalgae for the enrichment of live-feed may help to improve larval survival.

CHAPTER IV

Use of differently enriched rotifers, *Brachionus plicatilis*, during the larviculture of haddock, *Melanogrammus aeglefinus*: Effects on early growth, survival and body lipid composition.

IV.1. Introduction

Haddock, *Melanogrammus aeglefinus*, is a bottom-living species from the Gadidae family that inhabits cool temperate waters from inshore areas to the edge of the continental shelf. Haddock is found on both sides of the North Atlantic. On the North American coast, it ranges from the Strait of Belle Isle to as far south as Cape Hatteras, with a major concentration on eastern Georges Bank (Scott and Scott, 1988). Haddock stocks were greatly overexploited in the mid-1960s and the stocks are still recovering. The average Canada/USA combined catch declined from 9,200 tones in the late 1980s, to about 4,000 tones in 1999 (DFO, 2002). Interest in haddock aquaculture in Canada began in the late 1980s, when salmon growers recognized the necessity to diversify into alternate species. Along with Atlantic halibut, haddock was identified as a potential alternate species that would require minimal change to the grow-out technology used for salmon, and would be suitable for rotation with salmon on a given site (Harmon, 2003). A combined effort between two government hatcheries and a private salmon aquaculture company resulted in the production of 180 metric ton of haddock in 2002. After the private partner lost interest, the project was discontinued in 2004. Outside of Canada, research into haddock aquaculture is underway at several institutions in the United States, Scotland, and Norway (Harmon, 2003).

As with many new marine species, the early rearing of haddock is hampered by poor growth and low survival rates. One of the main factors contributing to this situation is the fact that the live-food organism normally used

during the first feeding period of marine species— the rotifer *Brachionus* spp.—is considered nutritionally inadequate. In this context, lipids play a central role. The importance of lipids for the early development of marine fish larvae has long been recognized (Sargent et al., 1989; Rainuzzo et al., 1997; Sargent et al., 1999b; Izquierdo et al., 2000). The natural diet of many marine species, including haddock, is composed mainly of copepods, which are rich in eicosapentaenoic acid (20:5ω3, EPA) and docosahexaenoic acid (22:6ω3, DHA). Studies that have examined fatty acid trophic transfer in marine planktonic systems—by comparing the relative fatty acid composition of different trophic levels—reveled that EPA and DHA were markedly concentrated in copepods and fish larvae relative to the phytoplankton (Brett and Müller-Navarra, 1997). These two fatty acids along with arachidonic acid (20:4ω6, AA) are considered to be essential for the nutrition of marine fish species (Sargent et al., 2002). On the other hand, rotifers are naturally poor on EPA and DHA, and if used without supplementation as the live food for cold-water marine fish larvae, often only provide suboptimum nutrition (Rainuzzo et al., 1997). The essential fatty acid requirements of haddock larvae have not been identified yet, however the enrichment or supplementation of rotifers with lipid rich diets is a common practice in the production protocol of many cold-water marine species (Olsen, 2004), including Atlantic cod, another gadoid closely related to haddock (Brown et al., 2003).

The present study was aimed at evaluating the effects of commercial products used for the enrichment of rotifers on the growth, survival and lipid composition of haddock larvae.

IV.2. Material and Methods

IV.2.1. Rotifer culture

Rotifers were cultured in 3,000 litre (L) rectangular tanks on a combination of baker's yeast (0.5 g million^{-1}, five feedings a day) and algae (*Nannochloropsis* sp. and *Isochrysis* sp.), on a five day cycle. From day 1 to day 3, Cultured Selco® (Inve Americas, Salt Lake City, UT, USA) was added to the culture tank (33.3 g day^{-1}) as supplemental food. The culture started with a volume of 900 L, a salinity of 15, and a density of 17 x 10^4 rotifers L^{-1}; and progressively increased in volume (final volume of 3000 L), density (final density of 13 x 10^4 rotifers L^{-1}), and salinity (final salinity 25 pus). The temperature was maintained around 20°C. Population and egg counts were performed daily on a number per millilitre basis. After five days, rotifers were removed from the tank, washed, concentrated, and placed in a 300 L conical tank for enrichment.

IV.2.2. Larval rearing and rotifer enrichments

Haddock eggs were obtained form captive broodstock maintained at the Department of Fisheries and Oceans Biological Station, St. Andrews, New Brunswick, Canada. Eggs were incubated at 6°C in 250 L upwelling conical-bottom incubators. Newly hatched haddock larvae were stocked in 1,000 L tanks at a density of 75 larvae L^{-1} (two replicates per treatment), and reared up to 29

days post-hatch (dph), which is equivalent to 273.2 degree-days (dd) on differently enriched rotifers. The initial water temperature was set to match that of the egg incubator (5-6°C) to prevent temperature shock to the larvae during the stocking phase. Once the tanks were stocked, the water temperature was increased gradually and maintained between 9.2 and 10.5°C, over the course of the experiment. Flow rates in the experimental tanks were initially set to 3 L min^{-1}. At approximately 24 dph, the flow rates were increased to 5 L min^{-1}. Each tank was supplied with constant aeration and equipped with a floating surface skimmer that collects oil and foam from the water surface. Lighting was supplied on a 24:0 light:dark photoperiod. Light intensity at the surface of the water in the experimental tanks was initially 100 Lux, and progressively increased to 300 Lux over the first 15 days of culture. After 20 days, the light intensity was reduced to approximately 60 Lux in order to reduce stress. Three different treatments were tested. These were commercially available products that were used as a single enrichment or in combination with a commercial algae paste. The products tested differed in their proximate and lipid composition (Tables IV.1 and IV. 2). The treatments were: 1) AlgaMac 2000® (Aquafauna Bio-Marine Inc., Hawthorne, CA, USA) 2) AquaGrow® Advantage (Advanced BioNutrition Corp., Columbia, MD, USA), and 3) *Pavlova* sp. paste (Reed Mariculture, Campbell, CA, USA) combined with AlgaMac 2000®.

Table IV.1. Proximate composition of the enrichments tested.

	Enrichments		
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. paste
Proximate composition[1] (% dry weight basis)			
Protein	20.2	10.0	4.6
Lipids	38.1	23.0	1.76
Carbohydrate	17.1	9.0	1.9-2.1
Ash	20.4	44.0	*
Moisture	4.2	4.0	91.0
Vitamin A (IU/100 g)	<100	737.5	*
Vitamin C (mg/100 g)	4.8	*	*
Vitamin D (IU/100 g)	457.0	*	*
Vitamin E (IU/100 g)	12.0	*	*
Calcium (%)	*	0.3	*
Potassium (%)	*	0.6	*
Phosphorus (%)	*	0.2	*

[1] Values according to manufacturer's information. *N.b.* some of this information has changed over time.

* Information not available.

Rotifers were enriched in 300 L conical tanks at a density of $5x10^5$ rotifers L^{-1}, at temperature of 22°C for a period of 24 hours. Enrichments were added to the rotifer tanks twice in an eight-hour period, at 9:00 *a.m.* and 5:00 *p.m.*, with the amounts added each time as per manufacturer's directions. Larvae from treatment 3 were fed with *Pavlova* sp.-enriched rotifers during the morning and *Pavlova* sp + AlgaMac 2000-enriched rotifers during the afternoon. For this treatment, rotifer batches were enriched following the protocol for treatment 3

described in Chapter II. Enriched rotifers were supplied to the larvae from 1 to 29 dph (8.8 to 273.2 dd) at a density of 5000 L^{-1}. After the enriched rotifers were harvested, part was directly supplied to the larvae for the morning feeding, and the remainder set aside in cold storage (~6°C) to minimize nutritional loss until the afternoon feeding. Larvae were fed twice a day, between 9:00 and 10:00 *a.m.,* and 4:00 and 5:00 *p.m.*

Table IV.2. Total lipid, lipid class and fatty acid composition of the enrichments tested.

	Enrichments		
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. paste
Total lipid (mg g^{-1} dw)	301.9 ± 5.1[a]	214.6 ± 9.4[b]	55.6 ± 3.7[c]
Lipid Class (% total lipids)			
Hydrocarbons	0.3 ± 0.1[a]	1.7 ± 0.3[b]	0.7 ± 0.1[a]
Steryl Esters/Wax Esters	3.5 ± 0.4[a]	0.1 ± 0.1[b]	0.0 ± 0.0[b]
Ketones	6.4 ± 0.5[a]	3.0 ± 0.3[b]	0.6 ± 0.1[c]
Triacylglycerols	71.9 ± 1.6[a]	63.7 ± 0.4[b]	35.7 ± 0.3[c]
Free fatty acids	1.5 ± 0.4[a]	2.9 ± 0.4[a]	12.2 ± 1.1[b]
Alcohols	1.9 ± 0.4[a]	1.1 ± 0.1[a]	2.2 ± 0.2[a]
Sterols	1.2 ± 0.2[a]	4.0 ± 0.1[b]	0.9 ± 0.2[a]
Acetone Mobile Polar Lipids	7.5 ± 0.4[a]	4.5 ± 0.9[b]	26.6 ± 0.3[c]
Phospholipids	6.0 ± 0.3[a]	18.9 ± 0.2[b]	21.1 ± 0.8[c]
Fatty acids (% total fatty acids)			
14:0	18.6 ± 0.1[a]	17.0 ± 0.5[ab]	14.2 ± 1.2[b]
16:0	37.4 ± 0.4[a]	18.2 ± 0.4[b]	10.0 ± 0.9[c]
18:0	1.0 ± 0.0[a]	1.2 ± 0.1[a]	0.2 ± 0.0[b]
ΣSFA[1]	58.1 ± 0.3[a]	36.9 ± 0.8[b]	26.9 ± 1.9[c]

16:1ω7	4.4 ± 0.0[a]	1.0 ± 0.0[b]	7.3 ± 0.6[c]
18:1ω9	0.1 ± 0.0[a]	14.4 ± 0.3[b]	9.0 ± 0.8[c]
18:1ω7	2.5 ± 0.1[a]	0.1 ± 0.0[b]	0.7 ± 0.1[c]
ΣMUFA[2]	7.4 ± 0.3[a]	16.7 ± 0.3[b]	25.5 ± 1.0[c]
18:2ω6	0.1 ± 0.0[a]	0.1 ± 0.1[a]	6.3 ± 0.6[b]
18:3ω3	0.0 ± 0.0[a]	0.0 ± 0.0[a]	7.4 ± 0.2[b]
18:4ω3	0.2 ± 0.0[a]	0.1 ± 0.0[a]	12.3 ± 3.5[b]
18:5ω3	0.0 ± 0.0[a]	0.0 ± 0.0[a]	6.1 ± 0.1[b]
20:3ω6	0.2 ± 0.0[a]	0.0 ± 0.0[b]	0.1 ± 0.0[c]
20:4ω6 (AA)	0.6 ± 0.0[a]	0.0 ± 0.0[b]	0.2 ± 0.0[c]
20:4ω3	0.4 ± 0.0[a]	0.0 ± 0.0[b]	0.1 ± 0.0[c]
20:5ω3 (EPA)	0.6 ± 0.0[a]	0.0 ± 0.0[b]	0.8 ± 0.0[c]
22:5ω6 (ω6DPA)	7.4 ± 0.2[a]	0.0 ± 0.0[b]	1.3 ± 0.2[c]
22:5ω3	0.1 ± 0.0[a]	0.2 ± 0.0[b]	0.2 ± 0.1[b]
22:6ω3 (DHA)	24.7 ± 1.0[a]	45.8 ± 0.4[b]	16.5 ± 0.5[c]
ΣPUFA[3]	34.4 ± 0.7[a]	46.3 ± 0.5[b]	53.6 ± 2.9[b]
Σω3	26.0 ± 0.9[a]	46.0 ± 0.5[b]	43.5 ± 3.8[b]
Σω6	8.3 ± 0.3[a]	0.3 ± 0.0[b]	9.6 ± 0.9[a]
ω3/ω6	3.1 ± 0.2[a]	177.2 ± 2.1[b]	4.7 ± 0.8[a]
DHA/EPA	43.2 ± 3.0[a]	-	20.5 ± 1.3[b]
EPA/AA	1.0 ± 0.0[a]	0.0 ± 0.0[b]	4.9 ± 0.4[c]

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids, which also includes: *i*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 19:0, 20:0, 22:0, and 23:0 at ≤ 0.54% each.

[2]Sum of monounsaturated fatty acids, which also includes: 14:1, 15:1, 16:1ω9, 16:1ω5, 17:1, 18:1ω11, 18:1ω6, 20:1ω11, 20:1ω7, 22:1ω11, 22:1ω9, 22:1ω7, and 24:1 at ≤ 0.91% each.

[3]Sum of polyunsaturated fatty acids, which also includes: 16:2ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 20:2ω6, 20:3ω3, 21:5ω3, and 22:4ω6 at ≤ 1.54% each.

IV.2.3. Sampling, growth and survival measurements

Pooled samples of larvae were collected at 1, 7, 14, 21, and 29 dph (equivalent to 9.2, 64.8, 129, 193.2, and 273.2 dd, respectively) for dry weight measurements (three replicates $tank^{-1}$). Growth measurements were performed as described in Chapter II. Briefly, between 150 (1 dph) and 20 (29 dph) larvae were collected on pre-weighed glass microfibre filters and dried at 60°C for 48 h. Filters were re-weighed using a microbalance (UMT2, Mettler Toledo, Switzerland), and growth was then calculated as specific growth rate (SGR; % d^{-1} = [ln (final dry weight) – ln (initial dry weight)/days] x 100).

Survival measurements were based on visual observations and performed as described in Chapter II. A nominal scale (0 to 5) was used to measure the weekly survival rates. The scale used considered 0 as total mortality (or 0% survival), 1 as poor survival, 2 as reasonable survival, 3 as good survival, 4 as very good survival, and 5 as total survival (or 100% survival). Under the circumstances in which the present study was performed, around 35% of survival at the end of the rotifer phase was considered a good survival rate for haddock larvae.

IV.2.4. Lipid analysis

Pooled samples of larvae were collected at 1,7,14, 21, and 29 dph for lipid analysis (total lipids, lipid classes, and fatty acid composition). Samples of the

experimental enrichments, algae paste and enriched live-food were collected in triplicate for lipid analysis. Lipid extraction, and lipid class and fatty acid analyses were performed as described in Chapter II. Briefly, lipids were extracted in chloroform/methanol according to Parrish (1998) using a modified Folch procedure (Folch et al., 1957). Lipid classes were determined using thin layer chromatography with flame ionization detection (TLC/FID) with a MARK V Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel coated Chromarods and a three-stage development system was used to separate lipid classes. Fatty acid methyl esters (FAME) were prepared by transesterification as described by Budge and Parrish (2003). A Varian model 3400 GC equipped with a Varian 8100 autosampler was used for fatty acid analysis (Varian, Palo Alto, CA, USA). Peaks were detected by flame ionization. Fatty acid peaks were integrated using Varian Star Chromatography Software (version 5.50) and identification was made with reference to known standards (PUFA 1, PUFA 3, BAME and 37 Component FAME Mix, Supleco Canada, ON).

IV.2.5. Statistical analysis

Growth and lipid composition data were analyzed as described earlier in Chapter II. The General Linear Model (Minitab Version 13.1) was employed and in all cases, the effect of tanks was not significant. Data from treatments were pooled. Linear regression was used to describe the relationship between larval

dry weight and the larval concentration of total lipids and phospholipids. Significance was set at $\alpha = 0.05$ for all analyses.

IV.3. Results

IV.3.1. Total lipid, lipid class and fatty acid composition of rotifers

Total lipid concentration of rotifers in all treatments increased after 24 h enrichment, being significantly higher than the concentration observed in unenriched rotifers ($F_{4,10}=191.91$, $p<0.0001$). The AlgaMac 2000 treatment resulted in the highest increase (96.3%), and the AquaGrow Advantage treatment resulted in the lowest (24.7%). The total lipid concentration of AquaGrow Advantage-enriched rotifers was similar to *Pavlova* sp.-enriched rotifers ($F_{1,4}=0.93$, $p=0.38$). With the exception of *Pavlova* sp.-enriched rotifers, rotifers from the other treatments experienced a significant decrease in the phospholipid (PL) percentage ($F_{4,10}=33.87$, $p<0.0001$). The acetone mobile polar lipid (AMPL) proportion observed in AquaGrow Advantage-enriched rotifers (34.9%) was significantly higher than in unenriched rotifers ($F_{4,10}=5.44$, $p=0.013$). The triacylglycerol (TAG) percentage showed some variation among treatments. However, the values observed in enriched rotifers were not different from those observed in unenriched rotifers (Table IV.3). The sterol percentage (ST) showed little variation among treatments with only AlgaMac 2000-enriched rotifers having

a ST percentage that was significantly lower than the other groups ($F_{4,10}$=21.14, p<0.0001).

Table IV.3. Total lipid (mg g^{-1} dry weight) and percentage lipid class composition of rotifers before and after experimental enrichments.

	Treatments				
	Unenriched	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp.	*Pavlova* sp. + AlgaMac 2000®
Total lipid (mg g^{-1} dw)	26.7 ± 0.5^{a}	52.4 ± 0.7^{b}	33.3 ± 1.1^{c}	34.5 ± 0.6^{c}	40.4 ± 0.3^{d}
Lipid Class (% total lipids)					
Hydrocarbons	0.4 ± 0.1^{a}	0.5 ± 0.1^{a}	0.5 ± 0.2^{a}	0.5 ± 0.1^{a}	0.8 ± 0.0^{a}
Steryl Esters/ Wax Esters	6.2 ± 1.7^{a}	3.3 ± 0.8^{a}	4.1 ± 1.1^{a}	5.4 ± 1.0^{a}	1.2 ± 0.1^{b}
Triacylglycerols	12.6 ± 1.7^{abc}	17.0 ± 0.6^{c}	11.9 ± 0.4^{ab}	8.9 ± 0.6^{a}	13.4 ± 1.7^{bc}
Free fatty acids	4.0 ± 0.3^{ab}	4.2 ± 0.3^{ab}	2.9 ± 0.1^{a}	1.9 ± 1.2^{a}	6.7 ± 0.9^{b}
Sterols	3.5 ± 0.2^{ac}	1.6 ± 0.2^{b}	3.3 ± 0.1^{ac}	3.7 ± 0.1^{a}	2.6 ± 0.4^{c}
Acetone Mobile Polar Lipids	17.5 ± 5.0^{a}	24.7 ± 1.4^{a}	34.9 ± 2.4^{b}	28.2 ± 2.7^{a}	30.5 ± 0.6^{a}
Phospholipids	55.8 ± 1.4^{a}	48.7 ± 0.3^{bc}	42.5 ± 1.0^{d}	51.5 ± 0.5^{ab}	44.9 ± 1.8^{cd}

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different (p< 0.05).

AlgaMac 2000-enriched rotifers had high levels of myristic acid (14:0), and levels of palmitic acid (16:0) significantly higher than rotifers from the other treatments ($F_{4,10}$=13.44, p=0.0005). Consequently, these rotifers had the highest sum of saturated fatty acids (ΣSFA) ($F_{4,10}$=15.20, p=0.0003). *Pavlova* sp.-enriched rotifers had significantly lower levels of 14:0 and 16:0 than rotifers from the other treatments ($F_{4,10}$=9.16, p=0.0022, and $F_{4,10}$=13.44, p=0.0005, respectively) resulting in the lowest ΣSFA ($F_{4,10}$=15.20, p=0.0003). On the other

hand, this treatment resulted in rotifers with significantly higher levels of 16:1ω7 ($F_{4,10}$=14.10, p=0.0004), 18:1ω11 ($F_{4,10}$=13.77, p=0.0004), 20:1ω9 ($F_{4,10}$=37.20, p<0.0001), and sum of monounsaturated fatty acids (ΣMUFA, $F_{4,10}$=23.57, p<0.0001) than rotifers from the other treatments. Levels of polyunsaturated fatty acids (PUFA) increased significantly (increment between 200 and 376%) in rotifers from all treatments after enrichment ($F_{4,10}$=17.80, p=0.0002) (Table IV.4). AlgaMac 2000 enriched-rotifers had the highest levels of EPA and ω6DPA ($F_{4,10}$=39.18, p<0.0001, and $F_{4,10}$=23.40, p<0.0001, respectively), however, the percentage of these fatty acids in AlgaMac 2000-enriched rotifers was not different to that in *Pavlova* sp. + AlgaMac 2000-enriched rotifers. AquaGrow Advantage-enriched rotifers showed the highest levels of DHA ($F_{4,10}$=112.35, p<0.0001). This treatment also resulted in the highest sum of fatty acids from the ω3 family (Σω3, $F_{4,10}$=43.27, p<0.0001) and in the lowest sum of fatty acids from the ω6 family (Σω6, $F_{4,10}$=15.22, p=0.0003). Consequently, AquaGrow Advantage-enriched rotifers had a significantly higher ω3/ω6 ratio ($F_{4,10}$=5.64, p=0.012). Conversely, *Pavlova* sp.-enriched rotifers had low Σω3, high Σω6, and a significantly lower ω3/ω6 ratio ($F_{4,10}$=5.64, p=0.012). The DHA/EPA ratio ranged from 0.8 in *Pavlova* sp.-enriched rotifers to 70.2 in AquaGrow Advantage-enriched rotifers, with the latter value being significantly higher than the other treatments ($F_{4,10}$=97.42, p<0.0001). The EPA/AA ratio showed less variation, with only AlgaMac 2000-enriched rotifers having an EPA/AA ratio different from initially or the other treatments ($F_{4,10}$=20.46, p<0.0001).

Table IV.4. Percentage fatty acid composition of rotifers before and after experimental enrichments.

