Aquaculture 561 (2022) 738586

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture

First feed matters: The first diet of larval fish programmes growth, survival, and metabolism of larval ballan wrasse (*Labrus bergylta*)

Arne M. Malzahn [a,*], Deni Ribičić [a], Bjørn Henrik Hansen [b], Antonio Sarno [a], Elin Kjørsvik [c], Anna Sigrid Norberg Aase [c], Luciana Alves Musialak [c], Laura García-Calvo [c,1], Andreas Hagemann [a]

[a] SINTEF Ocean, Fisheries and New Biomarine Industry, Trondheim, Norway
[b] SINTEF Ocean, Climate and Environment, Trondheim, Norway
[c] Department of Biology, Norwegian University of Science and Technology, Trondheim, Norway

ARTICLE INFO

Keywords:
Nutritional programming
Cleaner fish
Gene expression
Lipidomics
Fish health

ABSTRACT

The use of cleaner fish, such as the ballan wrasse (*Labrus bergylta*), is important for combatting the sea lice problem in salmonid cage farming. Ballan wrasse is the only wrasse species that is cultivated, though only about 50% of the approx. 3 million ballan wrasse used in 2020 was produced by aquaculture. The major obstacle for large scale cultivation of ballan wrasse is the difficult first feeding phase and a lack of functional feeding protocols.

Like most pelagic marine fish larvae, ballan wrasse do not accept inert diets as first feed, and feeding regimes based on rotifers and *Artemia* (brine shrimps) usually lead to mixed results and are far from being optimal nutrition for the larvae.

In a 48-day start feeding experiment, we studied the feasibility of replacing rotifers by an Experimental cirriped diet or copepod nauplii (*Acartia tonsa*) and replacing *Artemia* by nauplii of the cirriped *Semibalanus balanoides*. Later, all treatments received the same formulated diets. We sampled larvae at each feed transition to analyze different response variables such as growth, morphometry, gene expression, lipidomics, histology, and microbiology.

We found significant differences in survival rates and growth. Larvae fed copepods as the first diet had significantly higher survival rates than larvae start-fed on either rotifers or small experimental cirripeds, and this pattern was also reflected in early growth and bone development. Gut histology at the end of the experiment (48 days after hatching) showed a more developed intestinal tissue in the larval group fed copepods first and cirripeds as the second diet compared to the other larval groups.

Gene expression at day 48 post hatch still revealed pronounced differences between the larval group first fed on rotifers and larvae from the other three feeding regimes which received natural, unenriched diets. Even weeks after receiving the same formulated diets, lipidomics analyses revealed that several lipid species correlated either negatively or positively with larval growth rates or mortality.

Our results are a clear indication for nutritional programming, pointing towards the importance the first diet has for the further life of a fish.

1. Introduction

Species diversification in marine aquaculture is an ongoing topic (François et al., 2010; Metian et al., 2020), and one of the reasons for the cultivation of rather few of the existing fish species is the difficulty of producing juveniles. Many promising species have in common that they are altricial and cannot be fed on formulated diets during the first period of their lives but need very small and nutritious live feeds. Virtually all marine fish larvae are planktivorous for at least the early life stages (Moser, 1984). Providing such plankton-based diets is complicated.

* Corresponding author.
E-mail address: arne.malzahn@sintef.no (A.M. Malzahn).
[1] Present address: Department of Biotechnology and Food Science, Norwegian University of Science and Technology, Trondheim, Norway.

https://doi.org/10.1016/j.aquaculture.2022.738586
Received 12 February 2022; Received in revised form 2 June 2022; Accepted 2 July 2022
Available online 18 July 2022

Using natural plankton makes it necessary for the hatcheries to be located directly by the sea, having harvesting technology in place and is prone to failures due to seasonal and regional differences in plankton availability and nutritional composition. Preserved inert plankton, such as deep-frozen plankton, is in many species not accepted as a diet, as it lacks movement, which acts as a hunting trigger.

The ballan wrasse (*Labrus bergylta* Ascanius, 1767) is a recent addition to the aquaculture species portfolio, due to its potential as a cleaner fish to combat salmon lice infestations in the marine salmon industry. Of the four wrasse species used as cleaner fish in Norway, so far only the ballan wrasse is cultivated. Between 50 and 70% of the 1 to 3 million ballan wrasse which were used as cleaner fish between 2015 and 2020 were sourced from capture fisheries.

Poor nutrition during the larval phase has several adverse effects in fish. Malnutrition causes mortality in many ways in nature, e.g. indirectly by reducing growth rate, which results in a longer larval phase, possibly poorer swimming ability, and thus an increased likelihood of being eaten by predators (Houde, 1987; Leggett and Deblois, 1994). Important developmental processes are taking place during this period, such as the maturation and development of the digestive system and ossification of the skeleton (Balon, 1999). These processes are pivotal for the later well-being of the fish. Malnutrition causes bone and other organ deformities (Cahu et al., 2003), affects muscle development (Vo et al., 2016), and also interferes with larval metabolism (Zambonino-Infante and Cahu, 2010).

Especially the less direct effects of nutrition during early life history of fish, such as priming metabolic capacities, may have a lasting effects on growth, health and welfare (Imsland et al., 2006; Øie et al., 2017). This is called 'nutritional- or metabolic programming'. In mammals, malfunctions in growth, body mass index, neural development, and metabolism, have been linked to malnutrition during critical phases, such as the breast-feeding phase (Fall and Kumaran, 2019). Many important developmental processes such as organogenesis in larval fish take place during the period of external nutrition (von Herbing et al., 1996). Thus, it is evident that nutritional programming plays an important role during early larval life when they switch to exogenous feeding (Hou and Fuiman, 2019). Successful onset of external optimal nutrition and feeding is not only important for instantaneous growth and survival, but also lays the foundation for a healthy life.

In fish, nutritional or metabolic programming is an emerging field of research which can be expected to lead to important advances in aquaculture. In pike perch (*Sander lucioperca*), provision or deprivation of merely one single fatty acid (docosahexaenoic acid; 22:5n-3, DHA) at a critical time of development influenced brain development and stress tolerance (Lund et al., 2012). Larvae were fed on a DHA deficient or DHA enriched diet from 7 to 29 days post-hatch, after which they received a common diet for another 150 days. Those fish receiving a DHA deficient diet showed a significantly smaller brain size, a lower brain DHA content and a lower stress tolerance than fish fed on a DHA supplemented diet (Lund et al., 2012). Similar results have been shown for Atlantic cod (*Gadus morhua* L.) (Øie et al., 2017), demonstrating the role of metabolic programming.

However, despite our knowledge that for example essential lipids fed at a critical stage during the early life of a fish are of paramount importance, current common practice in cold-water marine finfish aquaculture does not reflect it. To date, rotifers or *Artemia,* which are fundamentally nutrient deficient organisms which are solely used to feed the early critical larval stages because of their suitable size and relative ease of large-scale production. To improve the nutritional value of these two organisms, they are enriched with emulsified lipids and micronutrients. This leads to, at best, barely acceptable fish quality in terms of growth, survival, health, and stress resistance, but these fish perform inferiorly compared to fish larvae reared on natural diets (Karlsen et al., 2015; Øie et al., 2017).

To investigate the role of different live feeds such as rotifers and *Artemia*, but also copepod and cirriped nauplii on ballan wrasse wellbeing and performance, we conducted a start feeding experiment lasting until 14 days after weaning the larvae on formulated diets, up to 48 days post hatching.

2. Materials and methods

2.1. Origin of larvae, transport, and general experimental procedures

To evaluate the effect of different live feed species on growth, survival, biochemistry and microbiology of ballan wrasse larvae, we conducted a 7-week experiment from start-feeding until successful weaning. This study was carried out at NTNU Centre for Fisheries and Aquaculture (National Animal Experimental Facility No. 154), in accordance with the Animal Welfare Act of 20th December 1974, amended 19th June 2009. The experiment was approved by the Norwegian Food Safety Authority (project approval FOTS ID 23022).

Ballan wrasse larvae ($n = 736{,}000$) were supplied by Mowi Rensefisk (Stord, Norway). The larvae were hatched during two consecutive nights at the hatchery and were thereafter concentrated and sent in oxygenated plastic bags ($n = 8$; 20 L volume) placed in Styrofoam boxes at a concentration of 4.6 larvae mL^{-1}. The eggs came from a light manipulated broodstock and were incubated at around 11 °C. Temperature upon arrival was 11.9 °C, and oxygen saturation in the bags was between 150 and 306%. The larvae were gently acclimated to the new water over a period of 2 h by slowly adding water to the bags. Larvae were then stocked into one 200-L tank per hatch date. Dead larvae were allowed to settle for 20 min before they were collected by siphoning them from the bottom of the tanks. Dead larvae were counted. The tanks containing live larvae were gently stirred to evenly distribute the larvae in the water column and 5 sub-samples were scooped from each tank and larvae were counted. Survival during transport was 36 and 40% for the two hatch dates. All surviving larvae were stocked to the experimental tanks, which resulted in approx. 19,700 larvae per tank (~ 100 larvae L^{-1}). Larvae were kept in darkness until the first feeding commenced on day 4 post-hatch, from when on they were kept at constant dimmed light. Tanks were randomly assigned to treatments using the random number function of MS Excel.

The temperature in the tanks was adjusted to 12 °C, water flow in each tank was set to 0.2 L per minute in the beginning of the experiment and gradually increased to approx. 1 L per minute at day 32, upon the initiation of weaning onto formulated feeds. The tanks were slightly aerated from the bottom through an air hose attached to the water outlet. Every morning throughout the experiment, the tanks were cleaned using inbuilt cleaning arms that covered the tank bottom and –sides, which was allowed to rotate for approx. 20 min, resulting in several rotations of the cleaning arm. Debris was manually siphoned from the tank bottom.

Feeding regimes: Larvae were fed four different feeding regimes in triplicates. The first food item was either rotifers (*Brachionus plicatilis*), or live-feed organisms meant to replace rotifers, namely copepod nauplii (*Acartia tonsa* Dana) or an Experimental cirriped diet. The Experimental cirriped diet was comprised of two different cirripede species (*Balanus crenatus* and *Balanus balanus*) in approximately equal amounts by abundance. Nauplii of the first species were 267 ± 18.8 μm long and 154 ± 26 μm wide, the latter being 339 ± 21.8 μm long and 194 ± 22.9 μm wide (average length ± standard deviation based on 70 measurements). The larvae received this first live feed type for 19 days (day 3 till day 22 post-hatch). This was followed by either *Artemia salina* nauplii or CryoPlanktonLarge (cryopreserved larvae of the barnacle *Semibalanus balanoides,* Planktonic AS, Norway), replacing *Artemia* for 19 days with an overlap of 5 days with rotifers/replacers in the beginning (day 18–36 post-hatch). From day 32 post-hatch on, all tanks were fed on the same formulated diets. Formulated diets were Gemma Micro 150 μm (Skretting) and AgloNorse Extra 2 (Tromsø Fiskeindustri AS, Tromsø, Norway) in a 1:1 mix from day 32 – day 37 post-hatch, followed by Gemma Micro 150 μm, Gemma Micro 300 and AgloNorse Extra 2 in a 1:1:2 mix (day 38

- day 42 post-hatch). From day 42 to day 48 post-hatch (end of experiment) larvae received Gemma Micro 300 and AgloNorse Extra 2 in a 1:1 mix. All feeds were applied ad libitum. More details can be found in Table 1.

2.2. Live feed preparation & procedures

Green water: Larvae were kept in green water to ensure a good light and microbial climate and allow the live feed organisms to continue to feed in the tanks and maintain their nutritional quality. The cryptophyte *Rhodomonas baltica* (clone NIVA 5/91: Cryptophyceae: Pyrenomonadales) was added to the tanks at a concentration of 30,000 cells mL $^{-1}$ in three doses per day (approx. 1 mg C L^{-1} day^{-1}) and *Nannochloropsis* Frozen Paste (BlueBioTech Gmbh, Germany) was added on top to reach 6 mg C L^{-1} day^{-1}, in three doses. *R. baltica* was cultivated semi-static, using Conwy medium (Walne, 1970) to fertilize the water. Filtered water from the Trondheim Fjord was sterilized using chlorine and neutralized using sodium thiosulfate. Algae were cultivated in 100-L acryl cylinders, continuously illuminated with fluorescence tubes at a light intensity of 150 μmol m^{-2} s^{-1} measured at the surface of the cylinder. Cultures were aerated using CO_2 enriched air (1–2% CO_2).

2.2.1. Rotifers & rotifer replacers

Rotifers: Rotifers were produced in 100-L tanks at 20–22 °C and fed on a 3:1 mix of bakers' yeast and Rotifer diet (Instant algae, Reed Mariculture), respectively. Rotifers were enriched with Larviva Multi-Gain (Biomar) at 0.15 g per million rotifers at 24 °C for two hours, after which enriched rotifers were washed and fed to the larvae or stored for a later feeding the same day. The rotifers stored for later feedings were kept at 4 °C for not longer than 16 h. Rotifers were fed at increasing densities from 3 to 12 ind. mL^{-1} day^{-1}.

