Received: 8 February 2020 | Revised: 26 April 2020 | Accepted: 29 April 2020

DOI: 10.1111/anu.13101

ORIGINAL ARTICLE

Aquaculture Nutrition WILEY

Change in nutrient composition of *Artemia* grown for 3–4 days and effects of feeding on-grown *Artemia* on performance of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae

Kristin Hamre[1] | Børre Erstad[2] | Jori de Kok[2,3] | Birgitta Norberg[4] | Torstein Harboe[4]

[1]Institute of Marine Research, Bergen, Norway

[2]Sterling White Halibut, Rørvik, Norway

[3]Aquaculture and Marine Resource Management, Wageningen Agricultural University, Wageningen, The Netherlands

[4]Institute of Marine Research, Storebø, Norway

Correspondence
Kristin Hamre, Institute of Marine Research, PO Box 1870, Nordnes, NO-5817 Bergen, Norway.
Email: kha@hi.no

Funding information
FP7 Food, Agriculture and Fisheries, Biotechnology

Abstract

Major challenges in culture of Atlantic halibut larvae have been slow growth during the late larval stages and inferior juvenile quality due to pigmentation errors and incomplete eye migration during metamorphosis. The hypothesis of this study was that feeding on-grown *Artemia* would alleviate these problems. *Artemia* were grown for 3–4 days on Origreen or Origo. The growth and nutrient composition of *Artemia* nauplii and on-grown *Artemia* were analysed, and both *Artemia* types were fed to Atlantic halibut larvae, on-grown *Artemia* from 15 days post-first feeding (dpff). The body length of *Artemia* increased with 20%–70% in response to on-growing. In all experiments, protein, free amino acids and the ratio of phospholipid to total lipid increased, while lipid and glycogen decreased. The fatty acid composition improved in some cases and not in others. The micronutrient profiles were not negatively affected in on-grown *Artemia*. All these changes are thought to be beneficial for marine fish larvae. The final weight of Atlantic halibut postlarvae was similar, and 90% of the juveniles had complete eye migration in both groups. It is concluded that the present version of *Artemia* nauplii probably covers the nutrient requirements of Atlantic halibut larvae.

KEYWORDS

Artemia nauplii, Atantic halibut, eye migration, fish larvae, on-grown *Artemia*, pigmentation

1 | INTRODUCTION

Atlantic halibut (*Hippoglossus hippoglossus* L.) is an emerging candidate for commercial aquaculture, with high quality meat and a high market value. It is a cold-water flatfish, which can reach a body weight of several hundred kg in the wild. In captivity, the females are slaughtered at about 5 kg and 4–5 years after fertilization, before they reach sexual maturation at 5–7 years of age. The males grow slower and mature at 2–3 years and 1–3 kg (Norberg, Weltzien, Karlsen, & Holm, 2001). This is the reason that halibut farmers have developed an all-female broodstock in recent years (Babiak et al., 2012; Hendry, Martin-Robichaud, & Benfey, 2003). Trials with Atlantic halibut farming started in the mid 1980's, when the first two juveniles survived larval rearing trials at the Institute

of Marine Research in Austevoll, Norway. Juvenile production of Atlantic halibut is complicated, partly due to the long yolk-sac period (30–40 days) and that the larvae are sensitive to bacterial growth and suboptimal culture conditions (Harboe & Mangor-Jensen, 1998; Harboe, Mangor-Jensen, Naas, & Næss, 1998). Furthermore, early rearing trials had low survival rates and resulted in juveniles with malpigmentation and incomplete eye migration, which was hypothesized to have been caused by malnutrition (Hamre et al., 2002). Another challenge has been unpredictable events of very high mortality. This has later been shown mainly to have been caused by Atlantic halibut reovirus (AHRV) (Blindheim et al., 2015) and is now prevented by strict hygiene measures. These obstacles are the reasons that development of commercial farming has taken such a long time; however, from 2017 the first halibut farm started to make profits and a few other farms are developing in the right direction.

Atlantic halibut larvae are approximately 12 mm in standard length (SL) at first-feeding and, because of their relatively large larval size, they are first-fed on *Artemia*. The live period lasts until 50–60 days post-first feeding, when the postlarvae are weaned onto formulated feed. Slow growth at late larval stages is a challenge (Erstad, personal observations) perhaps due to the long live feed period. Although still a challenge, pigmentation disorders and incomplete eye migration have improved in recent years. One reason is improved light and feeding regimes (Harboe, Mangor-Jensen, Moren, Hamre, & Ronnestad, 2009), but improvement in other factors, such as *Artemia* enrichment (Hamre & Harboe, 2008a, 2008b), tank design and other rearing conditions affecting especially water quality (Harboe & Mangor-Jensen, 1998; Harboe et al., 1998), are also of importance.

A potential strategy to alleviate the slow growth of later stage Atlantic halibut larvae is to feed them on-grown *Artemia*. On-grown *Artemia* are larger and have a lower carbon to nitrogen ratio, meaning that they probably contain more protein and less lipids (Olsen, Attramadal, Jensen, & Olsen, 1999). Because of the larger size, they will probably also have a lower shell-to-soft tissue ratio. These differences may explain why Atlantic halibut fed on-grown *Artemia* grew faster and developed into juveniles with better pigmentation and eye migration than larvae fed *Artemia* nauplii (Olsen et al., 1999).

Since *Artemia* is a whole food with all nutrients incorporated, our approach to this study is holistic in analysing as many nutrients as possible and relating the results to hypotheses on larval nutrition known from the literature. We show that on-grown *Artemia* is a better food in many aspects, according to the literature. Still, we did not get an improvement in growth performance of Atlantic halibut larvae fed the on-grown *Artemia*. The larvae fed *Artemia* nauplii, enriched in the same way as in most halibut hatcheries, had virtually no deformities. It is concluded that *Artemia* nauplii enriched in this way, probably covers the nutrient requirements of Atlantic halibut and that other variables than nutrition may be responsible for malformations found in many hatcheries.

2 | MATERIALS AND METHODS

The experiments were performed at IMR and at Sterling White halibut (SWH), Rørvik, Norway in 2015. At IMR, *Artemia* cysts (EG, INVE Aquaculture) were hatched in a separate tank, held for 24 hr and then transferred to either short-term enrichment or on-growing tanks. Conical 300-L fibreglass tanks were used both for hatching, short-term enrichment and on-grown *Artemia*. All tanks were equipped with temperature (500 W, and Carlo Gavazzi 600+ temperature regulator) and oxygen control systems (Ocea). Hatching and short-term enrichment were performed at stagnant conditions, while on-growing tanks had an open flow-through system.

Seawater was pumped from 160 m depth subjected to sand filtration. For hatching and short-term enrichment, the water was treated with chlorine and thereafter thiosulphuric acid for at least 18 hr. For the on-growing tanks, the 160 m depth water was only filtered down to 5 µm before being connected to the tanks. Flow rate was 15 L/hr for the entire period. The disinfectant Sanocare ACE (100g, INVE Aquaculture) was mixed with 1 L of freshwater using a blender (Hamilton Beach commercial) for 2 min and added to the tanks daily.

OriGreen from Skretting AS was used for grow-out of *Artemia* nauplii. In a pilot trial, *Artemia* was fed using a belt feeder, but due to variation in how the feed dispersed in the *Artemia* on-growing tanks, the feed was mixed with 1 L of freshwater using a blender (Hamilton Beach commercial) for 2 min and added to the tanks twice a day. The *Artemia* were fed 20 g of OriGreen in each meal.

Larviva Multigain (Biomar) was used for short-term enrichment of both nauplii and on-grown *Artemia*, using the manufacturer's standard procedure for short-term enrichment. The enrichment period was 12 hr, and the density of *Artemia* was 200 ind/ml.

Sampling was performed by siphoning *Artemia* onto a 250-µm plankton screen. *Artemia* for nutrient analyses (in total approximately 30 g divided in separate tubes for each nutrient) were washed in freshwater, and the screen was dried thoroughly from underneath with a paper towel. The samples were then frozen at −80°C and transported to IMR, Bergen on dry ice, where they were stored again at −80°C until analysis. For measuring *Artemia* size, live *Artemia* were photographed using a dissecting microscope.