	Treatments				
	Unenriched	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp.	*Pavlova* sp. + AlgaMac 2000®
Fatty acids (% total fatty acids)					
14:0	6.7 ± 0.1^{a}	12.0 ± 1.2^{b}	11.0 ± 2.1^{ab}	3.8 ± 0.1^{c}	9.4 ± 0.5^{ab}
16:0	19.6 ± 0.1^{ab}	24.7 ± 1.9^{b}	16.1 ± 0.4^{ac}	10.5 ± 0.7^{c}	17.2 ± 2.4^{a}
17:0	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}	0.4 ± 0.3^{a}	0.2 ± 0.0^{a}	0.5 ± 0.2^{a}
18:0	6.1 ± 0.0^{a}	3.8 ± 0.2^{b}	5.6 ± 0.8^{ab}	4.7 ± 0.1^{ab}	4.3 ± 0.5^{ab}
ΣSFA[1]	41.2 ± 0.2^{ab}	46.8 ± 0.7^{b}	41.2 ± 3.1^{ab}	27.7 ± 1.2^{c}	36.7 ± 2.2^{a}
16:1ω7	16.6 ± 0.1^{ac}	13.0 ± 1.2^{ab}	9.2 ± 1.4^{b}	18.2 ± 0.5^{c}	13.0 ± 0.9^{ab}
18:1ω11	4.6 ± 0.1^{a}	2.2 ± 0.0^{b}	2.8 ± 0.1^{b}	4.7 ± 0.1^{a}	2.8 ± 0.7^{b}
18:1ω9	16.5 ± 0.2^{a}	8.5 ± 0.5^{b}	14.6 ± 2.5^{ab}	19.7 ± 0.8^{a}	14.5 ± 1.5^{ab}
18:1ω7	4.9 ± 0.0 ^{a}	5.7 ± 0.3^{a}	2.8 ± 0.4^{b}	5.1 ± 0.0^{a}	4.4 ± 0.4^{a}
20:1ω9	3.1 ± 0.1^{a}	0.1 ± 0.0^{b}	1.9 ± 0.3^{c}	3.2 ± 0.1^{a}	2.1 ± 0.4^{c}
ΣMUFA[2]	54.1 ± 0.0^{ac}	32.4 ± 2.2^{b}	36.3 ± 4.2^{b}	58.1 ± 0.8^{c}	43.8 ± 1.7^{a}
18:2ω6	0.3 ± 0.0^{a}	0.2 ± 0.1^{a}	0.3 ± 0.1^{a}	5.5 ± 1.3^{b}	3.0 ± 1.4^{ab}
18:3ω3	0.2 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.1^{a}	0.8 ± 0.3^{a}	0.5 ± 0.2^{a}
20:3ω6	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}
20:4ω6 (AA)	0.2 ± 0.0^{a}	0.6 ± 0.0^{ab}	0.2 ± 0.0^{a}	0.5 ± 0.2^{ab}	0.7 ± 0.0^{b}
20:4ω3	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.0 ± 0.0^{a}	0.4 ± 0.1^{a}	0.4 ± 0.2^{a}
20:5ω3 (EPA)	0.4 ± 0.0^{a}	1.8 ± 0.1^{b}	0.3 ± 0.0^{a}	0.6 ± 0.2^{a}	1.2 ± 0.1^{b}
22:5ω6 (ω6DPA)	0.1 ± 0.0^{a}	4.8 ± 0.9^{b}	0.1 ± 0.0^{a}	0.4 ± 0.0^{a}	2.5 ± 0.3^{b}
22:5ω3	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.2 ± 0.0^{a}	0.3 ± 0.1^{a}
22:6ω3 (DHA)	0.9 ± 0.1^{a}	11.7 ± 0.9^{b}	19.8 ± 1.1^{c}	0.4 ± 0.0^{a}	7.2 ± 0.9^{d}
ΣPUFA[3]	4.7 ± 0.1^{a}	20.3 ± 1.5^{bc}	22.4 ± 1.2^{b}	14.1 ± 1.9^{c}	19.5 ± 2.6^{bc}
Σω3	2.8 ± 0.2^{a}	14.4 ± 0.8^{b}	21.3 ± 1.4^{c}	4.3 ± 1.2^{d}	10.7 ± 1.6^{b}
Σω6	1.0 ± 0.1^{a}	5.7 ± 0.8^{b}	0.9 ± 0.3^{a}	9.3 ± 0.5^{b}	6.8 ± 1.9^{b}
ω3/ω6	2.7 ± 0.2^{a}	2.6 ± 0.2^{a}	30.1 ± 11.9^{b}	0.4 ± 0.1^{a}	1.9 ± 0.5^{a}
DHA/EPA	2.3 ± 0.1^{a}	6.6 ± 1.0^{a}	70.2 ± 6.6^{b}	0.8 ± 0.2^{a}	5.9 ± 0.9^{a}

EPA/AA	1.7 ± 0.2[a]	3.2 ± 0.0[b]	1.4 ± 0.2[a]	1.3 ± 0.2[a]	1.7 ± 0.1[a]
DHA + ω6DPA	1.0 ± 0.1[a]	16.5 ± 1.8[b]	19.9 ± 1.1[b]	0.8 ± 0.0[a]	9.7 ± 1.0[c]
DHA/ω6DPA	10.8 ± 2.6[a]	2.6 ± 0.3[a]	159.4 ± 44.4[b]	1.0 ± 0.0[a]	2.9 ± 0.5[a]
ω6DPA/AA	0.4 ± 0.1[a]	8.6 ± 2.1[b]	0.5 ± 0.1[a]	1.1 ± 0.4[a]	3.4 ± 0.5[a]

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids, which also includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 19:0, 20:0, 21:0, 22:0, 23:0, and 24:0 at ≤ 2.7% each.

[2]Sum of monounsaturated fatty acids, which also includes: 14:1, 15:1, 16:1ω9, 16:1ω5, 17:1, 18:1ω6, 18:1ω5, 20:1ω11, 20:1ω7, 22:1ω11, 22:1ω9, 22:1ω7 and 24:1 at ≤ 2.2% each.

[3]Sum of polyunsaturated fatty acids, which also includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:3ω4, 18:4ω3, 18:4ω1, 18:5ω3, 20:2β, 20:2ω6, 20:3ω3, 21:5ω3, 22:4ω6, and 22:4ω3 at ≤ 2.3% each.

IV.3.2. Larval survival and growth

The combination of *Pavlova* sp. and AlgaMac 2000 resulted in the best survival during the experimental period. Larvae from the AlgaMac 2000 treatment showed the poorest survival, with both tanks from this treatment producing very small numbers of larvae (Figure IV.1). For this reason, this treatment was terminated at the end of the rotifer phase and it was not tested during the *Artemia* phase (Chapter V).

The treatments did not influence the larval dry weight throughout the experimental period ($F_{2,65}=0.51$, $p=0.60$). At 29 dph (273.2 dd), larvae from all treatments had approximately 1 mg of dry weight (Figure IV.2; Table IV.5). Similarly, there were no differences in the growth rates produced by the different

treatments, with larvae from all treatments growing approximately 8% of their dry weight per day ($F_{2,15}$=0.36, p=0.70) (Table IV.5).

Table IV.5. Growth of haddock larvae fed differently enriched rotifers. Dry weights of 20 larvae were determined at 29 dph (273.2 dd). Dry weight SGR were calculated between 1 and 20 dph (n=6).

	Treatments		
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®
Dry weight at 29 dph (mg)	1.03 ± 0.07[a]	1.09 ± 0.08[a]	1.10 ± 0.05[a]
Specific growth rate (% d^{-1})	8.28 ± 0.10[a]	8.36 ± 0.14[a]	8.41 ± 0.08[a]

Values (mean ± SE) with different superscript are significantly different (p<0.05).

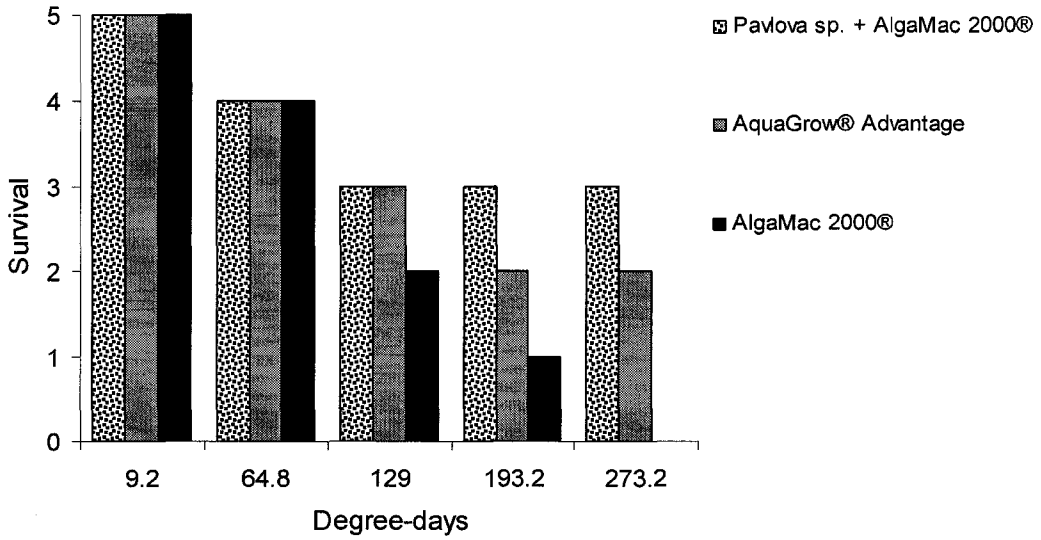

Figure IV.1. Survival of haddock larvae fed differently enriched rotifers at 9.2, 64.8, 129, 193.2, and 273.2 degree-days (equivalent to 1, 7, 14, 21, and 29 days post hatch, respectively). Values are expressed as mean of twelve observations per treatment.

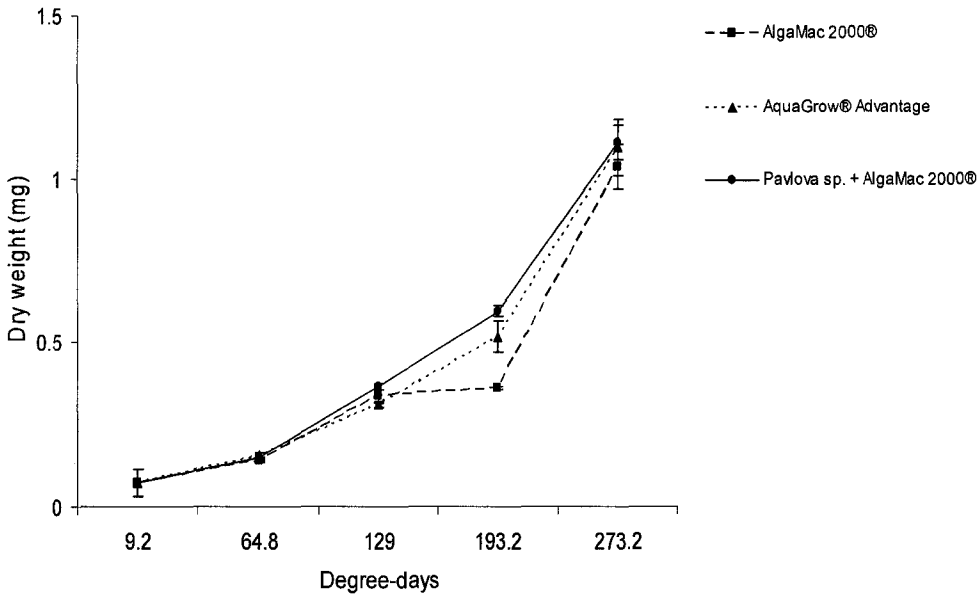

Figure IV.2. Dry weight of haddock larvae fed differently enriched rotifers at 9.2, 64.8, 129, 193.2, and 273.2 degree-days (equivalent to 1, 7, 14, 21, and 29 days post hatch, respectively). Values expressed as mean ± SE of 6 samples (except at 9.2 dd where n=3).

IV.3.3. Larval total lipid, lipid class and fatty acid composition

All treatments caused the larval tissue concentrations of total lipid and phospholipid to decrease (Table IV.6). At the end of the experiment, only larvae from the *Pavlova* sp. + AlgaMac 2000 treatment had total lipid concentrations similar to those observed in newly hatched larvae ($F_{1,5}$=2.02, p=0.21). Larvae from all treatments had lower PL concentrations (Appendix 1) and percentages at 29 dph (273.2 dd) than at 1 dph (9.2 dd) ($F_{3,11}$=32.69, p<0.0001 and $F_{3,11}$=19.28,

p=0.0001, respectively). Conversely, the larval ST concentration increased significantly during the experimental period, with larvae from all treatments having higher ST concentrations at the end than at the start of the experiment ($F_{3,11}$=39.79, p<0.0001) (Appendix 1). At 29 dph (273.2 dd), larvae from the AlgaMac 2000 treatment had the highest and the lowest TAG and ST concentrations, respectively (Figures IV.3A and IV.3B). Although the treatments did not affect the larval dry weights, there was a strong correlation between larval dry weight and larval total lipid ($F_{1,70}$=144.03, p<0.001, r^2=67.3%) and phospholipid ($F_{1,70}$=177.56, p<0.001, r^2=71.7%) concentration over all diets in all time points (Figures IV.4A and IV.4B). There were no significant correlations between larval dry weight and major fatty acids.

Table IV.6. Total lipid (mg g^{-1} dry weight) and percentage lipid class composition of haddock larvae at the start (newly hatched larvae) and the end of the experiment (29 days post-hatch).

		Treatments		
	Newly hatched larvae (1dph)	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®
Total lipids (mg g^{-1} dw)	52.9 ± 1.6[a]	39.9 ± 1.7[b]	39.7 ± 0.9[b]	50.8 ± 0.5[a]
Lipid classes[1] (% total lipids)				
Hydrocarbons	0.3 ± 0.0[a]	0.2 ± 0.0[a]	0.3 ± 0.1[a]	0.3 ± 0.0[a]
Triacylglycerols	3.7 ± 0.8[a]	7.8 ± 1.2[b]	4.4 ± 0.3[a]	3.0 ± 0.5[a]
Free fatty acids	0.3 ± 0.1[a]	0.5 ± 0.1[a]	1.5 ± 0.4[b]	0.5 ± 0.0[a]
Sterols	6.7 ± 0.9[a]	19.0 ± 1.0[b]	22.7 ± 1.2[b]	20.5 ± 0.6[b]
Acetone Mobile Polar Lipids	2.7 ± 0.8[a]	1.7 ± 0.5[a]	2.2 ± 0.7[a]	0.7 ± 0.4[a]
Phospholipids	84.6 ± 2.4[a]	70.1 ± 1.5[bc]	67.7 ± 1.6[b]	74.5 ± 1.0[c]

Values (mean ± SE of four replicates, except initial n=3) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] May also contain steryl esters, ketones, and alcohols at $\leq$ 1.6% each.

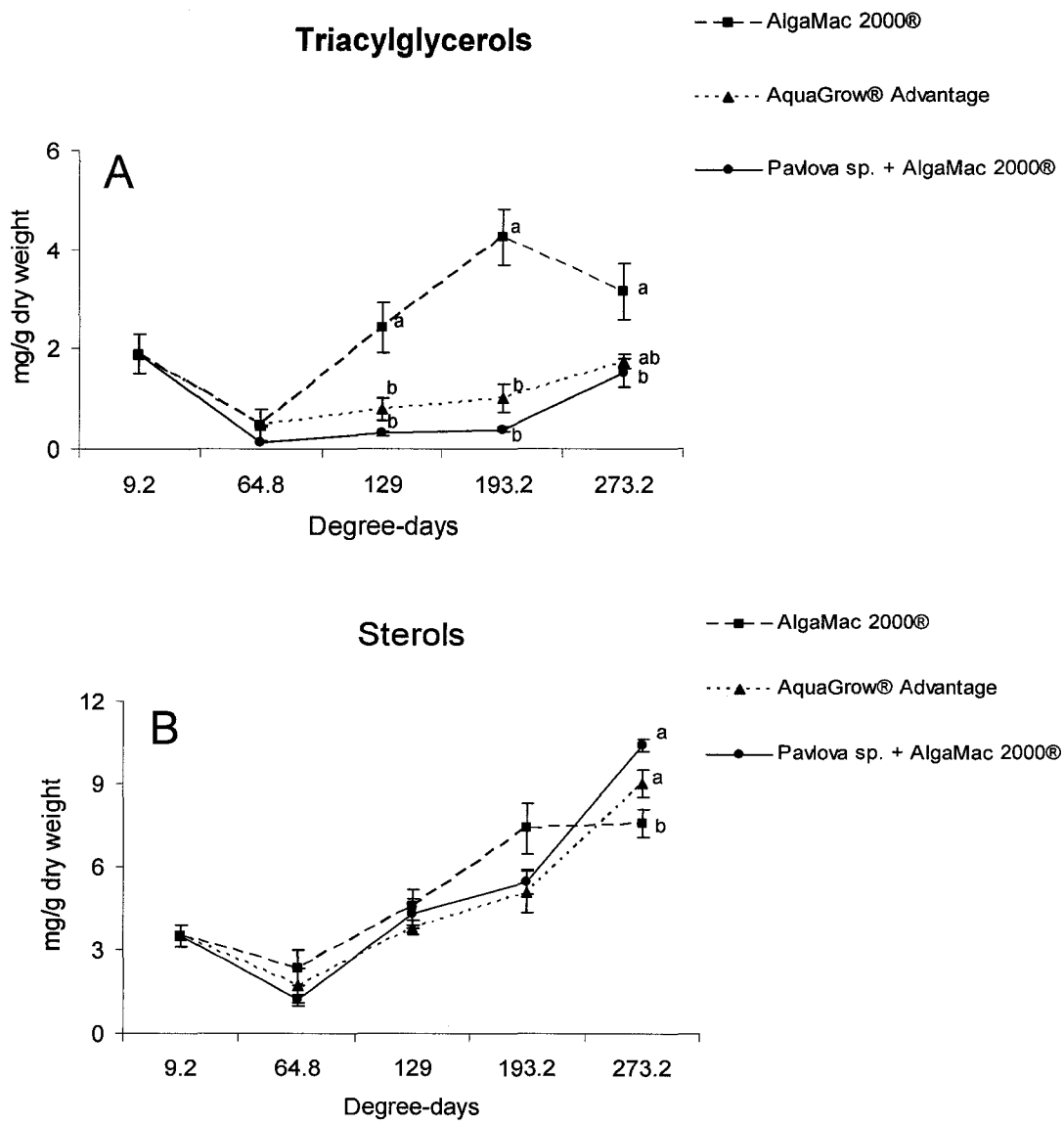

Figure IV.3. Changes in the concentrations of (A) triacylglycerols and (B) sterols in haddock larvae fed differently enriched rotifers. Samples were collected at 9.2 dd (1 dph), 64.8 dd (7dph), 129 dd (14 dph), 193.2 dd (21 dph), and 273.2 dd (29 dph). Values are expressed as mean ± SE of 6 samples, except at 9.2 dd (1 dph) where n=3. Data points with different superscripts are significantly different at $p<0.05$.

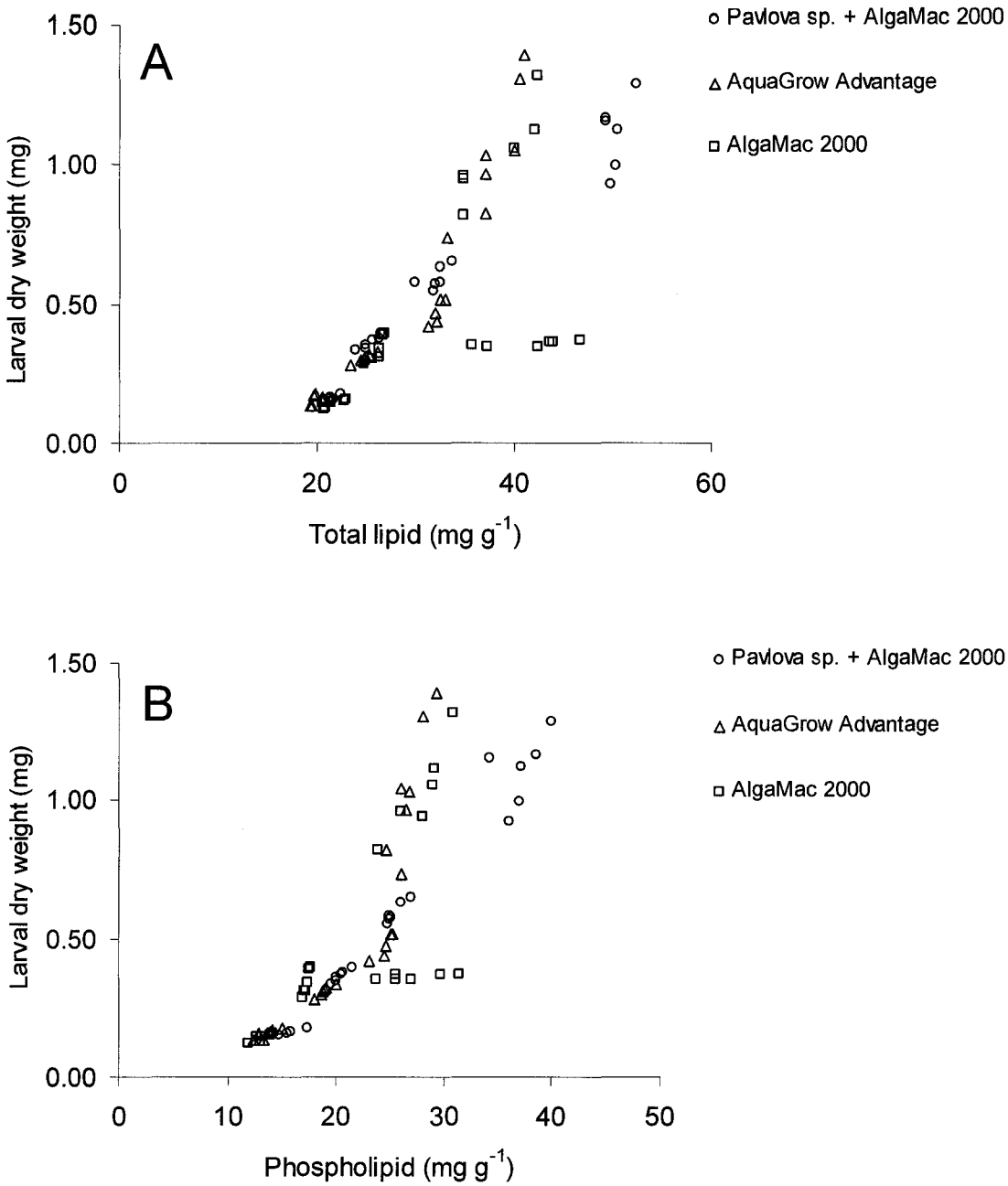

Figure IV.4. The relationship between larval dry weight and (A) larval total lipid concentration, and (B) larval phospholipid concentration, over the experimental period.

Larvae from all treatments reached 29 dph (273.2 dd) with significantly lower levels of palmitic acid (16:0) than those observed in newly hatched larvae ($F_{3,11}$=53.88, p<0.0001). The percentage of this fatty acid decreased from approximately 23% at 1 dph to 13% at 29 dph. As a result, the ΣSFA decreased approximately 6% in larvae from all treatments (Table IV.7). At 29 dph, larvae from all treatments had similar ΣSFA ($F_{2,9}$=0.23, p=0.79). In contrast, the larval ΣMUFA increased in all treatments during the experimental period, and at 29 dph, larvae from all treatments had significantly higher ΣMUFA than initially ($F_{3,11}$=113, p<0.0001). Among individual monounsaturated fatty acids, 16:1ω7 showed the highest increment, with larval levels at 29 dph being between 2 and 5% higher than the levels observed in newly hatched larvae ($F_{3,11}$=34.70, p<0.0001). Furthermore, levels of 18:1ω9 increased significantly in larvae from the AquaGrow Advantage and *Pavlova* sp. + AlgaMac 2000 treatments; however, in larvae from the AlgaMac 2000 treatment this fatty acid decreased significantly after 29 days of larviculture ($F_{3,11}$=128.14, p<0.0001). The percentages of AA and ω6DPA in larvae from AlgaMac 2000 and *Pavlova* sp.+ AlgaMac 2000 treatments at 29 dph were significantly higher than in newly hatched larvae ($F_{3,11}$=217.28, p<0.0001, and $F_{3,11}$=988.74, p<0.0001, respectively). Conversely, the percentage of EPA (20:5ω3) decreased in larvae from all treatments, with larvae from the AlgaMac 2000 treatment having significantly lower percentage ($F_{3,11}$=226.08, p<0.0001) and concentration (Figure IV.5A: $F_{2,9}$=119.71, p<0.0001) of this fatty acid than the larvae from other treatments.

Table IV.7. Percentage fatty acid composition of haddock larvae at the start (newly hatched larvae) and end of the experiment (29 days post hatch).