Copepods: Copepod eggs and live copepods (*A. tonsa*) were supplied by CFEED AS (Vanvikan, Norway). Larval ballan wrasse were fed naupliar stages 1–3 from day 4 to day 10 post-hatch. Afterwards, larvae received naupliar stages 4–6 till day 22 post-hatch. Naupliar stages 4–6 were supplied by CFEED in 1000-L IBC containers, whereas nauplii stage 1–3 were produced on site; eggs were hatched in seawater (22 °C) in 100-L conical tanks which were heavily aerated from the bottom through an air-stone to keep the eggs in suspension. After 24 h, 10 L of *R. baltica* (approx. 1.5 million cells mL^{-1}) was added to the tanks to feed the hatched copepods. Before harvesting, the aeration was stopped and the empty eggshells and debris (faecal matter and exoskeletons) were allowed to settle for 15 min. The tanks were then drained and the first 5 L in each tank containing debris were discarded. Copepod nauplii were fed to the larvae at increasing concentrations from 10 to 20 ind. mL^{-1} day^{-1} in three doses per day.

Experimental small cirriped diet: We used an Experimental cirriped diet supplied by Planktonic AS (Trondheim, Norway) as a rotifer replacer. This diet comprised a mixture of *Balanus crenatus* and *Balanus balanus* nauplii I and II, both species comprising ca. 50% of the prey items by numbers. *B. balanus*, which is approx. 340 μm long and 190 μm wide, was probably too large for the larvae to ingest at the onset of exogenous feeding. The diet was thawed in cold sea water for several minutes, followed by five minutes of rinsing in a sieve with running sea water. The washed plankton was then allowed to revitalize in aerated sea water at 4 °C for 6 h. The diet was stored in heavily aerated 5-L canisters next to the respective tanks and pumped to the larvae using peristaltic pumps over a period of approx. 4 h, followed by a 4-h pause, three times per day, at volumes intended to reach a density of 20 ind. mL^{-1} day^{-1}.

2.2.2. Artemia & Artemia replacer

Artemia: Cysts (EG SepArt 225) were purchased from INVE (Belgium) and were hatched and separated from the cysts following INVE's protocols. Hatched nauplii were enriched with Larviva Multigain (MG; Biomar), using the following long-term enrichment strategy; 1 g MG per million newly hatched *Artemia* nauplii was added to the enrichment tanks (500 ind. mL^{-1}). After 18 h, a second dose of MG at the same concentration was added, and the *Artemia* were allowed to ingest the enrichment for another 6 h. *Artemia* were added at a density of 9 ind. mL^{-1} day^{-1} to the tanks with the larvae.

Cryoplankton: The commercial product CryoPlanktonLarge was purchased from Plankonic AS and was used as an *Artemia* replacer. These are newly hatched barnacle nauplii of the species *S. balanoides*, which resemble the size of an *Artemia* nauplii. The diet was thawed, revitalized, and introduced to the larval tanks using the same procedure as described above for the "Experimental cirriped diet". The diet was fed to the larval tanks at a density of 6–12 ind. mL^{-1} day^{-1}.

2.2.3. Formulated diets

Larvae were fed formulated diets from day 32 post-hatch on, with a period of co-feeding with the previous feed until day 37 post-hatch. Larvae were fed a 1:1 mix of Gemma micro 150 and AgloNorse 2 for the first 6 days. From day 7 to day 12 we increased the particle size by replacing half the Gemma Micro 150 (150 μm particle size) by Gemma Micro 300 (300 μm particle size). The last 6 days (day 43 till day 48 post-hatch) larvae received a 1:1 mix of Gemma Micro 300 and AgloNorse 2 (200-300 μm). Formulated diets were supplied *ad libitum*, using automated feeder. Larvae received 12 doses, 0.4 g each, per day.

2.3. Sampling & analysis

Samples of fish and tank water were taken at each day a new feed type was introduced. Samples were always retrieved in the morning, before the new feed was added to the tanks. This resulted in 8 sampling days occurring roughly weekly over the 48-day experimental period. Before further treatment, all larvae were randomly sampled from each tank, anesthetised and euthanised with 500 mg L^{-1} MS222, then thoroughly rinsed with fresh and deionized water.

Mortality estimations: Approximately 500 to 900 larvae were sampled in total per tank during the experiment, which amounted to about 2.5–5% of the initially stocked larvae. In order to correct mortality estimates for the sampled larvae, we followed the approach of Kotani et al. (2011). This approach assumes a constant mortality over the experimental duration and assigns this to both non-sampled and sampled larvae (Eq. 1):

$$N_t = e^{-mt}(N_0 - \sum N_{Sn} e^{mdn}) \quad (1)$$

The equation is based on an exponential population decline but includes a term allowing for a theoretical mortality of sampled larvae (N_{Sn}) as well. Mortality coefficients were then used as entry data for an ANOVA, testing for differences in mortality between treatments, followed by the Holm Sidak post hoc test.

Length and weight measurements: 10 larvae were frozen at −20 °C until analysed. Larvae were thawed and imaged under a calibrated stereomicroscope (Olympus SZX10). Larvae were measured for standard length on the images using the software package Infinity Analyze (Teledyne Lumenera). After imaging, larvae were rinsed in distilled water put into pre-weighed tin capsules. Larvae were dried at 60 °C and weighed using a UMX2 Ultra-microbalance (Mettler-Toledo). Length – weight relationships were compared using the exponent of a power function $y = ax^b$ calculated to length-weight data from each of the tanks. The exponents were compared by one-way ANOVA followed by Holm-Sidak post-hoc test.

Biometry: Larvae were imaged following a standard procedure using a macroscope (model Z6APO, Leica Microsystems, Germany) equipped with a CMOS camera (MC170HD, Leica Microsystems, Germany). Biometric analysis of larval length and body area were automatically determined using AutoMOMI (Automated Morphometrics On Microscope Images, (Kvæstad et al., 2022). AutoMOMI utilizes the MASK-R CNN neural net architecture (He et al., 2017) and was trained on manually annotated images of ballan wrasse larvae. Body area was calculated using automated image processing techniques, such as

Table 1
Summary of experimental parameters during the ballan wrasse start -feeding experiment.

Date	Day post hatch	Rotifers (feedings/ day * ind/ mL)	*Artemia* (feedings/ day * ind/ mL)	Dry feed	Sive mesh size (μm)	*Artemia* (feedings/ day * ind/ mL)	Dry feed	*Acartia tonsa* n1-n2 (feedings/ day * ind/ mL)	*Acartia tonsa* n4-n6 (feedings/ day * ind/ mL)	Sive mesh size (μm)	Dry feed	Cirriped, small (feedings/ day * ind/ mL)	Cirriped, large (feedings/ day * ind/ mL)	Sive mesh size (μm)	Dry feed	Cirriped, large (feedings/ day * ind/ mL)	*Acartia tonsa* n1-n2 (feedings/ day * ind/ mL)	*Acartia tonsa* n4-n6 (feedings/ day * ind/ mL)	Sive mesh size (μm)	Water exchange (%/day)	Light (hours on)	Temperature (°C)
21.2.20	1				250					64				250					64	200	0	12
22.2.20	2				250					64				250					64	200	0	12
23.2.20	3				250					64				250					64	200	0	12
24.2.20	4	1 * 3			250			1 * 20		64		4* 5		250			1 * 20		64	200	24	12
25.2.20	5	1 * 3			250			3*3.3		64		4* 5		250			3*3.3		64	200	24	12
26.2.20	6	2 * 3			250			3*3.3		64		4* 5		250			3*3.3		64	200	24	13
27.2.20	7	3 * 3			250			3*4		64		4* 5		250			3*4		64	200	24	13
28.2.20	8	3 * 3			250			3* 5.3		64		4* 5		250			3* 5.3		64	200	24	13
29.2.20	9	3 * 3			250			3* 5.3		64		4* 5		250			3* 5.3		64	200	24	13
1.3.20	10	3 * 3			250			3* 7		64		4* 5		250			3* 7		64	200	24	13
2.3.20	11	3 * 3			250				3* 7	64		4* 5		250				3* 7	64	200	24	13
3.3.20	12	3 * 3			250				3* 7	64		4* 5		250				3* 7	64	200	24	14
4.3.20	13	3 * 3			250				3* 7	64		4* 5		250				3* 7	64	200	24	14
5.3.20	14	3 * 3			250				3* 10	64		4* 5		250				3* 10	64	200	24	14
6.3.20	15	3 * 3			250				3* 10	350		4* 5		250				3* 10	350	200	24	15
7.3.20	16	3 * 3			250				3* 10	350		4* 5		250				3* 10	350	200	24	15
8.3.20	17	3 * 3			250				3* 13	350		4* 5		250				3* 13	350	200	24	15
9.3.20	18	3 * 3	1 * 3		350	1 * 3			3* 13	350		4* 5	2* 3	350		2* 3		3* 13	350	400	24	15
10.3.20	19	3 * 3	1 * 3		350	1 * 3			3* 10	350		4* 5	2* 3	350		2* 3		3* 10	350	400	24	15
11.3.20	20	3 * 3	2 * 3		350	2 * 3			3* 10	350		4* 5	4* 3	350		4* 3		3* 10	350	400	24	15
12.3.20	21	3 * 3	3 * 3		350	3 * 3			2* 10	350		4* 5	4* 3	350		4* 3		2* 10	350	400	24	15
13.3.20	22	3 * 3	3 * 3		350	3 * 3			1* 20	350		4* 5	4* 3	350		4* 3		1* 20	350	400	24	15
14.3.20	23		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	400	24	16
15.3.20	24		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	400	24	16
16.3.20	25		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	400	24	16
17.3.20	26		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	600	24	16
18.3.20	27		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	600	24	16
19.3.20	28		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	600	24	16
20.3.20	29		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	600	24	16
21.3.20	30		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	600	24	16
22.3.20	31		3 * 3		350	3 * 3				350			4* 3	350		4* 3			350	600	24	16
23.3.20	32		3 * 3	A	700	3 * 3	A			700	A		4* 3	700	A	4* 3			700	600	24	16
24.3.20	33		3 * 3	A	700	3 * 3	A			700	A		4* 3	700	A	4* 3			700	800	24	16
25.3.20	34		3 * 3	A	700	3 * 3	A			700	A		4* 3	700	A	4* 3			700	800	24	16
26.3.20	35		3 * 3	A	700	3 * 3	A			700	A		4* 3	700	A	4* 3			700	800	24	16
27.3.20	36		3 * 3	A	700	3 * 3	A			700	A		4* 3	700	A	4* 3			700	800	24	16
28.3.20	37			A	700		A			700	A			700	A				700	800	24	16
29.3.20	38			B	700		B			700	B			700	B				700	800	24	16
30.3.20	39			B	700		B			700	B			700	B				700	800	24	16
31.3.20	40			B	700		B			700	B			700	B				700	800	24	16
1.4.20	41			B	700		B			700	B			700	B				700	800	24	16
2.4.20	42			B	700		B			700	B			700	B				700	800	24	16
3.4.20	43			C	700		C			700	C			700	C				700	800	24	16
4.4.20	44			C	700		C			700	C			700	C				700	800	24	16

(*continued on next page*)

Table 1 (*continued*)

Date	Day post hatch	Rotifers (feedings/ day * ind/ mL)	*Artemia* (feedings/ day * ind/ mL)	Dry feed	Sive mesh size (μm)	*Artemia* (feedings/ day * ind/ mL)	Dry feed	*Acartia tonsa* n1-n2 (feedings/ day * ind/ mL)	*Acartia tonsa* n4-n6 (feedings/ day * ind/ mL)	Sive mesh size (μm)	Dry feed	Cirriped, small (feedings/ day * ind/ mL)	Cirriped, large (feedings/ day * ind/ mL)	Sive mesh size (μm)	Dry feed	Cirriped, large (feedings/ day * ind/ mL)	*Acartia tonsa* n1-n2 (feedings/ day * ind/ mL)	*Acartia tonsa* n4-n6 (feedings/ day * ind/ mL)	Sive mesh size (μm)	Water exchange (%/day)	Light (hours on)	Temperature (°C)
5.4.20	45			C	700		C			700	C			700	C				700	800	24	16
6.4.20	46			C	700		C			700	C			700	C				700	800	24	16
7.4.20	47			C	700		C			700	C			700	C				700	800	24	16
8.4.20	48			C	700		C			700	C			700	C				700	800	24	16
Sampling dates							Gemma Micro 150: Gemma Micro 300: AgloNorse 2, 200–300:	A 50%	B 25%	C 50%												
									25%	50%												
								50%	50%	50%												

Topological Structural Analysis (TSA) (Suzuki, 1985) ellipse fitting (Fitzgibbon and Fisher, 1995), and skeletonize (Zhang and Suen, 1984).