2.1 | Determining the culture period for on-grown *Artemia*, IMR

This pilot experiment was set up in order to decide the optimal culture period for on-grown *Artemia*, in order to decide the conditions for the main experiment. The cultures were started at a density of 100–110 ind/ml. *Artemia* were not enriched, and samples for size determination and nutrient analyses were taken daily at 13.00. The experiment was performed in triplicate tanks and lasted 4 days.

2.2 | Culture and enrichment of on-grown *Artemia*, IMR

Artemia were hatched and the nauplii transferred either directly to enrichment tanks or to on-growing tanks for 3 days and then to enrichment tanks, as described. On-growing and enrichment were performed in triplicate. After enrichment, the *Artemia* were pumped from the tanks to a 70 L washing tank with a 250-µm plankton mesh and heavy aeration to prevent clogging to the sieve. Thereafter, *Artemia* were flushed with warm seawater (22°C, chlorine and thiosulphuric acid treated) at 35 L/hr for 10 min and then freshwater until the salinity reached 0.5 ppt. *Artemia* were held below this salinity for another 5 min by continued flushing with freshwater. Samples were taken of unenriched and enriched nauplii and from unenriched and enriched on-grown *Artemia*; however, the unenriched on-grown *Artemia* were not analysed in triplicate, due to a limited amount of material. Samples of enriched *Artemia* were taken after completion of the washing procedure to mimic *Artemia* fed to halibut larvae.

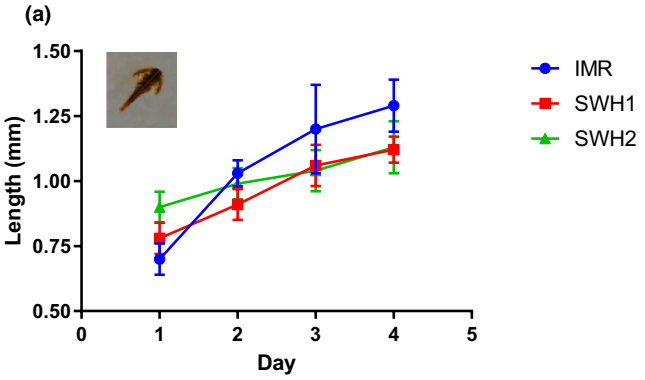

FIGURE 1 Lengthwise growth of *Artemia* cultured for 3 or 4 days from nauplii. at IMR and SWH (mean ± *SD*) [Colour figure can be viewed at wileyonlinelibrary.com]

2.3 | Culture and enrichment of on-grown *Artemia*, SWH

To document reproducibility when *Artemia* on-growing is performed in the industry, a trial on-growing Artemia was also performed at

TABLE 1 Analytical methods for the different nutrients

Analyte	Principle	Reference
Dry matter	Gravimetric after freeze drying	Hamre and Mangor-Jensen (2006)
Protein	N x 6.25, 4.7 or 5.6, Leco N Analyzer	Hamre and Mangor-Jensen (2006)
Total amino acids	Hydrolyses, derivatization and HPLC analyses	Espe, Lemme, Petri, and El-Mowafi (2006)
Free amino acids	HPLC and postcolumn derivatization	Srivastava, Hamre, Stoss, Chakrabarti, and Tonheim (2006)
Total lipids	Gravimetric after acid hydrolyses	EU directive 84/4 1983
Fatty acids	Transmethylation extraction and GC/FID	Lie and Lambertsen (1991)
Lipid classes	HPTLC	Jordal, Lie, and Torstensen (2007)
Glycogen	Hydrolysis and spectrometric detection	Hemre, Lie, Lied, and Lambertsen (1989)
Thiamine	HPLC	CEN (2003a)
Riboflavine	Microbiological	Maeland, Ronnestad, Fyhn, Berg, and Waagbo (2000)
Niacine	Microbiological	Maeland et al. (2000)
Folat	Microbiological	Maeland et al. (2000)
Vitamin B6	HPLC	CEN (2005)
Vitamin C	HPLC	Mæland and Waagbø (1998)
Vitamin A	HPLC	Moren, Naess, and Hamre (2002)
Vitamin D	HPLC	CEN (1999)
Vitamin E	HPLC	Hamre, Kolås, and Sandnes (2010)
Sum vitamin K	HPLC	CEN (2003b)
Microminerals	ICP-MS	Julshamn, Lundebye, Heggstad, Berntssen, and Boe (2004)
Macrominerals	ICP-MS	Liaset, Julshamn, and Espe (2003)
Iodine	ICPMS	Julshamn, Dahl, and Eckhoff, (2001)

SWH with slightly different methods due to different equipment and setup. *Artemia* (SepArt EG cysts > 240,000 npl/g) were obtained from INVE Aquacultuture Inc., hatched and grown at 200 ind/ml initial density, on OriOne (Skretting) for 3 days, using the procedure described above and removing excess feed every day before feeding. The seawater for the hatchery was pumped from 150 m depth, sand-filtered and treated with ozone and UV light. The *Artemia* tanks were conical 250 L cylinders, supplied at 20 L/hr with seawater heated to 22.5°C and aerated in a separate 2,700 L silo. Both inlet and outlet of water were mounted at the water surface in the tanks. The tanks were supplied with both oxygen and air. An outlet for debris was mounted at the bottom of the cone. Oxygen saturation was highly variable, between less than 50 and more than 250% saturation and varied between the replicates. The temperature varied between 20 and 22°C and pH between 7 and 8.

At day 3, samples of unenriched *Artemia* were first taken. Before sampling, *Artemia* were transferred to a washer and flushed with seawater until the water became clear. A 5 L sample was taken and sieved through plankton mesh, which was then dried from underneath with a paper towel. The *Artemia* were then enriched with 0.5 g Larviva Multigain and 0.01 g thiamine per million individuals for 15 min. The culture was washed with freshwater until the salinity reached less than 5 ppt and kept there for 10 min. Thereafter, the salinity was taken back to >31 ppt by flushing with seawater and the samples of enriched *Artemia* were taken as explained. The samples were frozen flat in plastic bags in liquid nitrogen and transported to IMR, Bergen, where they were kept at −80°C until analysis.

2.4 | Measurements of *Artemia* growth and survival

The length of *Artemia* was measured as shown in Figure 1, and survival was calculated from counts of *Artemia* in 3 samples of 200 µl per tank (IMR) or 7 samples of 100 µl per tank (SWH) at start and end of the *Artemia* culture period.

2.5 | Feeding experiment with Atlantic halibut larvae IMR

The feeding trial was conducted in accordance with Norwegian laws and regulations concerning experiments with live animals. Experiments are overseen by the Norwegian Food Safety Authority. The experiments in the present study were feeding experiments with normal larval feeds and not regarded as harmful to the experimental animals.

Halibut larvae from one single egg batch were hatched and further incubated in two 5 m^3 siloes until 260 day-degrees