	Treatments			
	Newly hatched larvae (1dph)	AlgaMac 2000®	AquaGrow Advantage®	*Pavlova* sp. + AlgaMac 2000®
Fatty acids (%)				
14:0	0.9 ± 0.1^{a}	2.2 ± 0.1^{b}	1.6 ± 0.3^{ab}	1.4 ± 0.0^{a}
16:0	22.6 ± 1.4^{a}	13.3 ± 0.1^{b}	13.8 ± 0.3^{b}	13.1 ± 0.2^{b}
18:0	5.0 ± 0.3^{a}	6.8 ± 0.3^{b}	6.6 ± 0.1^{b}	7.3 ± 0.1^{b}
22:0	0.2 ± 0.0^{a}	0.1 ± 0.0^{ab}	0.0 ± 0.0^{b}	0.1 ± 0.1^{ab}
ΣSFA[1]	30.1 ± 1.9^{a}	24.3 ± 0.6^{b}	24.3 ± 0.0^{b}	24.0 ± 0.3^{b}
16:1ω7	1.6 ± 0.0^{a}	4.5 ± 0.0^{b}	3.6 ± 0.5^{b}	6.1 ± 0.2^{c}
18:1ω9	9.3 ± 0.1^{a}	6.9 ± 0.1^{b}	10.6 ± 0.2^{c}	11.2 ± 0.2^{c}
18:1ω7	3.5 ± 0.0^{a}	4.0 ± 0.1^{a}	4.5 ± 0.9^{a}	4.7 ± 0.1^{a}
20:1ω9	2.4 ± 0.0^{a}	1.3 ± 0.0^{b}	1.5 ± 0.1^{b}	1.8 ± 0.0^{c}
ΣMUFA[2]	18.3 ± 0.1^{a}	21.3 ± 0.2^{b}	24.4 ± 0.6^{c}	29.1 ± 0.4^{d}
18:2ω6	1.1 ± 0.4^{a}	1.2 ± 0.0^{a}	2.6 ± 0.6^{ab}	3.0 ± 0.1^{b}
18:3ω3	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.4 ± 0.0^{b}
18:4ω3	0.8 ± 0.0^{a}	0.3 ± 0.1^{a}	0.6 ± 0.4^{a}	0.7 ± 0.1^{a}
20:3ω6	0.0 ± 0.0^{a}	0.4 ± 0.0^{b}	0.4 ± 0.0^{b}	0.8 ± 0.0^{c}
20:4ω6 (AA)	1.7 ± 0.0^{a}	3.6 ± 0.1^{b}	1.8 ± 0.1^{a}	3.0 ± 0.0^{c}
20:4ω3	0.2 ± 0.1^{a}	0.5 ± 0.0^{b}	0.5 ± 0.1^{b}	1.0 ± 0.0^{c}
20:5ω3 (EPA)	13.6 ± 0.6^{a}	3.7 ± 0.1^{b}	5.2 ± 0.2^{c}	6.1 ± 0.1^{c}
22:5ω6 (ω6DPA)	0.2 ± 0.0^{a}	8.0 ± 0.2^{b}	0.3 ± 0.0^{a}	4.7 ± 0.1^{c}
22:5ω3	0.7 ± 0.4^{a}	1.2 ± 0.1^{a}	1.4 ± 0.2^{a}	2.3 ± 0.0^{b}
22:6ω3 (DHA)	31.3 ± 1.1^{a}	33.9 ± 0.2^{ab}	35.4 ± 0.8^{b}	22.5 ± 0.5^{c}
ΣPUFA[3]	51.6 ± 1.8^{a}	54.4 ± 0.7^{a}	51.3 ± 0.6^{a}	46.9 ± 0.2^{b}
Σω3	47.9 ± 1.1^{a}	40.2 ± 0.4^{b}	44.5 ± 0.1^{c}	33.8 ± 0.3^{d}
Σω6	3.2 ± 0.6^{a}	14.0 ± 0.1^{b}	5.5 ± 0.7^{c}	12.3 ± 0.1^{b}

ω3/ω6	16.0 ± 2.7[a]	2.9 ± 0.0[b]	8.4 ± 0.9[c]	2.8 ± 0.0[b]
DHA/EPA	2.3 ± 0.2[a]	9.3 ± 0.3[b]	6.9 ± 0.4[c]	3.7 ± 0.2[d]
EPA/AA	7.9 ± 0.4[a]	1.0 ± 0.0[b]	2.9 ± 0.0[c]	2.0 ± 0.1[d]
DHA + ω6DPA	31.4 ± 1.1[a]	41.8 ± 0.4[b]	35.6 ± 0.8[c]	27.3 ± 0.6[c]
DHA/ω6DPA	210.4 ± 43.9[a]	4.3 ± 0.1[b]	138.0 ± 13.2[c]	4.8 ± 0.0[b]
ω6DPA/AA	0.1 ± 0.0[a]	2.2 ± 0.0[b]	0.2 ± 0.0[a]	1.6 ± 0.0[c]

Values (mean ± SE of four replicates, except for initial, n=3) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids, which also includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 19:0, 20:0, and 24:0 at $\leq$ 0.5% each.

[2] Sum of monounsaturated fatty acids, which also includes: 16:1ω9, 16:1ω5, 17:1, 18:1ω11, 18:1ω6, 18:1ω5, 20:1ω7, 22:1ω11, and 24:1 at $\leq$ 1.6% each.

[3]Sum of polyunsaturated fatty acids, which also includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω4, 20:2β, 20:2ω6, 20:3ω3, 21:5ω3, 22:4ω6, and 22:4ω3 at $\leq$ 0.8% each.

At 29 dph (273.2 dd), larvae from AquaGrow Advantage treatment had a higher percentage of DHA (22:6ω3) than newly hatched larvae ($F_{3,11}$=86.67, $p<0.0001$). In *Pavlova* sp. + AlgaMac 2000-larvae the DHA percentage at the end of the experiment was significantly lower than the DHA percentage in newly hatched larvae ($F_{3,11}$=86.67, $p<0.0001$). At 29 dph, larvae from this treatment had a significantly lower DHA concentration than the larvae from the other treatments (Figure IV.5B, $F_{2,9}$=34.94, $p<0.0001$). Furthermore, *Pavlova* sp. + AlgaMac 2000-larvae had a significantly lower sum of polyunsaturated fatty acids (ΣPUFA) and a significantly lower Σω3 than the larvae from the other treatments ($F_{3,11}$=13.83, $p=0.0005$, and $F_{3,11}$=144.91, $p<0.0001$, respectively). AquaGrow Advantage-larvae had a significantly lower Σω6 family than the larvae from the other treatments ($F_{3,11}$=137.35, $p<0.0001$), and as a result, had the highest ω3/ω6 ratio

($F_{3,11}$=25.94, p<0.0001). The larval DHA/EPA ratio ranged from 2.3 in newly hatched larvae to 9.3 in AlgaMac 2000-larvae, with the latter value being significantly higher than the DHA/EPA ratio observed in the larvae from the other treatments ($F_{3,11}$=25.94, p<0.0001). The larval EPA/AA ratio ranged from 1.0 in AlgaMac 2000-larvae to 7.9 in newly hatched larvae. The EPA/AA ratio observed in AlgaMac 2000-larvae was significantly lower than the ratio observed in the larvae from the other treatments ($F_{3,11}$=105.36, p<0.0001). Larval concentrations of EPA did not reflect the relative concentrations observed in the enriched rotifers and enrichments tested (Figure IV.5A). On the other hand, larval concentrations of DHA, AA, and ω6DPA mirrored the relative concentrations in their respective diets and in the enrichments used to enhance the nutritional value of rotifers (Figures IV.5B, IV.6A and IV.6B).

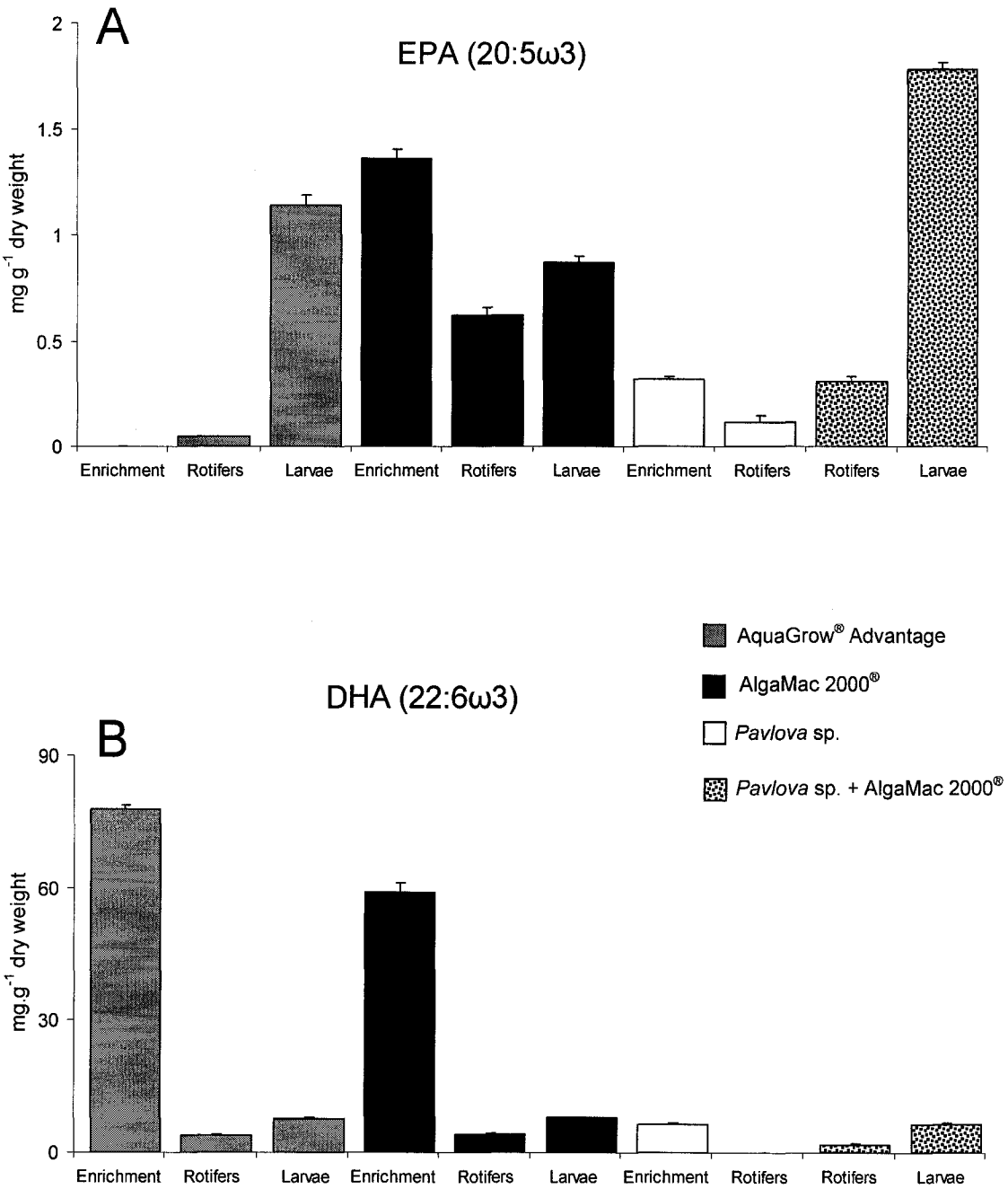

Figure IV.5. Concentrations of two fatty acids from the ω3 family, EPA (A) and DHA (B), in commercial enrichments, enriched rotifers, and haddock larvae at 29 days post hatch (273.2 dd) fed on them. Values are expressed as mean + SE of 3 samples, except for larvae where n=4.

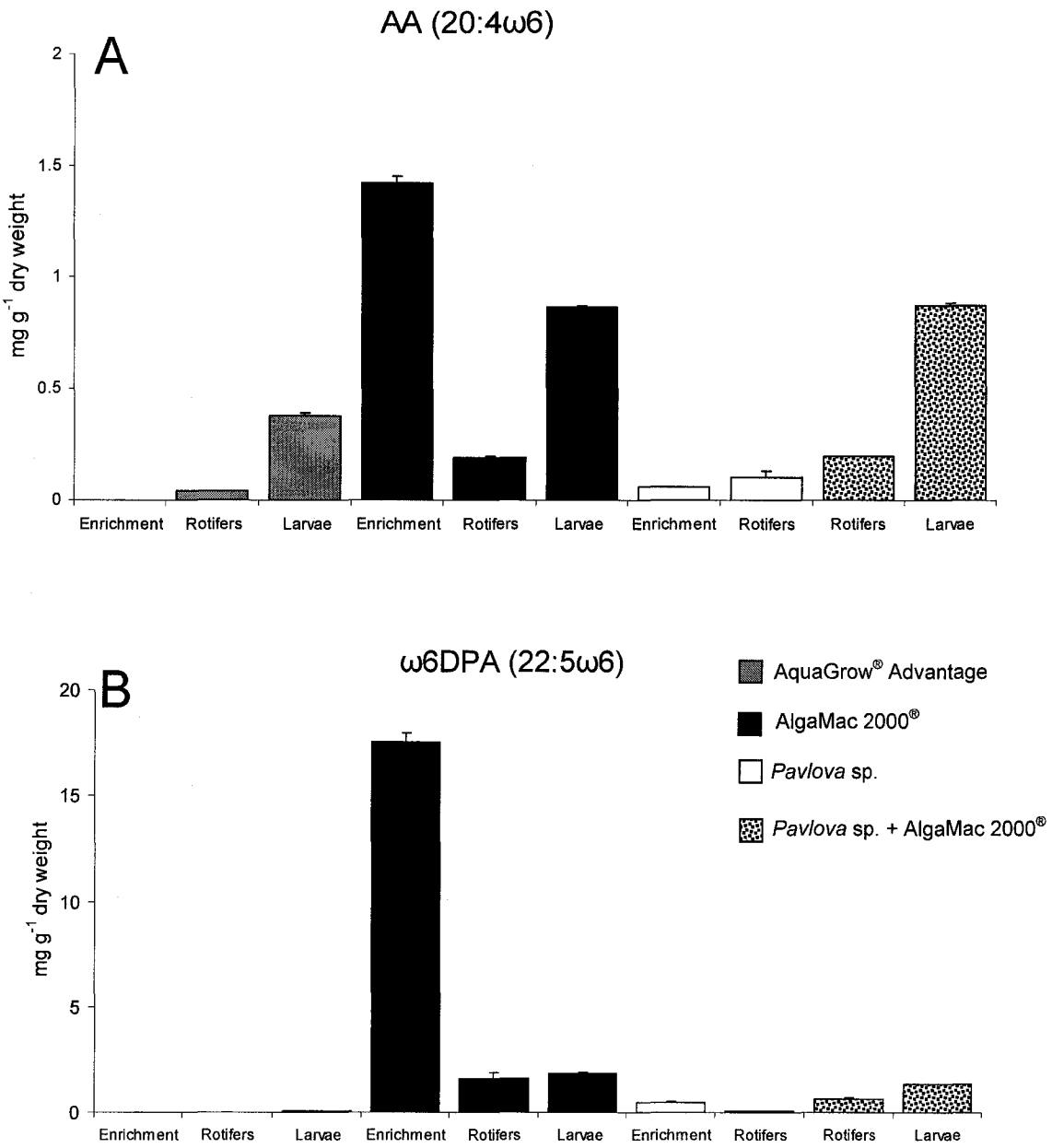

Figure IV.6. Concentrations of two fatty acids from the ω6 family, AA (A) and ω6DPA (B), in commercial enrichments, enriched rotifers, and haddock larvae at 29 days post hatch (273.2 dd) fed on them. Values are expressed as mean + SE of 3 samples, except for larvae where n=4.

IV.4. Discussion

All treatments were effective in increasing the total lipid content of rotifers after 24 h of enrichment. Regardless of the enrichment composition, rotifers from all treatments showed a significant increment of the AMPL percentage. Acetone mobile polar lipids (AMPL) is a common lipid class in aquatic samples, being often the major lipid class in seawater samples (Parrish, 1987). This lipid class is heterogeneous and can contain pigments and cellular membrane material: it can include monoacylglycerol, diacylglycerol, and glycolipids (Budge and Parrish, 2003). Although there was a slightly decrease in the proportion of PL in rotifers after the enrichment process, the higher total lipid content of enriched rotifers resulted in higher concentrations of PL being delivered to the larvae, with the only exception being AquaGrow Advantage-enriched rotifers, where the PL concentration was similar to that in unenriched rotifers. Similarly, rotifers from all treatments showed higher concentrations of TAG, with the only exception being *Pavlova* sp-enriched rotifers, which had TAG concentrations similar to those in unenriched rotifers. Phospholipids have been claimed by several authors to be the preferred vehicle to deliver polyunsaturated fatty acids to marine fish larvae (Coutteau et al., 1997; Sargent et al., 1999b; Izquierdo et al., 2000). It has been suggested that the beneficial effects of dietary PL in terms of larval survival, growth, resistance to stress, and occurrence of deformities is a result of a limited ability of fish larvae to biosynthesize phospholipids *de novo* (Fontagne et al., 1998). Nevertheless, the lack of a significant difference in growth between

haddock larvae reared in the different treatments suggests that PL levels in the diet may not play a central role in determining the rate of the growth of haddock larvae. Furthermore, the treatment that delivered the highest PL concentration to the larvae (AlgaMac 2000-enriched rotifers, PL= 25.5 ± 0.2 mg g^{-1} dry weight) resulted in the poorest survival during the experimental period. A similar trend was found with Atlantic cod larvae, where different dietary PL concentrations did not affect the larval growth (Park et al., 2006).

In broad terms, enriched rotifers from this experiment differed from those in the Atlantic cod experiment (Chapter II) by having higher total lipid concentrations, AMPL percentages, and ΣSFA and lower TAG percentages and ΣMUFA. These results are likely caused by differences in the lipid composition between the different batches of enrichments tested, differences in the lipid composition of unenriched rotifers, and differences in the tanks used for the rearing and enrichment of the rotifers.

According to Sargent et al. (1999a), the interactions between DHA and EPA and between EPA and AA have to be taken into account simultaneously when evaluating diets for marine fish larvae. Therefore, the DHA/EPA/AA ratio has to be considered as indicative of the nutritional value of a given diet rather than only the individual levels of these essential fatty acids. In the present study, the best performance (considering larval growth and survival) was rendered by a combination of *Pavlova* sp.-enriched rotifers and *Pavlova* sp. + AlgaMac 2000-enriched rotifers. Larvae from this treatment were slightly heavier at 29 dph (273.2 dd), and showed better survival and higher resistance to handling stress

than larvae from other treatments. The DHA/EPA/AA ratio in *Pavlova* sp.-enriched rotifers and *Pavlova* sp. + AlgaMac 2000-enriched rotifers were approximately 0.8/1.2/1 and 10/2/1, respectively. On the other hand, rotifers containing a DHA/EPA/AA ratio of 19.5/3/1 resulted in the poorest survival rates. In the study performed by Castell et al. (2003) haddock larvae fed rotifers containing a DHA/EPA/AA ratio ranging from approximately 1.6/2/1 to 12/13/1 showed similar growth and survival. However, the larval fatty acid composition was affected by the differently enriched rotifers, with larvae fed rotifers containing a DHA/EPA/AA ratio of approximately 2/1/1 having significantly higher levels of AA (Castell et al., 2003). Based on the proportions of DHA, EPA, and AA in the larvae relative to the live-food, these authors suggested that an optimal DHA/EPA/AA ratio in the diet for haddock larvae would be 10/1/1. Considering the results above, and the DHA/EPA/AA ratio of 10/5/1, proposed by Sargent et al. (1999a) as an optimal ratio for marine larvae in general, it seems that the DHA/EPA/AA ratio observed in *Pavlova* sp. + AlgaMac 2000-enriched rotifers is closer to an optimal ratio for the early development of haddock larvae, than the ratio observed in *Pavlova* sp.-enriched rotifers. Although *Pavlova* sp.-enriched rotifers had low levels of DHA relative to EPA, these rotifers were rich in monounsaturated fatty acids—particularly 16:1ω7 and 18:1ω9—possibly acting as a better source of energy, since larvae feeding on these rotifers could spare higher proportions of highly unsaturated fatty acids for membrane composition and/or eicosanoid production.

All treatments resulted in similar larval dry weight and SGR; averaging 1.07 mg and 8.35% dry weight d^{-1}, respectively at 29 dph (273.2 dd). These results are superior to those reported in other studies in which haddock larvae were raised at similar temperatures and fed with the same prey type and prey density. Castell et al. (2003) reported larval dry weight ranging from approximately 0.76 to 0.81 mg at 30 dph. In the study performed by Caldarone (2005), after 30 dph the SGR of haddock larvae averaged 7.2% protein d^{-1}. Growth rates based on protein or dry weight are comparable since the bulk of larval dry weight is comprised of protein, and the relative proportion of protein to dry weight is fairly constant during the larval period (Caldarone, 2005).

Larval DHA levels remained high from hatch (31% of total fatty acids) to the end of the experiment in all treatments (around 34.5% of total fatty acids, except in larvae from *Pavlova* sp. + AlgaMac 2000 treatment that had about 22% of this fatty acid). On the other hand, larval levels of EPA decreased significantly in all treatments after 29 days of larviculture. The larval levels of EPA dropped from 13.6% at hatch to less than 50% of this value at 29 dph (273.2 dd). These results suggest that DHA is specifically accumulated in the larval tissues during the early development of haddock larva, while EPA is selectively catabolized. Alternately, haddock larvae could be conserving the maternal DHA that is provided in the egg yolk. Haddock eggs are typically rich in DHA, with the eggs used in the present study having 32.6 ± 1.6% of their total fatty acids as DHA. Larval levels of AA were more stable, showing only a slight increment during the experimental period. Retention of DHA and AA and catabolism of EPA during the

early development of haddock larvae were reported earlier (Castell et al., 2003) A similar retention of DHA was observed during the early larval development of other marine species. Fraser et al. (1988) reported that about 33% of the DHA released during the catabolism of the lipid reserves in Atlantic cod eggs was incorporated in the fish tissues. In halibut larvae, the accumulation of DHA was even higher, with over 60% of the DHA released during the hydrolysis of the egg's lipid reserves being accumulated in the body (Rønnestad et al., 1995). Based on studies mainly with plaice, Rainuzzo (1993) suggested that after the onset of exogenous feeding, fish larvae selectively retain DHA and AA at the expense of other fatty acids, including EPA, which are mainly catabolized. Tocher (2003) proposed that selective oxidation of saturated and monounsaturated fatty acids, and also EPA, leading to selective retention of DHA may be a general feature of fish, at least during later stages of development, when swimming muscles are fuelled preferably by fatty acid oxidation. It is worth mentioning that although the larvae with the best overall performance had lower DHA proportions than the larvae from the other treatments, the higher total lipid and phospholipid content of *Pavlova* sp. + AlgaMac 2000-larvae resulted in a larval DHA concentration similar to the concentrations observed in the larvae from the other treatments.

From 14 dph (64.8 dd) to the end of the experimental period, larvae that had the poorest survival had a significantly higher body concentration of TAG, when compared to the larvae from the other treatments. Furthermore, at 29 dph (273.2 dd), these larvae had significantly lower ST body concentrations than the

larvae from the other treatments. These results seem contradictory considering that relative improvements in larval condition of aquatic organisms, including fish, has been attributed to elevated TAG per dry weight and elevated TAG/ST ratios (Fraser, 1989). However, Fraser (1989) suggested the TAG/ST ratio as a condition index for larvae of aquatic organisms in the wild. The biochemical basis for the application of the TAG/ST ratio as a condition index is that in the wild, larvae under environmental stress—which could be caused by scarcity of food—are often unable to obtain sufficient energy from exogenous sources and, as result, endogenous energy reserves are catabolized to maintain basal metabolism (Fraser, 1989). Invariably, most of the endogenous energy is in the form of TAG, the major storage lipid in animal cells (Lehninger et al., 1993). Well-nourished larvae will have their metabolic demands satisfied and excess energy will be stored in the tissues as TAG, which in turn can be catabolized under starvation. However, absolute TAG content is dependent on larval size and cannot be directly correlated with larval condition. For this reason, the larval condition index proposed by Fraser (1989) expresses TAG content relative to ST content, since the latter represents a constant proportion of the wet weight of a wide range of eukaryotic organisms (Nes, 1974).

Under the aquaculture situation, food is constantly supplied to the organism being cultivated, and higher TAG storage does not represent an advantage to the larvae, because starvation is unlikely to occur. Furthermore, free amino acids and phospholipids are also important sources of energy for marine fish embryos and larvae (Cahu and Zambonino Infante, 2001). In turbot

for instance, free amino acids become a significant energy substrate in the early stage of eggs and account for 100% of the aerobic energy dissipation 2 days after fertilization, decreasing to *ca* 60% at the time of hatching (Rønnestad et al. 1992). Few studies have examined the importance of free amino acids catabolism in exogenously feeding larvae, but there is some indication that they continue to be an important fuel at this stage (Rønnestad et al., 2003). Catabolism of phospholipids for energy may be a common characteristic of fish eggs that are rich in phospholipids (Sargent et al., 1989), such as Atlantic cod and haddock eggs. It has been reported that in Atlantic cod, phospholipid was utilized during both embryogenesis and to a great extent during early larval development (Fraser et al., 1988). On the other hand, low ST concentrations could impair larval development and even lead to mortality, since sterols are precursors of hormones, and are important structural components of cell membranes, both from a qualitative and quantitative point of view (Lehninger et al., 1993). Additionally, the contemporary view regarding sterol function in biological membranes is that they play a crucial role regulating the membrane trafficking mechanisms (Bankaitis and Morris, 2003) and are associated with the function of a number of membrane proteins. Signal transduction events mediated by membrane proteins are the primary means by which cells communicate with and respond to their external environment (Pucadyil and Chattopadhyay, 2003).