Bone development: At the end of the experiment (48 days post-hatch), 50 larvae from each treatment larvae were fixed in 4% paraformaldehyde in phosphate buffer (pH 7.4; Apotekproduksjon AS, Norway). They were stained with Alizarin Red according to Balon (1985) with modifications for cod larvae by Kjørsvik et al. (2009). Photographs were taken with a dissecting microscope (Leica M205, Leica Microsystems, Germany) equipped with a camera (AxioCam ERc 5 s, Zeiss Inc., Germany) and images were analysed for the degree of ossification, namely 'Fully ossified', "Compact", "Partly ossified" and "Transparent". We then calculated a weighted average ossification, by appointing weights to the ossification scores. Fully ossified vertebrae scored 1.0; compact 0.8; partly ossified 0.2; and transparent score 0.0.

Gut histology: Five larvae from each treatment were fixed in 4% paraformaldehyde in phosphate buffer, dehydrated, and embedded in Technovit 7100 before being cut into semi-thin sections (2 μm) with a Leica Reichert Ultracut microtome (Leica Microsystems, Germany) equipped with a glass knife. The sections were stained with a 0.05% Toluidine blue solution, and some sections were also stained with Periodic Acid Schiff (ABPAS) for visualization of possible glycogen in the liver. Sections were mounted and scanned (NanoZoomer). Images were examined using the image analysis software QuPath and ImageJ. In the gut tissue, villus height, microvillus height and gut epithelial height was measured.

Fatty acid (FA) composition: Feed and larval samples were collected as described above for lipidomics analyses. FA composition was studied by fatty acid transmethylation to fatty acid methyl esters (FAMEs) and gas chromatography with flame ionization detection (GC-FID) analysis. Lipids were extracted after Folch (Folch et al., 1957). Larvae or feeds were freeze-dried, resuspended in 10 mL 2:1 chloroform:methanol and homogenized using a blender with a stainless steel tip (IKA T-10 basic ULTRA-TURRAX). Crude extracts were washed with a 0.88% KCl solution. The organic (lower) phase was recovered, dried under a gentle N_2 stream and resolved in 2:1 chloroform:methanol to a final concentration of 10 mg mL^{-1}. Total lipid content was determined gravimetrically by weighing the lipid extract after drying. Next, samples were spiked with 23:0 from NuChecPrep (Chiron) as internal standard and methylated by adding 1 mL chloroform and 2 mL 1% H2SO4: MeOH and incubating at 50 °C for 16–18 h. Lipophobic phase separation was achieved by adding 6 mL isooctane and 5 mL saturated NaCl, followed by centrifugation at 4000 rpm for 3 min at 4 °C. The upper (organic) phase was recovered and dried under a gentle nitrogen stream. Finally, samples were reconstituted in 150 μL isooctane and stored at −80 °C until analysis.

The FAMEs were analysed according to Daukšas et al. (2005) with the following modifications: an Agilent Technologies 7890B GC–FID equipped with a 7693A autosampler was used. The detector temperature was held at 280 °C, and the flame was maintained with 30 mL min^{-1} H_2 gas and 400 mL min^{-1} filtered air. Chromatography was carried out using a CP-wax 52 CB, 25 m, 250 μm with i.d. 0.2 μm column (Agilent Technologies). Helium was used as the carrier gas at a flow rate 3 mL min^{-1}. GC inlets were held at 250 °C. The initial oven temperature was held for 2 min at 50 °C and increased to 150 °C at 30 °C min, after which the temperature was increased to 230 °C at 2.5 °C/min, followed by a increase to 240 °C at 10 °C/min, and a 23 min final hold. Free FAMEs were identified comparing their retention times with those of a commercial standard mix chromatographed at identical conditions. Quantification was based on a commercial standard mix (68D from NuChekPrep, Chiron), and using tricosanoic acid (C23:0, NuChekPrep, Chiron) as internal standard. Selected fatty acids and fatty acid groups as well as total lipids of the live feeds were compared according to the phase in which they were fed to the larvae (Phase 1: Rotifers, Exp. Cirripeds and copepods, and phase 2: *Artemia* and cirripeds). Proportional data was arcsine-transformed before running statistics. Most measures failed the test for normality test (Shapiro-Wilks,) or the equal variance test (Brown-Forsythe) and were consequently analysed by

Kruskal Wallace ANOVA on ranks (comparing rotifers, Exp. Cirripeds and copepods or a student *t*-test (comparing *Artemia* and Cirripeds).

Lipidomics: Sampled larvae and the different feed types were transferred to cryotubes and flushed with N_2, immediately snap-frozen with liquid N_2 and stored at −80 °C until analysis. Lipids were extracted with a high throughput extraction method using methyl-*tert*-butyl ether (MTBE), chloroform and ceramic beads, according to Abbott et al. (2013), with the following modifications. Prior to extraction, samples were spiked with an internal standard (SPLASH® II LIPIDOMIX® Mass Spec Standard, Avanti, Sigma). Extraction was performed for 1 h at room temperature, followed by addition of 0.15 M ammonium acetate (MTBE/methanol/0.15 M ammonium acetate 20:6:5). After a 10 min incubation, samples were centrifuged for 10 min at 4000 relative centrifugal force (RCF) and 4 °C. The organic phase (upper phase) was collected, dried under N_2, resuspended in 500 μL $CHCl_3$ and stored at −80 °C until analysis.

For lipidomics analysis, samples were dried under nitrogen and redissolved in 100 μL acetonitrile-water (40:60) containing 10 mM ammonium formate and 0.1% formic acid. Samples were analysed on an Agilent 1260 HPLC coupled to a 4670 triple quadrupole mass spectrometer with an electrospray ion source. The HPLC column was a Waters Acquity CSH C18 column (2.1 × 100 mm, 1.7 μm particle size) kept at 45 °C using a flow rate of 0.25 mL/min and to which 5 μL sample were injected per run. The mobile phase consisted of a 60-min gradient of (A) acetonitrile-water (40:60) and (B) isopropanol-acetonitrile (90:10), both containing 10 mM ammonium formate and 0.1% formic acid. Each sample was injected three times with each injection analysing a different set of lipids (phospholipids, glycerides and free fatty acids, and sphingolipids). All data was acquired in multiple reaction monitoring (MRM) mode with mass transitions obtained from the literature (Jouhet et al., 2017; Takeda et al., 2018; Tsugawa et al., 2015; Woodfield et al., 2018; Xuan et al., 2018). All MRMs were scanned on a sample of lipid extract from different pooled sources to obtain retention times using the observation that acyl chain length increases and desaturation decreases with increased retention time on a reverse phase column (Bromke et al., 2015; Giavalisco et al., 2011). Data from LC-MS/MS analyses were analysed using the Agilent MassHunter Quantitative Analysis software package. All peaks were controlled for retention time drift, peak symmetry, and minimum intensity. An exogenous standard comprised of a small aliquot from each sample was also run with different injection volumes to run as a surrogate standard curve, which was used to determine the response for each mass transition. A regression curve was generated for each transition and a r^2 cut-off of 0.7 was used, under which samples were excluded. Next, the peak intensities were normalized to heavy isotope-labelled standards of the same lipid class in each sample, which controls for both instrument response drift and extraction efficiency. Finally, signals were normalized to total sample weight.

Gene expression: Larvae ($n = 5$–10) were collected from each tank on day 48 post-hatch. Larvae were euthanised and rinsed using MS222 (500 mg mL^{-1}) before storing in cryo-vials containing DNA/RNA Shield and mechanical beads for RNA extraction. Samples were immediately frozen at −80 °C. RNA extraction was conducted on all samples using a commercial RNA extraction kit (Quick-RNA Miniprep Plus Kit, ZymoResearch). Obtained RNA was quantified for quality and quantity (NanoDrop and Qubit) and was sent on dry ice to BGI for sequencing.

Gene expression bioinformatics: Sequencing was performed on DNBSeq platform, using a standardized protocol at BGI. Raw sequences were treated as follows. Firstly, we removed the reads mapped to rRNA. Then, the sequencing reads containing low-quality, adaptor-polluted, and high content of unknown base(N) reads, were removed before downstream analyses. After reads filtering, clean reads were mapped to reference genome (NCBI accession# PRJEB13687) using HISAT2. On average 92.03% reads were mapped, and the uniformity of the mapping result for each sample suggests that the samples were comparable. After genome mapping, StringTie was used to reconstruct transcripts, and with genome annotation information it was possible to identify novel transcripts by using Cuffcompare (a tool of Cufflinks) and predict the coding ability of those new transcripts using CPC. Novel transcripts were merged with reference transcripts to obtain complete reference. Subsequently, the mapping of clean reads to complete reference was performed using Bowtie2, and gene expression level for each sample was conducted with RSEM.

Microbiology: Samples for the microbial community of the water were taken by pre-filtering tank water (1 L) through a 64 μm sieve (SEFAR Nitex), followed by a 0.2 μm filtration (MF Millipore membrane filter). Larvae were sampled from the tanks and euthanized using 500 mg L^{-1} MS-222. Larvae were rinsed three times in distilled water. Appropriate amounts of the different feed items were washed using a 64 μm sieve and filtered seawater. Samples were stored at −20 °C until extraction. The DNA of larvae, water and feed organisms was extracted from the filters using a ZymoBIOMICS 96 MagBead DNA kit. DNA was then eluted in in DNase/RNase free water and concentrations and quality were analysed using NanoDrop and Qubit. Ilumina sequencing was done at Beijing Genomic Institute (BGI) and sequence data was further processed by using the QIIME2TM pipeline version: 2020.2.0.

Microbiology bioinformatics: Raw reads were treated using QIIME2 pipeline (v. 2021.2). Shortly, raw reads were imported into pipeline as qiime2 artifacts, quality thresholds were chosen based on median value of quality Phred score ($P > 20$). The dada2 algorithm was used to infer amplicon sequence variants (ASVs) based on generated sequence error model. Representative sequences for ASV were obtained and used for sequence alignment to generate a phylogenetic tree (unrooted and rooted). Finally, taxonomic assignment was conducted on representative ASV based on Silva 16S rRNA database (v.138). Downstream statistical analysis and graphical representation was conducted in R language employing various bioinformatic packages (phyloseq, microbiomeSeq). Mitochondria and chloroplast sequences were filtered out. ASVs accounting for ten sequences or less were also filtered out, and the dataset was rarefied down to 15,000 reads. Alpha and beta diversity was calculated and statistically tested on the cleaned dataset. Chao1 and the Simpson index was used to evaluate alpha diversity, while the Bray-Curtis diversity index was used to evaluate beta diversity by the means of principal coordinate analysis. Kruskal-Wallis and pairwise Dunn's statistical tests were applied to test for differences in alpha diversity ($p < 0.01$), and PERMANOVA statistical test was applied for testing for differences in beta diversity ($p < 0.01$).

3. Results

Larvae from the two treatments receiving copepod nauplii as the first diets had significantly lower mortality rates than those receiving rotifers or the experimental cirriped diet (Fig. 1, ANOVA, $p < 0.05$, followed by Holm Sidak post hoc test). There were no differences between the two groups receiving copepods first, with a final survival of 8%, and no differences between the rotifer and Experimental cirriped started groups (approx. 1% final survival). Mortality peaked in all tanks around 14 days post-hatch, with higher mortality of larvae in the Experimental cirriped and rotifer treatments than for larvae in the copepod-started tanks, likely marking the point of maternal reserve depletion for those that were not able to initiate growth by utilizing external nutrition.

Growth & ossification: Larvae from three of the four treatments reached the same size by the end of the experiment (9 mm SL), whereas those that were start-fed with the Experimental cirriped diet were not only fewer, but also significantly smaller than larvae from the other groups at the end of the experiment (7 mm SL) (Fig. 2). Applying ANCOVA to the length vs. age data revealed that the slope of larvae from the Exp. Cirriped/Cirriped treatment was significantly lower than the slope of larvae from the other three treatments, while the slopes of the other three did not differ from each other (ANCOVA $p < 0.05$). Ballan wrasse larvae drastically changed their growth pattern during larval development; they grew predominantly in body length during the very

early phase up till they reached approx. 5.5 mm SL, thereafter they switched to also growing in body height. The increase in body area vs. SL did not differ between treatments, suggesting that diet had no effect on this growth pattern (Fig. 3).

At the end of the experiment, the Exp. Cirripeds/Cirripeds fed larvae was the only group with a substantial number of larvae small enough not to be ossified (~6–7 mm); and 84% of the stained larvae were in the 6 to 8 mm range, which is the size in which the main vertebrae ossification is taking place (Fig. 4). Only 8 out of the 50 sampled larvae in this group were fully ossified. Larvae from the groups start-fed on copepods or rotifers had <20% larvae being smaller than 8 mm and having only partly ossified vertebrae. It must be noted that both copepod-started groups showed a tendency of lesser vertebrae ossification, where a considerable number of vertebrae being were compact but not yet fully ossified between 10 and 12 mm SL. In this range, all larvae start-fed on rotifers had fully ossified vertebrae, while the Exp. Cirriped treatment did not produce larvae of this size during the experimental duration.