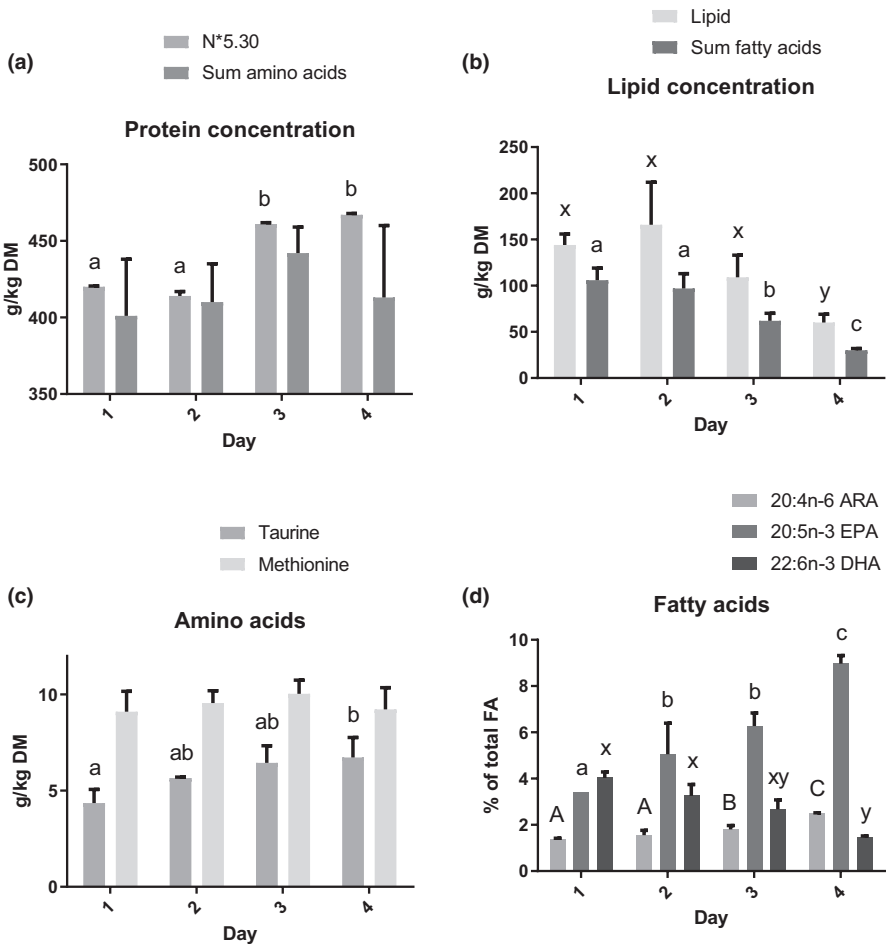

FIGURE 2 Daily change in nutrient concentrations in *Artemia* grown for 4 days on OriGreen at IMR. (a) Protein concentration (g/kg DM) expressed as the sum of amino acids, cysteine and tryptophan excluded, or as nitrogen (N) * 5.30 (the average protein to nitrogen factor for *Artemia*, (Hamre et al., 2013). (b) The amino acid methionine and the aminosulfonic acid taurine (g/kg DM). (c) Lipid concentration (g/kg DM) measured as the sum of fatty acids or as total lipid after acid hydrolyses. (d) Arachidonic (ARA), eicopentaenoic acid (EPA) and docosahexaenoic acid (DHA) in % of total fatty acids. Mean and *SD*, data were analysed with one-way ANOVA, and differences between days are indicated with different letters ($p < .05$)

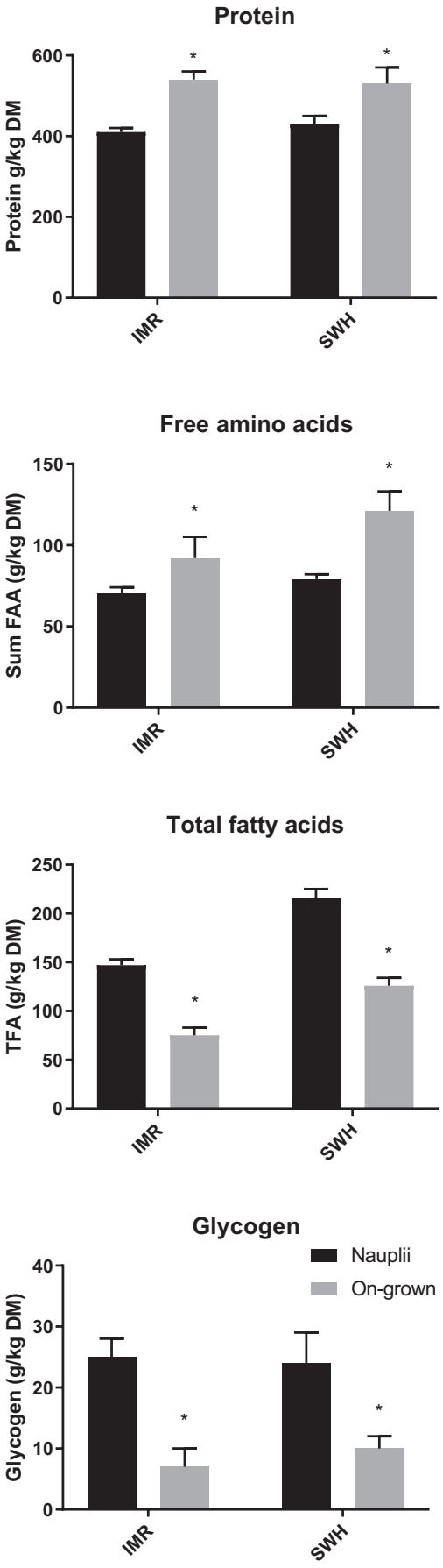

FIGURE 3 Macronutrients (g/kg DM) and free amino acids (g/kg DM) in enriched *Artemia* nauplii and *Artemia* grown for 3 days on OriGreen® (IMR) or OriGo® (SWH). An asterix indicates significant differences between nauplii and on-grown *Artemia* ($p < .05$, ns, not significant)

posthatch. Then, they were transferred to 6 first-feeding tanks and stocked at approximately 3,000 larvae tank^{-1}. The first-feeding tanks were 1.5 m in diameter and 0.8 m in height. The tanks had continuous water supply entering near the surface and outlet sieve in the middle of the tank. Each tank had a fluorescent light above the centre of the tank and was equipped with a shadow frame, to reduce light reflections from the tank wall, which attract the larvae. The tanks also had central aeration from near the bottom and an automatic cleaning system consisting of a cleaning arm (car windshield wiper) that rotates slowly at the bottom by use of an electric engine.

Water flow started at 1 L/min immediately after incubation and increased within the next 4 days to 5 L/min, where it was held for the rest of the experiment. Dissolved clay (30 g morning and 30 g evening) was added to each tank daily to keep turbidity high during the live feed period (2NTU). Live feed was added three times a day at 10.00, 15.00 and 21.00. Light was on from 07.00 to 24.00 thereafter darkness (photoperiod L:D = 17:7).

Artemia (EG, INVE Aquaculture) nauplii and on-grown Artemia were produced and enriched as described above for IMR. The larvae in all six tanks were fed *Artemia* nauplii from 1 until 14 dpff (days post-first feeding). Then, one group of larvae was fed nauplii, and another group on-grown *Artemia* (2 out of 3 meals) in triplicate tanks until 28 dpff. The amount of *Artemia* fed in each meal was based on the clearance rate of *Artemia* in each larvae tank. This was done by examining 100 ml of rearing water from each tank for *Artemia* content, which should be zero at least 1 hr before next meal. Parameters such as the rest number of *Artemia*, water flow, temperature and number of *Artemia* fed the larvae were recorded daily.

2.6 | Nutrient analyses

The nutrient composition of *Artemia* and larvae was measured by ISO certified routine methods at IMR, Bergen. Table 1 presents an overview over the biochemical methods with analysis principles and references. The protein concentration was measured by two different methods, total hydrolysed amino acids (TAA) and N*4.7 for day 1 and 2, N*5.30 for day 3 and 4. The TAA method does not detect cysteine, cystine and tryptophan and therefore underestimates the protein. In animal protein, protein concentration is generally assumed to be N*6.25. In reality, every organism has its own ratio and previous results have found the protein to N factor in *Artemia* nauplii to be 4.7 and that in on-grown *Artemia* to be 5.30 (Hamre et al., 2013).

2.7 | Statistics

Statistica (ver11, Statsoft Inc.) was used for the statistical treatment, and data are given as mean ± standard deviation. Means were assumed to be different at $p < .05$.

Data on nutrient composition of *Artemia* cultured for an increasing number of days were subjected to one-way ANOVA, after use of Levene's test for check of homogenous variances. Variables with significant results in Levene's test were Box-Cox transformed. Differences between days were determined using Tukey's HSD test.