Nevertheless, the low ST concentration in AlgaMac 2000-larvae at the end of the experiment does not fully explain the higher mortality observed in this treatment at 14 and 21 dph (129 and 193.2 dd, respectively), when larvae from

this treatment had ST concentrations as high as the concentrations in the larvae from the other treatments. However, the larval fatty acid composition can help to further explain the high mortality observed in the AlgaMac treatment. From 14 dph (129 dd) up to the end of the experiment, larvae from this treatment had significantly higher levels of AA and significantly lower levels of EPA when compared to the larvae from the other treatments. Both EPA and AA are precursors of eicosanoids and the interactions between them and the production of eicosanoids was discussed earlier in Chapter II. Arachidonic acid is considered one of the most important polyunsaturated fatty acids associated with membrane phospholipids. When hydrolyzed from the membrane, AA can be oxidatively metabolized to a variety of eicosanoids, many of which can have deleterious effects when chronically or excessively produced. In mammalian tissues, excessive production of AA-derived eicosanoids results in proinflamatory, prothrombotic, and proatherogenic action (Kinsella et al., 1990). The amounts synthesized are influenced by the levels of AA in membrane phospholipids, and even low levels of dietary AA can have a significant impact on tissue AA content, and thus increment AA-derived eicosanoid production (Li et al., 1994). Conversely, long-chain fatty acids form the ω3 family tend to attenuate AA levels in the tissues, and many of the beneficial effects of ω3 HUFA have been linked to their ability to inhibit the AA cascade and control eicosanoid production (Kinsella et al., 1990; Li et al., 1994). AlgaMac 2000-enriched rotifers had 624 ± 43.6 μg g^{-1} of EPA and 196 ± 11.4 μg g^{-1} of AA, resulting in a EPA/AA ratio significantly higher than in rotifers from other treatments. It seems that AlgaMac 2000-

enriched rotifers had an excessive concentration of EPA in relation to AA, resulting in high accumulation of AA and depletion of EPA in the tissues of larvae fed on them. It has been suggested earlier that an increase or decrease in the proportions of DHA, EPA, and AA in the larvae relative to the live-food would indicate a deficiency or excess of that particular fatty acid, respectively (Castell, et al., 2003). It is possible that the low EPA and high AA tissue levels in AlgaMac 2000-larvae caused excessive production of AA-derived eicosanoids, making the larvae from this treatment unable to overcome the stress imposed by the morphological and physiological transformations experienced during the larval development. In healthy fish, AA is the preferred substrate for eicosanoid production, despite the preponderance of EPA in the tissue phospholipids (Tocher, 2003). This finding suggests that the enzymes involved in the production of the free precursor acid, phospholipase A_2, phospholipase C, and diacylglycerol lipase—and possibly the enzymes that oxidize the free precursor to eicosanoids, such as cyclooxygenases and lipoxygenases—have higher affinity for AA than for EPA. Therefore, an unbalanced ratio of EPA/AA would result in excessive eicosanoid production and damaging effects.

Larval growth and dry weight were not affected by the different treatments. However, the enrichments affected the fatty acid composition of the rotifers which was in turn reflected in the fatty acid composition of haddock larvae. AlgaMac 2000-enriched rotifers had low ST concentrations (0.8 ± 0.0 mg g^{-1}), high EPA/AA ratio, and their feeding resulted in high larval mortality. Results from the present study indicate that rotifers to be used as live food during the early development of

haddock larvae should contain ST in concentrations $\geq$ 1 mg g^{-1} and EPA/AA ratios < 2 in order to avoid high larval mortality.

Chapter V

Enrichment of *Artemia* metanauplii in essential fatty acids and their use during larviculture of haddock, *Melanogrammus aeglefinus*.

V.1. Introduction

Haddock, *Melanogrammus aeglefinus*, is one of the most highly prized fish caught for the north-eastern North American markets. Haddock is a very popular food fish, sold fresh, smoked, frozen, dried, and to a small extent canned. The commercial catch of haddock in North America had declined sharply in recent years but is now recovering with recruitment rates running around where they historically were from the 1930s to 1960s (Scott and Scott, 1988). Interest in haddock aquaculture had its origins in New Brunswick, Canada, in the late 1980s. Ever since, there has been a great amount of research and development of haddock aquaculture by industry, government laboratories, and the scientific community. The current production protocol is based on the use of live-food during the larval rearing, with the rotifer *Brachionus plicatilis* being supplied to the larvae during the early stages of development (from 1 to approximately 25 days post-hatch) and *Artemia* sp. during the later stages (Neil, 2003). Little is known about the nutritional requirements of haddock larvae (Harmon, 2003), and although the larval requirements for essential fatty acids—eicosapentaenoic acid (EPA, 20:5ω3), docosahexaenoic acid (DHA, 22:6ω3), and arachidonic acid (AA, 20:4ω6)—have not been determined yet, the live-food supplied during the larviculture of haddock are normally enriched with algae and/or commercial products. Rotifers and *Artemia* are naturally deficient in DHA, EPA and AA, and the enrichment process is a common practice in marine fish hatcheries, in an attempt to enhance their nutritional value (Sorgeloos et al., 2001). Although

hatchery technology follows similar protocols worldwide, modifications are necessary to meet specific needs. Verreth (1994) pointed out that the major bottlenecks in development of techniques for larval rearing of marine fish species have nutritional origins. In this context, the lack of knowledge of feeding behavior and nutritional requirements during the early larval stages play a crucial role. Improvements in feeding regimes and culture systems often result in higher fish survival and lower production costs (Lee, 2003). Although live-food deficient in essential fatty acids can cause poor growth and low survival rates during the larviculture of marine fish, excessive levels of highly unsaturated fatty acids (HUFA) also have been associated with low rearing performance (Støttrup, 1992). According to Planas and Cunha (1999) it is very likely that the live prey used for cultivation of marine fish larvae contain unbalanced ω3HUFA content in their various lipid classes or that their lipid content is excessive. It has been suggested that prey items containing high lipid levels are detrimental to the larvae. Overload of the digestive capacity and alterations of the protein absorption in the hindgut have been reported in larvae of marine species fed rotifers and *Artemia* nauplii with high lipid content (Kjørsvik et al., 1991a; Palnas and Cunha, 1999). To further advance the rearing technology of haddock larvae, specific experimental work must be undertaken on enrichment of live-food and its effects on the lipid composition of prey and predator in order to increase our knowledge of larval total lipid and essential fatty acids requirements.

The present study was aimed to evaluate commercial products and an algae paste for the enrichment of *Artemia* metanauplii to be used as live-food during the larviculture of haddock.

V.2. Material and Methods

V.2.1. Larval rearing and *Artemia* enrichments

Haddock larvae (29 days post-hatch (dph)) were stocked in 1,000 litre (L) circular tanks at similar density (two replicates per treatment) and reared up to 43 dph (equivalent to 417.7 degree-days (dd)) on *Artemia* enriched differently. Water management followed the protocol outlined previously in Chapter IV. Water temperature ranged from 10-11°C over the course of the experiment. Four different treatments were tested using commercially available products as a single enrichment or in combination with algae paste. The products tested differed in their proximate and lipid composition (Tables V.1 and V.2). The treatments were: 1) AlgaMac 2000® (Aquafauna Bio-Marine Inc., Hawthorne, CA, USA), 2) AquaGrow® Advantage (Advanced BioNutrition Corp., Columbia, MD, USA), 3) *Pavlova* sp. paste (Reed Mariculture, Campbell, CA, USA) combined with AlgaMac 2000®, and 4) DC DHA Selco (Inve Americas, Salt Lake City, UT, USA) combined with AlgaMac 2000®. Prior to the start of the experiment, larvae were reared from hatch up to 29 dph, in the same tanks, and fed rotifers enriched with the same treatments, except larvae from treatment 4. Feeding schedules

and rotifer enrichments used during the rotifer phase were as described in Chapter IV. At the end of the rotifer phase (29 dph; 273.2 dd) larvae fed with *Pavlova* sp. + AlgaMac 2000®-enriched rotifers had better survival rates than the larvae from the other treatments. Excess larvae from this treatment were used for treatment 4 (two replicates).

Table V.1. Proximate composition of the enrichments tested.

	Enrichments			
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. paste	DC DHA Selco®
Proximate composition[1] (%)				
Protein	20.2	10.0	4.6	*
Lipids	38.1	23.0	1.76	65.0
Carbohydrate	17.1	9.0	1.9-2.1	*
Ash	20.4	44.0	*	3.0
Moisture	4.2	4.0	91.0	30.0
Vitamin A (IU/100 g)	<100	737.5	*	150
Vitamin C (mg/100 g)	4.8	*	*	0.8
Vitamin D (IU/100 g)	457.0	*	*	150
Vitamin E (IU/100 g)	12.0	*	*	3.6
Calcium (%)	*	0.3	*	*
Potassium (%)	*	0.6	*	*
Phosphorus (%)	*	0.2	*	0.2

[1] Values according to manufacturer's information. *N.b.* some of this information has changed over time.

* Information not available.

Artemia were reared from decapsulated cysts using a two-day production cycle. *Artemia* cysts were hydrated and decapsulated according to standard practices. Cysts were set to hatch in 300 L tanks at a maximum density of 100 x 10^3 cysts L^{-1} in seawater (salinity 30 psu) under heavy aeration at 28°C for 24 hours. On day two, instarr I *Artemia* nauplii were harvested, concentrated, rinsed (water temperature ~6°C), restocked in the same tanks at a density of 10 x 10^4 nauplii L^{-1}, and enriched for 24 hours at 28°C. The enrichment of metanauplii for treatments 1 and 2 followed the manufacturer's directions. Larvae from treatment 3 were fed with *Pavlova* sp.-enriched *Artemia* during the morning and *Pavlova* sp. + AlgaMac 2000®-enriched *Artemia* during the afternoon. Larvae from treatment 4 were fed with DC DHA Selco®-enriched *Artemia* during the morning and with DC DHA Selco® + AlgaMac 2000®-enriched *Artemia* during the afternoon. For these treatments, *Artemia* metanauplii batches were enriched following the protocols described for treatments 3 and 4 in Chapter III. After enrichment, the *Artemia* metanauplii were again rinsed and concentrated prior to feeding. Larvae were fed twice a day, between 9:00 and 10:00 *a.m.*, and 4:00 and 5:00 *p.m.* The morning feeding was supplied to the larvae directly and the remainder set aside in cold storage (~6°C) to minimize nutritional loss until the afternoon feeding. Enriched *Artemia* were supplied to the larvae from 29 to 43 dph (273.2 to 417.7 dd) twice a day as necessary to sustain a prey density of 1,000 L^{-1}. In order to maintain the desired prey concentration within each experimental tank, before each feeding, three 10 mL water aliquots were sampled from each tank. The number of prey in

each sample was counted and the average number was used to adjust prey concentrations as needed.

Table V.2. Total lipid, lipid class and fatty acid composition of the enrichments tested.

	Enrichments			
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. paste	DC DHA Selco®
Total lipid (mg g^{-1} dw)	301.9 ± 5.1[a]	214.6 ± 9.4[b]	55.6 ± 3.7[c]	581.7± 6.1[d]
Lipid Class (% total lipids)				
Hydrocarbons	0.3 ± 0.1[a]	1.7 ± 0.3[b]	0.7 ± 0.1[a]	0.3 ± 0.1[a]
Steryl Esters/Wax Esters	3.5 ± 0.4[a]	0.1 ± 0.1[b]	0.0 ± 0.0[b]	3.2 ± 0.3[a]
Ketones	6.4 ± 0.5[a]	3.0 ± 0.3[b]	0.6 ± 0.1[c]	3.8 ± 0.2[b]
Triacylglycerols	71.9 ± 1.6[a]	63.7 ± 0.4[b]	35.7 ± 0.3[c]	65.5 ± 1.3[b]
Free fatty acids	1.5 ± 0.4[a]	2.9 ± 0.4[a]	12.2 ± 1.1[b]	1.9 ± 0.3[a]
Alcohols	1.9 ± 0.4[a]	1.1 ± 0.1[a]	2.2 ± 0.2[a]	2.7 ± 0.6[a]
Sterols	1.2 ± 0.2[a]	4.0 ± 0.1[b]	0.9 ± 0.2[a]	1.0 ± 0.1[a]
Acetone Mobile Polar Lipids	7.5 ± 0.4[a]	4.5 ± 0.9[b]	26.6 ± 0.3[c]	14.2 ± 0.3[d]
Phospholipids	6.0 ± 0.3[a]	18.9 ± 0.2[b]	21.1 ± 0.8[c]	7.3 ± 0.5[a]
Fatty acids (% total fatty acids)				
14:0	18.6 ± 0.1[a]	17.0 ± 0.5[ab]	14.2 ± 1.2[b]	4.2 ± 0.4[c]
16:0	37.4 ± 0.4[a]	18.2 ± 0.4[b]	10.0 ± 0.9[c]	18.4 ± 0.5[b]
18:0	1.0 ± 0.0[a]	1.2 ± 0.1[a]	0.2 ± 0.0[b]	4.9 ± 0.2[c]
ΣSFA[1]	58.1 ± 0.3[a]	36.9 ± 0.8[b]	26.9 ± 1.9[c]	30.1 ± 0.4[c]
16:1ω7	4.4 ± 0.0[a]	1.0 ± 0.0[b]	7.3 ± 0.6[c]	6.8 ± 0.6[c]
18:1ω9	0.1 ± 0.0[a]	14.4 ± 0.3[b]	9.0 ± 0.8[c]	0.0 ± 0.0[a]
18:1ω7	2.5 ± 0.1[a]	0.1 ± 0.0[b]	0.7 ± 0.1[c]	16.1 ± 0.4[d]
ΣMUFA[2]	7.4 ± 0.3[a]	16.7 ± 0.3[b]	25.5 ± 1.0[c]	27.4 ± 0.4[c]
18:2ω6	0.1 ± 0.0[a]	0.1 ± 0.1[a]	6.3 ± 0.6[b]	0.1 ± 0.0[a]

18:3ω3	0.0 ± 0.0[a]	0.0 ± 0.0[a]	7.4 ± 0.2[b]	0.1 ± 0.0[a]
18:4ω3	0.2 ± 0.0[a]	0.1 ± 0.0[a]	12.3 ± 3.5[b]	0.9 ± 0.1[a]
18:5ω3	0.0 ± 0.0[a]	0.0 ± 0.0[a]	6.1 ± 0.1[b]	0.0 ± 0.0[a]
20:3ω6	0.2 ± 0.0[a]	0.0 ± 0.0[b]	0.1 ± 0.0[c]	0.1 ± 0.0[c]
20:4ω6 (AA)	0.6 ± 0.0[a]	0.0 ± 0.0[b]	0.2 ± 0.0[b]	1.4 ± 0.1[c]
20:4ω3	0.4 ± 0.0[a]	0.0 ± 0.0[b]	0.1 ± 0.0[b]	0.7 ± 0.1[c]
20:5ω3 (EPA)	0.6 ± 0.0[a]	0.0 ± 0.0[b]	0.8 ± 0.0[c]	9.9 ± 0.0[d]
22:5ω6 (ω6DPA)	7.4 ± 0.2[a]	0.0 ± 0.0[b]	1.3 ± 0.2[c]	0.9 ± 0.1[c]
22:5ω3	0.1 ± 0.0[a]	0.2 ± 0.0[a]	0.2 ± 0.1[a]	1.2 ± 0.3[b]
22:6ω3 (DHA)	24.7 ± 1.0[a]	45.8 ± 0.4[b]	16.5 ± 0.5[c]	21.9 ± 0.1[d]
ΣPUFA[3]	34.4 ± 0.7[a]	46.3 ± 0.5[b]	53.6 ± 2.9[b]	42.4 ± 0.2[b]
Σω3	26.0 ± 0.9[a]	46.0 ± 0.5[b]	43.5 ± 3.8[bc]	35.5 ± 0.6[c]
Σω6	8.3 ± 0.3[a]	0.3 ± 0.0[b]	9.6 ± 0.9[a]	3.5 ± 0.3[c]
ω3/ω6	3.1 ± 0.2[a]	177.2 ± 2.1[b]	4.7 ± 0.8[a]	10.4 ± 0.9[c]
DHA/EPA	43.2 ± 3.0[a]	-	20.5 ± 1.3[b]	2.2 ± 0.0[c]
EPA/AA	1.0 ± 0.0[a]	0.0 ± 0.0[a]	4.9 ± 0.4[b]	7.0 ± 0.5[c]

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids, which includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 19:0, 20:0, 21:0, 22:0, and 23:0 at $\leq$ 0.54% each.

[2]Sum of monounsaturated fatty acids, which includes: 14:1, 15:1, 16:1ω9, 16:1ω5, 17:1, 18:1ω11, 18:1ω6, 18:1ω5, 20:1ω11, 20:1ω7, 22:1ω11, 22:1ω9, 22:1ω7, and 24:1 at $\leq$ 0.91% each.

[3]Sum of polyunsaturated fatty acids, which includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω4, 18:3ω6, 18:4ω1, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 21:5ω3, 22:4ω6, and 22:4ω3 at $\leq$ 1.54% each.

V.2.2. Sampling, growth and survival measurements

Pooled samples of larvae were collected at 29, 36 and 43 dph (273.2, 345.4, and 417.7 dd, respectively) for dry weight measurements (three replicates

tank^{-1}). Growth measurements were performed as described in Chapter II. Briefly, between 20 (29 dph) and 10 (43 dph) larvae were collected on pre-weighed glass microfibre filters dried at 60°C for 48 h. Filters were re-weighed, and growth was then calculated as specific growth rate for dry weight (SGR; % d^{-1} = [ln (final dry weight) – ln (initial dry weight)/days] x 100)

Survival measurements were based on visual observations and performed as described in Chapter II. A nominal scale (0 to 5) was used to measure the weekly survival rates. The scale used considered 0 as total mortality (or 0% survival), 1 as poor survival, 2 as reasonable survival, 3 as good survival, 4 as very good survival, and 5 as total survival (or 100% survival). Under mass production circumstances in the hatchery, around 25% survival at the end of the *Artemia* phase is considered a good survival rate for haddock larvae.

V.2.3. Lipid analysis

Pooled samples of larvae were collected at 29, 36, and 43 dph for lipid analysis (total lipids, lipid classes, and fatty acid composition). Samples of the experimental enrichments, algae paste and enriched live-feed were collected in triplicate for lipid analysis. Lipid extraction, and lipid class and fatty acid analyses were performed as described in Chapter II. Briefly, lipids were extracted in chloroform/methanol according to Parrish (1998) using a modified Folch procedure (Folch et al., 1957). Lipid classes were determined using thin layer chromatography with flame ionization detection (TLC/FID) with a MARK V

Iatroscan (Iatron Laboratories, Tokyo, Japan) as described by Parrish (1987). Extracts were spotted on silica gel coated Chromarods and a three-stage development system was used to separate lipid classes. Fatty acid methyl esters (FAME) were prepared by transesterification as described by Budge and Parrish (2003). A Varian model 3400 GC equipped with a Varian 8100 autosampler was used for fatty acid analysis (Varian, Palo Alto, CA, USA). Peaks were detected by flame ionization. Fatty acid peaks were integrated using Varian Star Chromatography Software (version 5.50) and identification was made with reference to known standards (PUFA 1, PUFA 3, BAME and 37 Component FAME Mix, Supleco Canada, ON).

V.2.4. Statistical analysis

Growth and lipid composition data were analyzed as described in Chapter II. The General Linear Model (Minitab Version 13.1) was employed and in all cases, the effect of tanks was not significant. Data from treatments were pooled. Linear regression was used to describe the relationship between larval dry weight and the larval concentration of total lipids, phospholipids, and selected fatty acids. Significance was set at $\alpha = 0.05$ for all analyses.

V.3. Results

V.3.1. Total lipid, lipid class and fatty acid composition of *Artemia*

The enrichment process resulted in an increase in total lipid content of *Artemia* in all treatments. Total lipid values ranged from 71.1 ± 0.8 mg g^{-1} in *Pavlova* sp.-enriched *Artemia* to 154.7 ± 1.3 in DC DHA Selco-enriched *Artemia* (Table V.3). All treatments resulted in *Artemia* having significantly higher concentrations of total lipids than unenriched *Artemia* ($F_{5,12}$=831.63, $p<0.0001$). The hydrocarbon (HC) and acetone mobile polar lipid (AMPL) percentages remained relatively constant after 24 h of enrichment, and the values observed in enriched *Artemia* were not different than in unenriched *Artemia* ($p>0.05$). The ketone (KET) percentage decreased in all treatments ($F_{5,12}$=24.78, $p<0.0001$), with the biggest reduction being observed in AquaGrow Advantage-enriched *Artemia* (8.4%). The percentage of triacylglycerol (TAG) also decreased in four out of five treatments after enrichment ($F_{5,12}$=16.87, $p<0.0001$), with the only exception being the DC DHA Selco + AlgaMac 2000 treatment, which caused the TAG percentage to increase. However, the TAG percentage of DC DHA Selco + AlgaMac 2000-enriched *Artemia* was not different from unenriched *Artemia* ($p>0.05$). The reduction in the TAG fraction ranged from 6.7% in *Pavlova* sp. + AlgaMac 2000-enriched *Artemia* to 19.6% in *Pavlova* sp.-enriched *Artemia*. On the other hand, all treatments caused the sterol (ST) percentage to increase after enrichment ($F_{5,12}$=18.05, $p<0.0001$). In *Artemia* enriched with *Pavlova* sp. and DC

DHA Selco, the PL percentages were significantly higher than in unenriched *Artemia* ($F_{5,12}$=9.86, p=0.0006).

The *Artemia* strain used in the present study had 16:0 (12.8 ± 0.0%), 18:1ω9 (20.2 ± 0.1%), and 18:3ω3 (32.0 ± 0.3%) as the major saturated, monounsaturated and polyunsaturated fatty acids, respectively (Table V.4). After 24 h of enrichment, the levels of palmitc acid (16:0) remained unchanged, with enriched *Artemia* having percentages of this fatty acid similar to those observed in unenriched *Artemia* (p>0.05). The stearic acid (18:0) was absent in unenriched *Artemia*, however levels of this fatty acid increased significantly after the enrichment ($F_{5,12}$=344.42, p<0.0001), ranging from 1.1 ± 0.1% in DC DHA Selco-enriched *Artemia* to 6.4 ± 0.1% in *Pavlova* sp.-enriched *Artemia*. In addition, enriched *Artemia* had higher levels of myristic acid (14:0) than unenriched *Artemia* ($F_{5,12}$=24.04, p<0.0001), with the only exception being *Pavlova* sp.-enriched *Artemia*, in which the percentage of this fatty acid was similar to the percentage observed in unenriched *Artemia*. However, only in AquaGrow Advantage-enriched *Artemia* and *Pavlova* sp. + AlgaMac 2000-enriched *Artemia* the ΣSFA was significantly higher than initially ($F_{5,12}$=15.81, p<0.0001). Levels of 18:1ω9 decreased in *Artemia* from four treatments, with the only exception being DC DHA Selco-enriched *Artemia*, where levels of 18:1ω9 were significantly higher than in unenriched *Artemia* ($F_{5,12}$=41.08, p<0.0001). This treatment also resulted in the highest sum of monounsaturated fatty acids (ΣMUFA, $F_{5,12}$=52.26, p<0.0001). Similarly, the percentage of 18:3ω3 decreased significantly in all treatments ($F_{5,12}$=300.85, p<0.0001), with the highest reduction being observed in

DC DHA Selco-enriched *Artemia* (28.3%). Levels of AA remained relatively constant, with the exception of DC DHA Selco enriched-*Artemia* which had higher levels than unenriched *Artemia* ($F_{5,12}$=21.47, p<0.0001). Similarly, EPA levels also remained relatively constant, with the exception of DC DHA Selco enriched-*Artemia* and DC DHA Selco + AlgaMac 2000-enriched *Artemia* in which an increase in the percentage of this fatty acid was observed ($F_{5,12}$=36.68, p<0.0001). In contrast, levels of DHA showed an increase ranging from 0.1% in *Pavlova* sp.-enriched *Artemia* to 9.1% in DC DHA Selco + AlgaMac 2000-enriched *Artemia.* However, only in three out of the five treatments was the increase significant (Table V4, $F_{5,12}$=15.81, p<0.0001). *Pavlova* sp. + AlgaMac 2000-enriched *Artemia* and DC DHA Selco-enriched *Artemia* had significantly lower sum of polyunsaturated fatty acids (ΣPUFA) than unenriched *Artemia* ($F_{5,12}$=27.52, p<0.0001). The reduction in the levels of PUFA in *Artemia* from these treatments was mainly due to a reduction in the levels of 18:3ω3.