Gut histology analyses revealed significant differences between treatments on day 48 post-hatch (Fig. 5). Larvae from the Copepod/Cirriped treatment had significantly higher villi (Fig. 5a), taller microvilli (Fig. 5b), and a significantly larger gut epithelial height (Fig. 5c) (Kruskal-Wallis test $p < 0.05$) than the three other groups. The three other treatments showed lower values and did not differ from each other in epithelial height (Fig. 5c). We found significant differences in villi height, where larvae from the Copepod/*Artemia*- and Rotifer/*Artemia*-treatments had similar villi height, falling between the Copepod/Cirriped-fed larvae and the significantly shorter villi height recorded for the Exp. Cirriped/Cirriped-fed larvae (Fig. 5a). The Copepod/Cirriped-fed larvae had the longest microvilli height, followed by the larvae of the Copepod/*Artemia*- and Rotifer/*Artemia*- treatments. The microvilli height of the larvae from the Exp. Cirriped/Cirriped-fed larvae were between the latter two, not differing from either (Fig. 5b, Wilcoxon test $p > 0.05$).

Liver histology analyses revealed that larvae from the Copepod/Cirriped treatment at day 48 post-hatch had larger hepatocyte cell areas, indicating a higher energy storage in the form of glycogen (data shown in Norberg Aase, 2022).

Total lipids and fatty acid profiles showed pronounced differences between the feed types (Table 2 & Fig. 6). The two enriched diets (*Artemia* and rotifers) contained more lipids than the two natural diets (cirripeds and copepods). Within these groups, *Artemia* was significantly richer in lipids than rotifers, and no differences were found between the cirripeds and copepods (ANOVA $p < 0.05$, followed by Holm Sidak post hoc test (Fig. 6).

During phase 1 of the experiment the fatty acids supplied with the rotifers, Exp. Cirripeds and copepods differed significantly. Larvae receiving rotifers also received a more lipid rich diet than the other two groups, and these organisms contained significantly higher amounts of saturated fatty acids and a lower amount of unsaturated fatty acids. Copepods had a higher proportion of omega-3 fatty acids. In general, cirripeds were higher in eicosapentaenoic acid (C20:5n3, EPA) than the other feeds, leading to a higher EPA/DHA ratio. Not surprisingly, *Artemia* showed similar patterns as rotifers in comparison with their counterpart, the cirripeds, during phase 2 of the experiment: higher total lipids, higher proportions of 16:0, 18:1n9, and saturated fatty acids, as well as lower EPA, DHA, omega-3 and unsaturated fatty acids and a lower EPA/DHA ratio.

Larvae did not show considerable changes in total lipid concentrations, apart from the expected decrease after yolk resorption (Table 3). Larvae grouped well according to the last diets they received using their fatty acid profiles as input for a Principal Component Analysis (PCA) (Fig. 7), as larvae differed in individual fatty acid content both between treatments and with age. The relative content of DHA, the percentage of saturated fatty acids, 16:0, the percentage of n-3 fatty acids, 22:0 and C17:1n7 contributed the most to PC1. The second principal component was mostly influenced by 20:3n3, 18:3n3, 15:0 and 20:1n9.

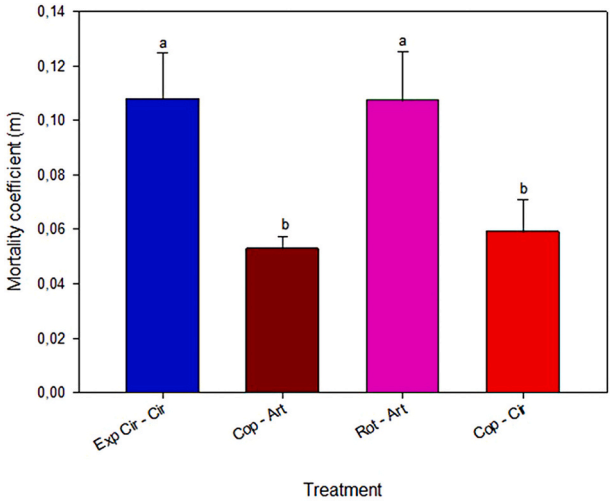

Fig. 1. Average mortality coefficient m of larval ballan wrasse during the 48 days start feeding experiment. Error bars are standard deviation of three tanks. a and b denote significant differences (ANOVA, followed by Holm-Sidak test, $p < 0.05$) between treatments. Treatment abbreviations: Exp. Cir - Cir: Experimental Cirriped/Cirriped. Cop – Art: Copepod/Artemia. Rot – Art: Rotifer/Artemia. Cop – Cir: Copepod/Cirripeds.

Considering individual fatty acids, the DHA concentration in the fish larvae was similar in all groups over time, declining from approx. 30% of the fatty acids at hatch to approx. 18% at day 48 post-hatch. However, the decline in the groups receiving *Artemia* as the second diet was more pronounced. Lower EPA concentrations were also present from day 12 post-hatch on in larvae receiving enriched diets from the beginning, and all groups showed a decrease in EPA when weaned on dry diets.

Lipidomics: The lipid profiles of the fish analysed on the last day of the experiment grouped the samples according to the whole feeding history of the larvae (i.e. treatment), and also according to the second feed type the larvae received (cirripeds or *Artemia*). The two groups receiving copepods as the first feed did not group anymore, suggesting that the lipid profile of the larvae follows the most recent supplied feed and that the lipid signature of the first feed items was too diluted to be detected.

A set of correlations between certain lipid species and the two response variables were revealed by using the lipidomics dataset and response variables such as mortality and growth rates to create a clustered image map (Fig. 8). The monoacylglycerides (MAGs) 18:0, 20:1 and 22:5 showed a strong positive correlation with the mortality coefficient, while several triacylglycerides (TAGs, such as 14:0–16:1–14:0, and three others not further identified TAGs with 44, 46 and 48 carbon atoms) were positively related to growth. Phosphatidylethanolamines (PEs) showed a strong negative correlation with growth rate.

Gene expression measured on the final experimental day (48 days post-hatch) showed pronounced differences between the group started with rotifers and the other three groups which received natural diets as the first feeds (Fig. 9). We found 4000–5000 genes differentially regulated between these groups, and the number of differentially regulated genes diminished when comparing the groups that received cirripeds or copepods (1500–2000) as the first feed items. Interestingly, almost no differences in gene expression were detected comparing the Copepod/*Artemia* and Copepod/Cirriped treatment, even though the fish larvae received very different diets as their second prey item for about two weeks' time (*Artemia* or cirripeds). This points towards a very pronounced effect of the first feeds the larvae received on gene expression patterns several weeks later. The differences in expressed genes between larvae fed on natural (Copepod/Cirriped) or enriched diets (Rotifers/*Artemia*) were most pronounced in genes involved in fatty acid elongation, glycerolipid metabolism, terpenoid synthesis. All genes involved in

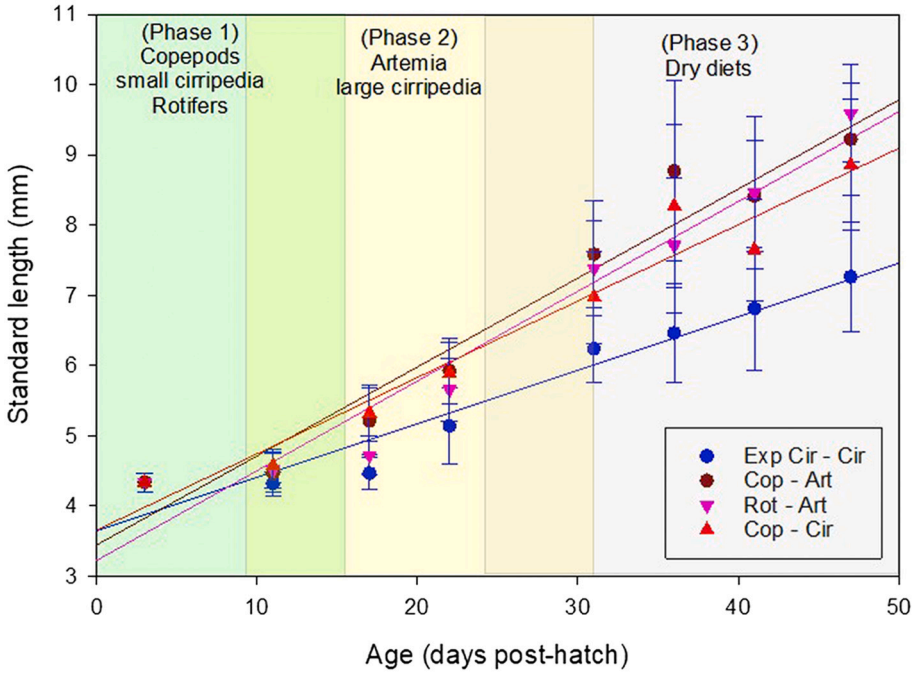

Fig. 2. Development of standard length over time of ballan wrasse larvae fed on 4 different feeding regimes. Solid lines are linear regressions. Error bars are standard deviation of the mean of the triplicate tanks. A minimum of 10 larvae were measured form each replicate at each sampling. Treatment abbreviations: Exp. Cir - Cir: Experimental Cirriped/Cirriped. Cop – Art: Copepod/Artemia. Rot – Art: Rotifer/Artemia. Cop – Cir: Copepod/Cirripeds.

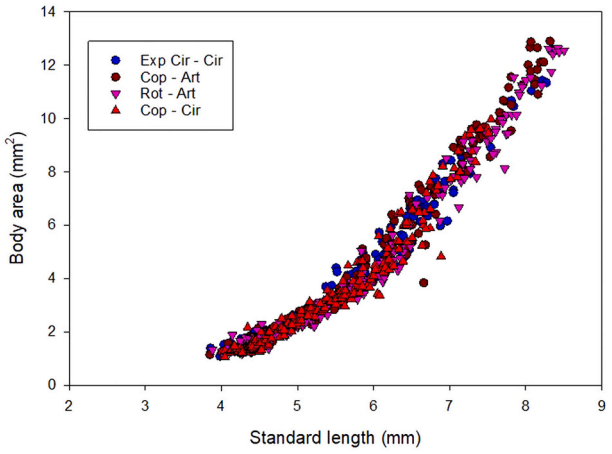

Fig. 3. Relationship between standard lengths and body area of ballan wrasse larvae fed on 4 different feeding regimes. Treatment abbreviations: Exp. Cir - Cir: Experimental Cirriped/Cirriped (n = 197 larvae). Cop – Art: Copepod/Artemia (n = 324 larvae). Rot – Art: Rotifer/Artemia (n = 210 larvae). Cop – Cir: Copepod/Cirripeds (n = 340 larvae).

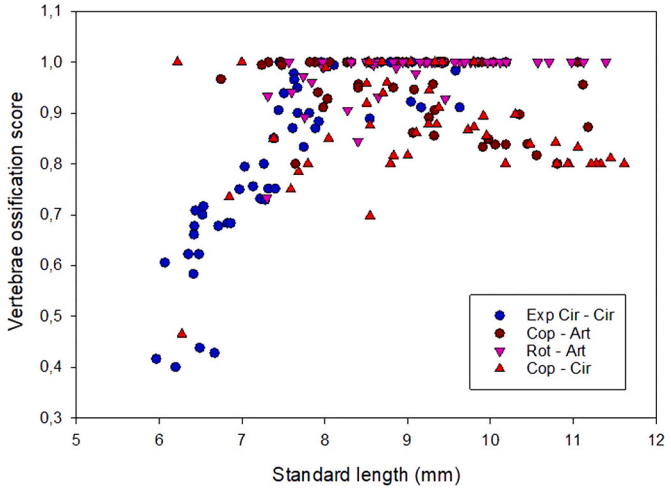

Fig. 4. Ossification score vs. larval standard length of ballan wrasse larvae fed on 4 different feeding regimes 48 days post-hatch. Ossification score is a weighted mean of the ossification status of the vertebral column. Fully ossified vertebrae score 1.0; compact 0.8; partly ossified 0.2; and transparent score 0.0. Treatment abbreviations: Exp. Cir - Cir: Experimental Cirriped/Cirriped. Cop – Art: Copepod/Artemia. Rot – Art: Rotifer/Artemia. Cop – Cir: Copepod/Cirripeds. N = 50 larvae per treatment.

these metabolic pathways were upregulated in larvae fed enriched diets. Expression of genes involved in purin and pyrimidin as well as amino acid metabolisms also showed pronounced differences but, in these pathways, different genes were upregulated in both groups. Comparing larvae fed either exp. Cirriped/Cirriped or Copepod/*Artemia* still revealed in approx. 1500 genes being expressed differently, only few differences in terpenoid synthesis and amino acid metabolism pathways could be detected. Comparing the two groups receiving copepods as their first diets (Copepod/*Artemia*, vs Copepod/Cirripeds) resulted again in approx. 1500 differently expressed genes, but no differences in metabolic pathways could be detected; a demonstration that even 3 weeks after these treatments were no longer receiving copepods, the impact of the first diets was still visible in the larval metabolism.