Variances of data on the nutrient composition of enriched *Artemia* nauplii and on-grown enriched *Artemia* at IMR and SWH were homogenous (Levene's tests) and were analysed with *t* tests. Samples of unenriched nauplii and unenriched on-grown *Artemia* at IMR were

TABLE 2 Nutrient composition of *Artemia* from IMR; nauplii, on-grown, enriched nauplii and enriched on-grown *Artemia*

	Unit	Nauplii unenriched	On-grown unenriched	Nauplii enriched	On-grown enriched	*p* Day 1–3
Dry matter	g/kg	103	102	84 ± 6	83 ± 4	.884
Protein[§]	g/kg	470	530	410 ± 10	540 ± 20	**.026**
TAA	g/kg	443.8	–	411 ± 10	452 ± 10	**.008**
Protein/TAA		0.119	–	0.11 ± 0.01	0.11 ± 0.004	.908
FAA	g/kg	62	–	70 ± 4	92 ± 13	**.044**
Taurine	g/kg	4.15	–	4.4 ± 0.2	5.5 ± 0.6	**.040**
Glycogen	g/kg	-	–	250 ± 30	71 ± 32	**.002**
Lipid	g/kg	20	–	17 ± 1	11 ± 1	**.004**
TFA	g/kg	168	93	147 ± 6	75 ± 8	**$<10^{-3}$**
PL	g/kg TL	2.5	2.1	2.4 ± 0.3	3.4 ± 0.3	**.013**
ARA	% TFA	1.6	1.8	2.4 ± 0.1	2.1 ± 0.1	**.016**
EPA	% TFA	1.5	6.3	4.1 ± 0.2	6.0 ± 0.7	**.010**
DHA	% TFA	<0.1	2.7	5.9 ± 0.6	17 ± 2	**.001**
Thiamine	mg/kg	10	12	10.8 ± 0.8	12.5 ± 1.1	.096
Vitamin C	mg/kg	824	307	1,037 ± 336	1,401 ± 166	.168
Vitamin D3	mg/kg	0.10	0.29	0.12 ± 0.01	0.24 ± 0.01	**$<10^{-3}$**
Vitamin E	mg/kg	129	775	580 ± 27	890 ± 224	.076
MK4	µg/kg	1.1	–	1,040 ± 137	102 ± 37	**$<10^{-3}$**
Phylloquinone (K1)	µg/kg	7.6	–	13 ± 1	281 ± 131	**.024**
MK6	µg/kg	0.0	–	nd	15 ± 7	**.024**
MK7	µg/kg	4.8	–	6.7 ± 0.7	75 ± 37	**.033**
MK8	µg/kg	0.0	–	nd	242 ± 111	**.020**
MK9	µg/kg	0.0	–	nd	22 ± 11	**.026**
MK10	µg/kg	0.0	–	nd	41 ± 22	**.031**
Total vitamin K	µg/kg	13.5	–	1,073 ± 124	778 ± 340	.231
Iodine	mg/kg	2.2	3.1	5.2 ± 0.5	8.2 ± 0.5	**.002**
Ca	g/kg	2.3	3.9	3.4 ± 0.5	3.1 ± 0.5	.460
K	g/kg	14.6	12.7	15 ± 1	14 ± 0.1	.152
Mg	g/kg	6.6	7.7	8.2 ± 0.8	7.1 ± 0.9	.165
P	g/kg	12.6	9.8	11.1 ± 0.9	10.9 ± 0.4	.420

Note: Data are on dry matter. *Artemia* were grown on OriGreen for 3 days in triplicate, and both nauplii and on-grown *Artemia* were enriched with Multigain. Only one sample of the unenriched *Artemia* types were taken; therefore, these samples were not included in the statistical analyses. Differences between enriched nauplii and on-grown enriched *Artemia* (Day 1–3) were analysed by *t* tests. Means were assumed to be different at $p < .05$ (bold font).

TAA, total amino acids, cysteine, cystine and tryptophan are not included; FAA, free amino acids; TFA, total fatty acids; TL, total lipid; PL, Phospholipids; ARA, arachidonic acid 20:4n-6; EPA, Eicosapentaenoic acid 20:5n-3; DHA, docosahexaenoic acid 22:6n-3; MK, menaquinone (vitamin K forms), number of isoprenoid units in the tail is indicated; —not analysed due to shortage of sample; nd, not detected.

[§]Protein: N*4.7 for nauplii, N*5.6 for on-grown *Artemia*.

not replicated and could not be included in the statistical analyses. At SWH, only one sample of the on-grown unenriched *Artemia* and two samples of unenriched nauplii were taken, therefore these samples were not compared by statistical analyses. Effects of feeding *Artemia* nauplii versus on-grown *Artemia* on final weight of Atlantic halibut postlarvae were tested using nested ANOVA on individual fish, with tanks nested in treatments.

3 | RESULTS

3.1 | Growth and survival of *Artemia* at IMR and SWH

Figure 1 shows the growth of *Artemia* over 4 days at both hatcheries. The nauplii used by IMR were smaller than those at SWH (n.s.

TABLE 3 Nutrient composition of *Artemia* from SWH; nauplii, on-grown, enriched nauplii and enriched on-grown *Artemia*

	Unit	Nauplii unenriched	On-grown unenriched	Nauplii enriched	On-grown enriched	*p*
Dry matter	g/kg	90	79 ± 3	90 ± 1	79 ± 2	**<10^{-3}**
Protein[§]	g/kg	420	580 ± 10	430 ± 20	580 ± 40	**.006**
TAA	g/kg	404	476 ± 22	397 ± 7	477 ± 15	**<10^{-3}**
FAA	g/kg	65	117 ± 13	79 ± 3	121 ± 12	**.000**
Taurine	g/kg	4.7	5.1 ± 0.3	4.7 ± 0.0	5.3 ± 0.3	**.022**
Glycogen	g/kg	18	10.8 ± 1.6	24 ± 5	10.3 ± 2.1	**<10^{-3}**
Lipid	g/kg	260	140 ± 10	270 ± 30	170 ± 10	**<10^{-3}**
PL	g/kg TL	2.4	3.8 ± 0.1	2.5 ± 0.1	3.3 ± 0.1	**<10^{-3}**
ARA	% TFA	1.5	3.6 ± 0.3	2.8 ± 0.2	3.2 ± 0.2	**.015**
EPA	% TFA	2.5	8.0 ± 0.2	5.9 ± 0.4	6.9 ± 0.3	**.005**
DHA	% TFA	<0.1	3.8 ± 0.3	18 ± 2	9.4 ± 0.9	**<10^{-3}**
TFA	g/kg	179	107 ± 7	216 ± 9	126 ± 8	**<10^{-3}**
Thiamine	mg/kg	24	18 ± 2	22 ± 1	20 ± 2	**.009**
Vitamin C	mg/kg	964	460 ± 53	920 ± 127	786 ± 254	.098
Vitamin D3	mg/kg	0.11	0.21 ± 0.08	0.28 ± 0.08	0.25 ± 0.01	.544
MK4	µg/kg	3,258	178 ± 14	2,776 ± 214	246 ± 28	**<10^{-3}**
K1	µg/kg	13	776 ± 42	14 ± 1	536 ± 101	**<10^{-3}**
MK6	µg/kg	nd	50 ± 19	nd	29 ± 17	**.013**
MK7	µg/kg	nd	145 ± 8	nd	105 ± 28	**<10^{-3}**
MK8	µg/kg	nd	243 ± 33	nd	140 ± 38	**.001**
MK9	µg/kg	nd	77 ± 14	nd	46 ± 14	**.001**
MK10	µg/kg	nd	60 ± 10	nd	36 ± 13	**.002**
Sum vitamin K	µg/kg	3,271	1529 ± 94	2,790 ± 215	1,137 ± 209	**<10^{-3}**
Vitamin E	mg/kg	733	792 ± 55	743 ± 18	869 ± 81	.072
Iodine	mg/kg	8.8	1.02 ± 0.06	5.8 ± 1.9	7.5 ± 1.1	.388
Mn	mg/kg	5.0	5.7 ± 0.6	3.7 ± 0.1	4.1 ± 0.3	.321
Fe	mg/kg	278	233 ± 15	123 ± 33	187 ± 13	.399
Co	mg/kg	0.26	0.41 ± 0.06	0.25 ± 0.02	0.37 ± 0.03	**.001**
Cu	mg/kg	12.2	24 ± 12	10.6 ± 2.3	21 ± 8	.071
Zn	mg/kg	178	178 ± 19	184 ± 22	177 ± 11	.641
Se	mg/kg	1.9	1.12 ± 0.0s9	1.7 ± 0.1	1.06 ± 0.17	**<10^{-3}**

Note: TAA, total amino acids, cysteine, cystine and tryptophan are not included; FAA, free amino acids; TFA, total fatty acids; TL, total lipid; PL, Phospholipids; ARA, arachidonic acid 20:4n-6; EPA, Eicosapentaenoic acid 20:5n-3; DHA, docosahexaenoic acid 22:6n-3; MK, menaquinone (vitamin K forms), number of isoprenoid units in the tail is indicated; nd, not detected.