Table V.3. Total lipid (mg g^{-1} dry weight) and percentage lipid class composition of *Artemia* before and after experimental enrichments.

	Treatments					
	Unenriched	AquaGrow® Advantage	*Pavlova* sp.	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco®	DC DHA Selco® + AlgaMac 2000®
Total lipid (mg g^{-1} dw)	60.0 ± 0.9[a]	71.1 ± 1.1[b]	71.1± 0.8[b]	93.4 ± 2.0[c]	154.7 ± 1.3[d]	110.9 ± 1.1[e]
Lipid class[1] (% total fatty acids)						
Hydrocarbons	0.3 ± 0.0[ab]	0.3 ± 0.0[ab]	0.5 ± 0.2[ab]	0.3 ± 0.0[a]	0.5 ± 0.2[ab]	0.6 ± 0.0[b]
Ketones	8.4 ± 1.1[a]	0.0 ± 0.0[b]	2.8 ± 0.7[b]	1.9 ± 0.8[b]	0.8 ± 0.1[b]	1.3 ± 0.0[b]
Triacylglycerols	70.9 ± 1.1[ab]	58.5 ± 0.3[c]	51.3 ± 4.4[c]	64.2 ± 0.8[b]	60.1 ± 2.0[c]	74.3 ± 0.2[a]
Free fatty acids	0.6 ± 0.0[a]	8.4 ± 2.3[b]	5.1 ± 1.5[a]	1.0 ± 0.2[a]	1.3 ± 0.2[a]	1.9 ± 0.2[a]
Sterols	0.7 ± 0.1[a]	2.8 ± 0.2[bc]	4.4 ± 0.7[d]	4.1 ± 0.1[cd]	2.7 ± 0.0[bc]	2.3 ± 0.2[b]
Acetone Mobile Polar Lipids	1.0 ± 0.1[a]	1.7 ± 0.5[a]	1.6 ± 0.1[a]	0.7 ± 0.3[a]	1.0 ± 0.1[a]	0.8 ± 0.3[a]
Phospholipids	15.0 ± 0.3[a]	26.4 ± 2.2[ab]	34.3 ± 5.1[b]	26.4 ± 1.4[ab]	33.6 ± 1.7[b]	18.8 ± 0.6[a]

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]May also contain alcohols at ≤ 1.94%.

Moreover, these treatments resulted in the lowest sum of fatty acids from the ω3 family (Σω3) ($F_{5,12}$=113.32, p<0.0001). AquaGrow Advantage-enriched *Artemia* and DC DHA Selco-enriched *Artemia* had the lowest and highest sum of fatty acids from the ω6 family (Σω6), respectively ($F_{5,12}$=247.14, p<0.0001). As a result, *Artemia* from these treatments had the highest and lowest ω3/ω6 ratio, respectively ($F_{5,12}$=200.22, p<0.0001). After the enrichment process, the DHA/EPA ratio ranged from 0.1 ± 0.0 in *Pavlova* sp.-enriched *Artemia* to 2.2 ± 0.1 in AquaGrow Advantage-enriched *Artemia*. The DHA/EPA ratio observed in AquaGrow Advantage-enriched *Artemia* and in DC DHA Selco + AlgaMac 2000-enriched *Artemia* were significantly higher than the ratio observed in *Artemia* from the other treatments ($F_{5,12}$=56.45, p<0.0001). These treatments also resulted in *Artemia* having an EPA/AA ratio significantly higher than unenriched *Artemia* ($F_{5,12}$=16.40, p<0.0001). The DHA/ω6DPA ratio showed great variation in enriched *Artemia*, ranging from 0.1 to 15.3. Conversely, the ω6DPA/AA ratio was more conservative, ranging from 0.04 to 0.8.

Table V.4. Percentage fatty acid composition of *Artemia* before and after experimental enrichments.

	Treatments					
Fatty acids (%)	Unenriched	AquaGrow® Advantage	*Pavlova* sp.	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco®	DC DHA Selco® + AlgaMac 2000®
14:0	1.0 ± 0.0^{ab}	2.4 ± 0.1^{d}	0.8 ± 0.0^{a}	1.9 ± 0.3^{cd}	1.4 ± 0.1^{bc}	1.8 ± 0.1^{c}
16:0	12.8 ± 0.0^{ab}	11.8 ± 0.4^{a}	11.2 ± 0.1^{a}	13.6 ± 0.6^{b}	14.0 ± 0.2^{b}	11.6 ± 0.5^{a}
17:0	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}	0.2 ± 0.0^{a}
18:0	0.0 ± 0.0^{a}	4.5 ± 0.2^{b}	6.4 ± 0.1^{c}	5.5 ± 0.1^{d}	1.1 ± 0.1^{e}	4.5 ± 0.2^{b}
Σ SFA[1]	20.4 ± 0.0^{a}	24.4 ± 0.6^{b}	23.2 ± 0.1^{ab}	26.9 ± 0.7^{c}	21.0 ± 0.8^{ab}	22. 2 ± 0.8^{ab}
16:1ω7	3.8 ± 0.0^{a}	3.0 ± 0.1^{b}	3.0 ± 0.0^{b}	3.9 ± 0.2^{a}	5.8 ± 0.2^{c}	4.2 ± 0.1^{a}
18:1ω9	20.2 ± 0.1^{a}	15.5 ± 0.6^{b}	18.7 ± 0.1^{a}	16.5 ± 0.1^{b}	23.8 ± 0.9^{c}	15.4 ± 0.6^{b}
18:1ω7	7.7 ± 0.2^{ab}	7.1 ± 0.3^{a}	9.7 ± 0.1^{b}	8.8 ± 0.2^{b}	11.4 ± 0.6^{c}	7.2 ± 0.3^{a}
20:1ω9	0.5 ± 0.0^{abc}	0.7 ± 0.2^{abc}	0.6 ± 0.0^{abc}	0.8 ± 0.1^{b}	0.3 ± 0.0^{c}	0.6 ± 0.1^{abc}
Σ MUFA[2]	34.2 ± 0.3^{a}	30.6 ± 1.4^{b}	33.4 ± 0.2^{ab}	32.2 ± 0.1^{ab}	43.3 ± 0.0^{c}	30.5 ± 0.7^{b}
18:2ω6	6.8 ± 0.0^{a}	4.5 ± 0.2^{b}	5.7 ± 0.1^{a}	5.0 ± 0.1^{ab}	10.4 ± 0.5^{c}	5.1 ± 0.2^{ab}
18:3ω3	32.0 ± 0.3^{a}	20.6 ± 0.9^{b}	25.2 ± 0.2^{c}	23.3 ± 0.5^{c}	3.7 ± 0.3^{d}	19.2 ± 0.8^{b}
18:4ω3	0.0 ± 0.0^{a}	3.2 ± 0.1^{b}	4.5 ± 0.1^{c}	3.3 ± 0.1^{b}	0.0 ± 0.0^{a}	3.5 ± 0.1^{b}
20:3ω6	0.1 ± 0.0^{a}	0.1 ± 0.0^{a}	0.0 ± 0.0^{ab}	0.1 ± 0.0^{a}	0.0 ± 0.0^{b}	0.1 ± 0.0^{a}
20:4ω6 (AA)	0.9 ± 0.0^{a}	0.8 ± 0.0^{a}	1.1 ± 0.0^{a}	1.1 ± 0.0^{a}	2.8 ± 0.4^{b}	1.1 ± 0.1^{a}
20:4ω3	0.8 ± 0.0^{a}	1.2 ± 0.7^{a}	0.5 ± 0.0^{a}	0.5 ± 0.0^{a}	0.4 ± 0.1^{a}	0.7 ± 0.0^{a}
20:5ω3 (EPA)	2.7 ± 0.0^{a}	3.4 ± 0.2^{ac}	3.1 ± 0.1^{a}	3.0 ± 0.1^{a}	7.6 ± 0.3^{b}	4.6 ± 0.6^{c}
22:5ω6 (ω6DPA)	0.05 ± 0.0^{a}	0.1 ± 0.1^{ab}	0.04 ± 0.0^{a}	0.6 ± 0.1^{bc}	0.5 ± 0.1^{abc}	0.9 ± 0.2^{c}

22:5ω3	0.1 ± 0.0^{a}	0.9 ± 0.5^{a}	0.1 ± 0.1^{a}	0.0 ± 0.0^{a}	0.6 ± 0.1^{a}	0.3 ± 0.1^{a}
22:6ω3 (DHA)	0.1 ± 0.0^{a}	7.7 ± 0.7^{b}	0.2 ± 0.0^{a}	2.0 ± 0.3^{a}	6.2 ± 0.3^{b}	9.2 ± 0.7^{b}
Σ PUFA[3]	45.7 ± 0.5^{a}	45.0 ± 1.1^{a}	43.4 ± 0.1^{ab}	40.9 ± 0.6^{b}	35.6 ± 0.8^{c}	45.0 ± 1.9^{a}
Σ ω3	37.3 ± 0.3^{ab}	38.7 ± 1.2^{a}	35.0 ± 0.2^{bc}	33.3 ± 0.4^{c}	19.7 ± 0.9^{d}	38.6 ± 0.5^{a}
Σ ω6	16.0 ± 0.4^{a}	5.7 ± 0.1^{b}	17.0 ± 0.3^{a}	15.8 ± 0.2^{a}	26.0 ± 0.4^{c}	14.8 ± 0.7^{a}
ω3/ω6	2.3 ± 0.1^{a}	6.8 ± 0.3^{b}	2.1 ± 0.0^{a}	2.1 ± 0.1^{a}	0.8 ± 0.0^{c}	2.6 ± 0.1^{a}
DHA/EPA	0.1 ± 0.0^{a}	2.2 ± 0.1^{b}	0.1 ± 0.0^{ac}	0.7 ± 0.1^{cd}	0.8 ± 0.0^{d}	2.1 ± 0.3^{b}
EPA/AA	3.1 ± 0.0^{a}	4.5 ± 0.2^{b}	2.9 ± 0.1^{a}	2.8 ± 0.1^{a}	2.8 ± 0.3^{a}	4.1 ± 0.3^{b}
DHA + ω6DPA	0.2 ± 0.0^{a}	7.9 ± 0.7^{b}	0.2 ± 0.0^{a}	2.5 ± 0.4^{c}	6.7 ± 0.2^{b}	10.1 ± 0.7^{d}
DHA/ω6DPA	3.2 ± 0.6^{a}	0.1 ± 0.1^{a}	3.0 ± 0.1^{a}	3.5 ± 0.0^{a}	15.3 ± 3.7^{b}	11.6 ± 2.3^{b}
ω6DPA/AA	0.1 ± 0.0^{a}	0.2 ± 0.1^{a}	0.04 ± 0.0^{a}	0.5 ± 0.1^{ab}	0.2 ± 0.1^{a}	0.8 ± 0.1^{b}

Values (mean ± SE of three replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] Sum of saturated fatty acids, which includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 19:0, 20:0, 22:0, 23:0, and 24:0 at ≤ 1.50% each.

[2] Sum of monounsaturated fatty acids, which includes: 14:1, 15:1, 16:1ω11, 16:1ω5, 17:1, 18:1ω5, 20:1ω11, 20:1ω7, 22:1ω11, 22:1ω9, and 24:1 at ≤ 1.34% each.

[3] Sum of polyunsaturated fatty acids, which includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:3ω4, 18:4ω1, 18:5ω3, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 22:2 NIMDa, 21:5ω3, 22:4ω6, and 22:4ω3 at ≤ 1.0% each.

V.3.2. Larval survival and growth

Larvae from the AquaGrow Advantage treatment had inferior survival performance when compared to the larvae from the other treatments. Larvae from the *Pavlova* sp. + AlgaMac 2000 and DC DHA Selco + AlgaMac 2000 treatments showed similar survival over the experimental period (Figure V.1). At 43 dph (417.7 dd), *Pavlova* sp. + AlgaMac 2000-larvae were significantly lighter than the larvae from the other treatments ($F_{2,47}$=6.67, p=0.003). Larvae from AquaGrow Advantage and DC DHA Selco + AlgaMac 2000 treatments had both dry weights of approximately 6 mg at 43 dph (Figure V.2). In addition, these treatments also resulted in superior growth rates, when compared to the growth rate produced by the *Pavlova* sp. + AlgaMac 2000 treatment ($F_{2,15}$=4.68, p=0.026) (Table V.5).

Table V.5. Growth of haddock larvae fed differently enriched *Artemia*. Dry weights of 10 larvae were determined at 43 dph (417.7 dd). Dry weight SGR were calculated between 29 and 43 dph (n=6).

	Treatments		
	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco® + AlgaMac 2000®
Dry weight at 43 dph (mg)	6.40 ± 0.49[a]	4.47 ± 0.30[b]	5.83 ± 0.38[a]
Specific growth rate (% d^{-1})	12.54 ± 0.52[a]	9.92 ± 0.52[b]	11.83 ± 0.50[a]

Values (mean ± SE) with different superscript are significantly different (p<0.05).

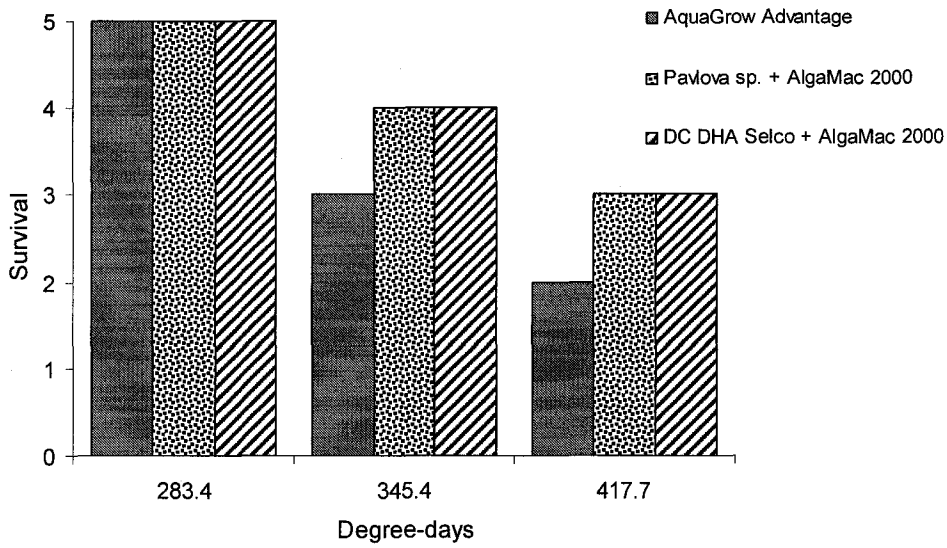

Figure V.1. Survival of haddock larvae fed differently enriched *Artemia* at 283.4, 345.4, 417.7 degree-days (equivalent to 30, 36 and 43 days post hatch, respectively). Values are expressed as mean of twelve observations per treatment.

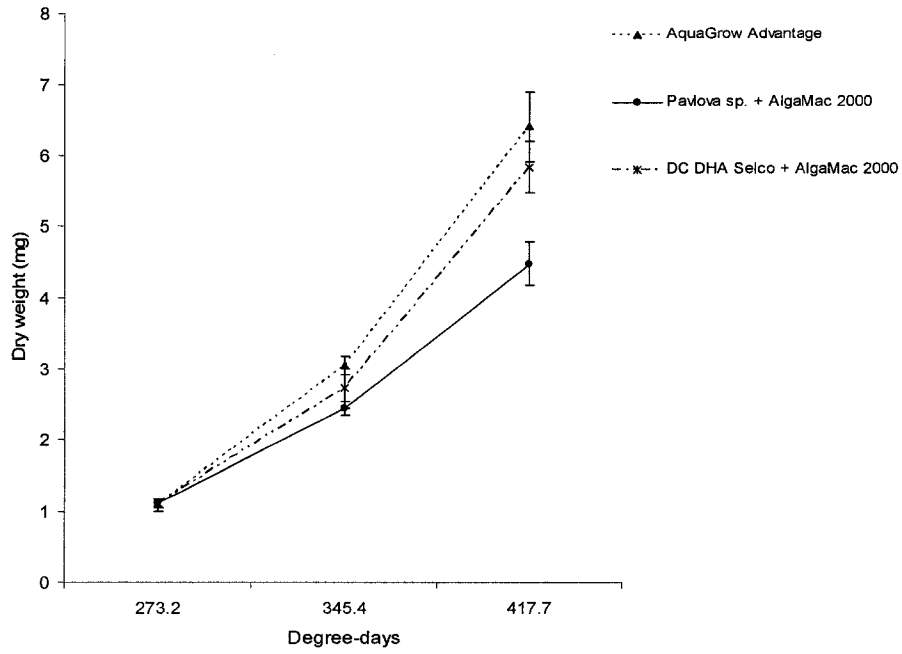

Figure V.2. Dry weight of haddock larvae fed differently enriched *Artemia* at 273.2, 345.4, and 417.7 degree-days (equivalent to 29, 36 and 43 days post hatch, respectively). Values are expressed as mean ± SE of 6 samples.

V.3.3. Larval total lipid, lipid class and fatty acid composition

At the end of the experiment, larvae from all treatments had higher total lipid content than at the start of the experiment (Table V.6). The different treatments resulted in larvae having different total lipid concentrations ($F_{4,15}$=130.05, $p<0.0001$). The DC DHA Selco + AlgaMac 2000 treatment resulted in the greatest increase in larval total lipid content (132.3%) while the lowest was produced by the *Pavlova* sp. + AlgaMac 2000 treatment (51.8%). The TAG

percentage increased in larvae from all treatments, with larvae from the DC DHA Selco + AlgaMac 2000 treatment having TAG percentage significantly higher than the larvae from the other treatments ($F_{4,15}$=9.43,p=0.0005). At 43 dph (417.7 dd), larvae from all treatments had similar ST levels ($F_{4,15}$=4.24, p=0.15). Larvae from the AquaGrow Advantage treatment had significantly lower PL percentage at 43 dph than at the start of the experiment ($F_{4,15}$=4.24, p=0.01). A strong correlation was found between larval dry weight and larval total lipid ($F_{1,46}$=175.28, p<0.001, r^2=79.2%) and PL ($F_{1,46}$=105.26, p<0.001, r^2=69.6%) concentrations over all treatments and time points (Figures V.3A and V.3B).

Table V.6. Total lipid (mg g^{-1} dry weight) and percentage lipid class composition of haddock larvae at the start (29 dph; 273.2 dd) and the end of the experiment (43 dph; 417.7 dd).

	Treatments				
	Start of the experiment (29 days post hatch)		End of the experiment (43 days post hatch)		
	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco + AlgaMac 2000
Total lipids (mg g^{-1} dw)	39.7 ± 0.9^{a}	50.8 ± 0.5^{a}	100.5 ± 5.6^{b}	77.1 ± 0.9^{c}	118.0 ± 2.9^{d}
Lipid classes[1] (% total lipids)					
Hydrocarbons	0.3 ± 0.1^{a}	0.3 ± 0.0^{a}	0.0 ± 0.0^{b}	0.1 ± 0.1^{ab}	0.1 ± 0.0^{b}
Triacylglycerols	4.4 ± 0.3^{ab}	3.0 ± 0.5^{a}	8.1 ± 1.0^{bc}	3.5 ± 0.8^{ab}	11.1 ± 2.1^{c}
Free fatty acids	1.5 ± 0.4^{a}	0.5 ± 0.0^{b}	1.6 ± 0.1^{a}	1.5 ± 0.2^{a}	1.2 ± 0.1^{ab}
Sterols	22.7 ± 1.2^{a}	20.5 ± 0.6^{a}	28.4 ± 3.6^{a}	26.4 ± 3.3^{a}	21.6 ± 1.7^{a}
Acetone Mobile Polar Lipids	2.2 ± 0.7^{a}	0.7 ± 0.4^{a}	1.1 ± 0.2^{a}	2.2 ± 0.9^{a}	0.5 ± 0.3^{a}
Phospholipids	67.7 ± 1.6^{ab}	74.5 ± 1.0^{a}	60.7 ± 2.8^{b}	66.1 ± 3.9^{ab}	65.5 ± 1.6^{ab}

Values (mean ± SE of four replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] May also contain steryl esters, ketones, and alcohols at ≤ 1.6% each.

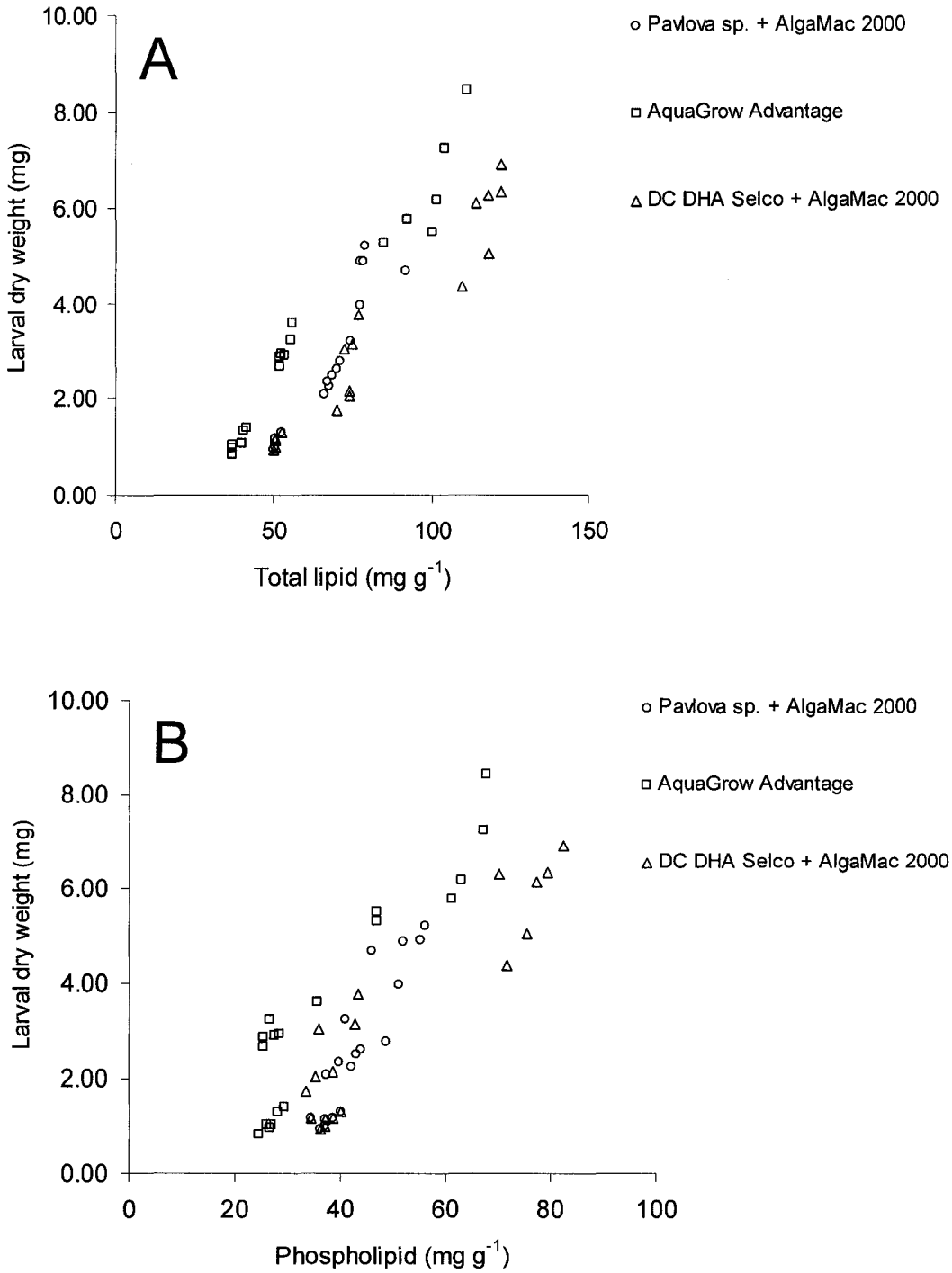

Figure V.3. The relationship between larval dry weight and (A) larval total lipid concentration, and (B) larval phospholipid concentration, over the experimental period.