Microbiology: The beta diversity of the bacterial community of larvae, the different feed items, and water were significantly different from each other (Fig. 10) (PERMANOVA $p < 0.001$). Feed microbiota showed similarities in microbial community to both larvae and water samples (Fig. 10a). Sampling date had a major impact of the microbial community in the water (Fig. 10b), as water was supplied as a flow through and not as a recirculating water supply, and changes can be expected to occur gradually. The microbial community of the larvae themselves differed in alpha diversity. The Exp. Cirriped/Cirriped-treatment differed from the three other treatments, while these

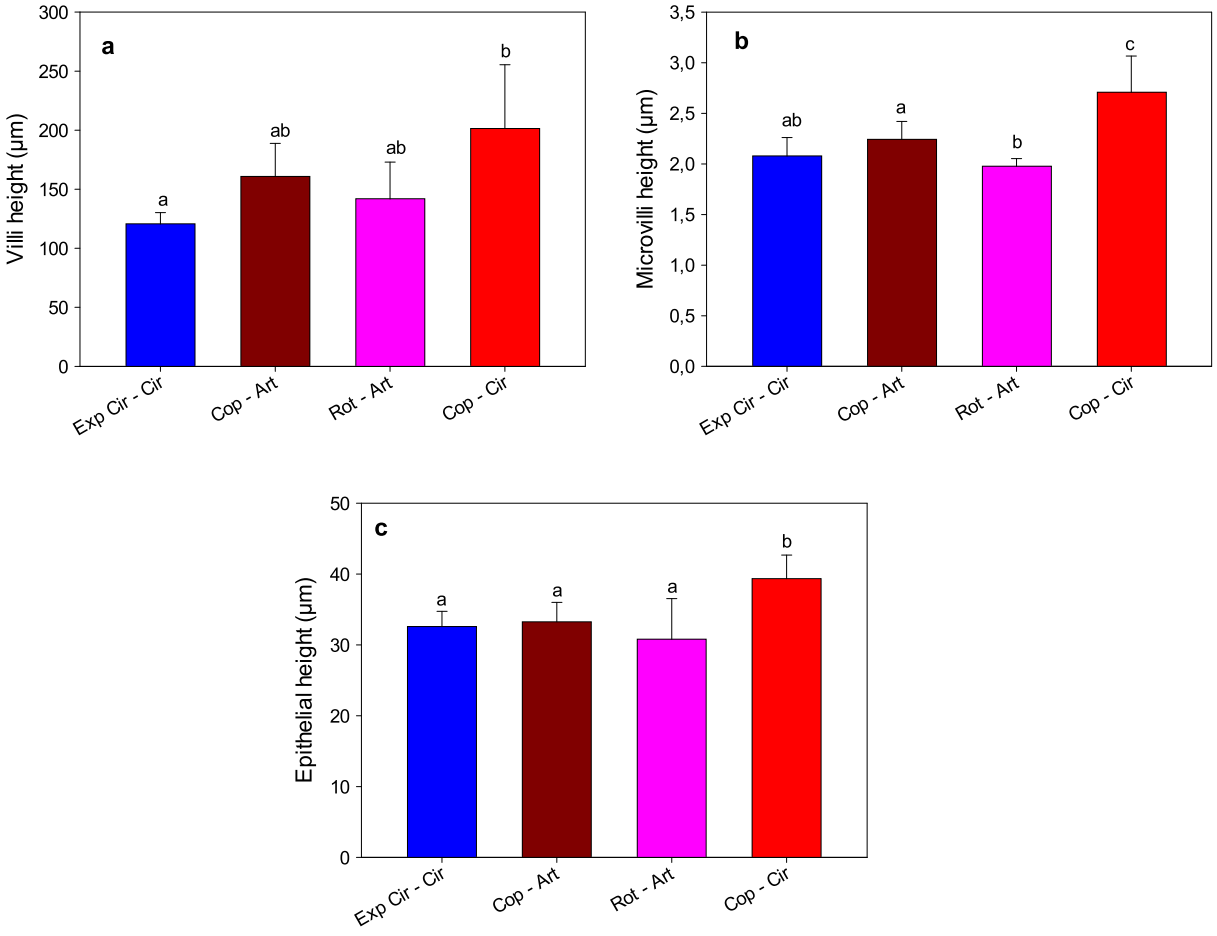

Fig. 5. Gut histology of 48 days post-hatch ballan wrasse larvae reared in four different feeding regimes. (a) Villus height (b) microvillus height, and (c) gut epithelial height. Error bars are standard deviations of the mean of the three replicate tanks. Treatment abbreviations: Exp. Cir - Cir: Experimental Cirriped/Cirriped. Cop – Art: Copepod/Artemia. Rot – Art: Rotifer/Artemia. Cop – Cir: Copepod/Cirripeds. N = 5 larvae.

treatments did not differ from each other (Fig. 11a) (median Shannon diversity of 4.15 vs 2.12, 2.39 and 2.22 for Cirriped/Cirriped, Copepod/*Artemia*, Copepod/Cirriped and Rotifer/*Artemia* treatment, respectively). This pattern was also found in beta-diversity of the microbial community of the larvae in the Exp. Cirriped/Cirriped-treatment being significantly different form the other groups, while the others did not differ (Fig. 11b) (PERMANOVA $p < 0.05$ for Exp. Cirripeds, vs other groups). We only had samples for larval microbiota available from day 32 on, and interestingly, the differences between the treatments are most pronounced on day 32 and diminish towards the end of the experiment. The Copepod/*Artemia*, Rotifer/*Artemia* and Copepod/Cirriped treatment did not differ, suggesting that the first days of Exp. Cirriped diet had a lasting effect on the microbiota that was not overridden by the second feed type until day 36 post-hatch.

As observed already in beta-diversity, microbial community composition showed variations over time for all the treatments (Fig. 12). Copepod/*Artemia* treatment was dominated at DPH 32 by *Sulfitobacter* and *Arcobacter* with 49.1 ± 66.9% and 31.8 ± 45% in relative abundance, respectively, exhibiting large variation between replicates. *Vibrio* was the next dominant genus at that time point (5.8 ± 4.5%). At DPH 37 *Sulfitobacter* remained the most abundant genus in all replicates (79.1 ± 12.5%), showing less variations compared to DPH 32. *Vibrio* followed up, exhibiting abundances of 8 ± 9.9%. By DPH 42, community shift could be observed, with *Vibrio* becoming most dominant genus (41.6 ± 40.8%). *Sulfitobacter* was still abundant at this time point (24.5 ± 1.7%), along *Pseudoalteromonas* which displayed significant increase (10.5 ± 16.8%). Other genera which were abundant included unassigned *Rhodobacteraceae* taxa (2.6 ± 4.5%) and *Aliiroseovarius* (1.6 ± 2.7%). At DPH 48 *Sulfitobacter* dominated community once again, with relative abundances of 19.3 ± 5.7%. It was followed by *Leucothrix* (10.7 ± 9%), *Sphingomonas* (6.6 ± 10.5%), *Vibrio* (3.3 ± 2.1%), *Acinetobacter* (3 ± 5.3%), *Aliiroseovarius* (2.6 ± 4.5%) and *Halocynthiibacter* (2 ± 3.4%).

Copepod/Cirriped treatment was abundant in *Leucothrix* (13.6 ± 11.9%), *Arcobacter* (10.6 ± 15.1%), *Vibrio* (9.8 ± 1.9%), *Pseudoalteromonas* (9.8 ± 2.7%), *Fusibacter* (7.5 ± 12.8%), *Sulfitobacter* (4 ± 3.9%) and unassigned genera of *Saprospiraceae* (3.9 ± 3.4%) and *Rhodobacteraceae* (3.3 ± 0.7%) at DPH 32. *Sulfitobacter* became the most abundant representative by DPH 37 (76.7 ± 12.1%). It was followed by *Psychrobacter* (4.7 ± 6.8%), while other genera remained lower than 3% in mean relative abundance. At DPH 42 *Sulfitobacter* was still dominating the community (58.2 ± 1.2%). *Marinomonas* was the second most abundant representative (5.5% ± 5.3%), which was followed by Vibrio (4% ± 6.7%). Finally, by DPH 48 *Sulfitobacter* remained most abundant genus (28.8 ± 30.1%), followed by *Shewanella* (16.1 ± 27.9%), *Pseudomonas* (8.7 ± 14.6%), *Alishewanella* (8.6 ± 14.2%), *Psychrobacter* (5.4 ± 5%) and *Aeromonas* (3.6 ± 6.2%).

Experimental Cirriped/Cirriped treatment showed abundance of *Sulfitobacter* in fish larvae at DPH 32 (23.7 ± 5.7%), similar to other treatments. *Psychrobacter* was the second most abundant genus across replicates of this treatment (12.2 ± 3.7%), followed by *Vibrio* (10.1 ± 6.4%), unassigned *Rhodobacteraceae* genus (7.3 ± 2.2%), *Aliivibrio* (5.2 ± 3.9%), *Pseudoalteromonas* (4.6 ± 2%), *Leucothrix* (3.9 ± 3%), *Halocynthiibacter* (3.5 ± 0.8%) and unassigned *Saprospiraceae* genus (3.2 ± 1.9%). A shift in community was observed by DPH 37, *Psychrobacter*

Table 2
Fatty acids of the different feeds used in the start feeding experiment with Ballan wrasse. Fatty acids are expressed as % of total fatty acids +/− standard deviation in brackets. Total lipids (last row) are expressed as % of dry weight.