Data are on dry matter. *Artemia* were grown for three days in triplicate on OriOne, and both nauplii and on-grown *Artemia* were enriched with LARVIVA MULTIGAIN. Only one sample of the on-grown unenriched *Artemia* and two samples of unenriched nauplii were taken; therefore, these samples were not subjected to statistical analyses. Differences between enriched nauplii and enriched on-grown *Artemia* were analysed by *t* tests. Means were assumed to be different at $p < .05$ (bold font).

[§]Protein: Nauplii N*4.7, On-grown *Artemia* N*5.6

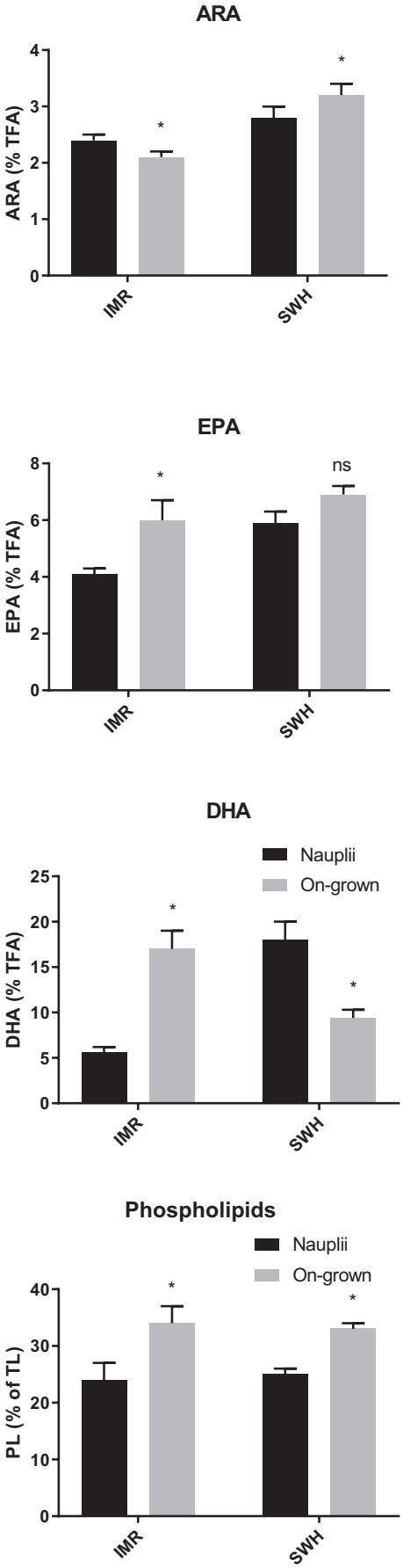

FIGURE 4 Arachidonic acid (ARA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA) (% of total fatty acids, TFA) and phospholipids (PL, % of total lipid) in enriched *Artemia* nauplii and *Artemia* grown for 3 days on OriGreen®. Mean and *SD*. An asterisk indicates significant differences between nauplii and on-grown *Artemia* within experiments ($p < .05$, ns, not significant)

for SWH1 and $p = .001$ for SWH2), but the growth and the resulting final length were higher ($p < .001$). In another experiment, on-grown *Artemia* was produced every day for 15 days at IMR. During this period, survival of *Artemia*, from start of incubation in on-growing tanks on day 1 until harvest on day 3, varied from 45% to 95% (data not shown). The survival of *Artemia* grown at SWH was 68 ± 30%, the large variation was probably due to flooding in two of the tanks when the outlet sieve became clogged.

3.2 | Change in nutrient composition during 4 days of culture

Artemia were cultured for 4 days at IMR, samples were taken for analyses each day and the results are given in Figure 2

For the N*4.7/5.30 method, the protein concentration was higher on day 3 and 4 than on day 1 and 2, while the TAA method showed no differences between days, due to a higher variation. Taurine increased gradually from day 1 to 4, while methionine was similar between days. Lipid was analysed by acid hydrolyses and as total fatty acids (TFA). TFA decreased gradually from day 2 until day 4, while lipid was lowered on day 4 compared with day 1–3. Arachidonic acid (ARA) increased gradually from day 2 until day 4, while DHA decreased in the same period and reached 2%–3% of TFA. EPA increased over the entire culture period from approximately 3%–9% of TFA. Based on a compromise between nutrient composition and workload, a culture period of 3 days was chosen for further experiments.

3.3 | Nutrient composition of Artemia nauplii compared to *Artemia* grown for 3 days

Protein concentration was higher in on-grown *Artemia* than in *Artemia* nauplii both at IMR and SWH ($p < .05$, Figure 3, Tables 2 and 3). The sum of free Amino acids was also higher in on-grown *Artemia* at IMR and SWH ($p < .05$), while total fatty acids and glycogen were both lower in on-grown *Artemia* than in nauplii in both experiments ($p < .05$, Figure 3, Tables 2 and 3). There were differences between nauplii and on-grown *Artemia* in ARA and DHA, but the direction of change varied between the experiments (Figure 4, Tables 2 and 3). EPA was higher in on-grown *Artemia* in the experiments at IMR ($p < .05$) and showed an insignificant tendency of being higher in the experiment at SWH. The ratio of phospholipids to total lipids was higher in on-grown *Artemia* than in nauplii, (Figure 4). The micronutrient concentrations were generally higher or unchanged in on-grown *Artemia* compared with nauplii, except for total vitamin K

and selenium in the *Artemia* from SWH, which were lower in the on-grown *Artemia* ($p < .05$, Tables 2 and 3).

3.4 | Performance of Atlantic halibut larvae fed on-grown *Artemia* or *Artemia* nauplii

There were no significant effects on the final weight of halibut postlarvae of feeding on-grown *Artemia* instead of *Artemia* nauplii (Figure 5). Eye migration was normal in approximately 90% of the fish fed both *Artemia* nauplii and on-grown *Artemia* (Figure 5). Concerning the macronutrient composition of postlarvae, there were no differences in glycogen, protein or lipid between the groups (Figure 6). There were no differences in the composition of other nutrients in the halibut larvae groups, except that eicosapentaenoic acid was slightly lower in larvae fed on-grown *Artemia* than in those fed nauplii ($p < .05$, Table 4).

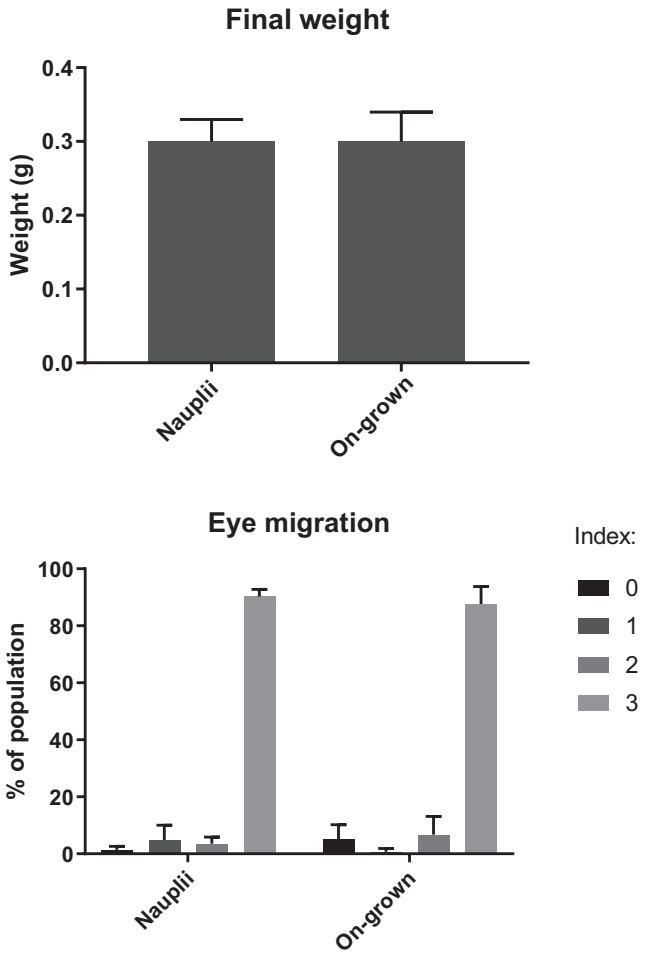

FIGURE 5 Atlantic halibut larval performance with respect to final weight (g) and eye migration. Atlantic halibut larvae were fed *Artemia* nauplii from 0 until 39 dpff or *Artemia* nauplii until 15 dpff and then transferred to on-grown *Artemia*. Mean and *SD*. The eye migration index ranges from 0 to 3, where 0 is no eye migration, 1 denotes fish where the eye has not passed the mid-dorsal ridge, 2 represents fish where the eye has passed the ridge but is not fully migrated and 3, fish with full eye migration