Larval levels of individual saturated fatty acids remained constant during the experimental period, with larvae from all treatments reaching the end of the experiment with very similar levels of saturated fatty acids (Table V.7). At 43 dph (417.7 dd), the ΣSFA in larvae from all treatments was not different from initial values, and there were not differences in the ΣSFA when larvae from different treatments were compared ($F_{4,15}$=1.96, p=0.15). Levels of 18:1ω9 and 18:1ω7 increased in larvae from all treatments, and the levels of these fatty acids observed at 43 dph were significantly higher than those at the start of the experiment ($F_{4,15}$=39.13, p<0.0001 and $F_{4,15}$=7.48, p=0.0016, respectively). On the other hand, levels of 16:1ω7 and 20:1ω9 decreased in larvae from all treatments, with larval levels at 43 dph (417.7 dd) being significantly lower than at 29 dph (273.2 dd) ($F_{4,15}$=77.88, p<0.0001 and $F_{4,15}$=70.51, p<0.0001, respectively). The ΣMUFA increased significantly in larvae from the AquaGrow Advantage treatment, while in the larvae from the other treatments, the ΣMUFA decreased significantly ($F_{4,15}$=16.57, p<0.0001). Among the polyunsaturated fatty acids, 18:3ω3 showed the highest accumulation in the larval tissues. At 43 dph, larval levels of this fatty acid were between 29 and 129 times higher than at 29 dph ($F_{4,15}$=768.64, p<0.0001). Levels of AA and EPA also showed a significant increase in larvae from all treatments. At 43 dph, larvae from the AquaGrow Advantage treatment had a significantly lower AA percentage than the larvae from the other treatments ($F_{4,15}$=137.21, p<0.0001), while EPA levels were significantly higher in larvae from the DC DHA Selco + AlgaMac 2000 ($F_{4,15}$=95.98, p<0.0001). Conversely, the DHA percentage decreased in all

treatments ($F_{4,15}$=387.09, p<0.0001). At 43 dph (417.7 dd), the ω6DPA percentage in larvae from the DC DHA Selco + AlgaMac 2000 treatment was significantly lower than in the larvae from the other treatments ($F_{4,15}$=19.51, p<0.0001). A positive correlation was found between larval dry weight and larval percentages of EPA, DHA, and AA, with the larval percentage of EPA showing a stronger correlation with larval dry weight ($F_{1,46}$=140.23, p<0.001, r^2= 75.3%) than the percentages of DHA or AA ($F_{1,46}$=29.67, p<0.001, r^2= 39.2% and $F_{1,46}$=10.54, p=0.002, r^2= 18.6%, respectively). However, when the larval concentration of these fatty acids was considered, only EPA ($F_{1,46}$=75.80, p<0.001, r^2=62.2%) and AA ($F_{1,46}$=22.28, p<0.001, r^2=36.2%) were positively correlated with larval dry weight (Figures V.4A and V.4B). Larvae from two out of three treatments had significantly higher ΣPUFA at 43 dph (417.7 dd) than at 29 dph (273.2 dd), with the exception of larvae from the AquaGrow Advantage treatment, which had lower ΣPUFA at 43 dph than at 29 dph ($F4,15=_{14.78}$, p<0.0001). In relation to the Σω3, at 43 dph only larvae from the *Pavlova* sp. + AlgaMac 2000 treatment had values similar to those observed at the start of the experiment. The Σω3 was higher and lower than initial values in larvae from DC DHA Selco + AlgaMac 2000 and AquaGrow Advantage treatments, respectively ($F_{4,15}$=73.39, p<0.0001). On the other hand, the Σω6 increased in larvae from two treatments, with the exception of larvae from the DC DHA Selco + AlgaMac 2000 treatment, which had Σω6 similar to initial values ($F_{4,15}$=117.59, p<0.0001). The larval DHA/EPA ratio decreased in all treatments, with values at 43 dph being around 1.0 ($F_{4,15}$=146.75, p<0.0001). Conversely, the larval EPA/AA ratio increased in all

treatments ($F_{4,15}$=108.16, $p<0.0001$), with values at 43 dph (417.7 dd) ranging from 2.4 ± 0.0 in *Pavlova* sp. + AlgaMac 2000-larvae to 3.5 ± 0.1 in AquaGrow Advantage-larvae. At the end of the experiment, larvae from the AquaGrow Advantage treatment had DHA/ω6DPA ratios significantly higher than the ratio observed in the larvae from the other treatments ($F_{4,15}$=64.71, $p<0.0001$), while larvae from the *Pavlova* sp. + AlgaMac 2000 treatment had ω6DPA/AA ratio significantly higher than the larvae from the other treatments ($F_{4,15}$=21.02, $p<0.0001$).

Table V.7. Percentage fatty acid composition of haddock larvae fed differently enriched *Artemia* at the start (29 dph; 273.2 dd) and the end of the experiment (43 dph; 417.7 dd).

	Start of the experiment (29 days post hatch)		End of the experiment (43 days post hatch)		
Treatments	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco® + AlgaMac 2000®
Fatty acids (% total fatty acids)					
14:0	1.6 ± 0.3^{a}	1.4 ± 0.0^{a}	0.5 ± 0.0^{b}	0.5 ± 0.0^{b}	0.6 ± 0.0^{b}
16:0	13.8 ± 0.3^{ab}	13.1 ± 0.2^{a}	13.7 ± 0.3^{ab}	14.3 ± 0.1^{b}	13.6 ± 0.3^{ab}
18:0	6.6 ± 0.1^{a}	7.3 ± 0.1^{b}	6.6 ± 0.1^{a}	6.7 ± 0.1^{a}	6.3 ± 0.1^{a}
22:0	0.0 ± 0.0^{a}	0.1 ± 0.1^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
Σ SFA[1]	24.3 ± 0.0^{a}	24.0 ± 0.3^{a}	24.5 ± 0.2^{a}	24.7 ± 0.2^{a}	24.0 ± 0.2^{a}
16:1ω7	3.6 ± 0.5^{a}	6.1 ± 0.2^{b}	1.2 ± 0.0^{c}	1.2 ± 0.0^{c}	1.0 ± 0.0^{c}
18:1ω9	10.6 ± 0.2^{a}	11.2 ± 0.2^{a}	15.8 ± 0.2^{b}	14.5 ± 0.2^{bc}	14.0 ± 0.6^{c}
18:1ω7	4.5 ± 0.9^{a}	4.7 ± 0.1^{a}	6.7 ± 0.4^{b}	7.1 ± 0.1^{b}	6.3 ± 0.2^{ab}
20:1ω9	1.5 ± 0.1^{a}	1.8 ± 0.0^{b}	1.1 ± 0.1^{c}	0.2 ± 0.0^{d}	0.9 ± 0.0^{c}
Σ MUFA[2]	24.4 ± 0.6^{a}	29.1 ± 0.4^{b}	27.9 ± 0.5^{b}	25.1 ± 0.4^{a}	24.7 ± 0.6^{a}
18:2ω6	2.6 ± 0.6^{a}	3.0 ± 0.1^{a}	4.9 ± 0.0^{b}	4.8 ± 0.1^{b}	4.7 ± 0.1^{b}
18:3ω3	0.1 ± 0.0^{a}	0.4 ± 0.0^{a}	12.9 ± 0.1^{b}	11.8 ± 0.2^{c}	11.6 ± 0.5^{c}
18:4ω3	0.6 ± 0.4^{a}	0.7 ± 0.1^{a}	1.1 ± 0.4^{a}	1.3 ± 0.0^{a}	1.6 ± 0.1^{a}
20:3ω6	0.4 ± 0.0^{a}	0.8 ± 0.0^{b}	0.4 ± 0.0^{a}	0.5 ± 0.0^{c}	0.3 ± 0.0^{d}

20:4ω6 (AA)	1.8 ± 0.1^{a}	3.0 ± 0.0^{b}	2.5 ± 0.1^{c}	3.8 ± 0.1^{d}	3.6 ± 0.1^{d}
20:4ω3	0.5 ± 0.1^{a}	1.0 ± 0.0^{b}	1.2 ± 0.0^{b}	1.3 ± 0.0^{b}	3.1 ± 0.1^{c}
20:5ω3 (EPA)	5.2 ± 0.2^{a}	6.1 ± 0.1^{a}	8.7 ± 0.2^{b}	8.9 ± 0.1^{b}	9.9 ± 0.3^{c}
22:5ω6 (ω6DPA)	0.3 ± 0.0^{ac}	4.7 ± 0.1^{b}	0.2 ± 0.0^{ac}	4.0 ± 1.0^{bc}	2.1 ± 0.2^{c}
22:5ω3	1.4 ± 0.2^{a}	2.3 ± 0.0^{b}	0.7 ± 0.0^{a}	1.2 ± 0.3^{a}	1.6 ± 0.4^{ab}
22:6ω3 (DHA)	35.4 ± 0.8^{a}	22.5 ± 0.5^{b}	11.7 ± 0.4^{c}	9.3 ± 0.1^{c}	11.7 ± 0.8^{c}
ΣPUFA[3]	51.3 ± 0.6^{a}	46.9 ± 0.2^{b}	47.6 ± 0.4^{b}	50.2 ± 0.5^{a}	51.0 ± 0.7^{a}
Σω3	44.5 ± 0.1^{a}	33.8 ± 0.3^{b}	37.8 ± 0.4^{c}	35.5 ± 0.6^{b}	39.2 ± 0.7^{c}
Σω6	5.5 ± 0.7^{a}	12.3 ± 0.1^{be}	9.4 ± 0.3^{ce}	21.6 ± 0.9^{d}	11.5 ± 0.3^{e}
ω3/ω6	8.4 ± 0.9^{a}	2.8 ± 0.0^{b}	4.0 ± 0.1^{b}	1.7 ± 0.1^{b}	3.4 ± 0.1^{b}
DHA/EPA	6.9 ± 0.4^{a}	3.7 ± 0.2^{b}	1.3 ± 0.0^{c}	1.0 ± 0.0^{c}	1.2 ± 0.1^{c}
EPA/AA	2.9 ± 0.0^{a}	2.0 ± 0.1^{b}	3.5 ± 0.1^{c}	2.4 ± 0.0^{d}	2.7 ± 0.1^{a}
DHA + ω6DPA	35.6 ± 0.8^{a}	27.3 ± 0.6^{b}	11.9 ± 0.4^{c}	13.3 ± 0.9^{c}	13.9 ± 0.8^{c}
DHA/ω6DPA	138.0 ± 13.2^{a}	4.8 ± 0.0^{b}	62.8 ± 9.7^{c}	2.8 ± 0.7^{b}	5.7 ± 0.7^{b}
ω6DPA/AA	0.2 ± 0.0^{a}	1.6 ± 0.0^{b}	0.1 ± 0.0^{a}	1.1 ± 0.3^{b}	0.6 ± 0.1^{a}

Values (mean ± SE of four replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] Sum of saturated fatty acids, which includes: *i*15:0, *ai*15:0, 15:0, *i*16:0, *ai*16:0, *i*17:0, *ai*17:0, 17:0, 19:0, 20:0, 21:0, and 24:0 at ≤ 0.90% each.

[2] Sum of monounsaturated fatty acids, which includes: 14:1, 15:1, 16:1ω9, 16:1ω5, 17:1, 18:1ω11, 18:1ω6, 18:1ω5, 20:1ω11, 20:1ω7, 22:1ω11, 22:11ω9, and 24:1 at ≤ 1.6% each.

[3] Sum of polyunsaturated fatty acids, which includes: 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2ω4, 18:3ω6, 18:3ω4, 20:2α, 20:2β, 20:2ω6, 20:3ω3, 22:2 NIMDa, 21:5ω3, 22:4ω6, and 22:4ω3 at ≤ 1.27% each.

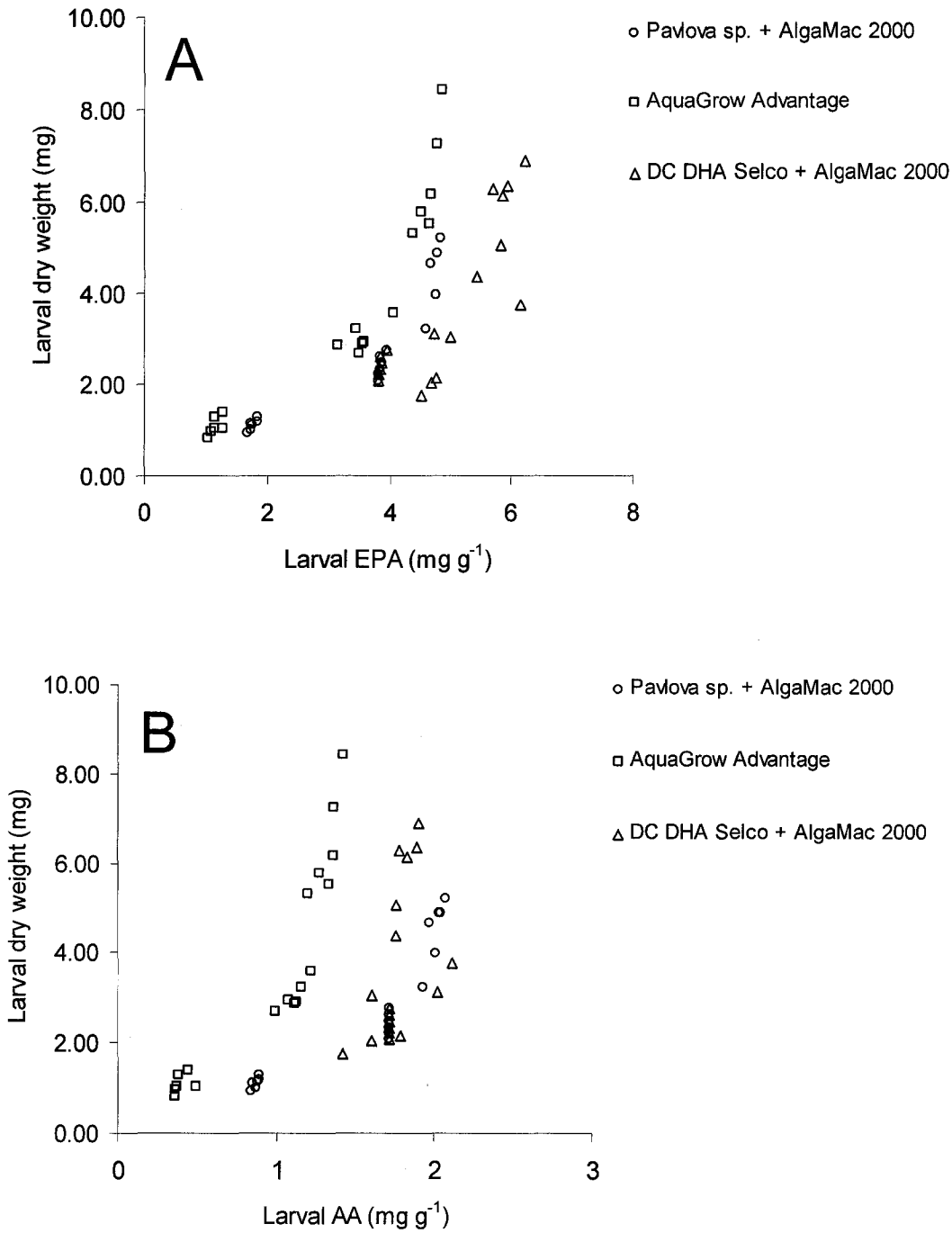

Figure V.4. The relationship of larval dry weight and (A) larval EPA concentration and (B) larval AA concentration, during the experimental period.

V.4. Discussion

All treatments resulted in a significant increase in the total lipid concentration of *Artemia*, with the increase in total lipids being mainly due to an accumulation of PL. Small but significant shifts in minor lipid components (KET and ST) occurred in all treatments. Studies on the lipid enrichment of *Artemia* nauplii have mainly focused on increasing the levels of ω3HUFA, and shifts of lipids classes due to the enrichment are poorly documented and their significance in terms of nutritional value not well understood (Sorgeloos et al., 2001). Similarly to what was observed before in Chapter III, *Artemia* actively changed the lipid composition of the diet during the enrichment process. The so-called "bioencapsulation" of *Artemia* is considered as the simple filing of the digestive tract of the animal with little biotransformation of the enrichment product in the digestive system (Tocher et al., 1997). However, there is enough information available in the literature indicating that incorporation of the diet without biotransformation seldom occurs in *Artemia* (Coutteau and Mourente, 1997; Navarro et al., 1999, Dhont and Van Stappen, 2003). In broad terms, the enriched *Artemia* from the present experiment differed from the enriched *Artemia* from the Atlantic cod experiment (Chapter III) for having lower total lipid concentrations and AMPL percentages, and higher PL percentages. These differences are probably due to differences in the lipid composition of the *Artemia* strains used, and to differences in the tanks used for the rearing and enrichment of the *Artemia*.

It is important to note that the larvae from the different treatments were not fed the same diet during the rotifer phase and had different biochemical composition at

the start of the *Artemia* phase. Therefore, these differences should also be considered whem interpreting the results from the present study. Nevertheless, at the end of the *Artemia* phase, larvae from the different treatments had a similar percentage of ST, PL, and DHA, and similar ΣSFA.

Larvae from the AquaGrow Advantage treatment showed inferior survival when compared to the larvae from the other treatments. In this treatment, larvae were supplied with *Artemia* containing lower total lipid and TAG concentrations than the *Artemia* supplied in the other treatments, with the exception of *Pavlova* sp.-enriched *Artemia*. In addition, in absolute terms, the AquaGrow Advantage-enriched *Artemia* had the lowest PL concentration (18.7 ± 1.4 mg g^{-1}) of all enriched *Artemia*. In the other treatments, the PL concentration ranged from 20.8 ± 0.6 mg g^{-1} in DC DHA Selco + AlgaMac 2000-enriched *Artemia* to 52.1 ± 3.0 mg g^{-1} in DC DHA Selco-enriched *Artemia*. As early as 36 dph (345.4 dd), differences between the survival of larvae from the AquaGrow Advantage treatment and the other larvae became evident. At 36 dph, AquaGrow Advantage-larvae had total lipid, PL, and AA concentrations significantly lower than the larvae from the other treatments (Appendices 2 and 3). Haddock larvae undergo metamorphosis at approximately 30 dph (Neil, 2003), and therefore, it is expected that some mortality will occur around this period. However, the lower supply of total lipids, TAG, and PL to the larvae from the AquaGrow Advantage-larvae, concomitant with low body concentrations of total lipids, PL, and AA, clearly puts these larvae at a disadvantage in relation to the larvae from the other treatments. The beneficial effects of dietary PL in terms of survival and resistance to stress in fish and crustacean larvae has long been recognized, and was

reviewed by Coutteau et al. (1997). Larval stages are very sensitive to PL deficiency. Even a small PL supplementation in the diet (as small as 2%) resulted in drastic improvements in the survival of larvae of species such as carp, goldfish, and red sea bream (Coutteau et al., 1997). Eicosanoids are autocrine compounds derived primarily from C_{20} PUFA—20:3ω6, 20:4ω6 and 20:5ω3—produced by virtually every cell in the body to act in their immediate vicinity in response to stressful situations (Sargent et al., 1999a). In mammals, AA is the chief fatty acid precursor of eicosanoids, with AA-derived eicosanoids being more biologically active than eicosanoids derived from 20:3ω6 or EPA. The competitive interactions between EPA and AA for the production of eicosanoids, and their effects in the stress response were discussed earlier in Chapter II. A large number of species of freshwater and marine fish have been found to produce the same range of eicosanoids as in mammals, and the evidence from fish is consistent with the situation described above for mammals (Tocher, 2003).

In terms of growth performance, the best results were observed in the AquaGrow Advantage and DC DHA Selco + AlgaMac 2000 treatments. Larvae from these treatments grew with a SGR of approximately 12% d^{-1}, and reached 43 dph (417.7 dd) with approximately 6 mg of dry weight. These results are superior to other results reported for haddock larvae raised on *Artemia* or microparticulate diets. Hamlin and Kling (2001) weaned haddock larvae to a microparticulate diet at 14, 21, 28 or 35 dph. By 42 dph, the average larval dry weight ranged between 1.4 ± 0.7 and 2.3 ± 0.6 mg. It is important to mention that the water temperature in the experiment performed by Hamlin and Kling (2001) was considerably lower (8.5 °C) than the water

temperature of the present study. Furthermore, the early replacement of live-feed by inert diets in the larval rearing of marine fish consistently results in poorer growth and survival performances when compared to larvae raised exclusively on live-feed (Cahu and Zambonino-Infante, 2001). However, even the control group from Hamlin and Kling's study (2001)—that was supplied with *Artemia* from 21 dph to end of the experiment—reached 42 dph weighing 3.6 ± 0.6 mg. Blair et al. (2003) evaluated microdiets versus live-feeds on the growth of haddock larvae. In this study, three different weaning diets and traditional live-food, enriched rotifers and enriched *Artemia*, were supplied to the larvae from 25 until 45 dph (average water temperature of 12 °C). Enriched *Artemia* resulted in the best larval growth, with larvae fed this diet weighing on average 2.1 ± 0.1 mg at 45 dph (Blair et al., 2003).

Considering both survival and growth, larvae from the DC DHA Selco + AlgaMac 2000 treatment showed the best overall performance. Larvae in this treatment were fed with prey containing DHA/EPA/AA ratios of approximately 2/3/1 and 8/4/1 (DC DHA Selco, and DC DHA Selco + AlgaMac 2000-enriched *Artemia*, respectively). There is a scarcity of information linking *Artemia* lipid composition and haddock larval growth, making comparisons with the results of the present study difficult. However, larvae from the AquaGrow Advantage treatment also had good growth and were fed *Artemia* containing a DHA/EPA/AA ratio of approximately 9.5/4/1. These results suggest that *Artemia* containing DHA/EPA/AA ratios around 9/4/1 result in good haddock larval growth. The high levels of fatty acids that could act as an energy source, such as 16:0, 18:1ω9 and 18:1ω7, observed in DC DHA Selco-enriched *Artemia*, may help to explain the contribution of this prey to the good

larval growth observed in the DC DHA Selco + AlgaMac 2000 treatment. When the DHA/EPA/AA ratio of 9/4/1 is compared with the ratio of 10/2/1, which was considered to be close to an optimum DHA/EPA/AA ratio for haddock larvae during the rotifer phase (Chapter IV), the main difference between them is a proportional increase in EPA. This observation is in agreement with the positive correlation found between larval dry weight and larval EPA levels. In fact, among the essential fatty acids, EPA was the only one that remained strongly correlated with larval dry weight when both percentage and concentration of fatty acids in the larval tissues, were considered. EPA is not only a major structural component of cell membranes but is also considered the main modulator of eicosanoid production and activity (James et al., 2000).