	Artemia	Cirripeds	Experimental cirripeds	Copepod (n4 - n6)	Copepod (n1−n3)	Dry feed	Rotifers
C14:0	1.45 (0.22)	1.59 (0.12)	2.58 (0.24)	1.97 (0.38)	2.69 (0.17)	2.48 (−)	2.82 (0.64)
C14:1n5	0 (0)	0.05 (0.01)	0.06 (0.01)	0.02 (0.03)	0.03 (0.01)	0.06 (−)	0.03 (0)
C15:0	0.12 (0.02)	0.28 (0.13)	0.35 (0.18)	0.2 (0.15)	0.19 (0.2)	0.08 (−)	0.43 (0.02)
C16:0	16.95 (0.32)	14.62 (0.8)	15.11 (0.29)	12.77 (2.39)	12.88 (0.18)	18.9 (−)	19.79 (5.44)
C16:1n5	0.26 (0.01)	0.04 (0.08)	0.11 (0.13)	0 (0)	0.09 (0.13)	0.33 (−)	0 (0)
C16:1n7	5.91 (0.46)	3.86 (0.24)	6.25 (0.09)	1.35 (1.7)	1.31 (0.2)	3.34 (−)	8.34 (3.28)
C16:1n9	0.21 (0.05)	0.17 (0.11)	0.27 (0.07)	0.39 (0.2)	0.27 (0.12)	0.36 (−)	0.6 (0.13)
C17:0	1.24 (0.08)	0.33 (0.02)	0.4 (0.15)	0.22 (0.09)	0.25 (0.01)	0.33 (−)	0.75 (0.18)
C17:1n7	0.2 (0.02)	0 (0)	0.04 (0.07)	0 (0)	0 (0)	0.18 (−)	0 (0)
C18:0	5.09 (0.32)	3.59 (0.5)	4.24 (0.33)	4.2 (0.43)	4.08 (0.68)	3.62 (−)	3.32 (0.48)
C18:1n7	8.02 (0.63)	9.14 (0.45)	7.79 (0.18)	4.17 (4.1)	3.06 (0.27)	3.38 (−)	3.09 (0.66)
C18:1n9	13.52 (0.46)	6.08 (0.25)	8.65 (0.14)	2.28 (2.91)	0.73 (0.31)	14.43 (−)	13.04 (3.81)
C18:2n6	5.46 (0.18)	0.9 (0.06)	1.54 (0.06)	7.01 (4.78)	12.04 (1.94)	26.85 (−)	4.34 (0.4)
C18:3n3	4.91 (0.4)	0.66 (0.04)	0.63 (0.02)	7.16 (4.38)	18.98 (4.47)	3.24 (−)	1.58 (0.51)
C18:3n6	0.33 (0.04)	0.15 (0.04)	0.17 (0.14)	0.36 (0.3)	0.58 (0.72)	0.09 (−)	0.2 (0.02)
C18:4n3	0.02 (0.03)	0.9 (0.61)	1.08 (0.72)	2.04 (1.76)	2.56 (3.5)	0 (−)	0.3 (0.04)
C20:0	0.19 (0.01)	0.12 (0.01)	0.14 (0.01)	0.17 (0.02)	0.7 (0.14)	0.35 (−)	0.18 (0.02)
C20:1n9	0.44 (0.09)	2.71 (0.13)	4.03 (0.12)	0.92 (1.49)	0.15 (0.04)	2.13 (−)	3.05 (0.75)
C20:2n6	0.15 (0)	0.62 (0.02)	0.55 (0.02)	1.54 (0.58)	3.63 (0.59)	0.26 (−)	0.62 (0.09)
C20:3n3	0.18 (0)	0.2 (0.01)	0.14 (0.01)	0.3 (0.08)	0.86 (0.18)	0.16 (−)	0.46 (0.13)
C20:4n3	0 (0)	0.28 (0.19)	0.36 (0.24)	2.73 (4.69)	4.38 (5.43)	0 (−)	1.63 (0.33)
C20:4n6	4.23 (1.93)	1.25 (0.03)	1.18 (0.03)	1.61 (0.25)	2.54 (0.17)	0.99 (−)	2.4 (0.55)
C20:5n3	12.55 (1.03)	31.92 (1.56)	27.82 (1.18)	17.7 (10.79)	7.96 (4.71)	6.97 (−)	4.36 (0.87)
C22:0	0.36 (0.06)	0.11 (0.01)	0.09 (0.02)	0.38 (0.17)	0.63 (0.02)	0.26 (−)	0.13 (0.02)
C22:1n9	0.03 (0)	0.17 (0.02)	0.23 (0.01)	0.09 (0.09)	0.13 (0.14)	0.07 (−)	0.36 (0.07)
C22:5n3	0 (0)	0.36 (0.24)	0.64 (0.23)	0.48 (0.33)	3.85 (4.37)	0 (−)	1.23 (0.27)
C22:5n6	0.03 (0.02)	0.18 (0.12)	0.22 (0.15)	0.83 (0.81)	0.66 (0.84)	0 (−)	9.52 (2.97)
C22:6n3	18.08 (0.31)	19.35 (0.49)	14.92 (0.59)	28.71 (10.58)	14.52 (6.41)	10.8 (−)	16.77 (2.28)
C24:1	0.08 (0.01)	0.37 (0.08)	0.43 (0.03)	0.38 (0.05)	0.25 (0.03)	0.35 (−)	0.65 (0.1)
Sum unsaturated fatty acids	25.4 (0.81)	20.64 (1.39)	22.9 (0)	19.91 (2.54)	21.42 (0.78)	26.02 (−)	28.46 (5.23)
Sum saturated fatty acids	74.6 (0.81)	79.36 (1.39)	77.1 (1.1)	80.09 (2.54)	78.58 (0.78)	73.98 (−)	71.54 (5.23)
Sum n3 fatty aicds	35.73 (1.14)	53.66 (2.33)	45.59 (1.1)	59.13 (7.51)	53.11 (0.19)	21.17 (−)	28.73 (5.42)
Sum n6 fatty aicds	10.21 (1.82)	3.11 (0.07)	3.66 (0.98)	11.35 (5.57)	19.45 (1.13)	28.19 (−)	15.33 (4.72)
EPA/DHA	0.69 (0.05)	1.65 (0.04)	1.86 (0.15)	0.9 (1.06)	0.53 (0.09)	0.65 (−)	0.24 (0.1)
Total lipids (% of dry weight)	21.28 (1.82)	9.56 (0.95)	12.61 (1.2)	9.14 (0.74)	11.69 (3.35)	16.4 (−)	16.25 (1.72)

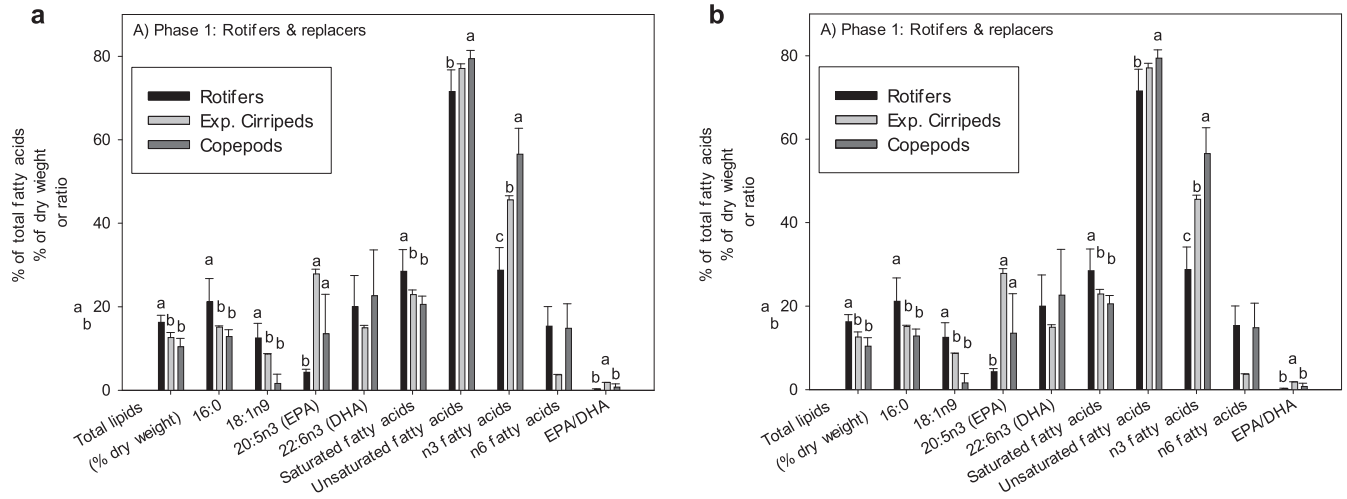

Fig. 6. Total lipids and selected fatty acids or groups of fatty acids of feeds grouped by the period of the experiment they were used. (A) Phase 1 rotifers and rotifer replacers. Letters denotes statistical differences between the feed types within each lipid measure. (B) Phase 2, Artemia and Artemia replacer. Asterisks in Fig. (B) denote statistically significant differences in lipid measures between the feed types.

became the most abundant genus (17.3 ± 7.5%), while *Sulfitobacter* did not exhibit >3% In mean relative abundance across replicates. Other abundant genera included *Psudoaltermonas* (7.1 ± 2.4%), unassigned *Rhodobacteraceae* (5 ± 2%), *Halocynthiibacter* (4.3 ± 7.2%) and *Vibrio* (4.3 ± 2.5%). At DPH 42, *Sulfitobacter* regained dominance (23 ± 27.7%). *Arcobacter* was the second most abundant genus (8.3 ± 6.7%), followed by *Pseudoalteromonas* (7.8 ± 4.5%), *Marinomonas* (5.8 ± 5.3%), *Vibrio* (5 ± 4.4%) and unassigned *Rhodobacteraceae* (3.2 ± 2.8%). There were not enough larvae for microbiological evaluation at DPH 48.

Rotifer/*Artemia* treatment exhibited different composition of the most abundant representatives at DPH 32 compared to other treatments, however only one replicate could be evaluated. *Marinomonas* was the most dominant taxa with 83.2% in relative abundance. It was followed by *Albirhodobacter* (11.5%), while other representatives did not show >3% in mean relative abundance. By DPH 37 *Sulfitobacter* became most

Table 3
Fatty acids of the ballan wrasse larvae fed on 4 different feeding regimes at different ages. Fatty acids are expressed as % of total fatty acids +/− standard deviation in brackets. Total lipids (last row) are expressed as % of dry weight.

Treatment	Experimental Cirripeds - Cirripeds					Rotifers - Artemia				
Age (days post hatch)	4	12	18	32	48	4	12	18	32	48
C14:0	0.73 (0.16)	0.59 (0.01)	0.92 (0.18)	0.88 (0.17)	0.99 (0)	0.56 (0.08)	0.6 (0.11)	0.69 (0.16)	0.75 (0.06)	0.76 (0.25)
C14:1n5	0 (0)	0 (0)	0 (0)	0.01 (0.02)	0 (0)	0 (0)	0 (0)	0 (0)	0.01 (0.02)	0 (0)
C15:0	0.34 (0.04)	0.3 (0)	0.27 (0.15)	0.31 (0.02)	0.31 (0)	0.28 (0.01)	0.31 (0.01)	0.29 (0.17)	0.26 (0.15)	0.31 (0.02)
C16:0	19.39 (0.54)	17.68 (0.3)	18.42 (0.37)	19.98 (0.7)	18.1 (0)	18.73 (0.39)	17.75 (0.41)	17.07 (1.99)	14.47 (0.59)	17.71 (0.71)
C16:1n5	0.21 (0.19)	0 (0)	0.25 (0.02)	0.08 (0.12)	0 (0)	0.2 (0.17)	0.08 (0.14)	0.15 (0.13)	0.15 (0.13)	0 (0)
C16:1n7	1.34 (0.09)	2 (0.06)	2.7 (0.38)	3.12 (0.26)	1.99 (0)	1.26 (0.04)	2.84 (0.14)	3.09 (1.16)	4.33 (0.05)	1.82 (0.15)
C16:1n9	1.64 (0.2)	0.85 (0.02)	0.58 (0.21)	0.23 (0.33)	0.36 (0)	1.54 (0.05)	1.01 (0.05)	0.61 (0.36)	0.47 (0.23)	0.47 (0.03)
C17:0	0.53 (0.04)	0.39 (0.04)	0.56 (0.08)	0.78 (0.15)	0.42 (0)	0.49 (0.04)	0.54 (0.1)	0.96 (0.25)	1.08 (0.14)	0.59 (0.01)
C17:1n7	0.17 (0.17)	0 (0)	0.07 (0.12)	0.51 (0.01)	0.37 (0)	0.23 (0.08)	0.11 (0.11)	0.49 (0.3)	0.83 (0.54)	0.51 (0.06)
C18:0	7.54 (0.22)	10.31 (0.3)	9.92 (0.65)	7.47 (0.36)	7.25 (0)	7.59 (0.12)	10.19 (0.57)	9.66 (1.33)	7.39 (0.63)	7.8 (0.37)
C18:1n7	2.31 (0.02)	4.09 (0.11)	5.05 (1)	8.46 (0.06)	3.13 (0)	2.33 (0.02)	3.07 (0.19)	5.1 (3.96)	8.83 (0.77)	3.51 (0.17)
C18:1n9	11.35 (0.09)	10.2 (0.22)	9.23 (1.2)	9.94 (0.1)	12.33 (0)	11.54 (0.15)	11.42 (0.49)	11.94 (2.63)	14.09 (0.78)	12.47 (0.29)
C18:2n6	3.89 (0.04)	2.11 (0.1)	2.05 (1.19)	0.96 (0.35)	17.41 (0)	3.86 (0.07)	3.14 (0.2)	3.62 (1.01)	4.57 (0.19)	16.11 (1.07)
C18:3n3	0.36 (0.08)	0.32 (0.14)	1.07 (1.42)	0.48 (0.13)	1.58 (0)	0.3 (0.01)	0.63 (0.07)	1.46 (1.7)	3.38 (0.06)	1.51 (0.03)
C18:3n6	0.36 (0.21)	0.15 (0.04)	0.19 (0.04)	0.24 (0.05)	0.91 (0)	0.25 (0.21)	0.14 (0.01)	0.6 (0.7)	0.29 (0.05)	0.32 (0.28)
C18:4n3	0.23 (0.03)	0.5 (0.03)	0.39 (0.34)	0.53 (0.03)	0.95 (0)	0.2 (0.02)	0.25 (0.02)	0.16 (0.15)	0.33 (0.28)	0.58 (0.04)
C20:0	0.17 (0.01)	0.2 (0)	0.2 (0.01)	0.2 (0.01)	0.16 (0)	0.17 (0.01)	0.17 (0.01)	0.18 (0.02)	0.18 (0.01)	0.2 (0.01)
C20:1n9	1.35 (0.01)	1.78 (0.08)	1.85 (0.66)	1.81 (0.03)	1.42 (0)	1.38 (0.02)	1.66 (0.13)	1.3 (0.48)	0.65 (0.09)	1.19 (0.03)
C20:2n6	0.96 (0.03)	0.78 (0.05)	0.75 (0.3)	0.58 (0.14)	0.59 (0)	0.97 (0.01)	0.83 (0.05)	0.57 (0.18)	0.3 (0.05)	0.57 (0.01)
C20:3n3	0.18 (0)	0.13 (0.01)	0.17 (0.14)	0.19 (0.05)	0.22 (0)	0.17 (0.01)	0.27 (0.02)	0.25 (0.02)	0.18 (0.12)	0.14 (0.01)
C20:4n3	0.21 (0.02)	0.16 (0.02)	0.12 (0.11)	1.22 (1.43)	0.49 (0)	0.2 (0.02)	0.67 (0.06)	2.9 (3.88)	2.74 (4.02)	0.19 (0.01)
C20:4n6	3.79 (0.07)	3.88 (0.02)	3.42 (0.27)	1.64 (0.01)	2.04 (0)	3.9 (0.03)	4.76 (0.26)	5.71 (1.37)	6.82 (0.4)	4.07 (0.21)
C20:5n3	10.3 (0.12)	11.73 (0.53)	13.44 (1.28)	17.97 (0.42)	7.59 (0)	10.34 (0.15)	5.76 (0.37)	4.85 (0.94)	9.82 (3.37)	6.53 (0.3)
C22:0	0.08 (0.01)	0.07 (0.01)	0.14 (0.07)	0.22 (0.16)	0.19 (0)	0.08 (0.02)	0.09 (0.03)	0.26 (0.24)	0.44 (0.08)	0.31 (0.03)
C22:1n9	0.07 (0.02)	0.08 (0.01)	0.09 (0.02)	0.23 (0.14)	0.09 (0)	0.05 (0.01)	0.09 (0.01)	0.09 (0.05)	0.03 (0)	0.07 (0)
C22:5n3	1.62 (0.05)	1.23 (0.02)	4.98 (6.14)	1.18 (0.18)	1.1 (0)	1.68 (0.08)	1.54 (0.1)	3.55 (3.25)	2.92 (3.79)	1.04 (0.04)
C22:5n6	0.45 (0.02)	0.46 (0.03)	0.16 (0.23)	0.4 (0.12)	0.79 (0)	0.44 (0.02)	4.76 (0.39)	4.25 (3.67)	3.66 (3.16)	2.8 (0.15)
C22:6n3	30.03 (0.86)	29.59 (0.37)	22.57 (5.03)	19.94 (0.98)	18.81 (0)	30.82 (0.07)	26.76 (3.02)	19.77 (11.51)	10.93 (3.89)	18.09 (0.1)
C24:1	0.41 (0.01)	0.42 (0.01)	0.44 (0.05)	0.46 (0.01)	0.41 (0)	0.44 (0.02)	0.56 (0.04)	0.45 (0.31)	0.1 (0)	0.32 (0.03)
Sum unsaturated fatty acids	28.77 (0.55)	29.54 (0.54)	30.44 (0.26)	29.82 (0.49)	27.41 (0)	27.9 (0.6)	29.65 (0.85)	29.1 (2.89)	24.58 (1.2)	27.68 (0.68)
Sum saturated fatty acids	71.23 (0.55)	70.46 (0.54)	69.56 (0.26)	70.18 (0.49)	72.59 (0)	72.1 (0.6)	70.35 (0.85)	70.9 (2.89)	75.42 (1.2)	72.32 (0.68)
Sum n3 fatty aicds	42.92 (0.68)	43.66 (0.38)	42.75 (1.94)	41.5 (0.9)	30.73 (0)	43.72 (0.31)	35.88 (2.44)	32.94 (1.89)	30.3 (0.43)	28.09 (0.19)
Sum n6 fatty aicds	9.44 (0.13)	7.39 (0.11)	6.57 (1.18)	3.82 (0.66)	21.75 (0)	9.42 (0.28)	13.62 (0.88)	14.74 (1.91)	15.64 (2.57)	23.88 (1)
EPA/DHA	0.34 (0.01)	0.4 (0.02)	0.61 (0.13)	0.9 (0.07)	0.4 (0)	0.34 (0)	0.22 (0.04)	0.41 (0.43)	0.9 (0.05)	0.36 (0.02)
Total lipids (% of dry weight)	20.56 (3)	16.48 (0.64)	17.86 (1.1)	15.62 (0.91)	23.04 (0)	19.66 (2.06)	14.66 (1.03)	16.47 (1.97)	20.45 (0.22)	15.78 (2.67)