4 | DISCUSSION

The hypothesis of the present study was that on-grown *Artemia* as feed for Atlantic halibut larvae would alleviate problems with reduced growth in postlarvae and inferior juvenile quality. Incomplete eye migration has been a large challenge in halibut farming, affecting up to 90% of the larval population (Hamre et al., 2002). It has been shown before that feeding on-grown *Artemia* can improve eye migration and growth in Atlantic halibut larvae (Olsen et al., 1999). We wanted to confirm the earlier findings and compare the nutrient composition of on-grown *Artemia* to that of *Artemia* nauplii in order to identify possible causes of the anticipated improved performance.

The results show that *Artemia* can increase in length by 20%–70% if they are cultured for 4 days after the instar II Nauplius stage. The growth rate seems to differ between *Artemia* strains, although in the present study the differences could also be connected to differences in culture conditions between the two hatcheries that participated in the study. *Artemia* grown for 3 or 4 days, increased the protein content and lowered lipid and glycogen contents. One can hypothesize that these changes are beneficial for fish larvae which probably have protein requirements above the level found in *Artemia* nauplii and an optimal lipid level at about 150 g/kg DM, while carbohydrates are present at very low levels in natural diets (Hamre et al., 2013; Karlsen et al., 2015). The amounts of free amino acids and fraction of phospholipids (PL) to total lipids were also higher in on-grown *Artemia* than in nauplii, and both these traits have been shown to improve fish larval performance (Kvale, Nordgreen, Tonheim, & Hamre, 2007; Cahu et al., 2009). Furthermore, the aminosulfonic acid, taurine, increased in response to on-growing. Taurine has been identified as a strong growth stimulant in fish larvae (Hawkyard, Laurel, Barr, Hamre, & Langdon, 2015; Hawkyard, Laurel, & Langdon, 2014), but the exact requirement could be lower than the level in *Artemia* nauplii. The levels of the above mentioned nutrients are probably more dependent on the endogenous metabolism of the *Artemia* than a result of dietary variation.

On the other hand, levels of fatty acids and vitamins in *Artemia* depend largely on the dietary composition. OriGreen used at IMR would have contained n-3 fatty acids, which can be seen on the fatty acid composition in unenriched on-grown *Artemia* compared with unenriched nauplii. A further enrichment with Multigain increased the omega-3 fatty acids in both *Artemia* nauplii and on-grown *Artemia*. At the commercial hatchery, the trend was similar for unenriched and enriched nauplii, and for on-grown unenriched *Artemia*, while the enrichment of on-grown *Artemia* was not successful, probably due to suboptimal enrichment conditions. The requirement of DHA for good growth and survival in Atlantic halibut seems to be around 7% of total fatty acids, while that for normal pigmentation is in the range of 15% (Hamre & Harboe, 2008a, 2008b). Therefore, DHA in the enriched on-grown *Artemia* in the present study appears to be sufficient in some cases and too low in others. The micronutrient profiles did not seem to be negatively affected by culture of

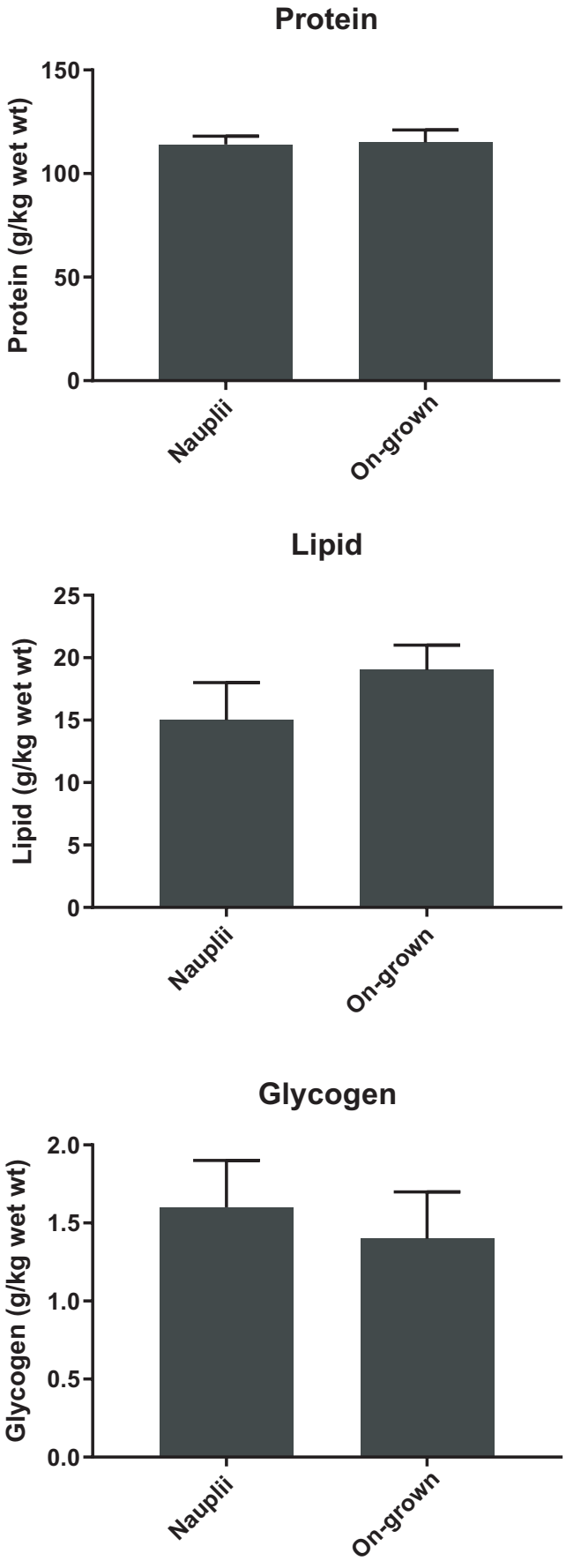

FIGURE 6 Protein, total fatty acids and glycogen (g/kg WW) in Atlantic halibut larvae fed *Artemia* nauplii from 0 until 39 dpff or *Artemia* nauplii until 15 dpff and then transferred to on-grown *Artemia*

Artemia on either OriGreen or OriOne. Furthermore, on-grown enriched *Artemia* did not seem to be deficient in micronutrients when compared to copepod levels and the requirement in fish as given by NRC (2011) (Table 5).

Taken together, the on-grown *Artemia* were larger and appeared to have an improved nutritional composition compared with nauplii, with more protein, PL and free amino acids and less lipids and carbohydrates. The micronutrient concentrations in on-grown *Artemia* also appeared sufficient, when compared to copepod concentrations of nutrients and the requirements of fish (Hamre et al., 2013; Karlsen et al., 2015; NRC, 2011). However, feeding on-grown *Artemia* did not improve growth in the late larval stages as had been expected. Approximately 90% of the fish in both groups had perfect eye migration (Index 3). It is therefore tempting to conclude that the improvement in nutrient composition of on-grown *Artemia* was not needed for delivery of sufficient amounts of nutrients to Atlantic halibut.