The percentages of DHA and EPA in the larval tissues showed an opposite trend when compared to the trend observed during the rotifer phase (Chapter IV). From hatch to 29 dph (273.2 dd), larval levels of DHA remained high (between 22 and 35% total fatty acids) while larval levels of EPA decreased progressively from 13.6% at hatch to around 4-6% at 29 dph (Chapter IV). During the *Artemia* phase, larval levels of DHA decreased progressively, being between 9 and 12% at 43 dph (417.7 dd). Conversely, EPA was accumulated during the *Artemia* phase, increasing from approximately 5 to 9% in AquaGrow Advantage-larvae, and from 6 to 9 and 10% in *Pavlova* sp. + AlgaMac 2000 and DC DHA Selco + AlgaMac 2000-larvae, respectively. Larval levels of AA were conserved during the rotifer phase, with only larvae from *Pavlova* sp. + AlgaMac 2000 treatment having higher levels of this fatty acid at 29 dph than newly hatched larvae (Chapter IV). From 29 to 43 dph, larvae

from all treatments accumulated AA in their tissues, and the levels observed at the end of the experiment were significantly higher than initial levels. Although haddock larvae accumulated both EPA and AA during the *Artemia* phase, the accumulation of EPA was higher than the accumulation of AA. On average, larval EPA levels increased 67%, while AA levels increased approximately 39%. The levels of essential fatty acids found in haddock larvae at 43 dph in the present study differ somewhat from those reported by Blair et al. (2003) for haddock larvae at 45 dph. These authors found larval levels of DHA ranging from 13.4 to 28.8%, EPA from 3.5 to 8.8%, and AA from 2.7 to 5.4%. However, differences should be interpreted cautiously since differences in water temperature and final dry weight suggest that at the end of each experiment, larvae from these two studies were in different stages of development. Nevertheless, the larvae with best overall performance (growth and survival) in the study performed by Blair et al. (2003), had body levels of DHA (13.4 ± 2.5%) and EPA (8.8 ± 0.6%) very close to the DHA and EPA levels observed in larvae at 43 dph in the present study. The authors did not report the fatty acid composition of the larvae at the start of the experiment (25 dph), making it therefore impossible to discuss possible accumulation or reduction in the larval levels of essential fatty acids. It is worth mentioning that Blair et al. (2003) observed bioaccumulation of ω6DPA (22:5ω6) in haddock larvae in relation to their diet. Levels of this fatty acid were between 0.1 and 5.5% in the diet, and between 2.4 and 6.1% in the fish tissues at 45 dph (Blair et al., 2003). The authors suggested that ω6DPA was being retroconverted to AA, and speculated a possible role as an essential fatty acid for ω6DPA. This observation is particularly interesting because ω6DPA has been practically neglected

in nutritional studies involving marine fish larvae. The effects of the incorporation of ω6DPA in cell membranes and the possible role of this fatty acid as precursor of docosanoids were discussed earlier in Chapter II. Although a relationship between ω6DPA and Atlantic cod larvae growth was suggested in Chapters II and III, such a relationship was not found for haddock larvae. At the end of the rotifer phase (29 dph; 273.2 dd), the larvae with the highest body concentration of ω6DPA (1.9 ± 0.1 mg g^{-1}) showed the poorest overall performance, while larvae containing 0.05 ± 0.0 or 1.4 ± 0.0 mg g^{-1} of ω6DPA had similar growth performance (Chapter IV). At the end of the *Artemia* phase (43 dph; 417.7 dd), the same trend was observed. The larvae with 2.1 ± 0.5 mg g^{-1} of ω6DPA had the lightest dry weight, while larvae with concentrations of 0.1 ± 0.0 or 1.3 ± 0.1 mg g^{-1} of ω6DPA had similar growth performance. More experiments specifically designed to test different dietary levels of ω6DPA, and the effects of this fatty acid on larval growth and survival, would help to elucidate the role that ω6DPA plays on haddock larvae development.

Results from the present study indicate that from 29 to 43 dph (273.2 to 417.7 dd, respectively), larval total lipid, PL, and EPA concentrations and growth of haddock larvae are correlated, and that *Artemia* to be used as live-feed during this period should have PL concentrations $\geq$ 20 mg g^{-1} and a DHA/EPA/AA ratio around 9/4/1.

CHAPTER VI

Summary

VI.1. Summary of the main results

The main objective of the present study was to evaluate different lipid enrichments for rotifers and *Artemia*, and the use of these enriched live-feed during the larviculture of two species belonging to the Gadidae family: Atlantic cod, *Gadus morhua*, and haddock, *Melanogrammus aeglefinus*. These two species are considered to have great potential for cold-water aquaculture, and represent a most needed alternative for the culture of salmonids in Atlantic Canada. As with many new marine species, problems related to larval nutrition are a major obstacle for the mass production of both species. This study provides information about the lipid nutrition of Atlantic cod and haddock larvae, which can help to improve the current protocols adopted for the production of these species.

Dietary lipids have long been recognized as one of the most important nutritional factors to affect growth, survival, and stress resistance in marine finfish larvae (Izquierdo et al., 2000). In the wild, the natural diet of marine finfish larvae is composed mainly of copepods, which in general, contain very high levels of ω3 HUFA, in particular EPA and DHA (Olsen, 2004). Studies that have examined fatty acid trophic transfer in marine planktonic systems revealed that EPA and DHA were markedly concentrated in copepods and fish larvae relative to the phytoplankton (Brett and Müller-Navarra, 1997). Due to limitations in their ability to elongate and/or desaturate C_{18} PUFA, most marine finfish studied to date require pre-formed DHA, EPA and AA in their diet (Sargent et al., 1999a).

The production of marine fish juveniles in commercial hatcheries depends heavily on the supply of live-prey to the larval stages. Despite many advances in the development of artificial diets, the total replacement of live-feed by inert diets has proved almost impossible, due to high larval mortality, impaired growth, and in many cases, abnormal pigmentation (Cahu and Zambonino-Infante, 2001). Reasons for difficulties in successfully rearing the early stages of marine fish larvae on artificial diets are not specifically known but several factors—such as the immaturity of the digestive system of the larvae, inappropriate biochemical composition of the diet, and poor digestibility of the artificial diet—are thought to play an important role (Langdon, 2003). Although copepods are considered ideal from the nutritional point of view, the mass culture of copepods is very difficult and their application to larval rearing is characterized by small scale and limited duration (Støttrup, 2000). With few exceptions, live-feeds used in commercial hatcheries for the production of marine fish include microalgae, rotifers and *Artemia*. Microalgae are not strictly necessary for all species during larval feeding (Muller-Feuga et al., 2003). Rotifers and *Artemia* are used worldwide as live-feed for the culture of several marine fish species, mainly because the technology for the production of these live-feed is well established; making them easy to culture in high densities at relatively low cost (Lubzens and Zmora, 2003). From the qualitative point of view, rotifers and *Artemia* provide only sub-optimum nutrition, especially regarding lipid nutrition. Because rotifers and *Artemia* are naturally poor in the fatty acids considered essential to marine fish, a range of techniques has been developed to enhance their lipid and fatty acid

composition, in a process commonly know as enrichment (Coutteau and Sorgeloos, 1997).

The present study evaluated the effects of differently lipid-enriched rotifers (Chapters II and IV) and *Artemia* (Chapters III and V) during the larviculture of two fish species with potential for aquaculture in Atlantic Canada.

In Chapter II, the effects of differently lipid-enriched rotifers on growth, survival and lipid composition of Atlantic cod larvae were evaluated. It was shown that the AlgaMac 2000 treatment resulted in a significant increment in the total lipid concentration of rotifers. The TAG and AMPL were the lipid classes most affected by the enrichment process. Although in AlgaMac 2000-enriched rotifers the PL percentage was significantly lower than in rotifers from the other treatments, in absolute terms, rotifers from all treatments delivered similar PL concentrations to the larvae (approximately between 8 and 9 mg g^{-1}). All treatments caused the percentage of DHA and EPA to increase in rotifers after the enrichment, however in *Pavlova* sp.-enriched rotifers and in *Pavlova* sp. + AlgaMac 2000-enriched rotifers the increments in DHA and EPA percentages, respectively, were not significant. Conversely, the percentage of AA decreased significantly in rotifers from all treatments after 24 h of enrichment, with the exception of AlgaMac 2000-enriched rotifers, which had percentages of this fatty acid similar to those observed in unenriched rotifers. The combination of *Pavlova* sp. and AlgaMac 2000 for the enrichment of rotifers resulted in the best survival performance during the 37 days of larviculture. During the rotifer phase, larvae grew with a SGR ranging from 6.3 to 7.1% dry weight d^{-1}. At 37 dph (443.2 dd), larval dry weight ranged from 1.03 mg in AquaGrow Advantage-larvae to

1.50 mg in *Pavlova* sp. + AlgaMac 2000-larvae, with the latter being significantly heavier than the former. The *Pavlova* sp. + AlgaMac 2000 treatment resulted in the best overall performance. Larvae from this treatment were supplied with prey items containing DHA/EPA/AA ratio of 1/1.6/1 (*Pavlova* sp.-enriched rotifers) and 11/1.5/1 (*Pavlova* sp + AlgaMac 2000-enriched rotifers). However, values previously reported in the literature suggest that the DHA/EPA/AA ratio of 11/1.5/1 is closest to an optimum for Atlantic cod larvae during the rotifer phase. Growth data show that good results were obtained with rotifers containing DHA percentages between 0.5 and 2.8% or as high as 14.7%, EPA percentages between 0.5 and 1.2%, and AA percentages between 0.3 and 1.0%. Larval levels of DHA remained high (above 25% of total fatty acids) in all treatments, while the levels of EPA dropped from 16% in newly hatched larvae to around 3% at 37 dph in larvae from all treatments. Larval levels of AA were significantly higher at 37 dph than in newly hatched larvae. A marked accumulation of ω6DPA (22:5ω6) was observed in larvae from AlgaMac 2000 and *Pavlova* sp. + AlgaMac 2000 treatments, with larval levels of this fatty acid at 37 dph being approximately 30 and 22 times higher than in newly hatched larvae, respectively. Larval growth and the amounts of DHA, EPA, AA and ω6DPA per larva were strongly correlated.

Chapter III evaluated the use of differently lipid-enriched *Artemia* as live-feed during the later stages of the larviculture of Atlantic cod. *Artemia* enriched with AquaGrow Advantage and *Pavlova* sp. had total lipid concentrations similar to unenriched *Artemia*. The increase in the total lipid content observed in *Artemia* from the other treatments (between 54 and 159%) was due mainly to increases in the PL

concentration (between 3 and 27-fold enrichment). All treatments resulted in enriched *Artemia* with higher DHA and EPA levels than unenriched *Artemia*, with the exception of *Artemia* from the *Pavlova* sp. treatment, which had DHA levels similar to those observed in unenriched *Artemia*. After enrichment, *Artemia* had levels of AA ranging from 0.4 to 1.9% of total fatty acids. Results from this experiment confirmed previous studies that showed that *Artemia* could actively change the lipid composition of their diet. Similarly to what happened during the rotifer phase, the combination of *Pavlova* sp. + AlgaMac 2000 resulted in the best survival during the *Artemia* phase. On the other hand, the best growth performance was produced by the AlgaMac 2000 treatment. During the *Artemia* phase, larvae grew with a SGR ranging from 4.9 to 10.4% dry weight d^{-1}, and reached 59 dph (709.7 dd) with dry weight ranging from 5.3 in DC DHA Selco + AlgaMac 2000-larvae to 12.0 mg in AlgaMac 2000-larvae. *Artemia* with DHA/EPA/AA ratio of approximately 7/2/1 resulted in larvae with the heaviest final dry weight, while a combination of two different *Artemia*—*Pavlova* sp.-enriched *Artemia* (0.3/3.7/1) and *Pavlova* sp. + AlgaMac 2000-enriched *Artemia* (7/2.4/1)—resulted in the highest survival rate. These results together with the DHA/EPA/AA ratio observed in the *Artemia* that resulted in poor survival and growth indicate that Atlantic cod larvae require diets with relatively high proportions of DHA and AA. Prey items containing a DHA/EPA/AA ratio of 7/2/1 were considered appropriate for Atlantic cod larvae during the *Artemia* phase. Based only on growth data, *Artemia* should have DHA percentage between 4.4 and 13.2%, EPA percentage around 4.0%, and AA percentage between 0.4 and 1.9% in order to promote good larval growth. At 59 dph (709.7 dd), larvae from all treatments had

lower concentrations of total lipids than at 37 dph (443.2 dd). The reduction in the larval lipid concentration was interpreted as a higher biochemical emphasis in proteins, resulting from the rapid growth observed during the *Artemia* phase. Larval percentage of DHA also decreased in all treatments, dropping from 26-35.8% at 37 dph to 11-20.5% at 59 dph. Conversely, larval percentage of EPA increased in all treatments (increment of 100 to 224%), while larval levels of AA remained relatively constant. The larvae with best growth and survival performances had both relatively high ω6DPA levels. Larval dry weight correlated positively with the amounts of DHA, EPA, AA, and ω6DPA per larva over all time points. However, the correlation was stronger with EPA than with AA, DHA or ω6DPA.

In Chapter IV, the effects of differently lipid-enriched rotifers on the early development of haddock larvae were investigated. Total lipid content of rotifers in all treatments increased after 24 h enrichment, being significantly higher than in unenriched rotifers. The enrichment process caused the PL percentage to decrease in rotifers from all treatments. The percentages of DHA observed in enriched rotifers were significantly higher than initial values, with the exception of *Pavlova* sp.-enriched rotifers. Only rotifers enriched with AlgaMac 2000 and with *Pavlova* sp. + AlgaMac 2000 had higher EPA percentages than unenriched rotifers. The levels of AA remained stable in most treatments, with the exception of rotifers enriched with *Pavlova* sp. + AlgaMac 2000, in which the percentage of this fatty acid was higher than unenriched rotifers. The combination of *Pavlova* sp. and AlgaMac 2000 resulted in the best survival, while the AlgaMac 2000 treatment resulted in the poorest survival performance. The treatments did not influence the larval growth throughout the

experimental period. During the rotifer phase, larvae grew with SGR of approximately 8% of their dry weight d^{-1}. At 29 dph (273.2 dd), larvae from all treatments had approximately 1 mg of dry weight. All treatments caused the larval tissue concentrations of total lipid and phospholipid to decrease, and at 29 dph, only larvae from the *Pavlova* sp. + AlgaMac 2000 treatment had total lipid concentrations similar to those observed in newly hatched larvae. Larvae from all treatments had lower PL concentrations and percentages at 29 dph than at 1 dph. Although the treatments did not affect the larval dry weights, there was a strong correlation between larval dry weight and larval total lipid and phospholipid concentrations over all diets in all time points. At 29 dph, larvae from the AquaGrow Advantage and AlgaMac 2000 treatments had higher percentages of DHA than newly hatched larvae, however the increment observed in the AlgaMac 2000-larvae was not significant. In *Pavlova* sp. + AlgaMac 2000-larvae the DHA percentage at the end of the experiment was significantly lower than in newly hatched larvae. The percentage of EPA decreased in all larvae, with larvae from the AlgaMac 2000 treatment having significantly lower percentage and concentration of this fatty acid than the larvae from other treatments. Larval levels of AA were more stable, showing only a slight increment during the experimental period. Based on the results from this experiment and values previously reported in the literature, the DHA/EPA/AA ratio of 10/2/1 (*Pavlova* sp. + AlgaMac 2000-enriched rotifers) was considered good for the early development of haddock larvae. Growth data indicate that rotifers containing DHA percentage between 0.4 and 7.2% or as high as 19.8%, EPA percentage between 0.3 and 1.8%, and AA percentage between 0.2 and 0.7% result in good larval growth. The high mortality

observed in the AlgaMac 2000 treatment was linked to the fact that the rotifers supplied in this treatment had low ST concentration and high EPA/AA ratio.

Chapter V evaluated the use of differently lipid-enriched *Artemia* during the larviculture of haddock, and their effects on growth, survival and larval lipid composition. Although the efficiency of the enrichment process was affected by the different treatments, all treatments resulted in *Artemia* having significantly higher concentrations of total lipids than unenriched *Artemia*. The increment of total lipid was a result of an accumulation of PL, regardless the different composition of the enrichments tested. Levels of DHA increased in all treatments. However, only in three out of the five treatments was the increase significant. Levels of AA remained relatively constant, with the exception of DC DHA Selco enriched-*Artemia* which had higher levels than unenriched *Artemia*. Similarly, EPA levels also remained relatively constant, with the exception of the DC DHA Selco enriched-*Artemia* and DC DHA Selco + AlgaMac 2000-enriched *Artemia* in which a significant increase in the percentage of this fatty acid was observed. Larvae from the AquaGrow Advantage treatment had inferior survival performance when compared to larvae from *Pavlova* sp. + AlgaMac 2000 and DC DHA Selco + AlgaMac 2000 treatments. At 43 dph (418 dd), *Pavlova* sp. + AlgaMac-larvae (4.5 mg dry weight) were lighter than the larvae from the other treatments. During the *Artemia* phase, larvae from DC DHA Selco + AlgaMac 2000 and AquaGrow Advantage treatments grew with a SGR of approximately 12% dry weight d^{-1}, and reached 43 dph weighing approximately 6 mg dry weight. Considering both survival and growth, larvae from the DC DHA Selco + AlgaMac 2000 treatment showed the best overall performance. Larvae in this

treatment were fed with prey containing DHA/EPA/AA ratios of approximately 2/3/1 and 8/4/1. However, considering that *Artemia* with DHA/EPA/AA ratio of 9.5/4/1 (AquaGrow Advantage-enriched *Artemia*) also resulted in good larval growth, prey items containing a DHA/EPA/AA ratio around 9/4/1 were considered appropriated for haddock larvae during the *Artemia* phase. Larvae fed with *Artemia* containing DHA percentage between 6.2 and 9.2%, EPA percentage between 3.4 and 7.6%, and AA percentage between 0.8 and 2.8% showed good growth. Larval levels of DHA decreased in all treatments, being between 9 and 12% at 43 dph (417.7 dd). Conversely, EPA and AA were accumulated by larvae from all treatments. The larval accumulation of EPA was almost five-fold the accumulation of AA. A strong correlation was found between larval dry weight and larval total lipid and PL concentrations. In addition, larval dry weight and larval percentage and concentration of EPA were also strongly correlated over all time points.

VI.2. Comparing the rotifer phase of the larviculture of Atlantic cod and haddock

Under the conditions in which the experiments of the present study were performed, the rotifer phase during the larviculture of Atlantic cod lasted 37 days (443 dd), while during the larviculture of haddock it lasted only 29 days (273.2 dd). The average water temperature of the Atlantic cod experiment was 12.0 ± 0.1°C, and of the haddock experiment was 9.4 ± 0.1°C. During the rotifer phase, haddock larvae grew faster than Atlantic cod larvae. Both species had similar final dry weights, with

Atlantic cod larvae weighing on average 1.2 ± 0.1 mg, and haddock larvae weighing 1.1 ± 0.0 mg. However, haddock larvae grew on average 8.4 ± 0.1% of their dry weight d^{-1}, while Atlantic cod larvae had a smaller SGR, growing on average 6.7 ± 0.1% of their dry weight d^{-1}. The faster growth of haddock when compared to Atlantic cod larvae was demonstrated in laboratory and field studies. The pioneering work of Laurence (1978) showed that when raised in laboratory under similar conditions at different temperatures, haddock had higher growth rates than Atlantic cod larvae at all temperatures tested, but more significantly when the highest temperature tested for each species were compared. In his study, Atlantic cod larvae grew 8.8% d^{-1} at 10°C and haddock larvae grew 13.4% d^{-1} at 9°C. Furthermore, absolute oxygen consumption values for a given dry weight were higher for haddock larvae at 9 and 7°C compared to Atlantic cod at 10 and 7°C, but no statistical differences were found (Laurence, 1978). More recently, laboratory studies that used RNA/DNA ratios to estimate growth of Atlantic cod (Caldarone et al., 2003) and haddock larvae (Caldarone, 2005) confirmed that when raised under similar conditions, haddock larvae grow faster than Atlantic cod larvae. The protein-specific growth rate of Atlantic cod larvae fed rotifers at 9.3°C was estimated to be on average 6.9% protein d^{-1} (Caldarone et al., 2003), and for haddock larvae raised at 10°C and fed rotifers at similar density was estimated to be 9.1% protein d^{-1} (Caldarone, 2005). Similarly, growth models based on haddock and Atlantic cod larval samples from Georges Bank predicted haddock larvae growing slightly faster than Atlantic cod larvae (Leising and Franks, 1999; Buckley et al., 2004). During early development, the morphology of the digestive tract of Atlantic cod and haddock larvae is similar. The

comparisons made here are based on morphological evaluations of the digestive system of Atlantic cod larvae raised at 6-8°C (Kjørsvik et al., 1991b) and haddock larvae raised at 7.5-8.5°C (Hamlin et al., 2000). Newly hatched larvae from both species have the digestive tract as a strait tube dorsal to the yolk-sac (Kjørsvik et al., 1991b; Hamlin et al., 2000). Around 3 dph, the foregut of both species becomes wider, having a larger diameter than the rest of the digestive tract. Govoni et al. (1986) suggested that the enlargement of the foregut allows increased food storage capacity, and that this characteristic may be critical for pelagic marine larvae which encounter patchy food supplies. In both species, the gut epithelium is folded, the cells of the short esophagus are multilayered and the rest of the gut is lined by a simple columnar epithelium, which is bordered by a layer of microvilli at the apical surface (Kjørsvik et al., 1991b; Hamlin et al., 2000). It is interesting to note that the first signs of absorptive activity in the anterior part of the gut was noticed around 7-9 dph in Atlantic cod larvae (Kjørsvik et al., 1991b) and around 18 dph in haddock larvae (Hamlin et al., 2000). However, it was reported that around 17 dph, the epithelial cells of the fore- and midgut of Atlantic cod larvae contained increased signs of absorptive activity (Kjørsvik et al., 1991b). The posterior intestine was considered the most active site in the absorption of macronutrients in haddock larvae (Hamlin et al., 2000). Similarly, the high densities of mitochondria observed in the fore- and midgut epithelial cells in the Atlantic cod larvae suggest that this region of the digestive tract is energetically active and capable of active transport (Kjørsvik et al., 1991b). During the first feeding period, the gut of both larvae does not have a functional stomach. Such larvae have an immature digestive mechanism, characterized by lipid

absorption and storage in the anterior part of the gut, and food protein ingestion and intracellular digestion by the rectal epithelial cells (Govoni et al., 1986). The digestive capacity of both species is also very similar. Perez-Casanova et al. (2006) compared the development of the digestive capacity in larval haddock and Atlantic cod. General protease, trypsin-like enzymes, pepsin-like enzymes, general lipase, bile salt-activated lipase, and alkaline phosphatase were all detected in both species as early as at hatch. The main differences between the enzymatic activities during the early development of both species were higher activities of trypsin-like enzymes and bile salt-activated lipase in haddock larvae (Perez-Casanova et al., 2006). Based on their findings, the authors concluded that Atlantic cod and haddock larvae are capable of digesting proteins and lipids at the time of mouth opening.

The *Pavlova* sp. + AlgaMac 2000 treatment resulted in the best larval performance (growth and survival) in both experiments. DHA/EPA/AA ratios of 11/1.5/1 and 10/1/1 were considered appropriate for the first feeding of Atlantic cod and haddock larvae, respectively. The fact that these values are so close and are characterized by high proportions of DHA probably reflects the lipid composition of the natural diet of these species. Although there are differences in the dietary preferences between young stages of larval Atlantic cod and haddock, larger larvae of both species (> 7.0 mm length) feed on nearly identical prey items (Kane, 1984). Large larvae of both species prey heavily on copepods, which are rich in ω3HUFA and in DHA in particular.

At the end of the rotifer phase, the most remarkable difference observed between haddock and Atlantic cod larvae was the larval total lipid concentration. At

37 dph (443.2 dd), Atlantic cod larvae had approximately six-fold the total lipid concentration observed in haddock larvae at 29 dph (273.2 dd) ($F_{5,16}$=953.79, $p<0.0001$) (Table VI.1). From hatch to the end of the rotifer phase, the total lipid concentration of Atlantic cod and haddock larvae fallowed opposite trajectories. The total lipid concentration of Atlantic cod larvae was higher at hatch than at the end of the rotifer phase, while in haddock larvae the opposite was observed. The larval fatty acid composition of both species also showed some differences. Haddock larvae had higher percentages of EPA than Atlantic cod larvae ($F_{5,18}$=102.08, $p<0.0001$). The AquaGrow Advantage treatment produced the most consistent results in terms of larval fatty acid composition. Haddock and Atlantic cod larvae from this treatment had similar percentages of AA, DHA, ω6 DPA, and similar ΣSFA, ΣMUFA, and ΣPUFA ($p>0.05$ for all analyses). Besides larvae from the AquaGrow Advantage treatment, haddock larvae had lower levels of AA than Atlantic cod larvae ($F_{5,18}$=79.13, $p<0.0001$). Larval levels of DHA were in the same vicinity, ranging from 26.0 to 35.8% and from 22.5 to 35.4% in Atlantic cod and haddock larvae, respectively. The lowest percentage of DHA was observed in *Pavlova* sp. + AlgaMac 2000-haddock larvae ($F_{5,18}$=130.55, $p<0.0001$). Levels of ω6DPA ranged from 0.7 to 5.7% in Atlantic cod larvae, and from 0.3 to 8.0% total fatty acids in haddock larvae. The highest percentage of ω6DPA was observed in AlgaMac 2000-haddock larvae ($F_{5,18}$=235.45, $p<0.0001$). Although an enrichment of ω6DPA as high as forty-fold was observed in haddock larvae (AlgaMac 2000 treatment), a relationship between larval dry weight and larval content of ω6DPA could not be established. The lack of information about ω6DPA makes difficult to explain the different growth responses of Atlantic cod and

haddock larvae to this fatty acid. It is possible that the same factors considered responsible for the low survival performance of the AlgaMac 2000-haddock larvae (Chapter IV) also resulted in arrested growth. It is interesting to note that the larvae with the best overall performance in each experiment had virtually the same DHA/ω6DPA ratio (4.3 and 4.8 for Atlantic cod and haddock larvae, respectively) and ω6DPA/AA ratio (1.6 for both species), suggesting a possible interaction of ω6DPA with DHA and AA, and a consequent effect in the production of lipid mediators related to the stress response.