Treatment	*Copepods - Artemia*					*Copepods - Cirripeds*				
Age (days post hatch)	*4*	*12*	*18*	*32*	*48*	*4*	*12*	*18*	*32*	*48*
C14:0	0.62 (0.06)	0.54 (0.01)	0.78 (0.13)	0.77 (0.15)	0.73 (0.26)	0.55 (0.15)	0.8 (0.07)	0.76 (0.05)	0.96 (0.2)	0.91 (0.09)
C14:1n5	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0.01 (0.02)	0 (0.01)	0.02 (0.02)	0.01 (0.01)
C15:0	0.35 (0.05)	0.23 (0)	0.21 (0.01)	0.25 (0.14)	0.22 (0.13)	0.29 (0.03)	0.26 (0.04)	0.17 (0.09)	0.23 (0.13)	0.22 (0.12)
C16:0	19.55 (0.8)	18.28 (1.04)	18.58 (0.42)	14.78 (0.24)	17.86 (1.42)	18.92 (1.11)	19.06 (1.15)	18.72 (0.38)	19.79 (1.25)	18.2 (0.42)
C16:1n5	0.31 (0.03)	0 (0)	0.13 (0.11)	0.06 (0.11)	0.08 (0.14)	0.1 (0.17)	0.09 (0.13)	0.19 (0.01)	0.13 (0.12)	0.08 (0.14)
C16:1n7	1.28 (0.02)	1.77 (0.04)	2.26 (0.12)	4.27 (0.1)	1.82 (0.03)	1.24 (0.08)	1.92 (0.01)	2.15 (0.06)	3.2 (0.44)	1.96 (0.04)
C16:1n9	1.51 (0.01)	0.78 (0.13)	0.49 (0.01)	0.45 (0.24)	0.39 (0.1)	1.52 (0.1)	0.77 (0.07)	0.39 (0.09)	0.4 (0.08)	0.31 (0.06)
C17:0	0.5 (0.04)	0.45 (0.01)	1.78 (0.04)	1.06 (0.07)	0.64 (0.22)	0.48 (0.03)	0.97 (0.72)	1.34 (0.69)	0.95 (0.3)	0.42 (0.03)
C17:1n7	0.26 (0.11)	0.11 (0)	0.35 (0.3)	0.78 (0.53)	0.44 (0.32)	0.12 (0.2)	0.07 (0.09)	0.4 (0.24)	0.42 (0.36)	0.24 (0.21)
C18:0	8.06 (0.52)	9.82 (0.74)	8.55 (0.34)	7.6 (0.44)	7.84 (1.26)	7.85 (0.62)	9.45 (1.03)	8.86 (0.48)	8.43 (0.6)	6.81 (0.29)
C18:1n7	2.3 (0.03)	2.39 (0.17)	2.37 (0.01)	8.05 (0.69)	3.65 (0.58)	2.31 (0.02)	2.42 (0.21)	2.42 (0.15)	7.03 (1.36)	3.22 (0.23)
C18:1n9	11.35 (0.34)	7.97 (1.15)	6.08 (0.1)	13.27 (0.86)	12.54 (0.45)	11.45 (0.18)	7.75 (0.32)	6.32 (0.15)	10.61 (0.82)	12.8 (0.28)
C18:2n6	3.84 (0.12)	4.86 (0.06)	5.21 (0.04)	4.49 (0.27)	14.28 (4.92)	3.82 (0.13)	4.97 (0.38)	5.41 (0.5)	2.58 (0.92)	19.1 (0.28)
C18:3n3	0.32 (0.02)	3.02 (0.4)	2.87 (0.06)	3.13 (0.27)	1.45 (0.21)	0.3 (0.01)	3.24 (0.34)	3.02 (0.51)	0.93 (0.24)	1.72 (0.05)
C18:3n6	0.11 (0.1)	0.28 (0.01)	0.33 (0.04)	0.29 (0.05)	0.24 (0.23)	0.18 (0.31)	0.32 (0.01)	0.31 (0.05)	0.26 (0.06)	0.37 (0.23)
C18:4n3	0.21 (0.04)	1.03 (0.16)	0.92 (0.01)	0.29 (0.25)	0.33 (0.31)	0.2 (0.02)	1.19 (0.13)	0.59 (0.53)	0.41 (0.36)	0.56 (0.48)
C20:0	0.18 (0.01)	0.23 (0.01)	0.19 (0.01)	0.18 (0.01)	0.24 (0.05)	0.17 (0.01)	0.21 (0.02)	0.19 (0.01)	0.22 (0.01)	0.2 (0.01)
C20:1n9	1.33 (0.09)	0.77 (0.14)	0.48 (0.01)	0.57 (0.01)	1.15 (0.16)	1.34 (0.04)	0.7 (0.05)	0.46 (0.01)	1.04 (0.46)	1.49 (0.05)
C20:2n6	0.95 (0.04)	1.51 (0.04)	1.67 (0.01)	0.36 (0.02)	0.54 (0.1)	0.97 (0.01)	1.48 (0.13)	1.71 (0.11)	0.81 (0.08)	0.67 (0.03)

(*continued on next page*)

Table 3 (*continued*)

Treatment	Experimental Cirripeds - Cirripeds					Rotifers - Artemia				
Age (days post hatch)	4	12	18	32	48	4	12	18	32	48
C20:3n3	0.18 (0.01)	0.5 (0.04)	0.63 (0.02)	0.24 (0.02)	0.15 (0.01)	0.17 (0)	0.51 (0.06)	0.63 (0.05)	0.29 (0.02)	0.16 (0.01)
C20:4n3	0.19 (0)	0.35 (0.02)	0.33 (0.01)	1.99 (3.03)	0.19 (0.19)	0.23 (0.06)	0.38 (0.03)	0.22 (0.19)	2.17 (3.15)	0.32 (0.28)
C20:4n6	3.77 (0.15)	3.43 (0.51)	2.46 (0.05)	6.36 (0.46)	4.71 (1.74)	3.89 (0.14)	3.23 (0.2)	2.6 (0.17)	2.49 (0.52)	1.75 (0.1)
C20:5n3	10.21 (0.27)	9.77 (0.34)	9.05 (0.18)	9.36 (1.96)	6.51 (0.37)	10.39 (0.36)	9.78 (0.8)	9 (0.77)	9.97 (2.73)	7.9 (0.57)
C22:0	0.09 (0.04)	0.19 (0.02)	0.2 (0.04)	0.4 (0.08)	0.32 (0.07)	0.08 (0.04)	0.2 (0.02)	0.22 (0.06)	0.18 (0.01)	0.18 (0.02)
C22:1n9	0.05 (0.01)	0.04 (0)	0.04 (0.02)	0.03 (0)	0.07 (0.01)	0.05 (0.01)	0.04 (0.01)	0.06 (0.03)	0.1 (0.02)	0.08 (0)
C22:5n3	1.63 (0.03)	1.17 (0.11)	1.03 (0.01)	2.64 (3.4)	3.57 (4.51)	1.62 (0.04)	1.14 (0.07)	4.38 (5.81)	3.62 (4.49)	0.66 (0.57)
C22:5n6	0.28 (0.25)	0.93 (0.01)	1.17 (0.02)	3.6 (3.12)	2.26 (2.17)	0.44 (0.01)	0.92 (0.07)	0.77 (0.63)	0.67 (0.61)	0.58 (0.5)
C22:6n3	30.14 (0.48)	29.23 (3.68)	31.53 (0.25)	14.62 (3.43)	17.41 (4.59)	30.89 (0.75)	27.8 (3.91)	28.41 (5.66)	21.63 (2.09)	18.74 (0.71)
C24:1	0.41 (0.05)	0.33 (0.04)	0.33 (0.02)	0.11 (0.01)	0.35 (0.02)	0.42 (0.02)	0.31 (0.04)	0.31 (0.03)	0.44 (0.05)	0.36 (0.01)
Sum unsaturated fatty acids	29.36 (1.42)	29.74 (1.76)	30.3 (0.47)	25.05 (0.55)	27.86 (0.12)	28.34 (1.6)	30.95 (1.46)	30.25 (0.84)	30.76 (1.36)	26.93 (0.66)
Sum saturated fatty acids	70.64 (1.42)	70.26 (1.76)	69.7 (0.47)	74.95 (0.55)	72.14 (0.12)	71.66 (1.6)	69.05 (1.46)	69.75 (0.84)	69.24 (1.36)	73.07 (0.66)
Sum n3 fatty aicds	42.89 (0.78)	45.08 (3.86)	46.36 (0.3)	32.27 (1.09)	29.61 (0.51)	43.8 (1.04)	44.04 (2.47)	46.24 (0.99)	39.02 (3.94)	30.05 (0.18)
Sum n6 fatty aicds	8.95 (0.4)	11.02 (0.5)	10.84 (0.08)	15.09 (2.43)	22.03 (1.52)	9.31 (0.5)	10.92 (0.79)	10.81 (0.08)	6.82 (1.89)	22.47 (0.82)
EPA/DHA	0.34 (0)	0.34 (0.05)	0.29 (0.01)	0.64 (0.02)	0.4 (0.14)	0.34 (0.01)	0.36 (0.08)	0.33 (0.1)	0.46 (0.11)	0.42 (0.01)
Total lipids (% of dry weight)	23.84 (6.65)	14.98 (0.05)	15.42 (0.98)	18.8 (0.68)	14.94 (1.26)	22.18 (7.03)	18.6 (8.89)	14.46 (0.89)	15.75 (0.68)	18.7 (1.2)