Malpigmentation and incomplete eye migration have challenged Atlantic halibut farmers since the first intensive hatcheries feeding *Artemia* were established in the early 1990s. These malformations were not seen in larvae that had been fed copepods in semi-extensive hatcheries. Improved protocols have gradually decreased the malformed fraction of fish from intensive hatcheries, but batches

TABLE 4 Nutrient composition of Atlantic halibut larvae fed *Artemia* nauplii from first-feeding until 15 dpff and then on-grown *Artemia* from 15 until 38 dpff compared with larvae fed nauplii for the whole period

On wet wt		Start	Nauplii	On-grown
Protein	g/kg	–	11.4 ± 0.4	11.5 ± 0.6
Sum FAA	g/kg	3.5	4.4 ± 1.1	4.8 ± 0.3
Taurine	g/kg	1.4	1.8 ± 0.2	1.8 ± 0.1
Glycogen	g/kg	0.95	1.6 ± 0.3	1.4 ± 0.3
20:4n-6%	%TFA	5.2	6.2 ± 0.1	6.2 ± 0.2
20:5n-3 EPA %	%TFA	8.4	7.8 ± 0.3[b]	6.9 ± 0.2[a]
22:6n-3 DHA %	%TFA	14	9.6 ± 0.9	8.4 ± 0.7
Total FA	g/kg	16	15 ± 3	19 ± 2
Thiamin	mg/kg	2.6	2.1 ± 0.2	2.2 ± 0.2
Vitamin C	mg/kg	158	155 ± 31	136 ± 12
Vitamin-D3	mg/kg	0.02	0.01 ± 0.00	0.01 ± 0.00
Vitamin E	mg/kg	37	25 ± 2	23 ± 1
Vitamin A1	mg/kg	0.7	1.2 ± 0.1	1.1 ± 0.1
Iodine	mg/kg	0.26	0.28 ± 0.02	0.26 ± 0.01

Note: Different letters in superscripts indicate significant differences (*t* test, $p < .05$). Data on wet weight or % of total fatty acids (TFA).

TABLE 5 Nutrient composition of copepods (Hamre et al., 2013; Karlsen et al., 2015) and nutrient requirements in fish according to (NRC, 2011) (nd, not determined)

Macronutrients (g/kg DM)	TAA	P/N factor	FAA	Lipid (TL)	PL (% TL)	Glycogen
Copepods	634 ± 89	5.30 ± 0.44	79 ± 11	156 ± 31	50 ± 12	5 ± 2
Vitamins (mg/kg DM)	Thiamine	C	E	K	D3	
Copepods	13–23	500	110	0.21	nd	
NRC (2011)	1	50	50	0.5–2	0.01–0.06	
Macrominerals (g/kg DM)	P	Ca	Mg			
Copepods	12.4–15.0	1.1–2.4	2.4–3.1			
NRC (2011)	3–8	nd	0.4–0.6			
Microminerals (mg/kg DM)	Iodine	Manganese	Copper	Zinc	Selenium	Iron
Copepods	50–350	8–25	12–38	340–570	3–5	85–371
NRC (2011)	0.6–1.1	2–12	3–5	15–37	0.15–0.25	30–150

of fish from commercial hatcheries can still have high frequencies of malformations. The problem seems to be multifactorial, since nutrition (Hamreet al., 2002), thyroid hormone signalling (Power et al., 2001; Schreiber & Specker, 1999) and photoperiod (Harboe et al., 2009; Shao et al., 2017) all have been shown to affect eye migration and pigmentation in flatfish. Shao et al., (2017) recently published a study showing that light drives development of pigmentation and eye migration in Japanese flounder through stimulation of vitamin A signalling. The process of metamorphosis is evidently complex and we see that there is an interplay between many factors that can be adjusted through culture conditions, husbandry and nutrition. The nutrient requirements may have been high, or nutrient utilization low, in the experiment of Olsen et al. (1999), due to inferior rearing protocols. Improvement of protocols between 1999 and 2015 may have lowered the thresholds and made *Artemia* nauplii a sufficient feed for Atlantic halibut larvae.

5 | SUMMARY AND CONCLUSION

Artemia grown for 3 or 4 days on OriGreen or OriOne and enriched with Larviva Multigain, obtained an improved nutrient profile in many aspects, based on claims in the available literature. The protein, free amino acid, and taurine contents increased, lipid and glycogen decreased, while the ratio of phospholipid to total lipid increased. The fatty acid composition improved in one case and not in the other. The micronutrient profiles did not seem to be negatively affected by culture of *Artemia*, and on-grown *Artemia* was not deficient in micronutrients when compared to copepod levels and the requirement in fish as given by NRC (2011).

However, feeding on-grown *Artemia* to Atlantic halibut larvae did not improve growth compared with the control group fed *Artemia* nauplii. Atlantic halibut fed *Artemia* nauplii had very good eye migration, similar to larvae fed on-grown *Artemia*. We propose that many small improvements in rearing practices between 1999 and 2015 lowered the nutrient requirements of Atlantic halibut larvae, or increased their utilization, so that they were covered by the nutrients delivered by *Artemia* nauplii. Due to the increased workload of producing on-grown *Artemia*, it is therefore not recommended for use in Atlantic halibut hatcheries.

ACKNOWLEDGEMENTS

This project has received funding from the European Union's Seventh Framework Program for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY). The authors want to thank Rangfrid Mangor-Jensen and Margareth Møgster for taking care of the rearing experiments at Austevoll Aquaculture Research Station. Kjersti Ask at former NIFES, now IMR, is acknowledged for organizing the analytical work.

DATA AVAILABILITY STATEMENT

Data will be available from the corresponding author on request.

ORCID

Kristin Hamre https://orcid.org/0000-0002-8302-3827

REFERENCES

Babiak, J., Babiak, I., Harboe, T., Haugen, T., van Nes, S., & Norberg, B. (2012). Induced sex reversal using an aromatase inhibitor, Fadrozole, in Atlantic halibut (*Hippoglossus hippoglossus*). *Aquaculture*, *324–32*(5), 276–280.

Blindheim, S., Nylund, A., Watanabe, K., Plarre, H., Erstad, B., & Nylund, S. (2015). A new aquareovirus causing high mortality in farmed Atlantic halibut fry in Norway. *Archieves of Virology*, *160*, 91–102. https://doi.org/10.1007/s00705-014-2235-8

Cahu, C. L., Gisbert, E., Villeneuve, L. A. N., Morais, S., Hamza, N., Wold, P. A., & Infante, J. L. Z. (2009). Influence of dietary phospholipids on early ontogenesis of fish. *Aquaculture Research*, *40*(9), 989–999. https://doi.org/10.1111/j.1365-2109.2009.02190.x

CEN (1999). *Comitè Europèen de Normalisation: Foodstuffs - Determination of vitamin D by high performance liquid chromatography -Measurement of cholecalciferol (D_3) and ergocalciferol (D_2)*. EN12821.

CEN (2003a). *Comitè Europèen de Normalisation: Foodstuffs - Determination of Vitamin B1 by HPLC*. EN 14122.

CEN. (2003b). *Comite Europeen de Normalisation: Foodstuffs - Determination of Vitamin K1 by HPLC*. EN14148.

CEN (2005). *Comitè Europèen de Normalisation: Foodstuffs - Determination of vitamin B_6 (including it's glycosylated forms) by HPLC*. EN 14663.

Espe, M., Lemme, A., Petri, A., & El-Mowafi, A. (2006). Can Atlantic salmon (*Salmo salar*) grow on diets devoid of fish meal? *Aquaculture*, *255*(1–4), 255–262. https://doi.org/10.1016/j.aquaculture.2005.12.030

Hamre, K., & Harboe, T. (2008a). Critical levels of essential fatty acids for normal pigmentation in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Aquaculture*, *277*(1–2), 101–108. https://doi.org/10.1016/j.aquaculture.2008.02.020

Hamre, K., & Harboe, T. (2008b). *Artemia* enriched with high n-3 HUFA may give a large improvement in performance of Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae. *Aquaculture*, *277*(3–4), 239–243. https://doi.org/10.1016/j.aquaculture.2008.02.028

Hamre, K., Kolås, K., & Sandnes, K. (2010). Protection of fish feed, made directly from marine raw materials, with natural antioxidants. *Food Chemistry*, *119*(1), 270–278. https://doi.org/10.1016/j.foodchem.2009.06.024

Hamre, K., & Mangor-Jensen, A. (2006). A multivariate approach to optimization of macronutrient composition in weaning diets for cod (*Gadus morhua*). *Aquaculture Nutrition*, *12*(1), 15–24. https://doi.org/10.1111/j.1365-2095.2006.00377.x

Hamre, K., Opstad, I., Espe, M., Solbakken, J., Hemre, G.-I., & Pittman, K. (2002). Nutrient composition and metamorphosis success of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae fed natural zooplankton or *Artemia*. *Aquaculture Nutrition*, *8*, 139–148. https://doi.org/10.1046/j.1365-2095.2002.00201.x

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

Harboe, T., & Mangor-Jensen, A. (1998). Time of first feeding of Atlantic halibut larvae. *Aquaculture Research*, *29*, 913–919.