When Atlantic cod and haddock data were considered together, a weak correlation was found between larval dry weight and 1) larval percentage of EPA ($F_{1,124}$=54.03, p<0.001, and r^2=30.3%) and 2) larval concentration of AA ($F_{1,124}$=57.79, p<0.001, and r^2=31.8%) (Figure VI.1A and VI.1B). A positive relationship was found between larval dry weight and 1) larval total lipid concentration ($F_{1,124}$=9.41, p=0.03, and r^2=7.1%), 2) larval PL concentration ($F_{1,124}$=15.78, p<0.001, and r^2=11.3%), 3) larval DHA concentration ($F_{1,124}$=7.25, p=0.008, and r^2=5.5%), and 4) larval percentage of AA ($F_{1,124}$=4.36, p=0.04, and r^2=3.4%). However, in these cases the correlation was negligible.

Table VI.1. Total lipid (mg g^{-1} dry weight) and selected percentage fatty acid composition of Atlantic cod larvae at 443.2 degree-days (37 dph) and haddock larvae at 273.2 degree-days (29 dph) fed differently enriched rotifers.

	Atlantic cod larvae at 443 degree-days (37 dph)			Haddock larvae at 273.2 degree-days (29 dph)		
	AlgaMac 2000	AquaGrow Advantage	*Pavlova* sp. + AlgaMac 2000	AlgaMac 2000	AquaGrow Advantage	*Pavlova* sp. + AlgaMac 2000
Total lipid (mg g^{-1})	274.4 ± 9.9^{a}	215.3 ± 3.5^{b}	341.8 ± 6.4^{c}	39.9 ± 1.7^{d}	39.7 ± 0.9^{d}	50.8 ± 0.5^{d}
% Total fatty acids						
EPA (20:5ω3)	2.3 ± 0.0^{a}	3.4 ± 0.1^{bc}	3.0 ± 0.0^{b}	3.7 ± 0.1^{c}	5.2 ± 0.2^{d}	6.1 ± 0.1^{e}
AA (20:4ω6)	4.2 ± 0.1^{a}	2.2 ± 0.1^{b}	3.5 ± 0.2^{c}	3.6 ± 0.1^{c}	1.8 ± 0.1^{b}	3.0 ± 0.0^{d}
DHA (22:6ω3)	28.3 ± 0.6^{a}	35.8 ± 0.3^{b}	26.0 ± 0.2^{c}	33.9 ± 0.2^{b}	35.4 ± 0.8^{b}	22.5 ± 0.5^{d}
ω6DPA (22:5ω6)	4.2 ± 0.3^{a}	0.7 ± 0.1^{b}	5.7 ± 0.2^{c}	8.0 ± 0.2^{d}	0.3 ± 0.0^{b}	4.7 ± 0.1^{a}
ΣSFA[1]	31.5 ± 0.0^{a}	25.2 ± 0.3^{bd}	23.9 ± 0.2^{cd}	24.3 ± 0.0^{d}	24.3 ± 0.0^{d}	24.0 ± 0.3^{d}
ΣMUFA[2]	25.0 ± 0.0^{a}	24.7 ± 0.3^{a}	29.3 ± 1.0^{b}	21.3 ± 0.2^{c}	24.2 ± 0.6^{a}	29.1 ± 0.4^{b}
ΣPUFA[3]	43.2 ± 0.1^{a}	50.0 ± 0.6^{b}	47.5 ± 0.7^{c}	54.4 ± 0.7^{d}	51.3 ± 0.6^{b}	46.9 ± 0.2^{c}

Values (mean ± SE of four replicates, except for total lipid in AlgaMac 2000 and *Pavlova* sp.+ AlgaMac 2000 Atlantic cod larvae where n=3) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids; [2]Sum of monounsaturated fatty acids; [3]Sum of polyunsaturated fatty acids.

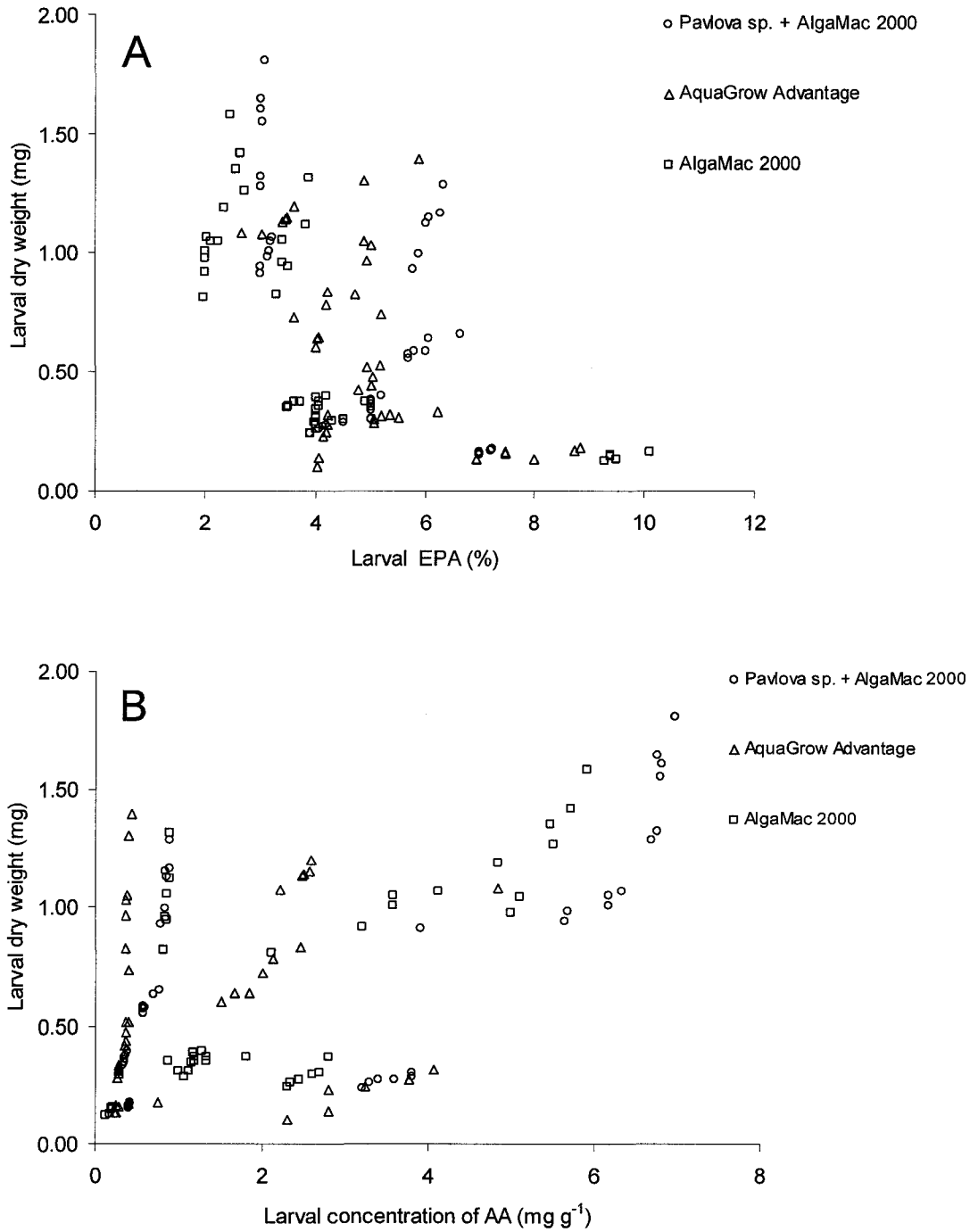

Figure VI.1. The relationship between larval dry weight and (A) larval percentage of EPA, and (B) larval concentration of AA. Graphs combine data from the rotifer phase of the Atlantic cod and haddock experiments.

VI.3. Comparing the *Artemia* phase of the larviculture of Atlantic cod and haddock

In the experiment with Atlantic cod larvae, the *Artemia* phase ended at 59 dph (709.7 dd), and in the experiment with haddock larvae it ended at 43 dph (417.7 dd). The average water temperature in which Atlantic cod larvae was raised was 12.0 ± 0.2°C, while haddock larvae were raised at an average water temperature of 10.4 ± 0.1°C. As with the rotifer phase, during the *Artemia* phase, haddock larvae grew faster than Atlantic cod larvae. The Atlantic cod larvae final dry weight ranged from 5.3 ± 0.2 to 12.1 ± 1.1 mg, and haddock larvae final dry weight ranged from 4.5 ± 0.3 to 6.4 ± 0.5 mg. Despite being heavier than haddock larvae, Atlantic cod larvae grew with SGR ranging from 4.9 ± 0.4 to 10.4 ± 0.4% dry weight d^{-1}, but haddock larvae grew with SGR ranging from 9.9 ± 0.5 to 12.5 ± 0.5% dry weight d^{-1}. These results indicate that, at least during the larval development, haddock grows faster than Atlantic cod when both species are raised in similar conditions. Studies that described the morphology of the digestive tract of Atlantic cod and haddock larvae showed that as the larvae grow bigger, the complexity of the digestive system increases. For instance, from hatching to 32 dph no stomach or pyloric caeca are present in haddock larvae. By 33 dph, the first gastric glands appear in the region of the digestive tract that will develop in the stomach (Hamlin et al., 2000). Perez-Casanova et al. (2006) reported that the gastric glands were first evident in haddock and Atlantic cod larvae at 266 and 243 dd, respectively. However, pepsinogen transcripts were first identified at 333 dd in haddock and 476 dd in Atlantic cod,

suggesting that the presence of gastric glands cannot be taken as indicative of pepsin digestive capability in these species (Perez-Casanova et al., 2006). In addition to the development of the stomach, the intestine convolutes and coils, providing more surface area for digestion, and gastric enzymes are secreted by the stomach and intestinal walls improving nutrient assimilation (Govini et al., 1986). According to Hunt von Herbing et al. (1996), growth rate of Atlantic cod larvae is influenced by the complexity of the larva's gut. Increased digestive tract complexity will increase growth rate through enhanced nutrient assimilation (Govini et al., 1986). These observations are in accordance with the fast growth rates recorded during the *Artemia* phase for both species. From hatch to the end of the larviculture, the fastest growth rates happened between 52 and 59 dph (630 and 709.7 dd, respectively) for Atlantic cod larvae, and between 36 and 43 dph (345 and 417.7 dd, respectively) for haddock larvae. Hunt von Herbing et al. (1996) pointed out that the degree and rate of the digestive tract development of Atlantic cod larvae is not age-dependent. According to these authors, the development of the digestive tract is dependent on a combination of intrinsic (genetic) and extrinsic (*e.g.* feeding success and environmental conditions) components. Therefore, it is possible that the different lipid composition of the prey supplied to Atlantic cod and haddock larvae in the present study had some influence in the development of the digestive system of the larvae, concomitantly affecting larval growth rates.

AlgaMac 2000-enriched *Artemia* resulted in the best larval performance in the Atlantic cod experiment, while a combination of DC DHA Selco-enriched *Artemia* and DC DHA Selco + AlgaMac 2000-enriched *Artemia* resulted in the best larval

performance in the haddock experiment. The DHA/EPA/AA ratio considered to be appropriated for Atlantic cod and haddock larvae during the *Artemia* phase were 7/2/1 and 9/4/1, respectively. Differences in the natural diet of juvenile Atlantic cod and haddock could explain why the best growth for each species was observed with a different DHA/EPA/AA ratio. Apart form the initial stages of larval development, when Atlantic cod is considered to be more aggressive feeders than haddock, larvae form both species have very similar diet. Later in the development, when the metamorphosis is completed and the young juveniles start to make the transition from a pelagic to a benthic life style, haddock and Atlantic cod seek different ecological niches. The natural diet of young juveniles of both species is composed of adult copepods, small euphausiids, adult hyperiid amphipods, mysids, cumaceans, fish larvae, and benthic invertebrates such as polychaetes and crustaceans (Perry and Neilson, 1988; Lough et al., 1989; Lough and Potter, 1993; Gaard and Reinert, 2003). However, haddock juveniles start to feed on larger prey at smaller sizes than the Atlantic cod juveniles (Gaard and Reinert, 2003). Perry and Neilson (1988) found little overlap in the diets of post-larval Atlantic cod and haddock in a thermally stratified water mass on the Northeast Peak of Georges Bank. Copepods (calanoids and the harpacticoid *Tisbe* sp.) were numerically abundant in Atlantic cod guts. In contrast, copepods formed only 1% of the diet of haddock, the major component instead being caridean shrimp, hyperiid amphipods, *Limacina* sp. and *Pagurus* sp. larvae (Perry and Neilson, 1988). Additionally, haddock appear to assume a more complete demersal life at a smaller size than Atlantic cod (Lough and Potter, 1993). Once they reach a demersal stage, both species stay close to the bottom by day and move off

bottom in the water column at night (Lough et al., 1989). However, according to Lough and Potter (1993) Atlantic cod make more extensive off-bottom migration at night than haddock, feeding on larger active pelagic prey, while haddock concentrate on smaller slower-moving or sedentary organisms.

In contrast with the end of the rotifer phase, when the total lipid concentration in Atlantic cod larvae was higher than in haddock larvae, at the end of the *Artemia* phase both species had comparable body lipid concentrations (Table VI.2). The highest total lipid concentration was observed in DC DHA Selco + AlgaMac 2000-Atlantic cod larvae ($F_{6,21}$=28.71, $p<0.0001$). Newly hatched Atlantic cod larvae had much higher total lipid concentrations than newly hatched haddock larvae. From hatch to the end of the *Artemia* phase, the total lipid concentration decreased in Atlantic cod larvae, while it increased steadily in haddock larvae. Haddock larvae had higher EPA percentages than Atlantic cod larvae, with the exception of AquaGrow Advantage-Atlantic cod larvae, which had higher EPA percentages than haddock larvae from the same treatment ($F_{6,21}$=42.56, $p<0.0001$). Conversely, Atlantic cod larvae had higher DHA percentage than haddock larvae ($F_{6,21}$=57.87, $p<0.0001$), with the exception of larvae from the AquaGrow Advantage treatment which had similar percentages of this fatty acid. Percentages of AA and ω6DPA were similar in larvae from both species.

Due to the great difference in the range of the final dry weight of Atlantic cod and haddock larvae, when data from both species were considered together no positive correlations were found between larval dry weight and any of the major lipid variables.

Table VI.2. Total lipid (mg g^{-1} dry weight) and selected percentage fatty acid composition of Atlantic cod larvae at 709.7 degree-days (59 dph) and haddock larvae at 417.7 degree-days (43 dph) fed differently enriched *Artemia*.

	Atlantic cod at 709.7 degree-days (59 dph)				Haddock at 417.7 degree-days (43 dph)		
	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco® + AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco® + AlgaMac 2000®
Total lipid (mg g^{-1})	90.8 ± 4.2^{ab}	93.0 ± 5.4^{ab}	84.5 ± 4.6^{ab}	142.4 ± 5.3^{c}	100.5 ± 5.6^{bd}	77.1 ± 0.9^{a}	118.0 ± 2.9^{d}
% Total fatty acids							
EPA (20:5ω3)	7.6 ± 0.3^{a}	10.1 ± 0.4^{b}	6.2 ± 0.1^{c}	6.3 ± 0.2^{c}	8.7 ± 0.2^{d}	8.9 ± 0.1^{de}	9.9 ± 0.3^{eb}
AA (20:4ω6)	6.0 ± 0.1^{a}	1.9 ± 0.0^{b}	3.5 ± 0.2^{c}	4.0 ± 0.0^{d}	2.5 ± 0.1^{e}	3.8 ± 0.1^{cd}	3.6 ± 0.1^{cd}
DHA (22:6ω3)	20.3 ± 0.8^{a}	11.8 ± 0.3^{b}	16.2 ± 0.8^{c}	20.5 ± 0.6^{a}	11.7 ± 0.4^{b}	9.3 ± 0.1^{b}	11.7 ± 0.8^{b}
ω6DPA (22:5ω6)	6.0 ± 0.1^{a}	1.6 ± 0.1^{b}	4.9 ± 0.3^{ca}	4.4 ± 0.1^{ca}	0.2 ± 0.0^{b}	4.0 ± 1.0^{c}	2.1 ± 0.2^{bc}
ΣSFA[1]	23.7 ± 0.4^{ab}	23.1 ± 0.2^{ab}	22.9 ± 0.7^{ab}	22.5 ± 0.5^{a}	24.5 ± 0.2^{bc}	24.7 ± 0.2^{c}	24.0 ± 0.2^{abc}
ΣMUFA[2]	19.7 ± 0.4^{a}	26.8 ± 0.2^{b}	24.7 ± 0.6^{b}	21.9 ± 0.1^{c}	27.9 ± 0.5^{d}	25.1 ± 0.4^{b}	24.7 ± 0.6^{b}
ΣPUFA[3]	56.8 ± 0.3^{a}	50.3 ± 0.2^{b}	52.4 ± 0.9^{b}	55.5 ± 0.5^{a}	47.6 ± 0.4^{c}	50.2 ± 0.5^{b}	51.0 ± 0.7^{b}

Values (mean ± SE of four replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids; [2]Sum of monounsaturated fatty acids; [3]Sum of polyunsaturated fatty acids.

VI.4. Conclusion

Results from the present study showed that two closely related gadoids raised under similar conditions have different growth, survival and lipid composition responses to lipid-enriched live-feed. During the rotifer phase, the treatments tested resulted in Atlantic cod and haddock larvae having similar growth and survival response (with the exception of the AlgaMac 2000 treatment, which resulted in high mortality of haddock larvae) but the larval lipid composition was not similar. In contrast, during the *Artemia* phase, the treatments tested affected the growth, survival and lipid composition of Atlantic cod and haddock larvae differently. These differences reflect the different ecological niches occupied by haddock and Atlantic cod in the wild.

VI.5. Recommendations for future research

Here I present some suggestions for future research that would build on the knowledge generated in the present study:

- An investigation of the effects of differently enriched live-feed on the stress resistance of Atlantic cod and haddock larvae.

- A study performed to investigate the effects of different dietary levels of essential fatty acids and ω6DPA on the production of lipid mediators (eicosanoids and/or docosanoids) in Atlantic cod and haddock larvae.

- A study specifically designed to test the effects of different dietary levels of ω6DPA on the growth and survival of Atlantic cod and haddock larvae.

- The use of algae for the enrichment of live-feed and its effects on the bacterial load and bacterial composition of prey and predators.

- Histological and morphological investigations on the effects of differently lipid-enriched live-feed on the development of the digestive system of Atlantic cod and haddock larvae.

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Appendix 1- Concentrations of lipid classes of newly hatched haddock larvae and haddock larvae at 273.2 dd (29 dph).

	Treatments			
	Newly hatched larvae	AlgaMac 2000®	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®
Lipid classes[1] (mg g^{-1} dw)				
Hydrocarbons	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]	0.2 ± 0.0[a]
Ketones	0.1 ± 0.0[a]	0.2 ± 0.1[a]	0.3 ± 0.1[a]	0.2 ± 0.0[a]
Triacylglycerol	1.9 ± 0.4[ab]	3.2 ± 0.6[a]	1.7 ± 0.1[ab]	1.5 ± 0.3[b]
Free fatty acids	0.2 ± 0.0[a]	0.2 ± 0.0[a]	0.6 ± 0.2[b]	0.3 ± 0.0[ab]
Sterols	3.5 ± 0.4[a]	7.6 ± 0.5[b]	9.0 ± 0.5[c]	10.4 ± 0.2[c]
Acetone Mobile Polar Lipids	1.4 ± 0.4[a]	0.4 ± 0.2[a]	0.8 ± 0.2[a]	0.4 ± 0.2[a]
Phospholipids	44.8 ± 2.6[a]	28.0 ± 1.5[b]	26.8 ± 1.0[b]	37.9 ± 0.8[c]

Values (mean ± SE of four replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1] May also contain steryl esters/wax esters and/or alcohols at $\leq$ 0.8 mg g^{-1}

Appendix 2- Concentrations of total lipids and lipid classes of haddock larvae at 345.4 dd (36 dph).

	Treatments		
	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco + AlgaMac 2000
Total lipids (mg g^{-1} dw)	53.6 ± 1.2[a]	68.4 ± 1.1[b]	73.6 ± 1.5[c]
Lipid classes[1] (mg g^{-1} dw)			
Hydrocarbons	0.04 ± 0.0[a]	0.1 ± 0.0[a]	0.1 ± 0.0[a]
Ketones	0.3 ± 0.0[a]	0.3 ± 0.0[a]	0.3 ± 0.1[a]
Triacylglycerol	9.8 ± 2.0[a]	7.8 ± 0.3[a]	20.3 ± 1.2[b]
Free fatty acids	1.0 ± 0.3[a]	0.8 ± 0.3[a]	1.4 ± 0.5[a]
Sterols	10.6 ± 1.3[a]	15.5 ± 1.0[b]	10.4 ± 0.7[a]
Acetone Mobile Polar Lipids	3.5 ± 0.6[a]	1.5 ± 0.6[a]	2.3 ± 0.3[a]
Phospholipids	28.4 ± 2.4[a]	43.0 ± 2.3[b]	38.6 ± 2.5[b]

Values (mean ± SE of four replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

Appendix 3- Concentrations of selected fatty acids of haddock larvae at 345.4 dd (36 dph).

	Treatments		
	AquaGrow® Advantage	*Pavlova* sp. + AlgaMac 2000®	DC DHA Selco + AlgaMac 2000
Fatty acids (mg g^{-1})			
16:0	8.4 ± 0.1[a]	7.7 ± 0.1[b]	7.3 ± 0.2[b]
18:1ω9	8.5 ± 0.3[a]	7.5 ± 0.1[b]	7.8 ± 0.3[ab]
18:3ω3	7.1 ± 0.1[ab]	6.3 ± 0.1[a]	7.5 ± 0.4[b]
20:4ω6 (AA)	1.1 ± 0.0[a]	1.7 ± 0.0[b]	1.8 ± 0.2[b]
20:5ω3 (EPA)	3.6 ± 0.2[a]	3.9 ± 0.0[a]	5.0 ± 0.4[b]
22:5ω6 (ω6DPA)	0.1 ± 0.0[a]	1.7 ± 0.1[b]	2.0 ± 0.4[b]
22:6ω3 (DHA)	9.2 ± 0.7[a]	6.9 ± 0.1[b]	9.1 ± 0.4[a]
Σ SFA[1]	15.5 ± 0.6[a]	13.9 ± 0.1[b]	15.4 ± 0.3[ab]
Σ MUFA[2]	17.2 ± 0.7[a]	15.3 ± 0.2[a]	16.7 ± 1.3[a]
ΣPUFA[3]	27.5 ± 0.8[a]	28.4 ± 0.3[a]	35.4 ± 1.4[b]

Values (mean ± SE of four replicates) in the same row not sharing a superscript are significantly different ($p < 0.05$).

[1]Sum of saturated fatty acids.

[2]Sum of mono unsaturated fatty acids.

[3]Sum of polyunsaturated fatty acids.