Harboe, T., Mangor-Jensen, A., Moren, M., Hamre, K., & Ronnestad, I. (2009). Control of light condition affects the feeding regime and enables successful eye migration in Atlantic halibut juveniles. *Aquaculture*, *290*(3–4), 250–255. https://doi.org/10.1016/j.aquaculture.2009.02.032

Harboe, T., Mangor-Jensen, A., Naas, K. E., & Næss, T. (1998). A tank design for first feeding of halibut, *Hippoglossus hippoglossus* L., larvae. *Aquaculture Research*, *29*, 919–925.

Hawkyard, M., Laurel, B., Barr, Y., Hamre, K., & Langdon, C. (2015). Evaluation of liposomes for the enrichment of rotifers (*Brachionus* sp.) with taurine and their subsequent effects on the growth and development of northern rock sole (*Lepidopsetta polyxystra*) larvae. *Aquaculture*, *441*, 118–125. https://doi.org/10.1016/j.aquaculture.2015.02.012

Hawkyard, M., Laurel, B., & Langdon, C. (2014). Rotifers enriched with taurine by microparticulate and dissolved enrichment methods influence the growth and metamorphic development of northern rock sole (*Lepidopsetta polyxystra*) larvae. *Aquaculture*, *424–425*, 151–157. https://doi.org/10.1016/j.aquaculture.2013.12.035

Hemre, G. I., Lie, Ø., Lied, E., & Lambertsen, G. (1989). Starch as an energy source in feed for cod (*Gadus morha*): Digestibility and retention. *Aquaculture*, *80*, 261–271.

Hendry, C. I., Martin-Robichaud, D. J., & Benfey, T. J. (2003). Hormonal sex reversal of Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture*, *219*, 769–781. https://doi.org/10.1016/S0044-8486(02)00344-7

Jordal, A. E. O., Lie, O., & Torstensen, B. E. (2007). Complete replacement of dietary fish oil with a vegetable oil blend affect liver lipid and plasma lipoprotein levels in Atlantic salmon (*Salmo salar* L.). *Aquaculture Nutrition*, *13*(2), 114–130. https://doi.org/10.1111/j.1365-2095.2007.00455.x

Julshamn, K., Dahl, L., & Eckhoff, K. (2001). Determination of iodine in seafood by inductively coupled plasma/mass spectrometry. *Journal of AOAC International*, *84*(6), 1976–1983. https://doi.org/10.1093/jaoac/84.6.1976

Julshamn, K., Lundebye, A. K., Heggstad, K., Berntssen, M. H. G., & Boe, B. (2004). Norwegian monitoring programme on the inorganic and organic contaminants in fish caught in the Barents Sea, Norwegian Sea and North Sea, 1994–2001. *Food Additives and Contaminants*, *21*(4), 365–376. https://doi.org/10.1080/02652030310001639512

Karlsen, O., van der Meeren, T., Ronnestad, I., Mangor-Jensen, A., Galloway, T. F., Kjorsvik, E., & Hamre, K. (2015). Copepods enhance nutritional status, growth and development in Atlantic cod (*Gadus morhua* L.) larvae – Can we identify the underlying factors? *PeerJ*, *3*, e902. https://doi.org/10.7717/peerj.902

Kvale, A., Nordgreen, A., Tonheim, S. K., & Hamre, K. (2007). The problem of meeting dietary protein requirements in intensive aquaculture of marine fish larvae, with emphasis on Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture Nutrition*, *13*(3), 170–185. https://doi.org/10.1111/j.1365-2095.2007.00464.x

Liaset, B., Julshamn, K., & Espe, M. (2003). Chemical composition and theoretical nutritional evaluation of the produced fractions from enzymic hydrolysis of salmon frames with Protamex (TM). *Process Biochemistry*, *38*(12), 1747–1759. https://doi.org/10.1016/S0032-9592(02)00251-0

Lie, Ø., & Lambertsen, G. (1991). Fatty acid composition of glycerophospholipids in seven tissues of cod (*Gadus morhua*), determined by a combined HPLC/GC method. *Journal of Chromatography*, *565*, 119–129.

Maeland, A., Ronnestad, I., Fyhn, H. J., Berg, L., & Waagbo, R. (2000). Water-soluble vitamins in natural plankton (copepods) during two consecutive spring blooms compared to vitamins in *Artemia franciscana* nauplii and metanauplii. *Marine Biology*, *136*(5), 765–772. https://doi.org/10.1007/s002270000280

Mæland, A., & Waagbø, R. (1998). Examination of the qualitative ability of some cold water marine teleosts to synthesise ascorbic acid. *Comparative Biochemistry and Physiology Part A*, *121*, 249–255. https://doi.org/10.1016/S1095-6433(98)10125-3

Moren, M., Naess, T., & Hamre, K. (2002). Conversion of beta-carotene, canthaxanthin and astaxanthin to vitamin A in Atlantic halibut (*Hippoglossus hippoglossus* L.) juveniles. *Fish Physiology and Biochemistry*, *27*(1–2), 71–80.

Norberg, B., Weltzien, F.-A., Karlsen, Ø., & Holm, J. C. (2001). Effects of photoperiod on sexual maturation and somatic growth in Atlantic halibut (*Hippoglossus hippoglossus* L.). *Comparative Biochemistry and Physiolology Part B*, *129*, 357–366.

NRC (2011). *Nutrient requirements of fish and shrimp*. Washington, DC: The Natioanal Academic Press.

Olsen, A. I., Attramadal, I., Jensen, A., & Olsen, Y. (1999). Influence of size and nutritional value of *Artemia fransiscana* on growth and quality of halibut larvae (*Hippoglossus hippoglossus*) during the live feed period. *Aquaculture*, *179*, 475–487.

Power, D. M., Llewellyn, L., Faustino, M., Nowell, M. A., Björnsson, B. T., Einarsdottir, I. E., … Sweeney, G. E. (2001). Thyroid hormones in growth and development of fish. *Comparative Biochemistry and Physiology C-Toxicology & Pharmacology*, *130*(4), 447–459. https://doi.org/10.1016/S1532-0456(01)00271-X

Schreiber, A. M., & Specker, J. L. (1999). Early larval development and metamorphosis in the summer flounder: Changes in per cent whole-body water content and effects of altered thyroid status. *Journal of Fish*

Biology, *55*(1), 148–157. https://doi.org/10.1111/j.1095-8649.1999.tb00664.x

Shao, C., Bao, B., Xie, Z., Chen, X., Li, B. O., Jia, X., … Chen, S. (2017). The genome and transcriptome of Japanese flounder provide insights into flatfish asymmetry. *Nature Genetics*, *49*(1), 119–124. https://doi.org/10.1038/ng.3732

Srivastava, A., Hamre, K., Stoss, J., Chakrabarti, R., & Tonheim, S. K. (2006). Protein content and amino acid composition of the live feed rotifer (*Brachionus plicatilis*): With emphasis on the water soluble fraction. *Aquaculture*, *254*(1–4), 534–543. https://doi.org/10.1016/j.aquaculture.2005.11.014

How to cite this article: Hamre K, Erstad B, de Kok J, Norberg B, Harboe T. Change in nutrient composition of *Artemia* grown for 3–4 days and effects of feeding on-grown *Artemia* on performance of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae. *Aquacult Nutr*. 2020;26:1542–1554. https://doi.org/10.1111/anu.13101