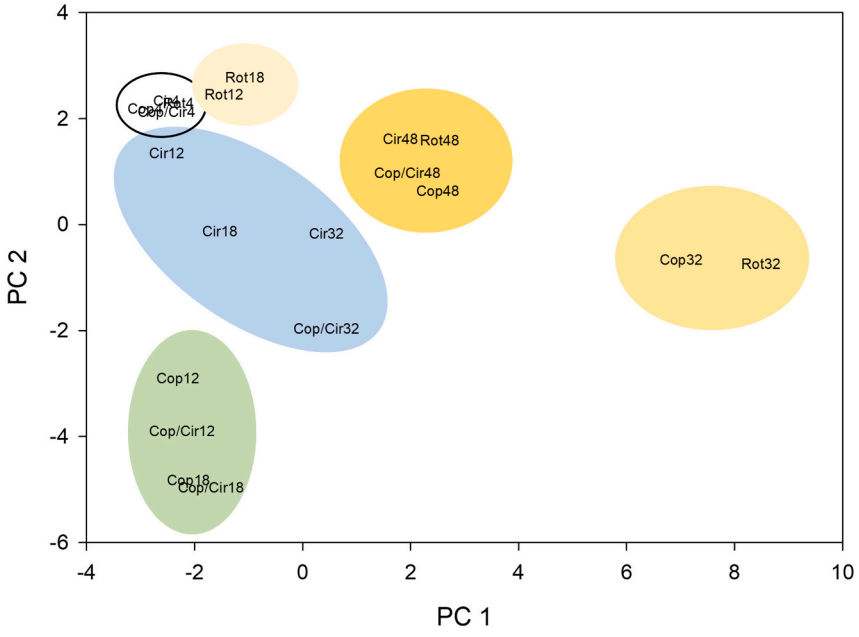

Fig. 7. Principal component analysis based on fatty acid profiles. The shaded background represents the feeds the fish received prior to sampling. Colour code is the same as in Table 1. Light yellow: Rotifers, yellow: Artemia, dark yellow: Dry feeds, Blue: Cirripeds, green: Copepods. Black circle encircles yolk sac larvae at day 4 post-hatch before the first feeding. Sample abbreviations: Cir: Experimental Cirriped/Cirriped. Cop: Copepod/Artemia. Rot: Rotifer/Artemia. Cop/Cir: Copepod/Cirripeds. The numbers following the treatment abbreviation is age in days post-hatch. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

abundant, reaching 54.2 ± 24% in relative abundance. *Marinomonas* was still abundant (8.7 ± 14%), and followed by *Oceanobacter* (7.7 ± 11.4%), *Arcobacter* (7.2 ± 11.5%) and *Pseudoalteromonas* (4.2 ± 6.2%). *Sulfitobacter* was still the most abundant genus by DPH 42 (50.9 ± 31.7%), as well as *Marinomonas* the next abundant genus (13.5 ± 15.9%). They were followed by *Maribacater* (12.7 ± 17.9%), *Oceanobacter* (4 ± 5.6%) and *Dokdonia* (3.7 ± 4.8%). By DPH 48, *Marinomonas* was dominating the microbial community (36.2 ± 48.2%). Unassigned *Rhodobacteraceae* showed to be next abundant genus (9.2 ± 11.5%), followed by *Aliiroseovarius* (6.9 ± 9.8%), *Litoreibacter* (4.3 ± 6.1%), Vibrio (4 ± 5.5%), *Psychrobacter* (3.8 ± 1.7%), *Amylbacter* (3.8 ± 5.3%), and *Sulfitobacter* (3.2 ± 2.4%).

4. Discussion

Larval fish display notoriously high mortality rates and understanding causes and effects of mortality is a formidable challenge in fisheries biology (Houde, 2008). The main agents acting on larval fish mortality in nature are nutrition, predation (Cury and Roy, 1989; Cushing, 1974; Lasker, 1981) and microbiota (Vadstein et al., 2013) and these are likely interconnected. Predation can be neglected in most experimental studies, leaving nutrition and host – microbiota interactions as the main factors explaining mortality in experiments. The large differences in mortality in our experiment were treatment (i.e. nutrition) dependent. Such results are in the range of what has been reported previously for comparable experiments in which larvae fed copepods showed higher survival rates (Støttrup and Norsker, 1997; Øie et al., 2017). This is usually explained by the fact that almost all marine

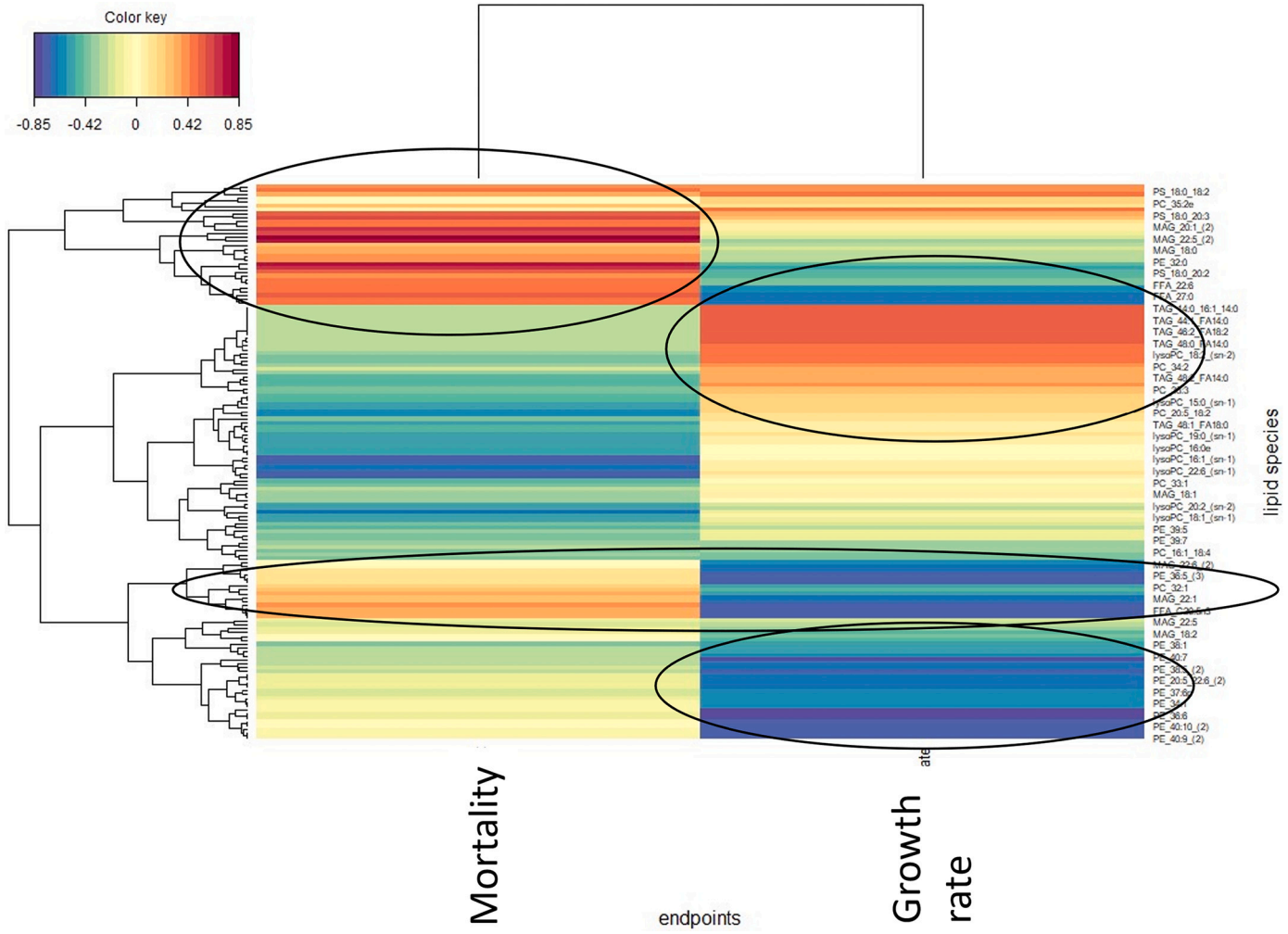

Fig. 8. Clustered image map of the lipidomics dataset analysed on larvae sampled on the last day of the experiment (day 48 post-hatch) and the corresponding mortality rates and growth rates of larvae fed on four different feeding regimes.

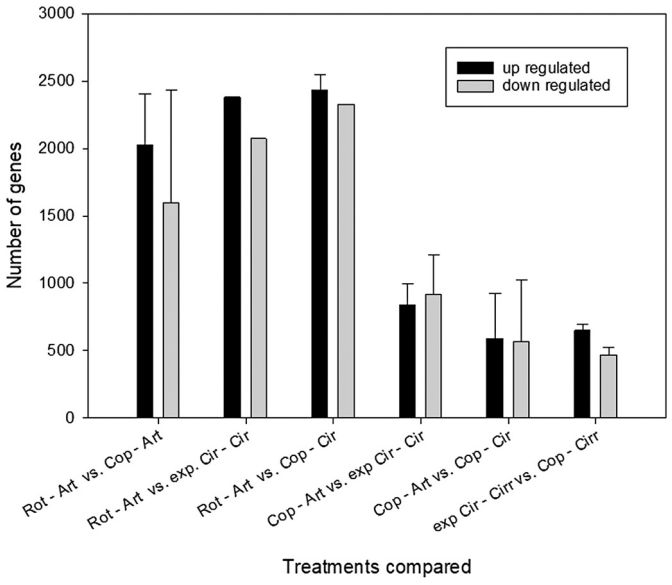

Fig. 9. Differential gene expression of larval ballan wrasse fed on 4 different feeding regimes on day 48 post-hatch. Treatment abbreviations: Exp. Cir - Cir: Experimental Cirriped/Cirriped. Cop – Art: Copepod/Artemia. Rot – Art: Rotifer/Artemia. Cop – Cir: Copepod/Cirripeds.

fish larvae feed on copepods during the first days of feeding, and that they are adapted to the nutritional composition of copepods. Further, the erratic movement of the copepods triggers a hunting response in many fish larvae. We expected similar high survival for the group started on the Exp. Cirripeds, as these nauplii also form a part of the natural diet of larval fish, but we found significantly higher mortality rates compared to the copepod started groups. This might be explained by an underfeeding of the larvae; contrasting to our expectations, the Experimental cirriped diet was not purely comprised of *B. crenatus* nauplii but contained approx. 50% nauplii of *B. balanus*. The latter species' nauplii are larger measuring approximately 190 μm in width and might have been too large to be ingested by the ballan wrasse larvae during the first days after initiation of feeding. Consequently, only one third of the supplied biomass of the Exp. cirriped diet was in the correct size range for the larvae.

The different feeding regimes used in the current study had pronounced effects on fish performance. Fish receiving copepods as their first diet showed significantly higher survival rates than those fed on either rotifers or experimental cirripeds for the first weeks of their lives. Length data presented by Hansen et al. (2013) were in a similar range as those presented here until approximately day 40 post-hatch, when the larvae in the Hansen study showed a steep increase in growth rates, which the authors explained as a response to successful weaning onto dry diets. This effect was not present in our study, and our larvae remained smaller, which might be explained by the higher and constant temperatures Hansen et al. used. This difference resulted in

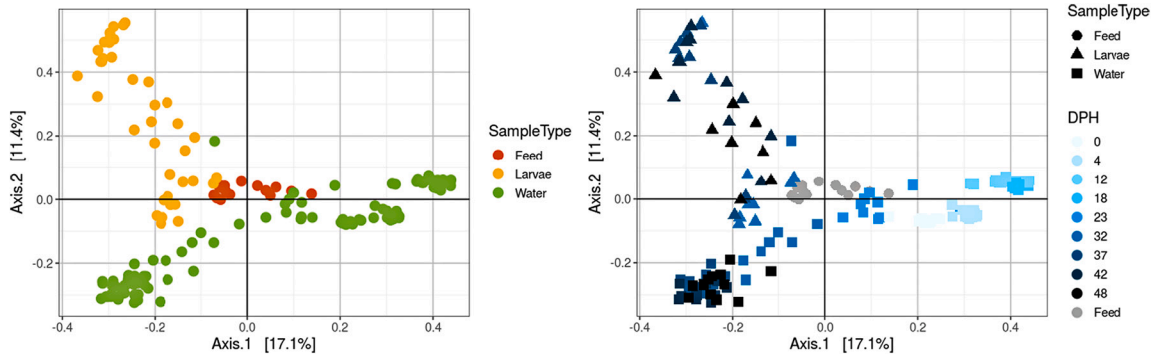

Fig. 10. Beta diversity of the microbial community of larvae, feed, and water. (A) By sample type, and (B) by sampling date.

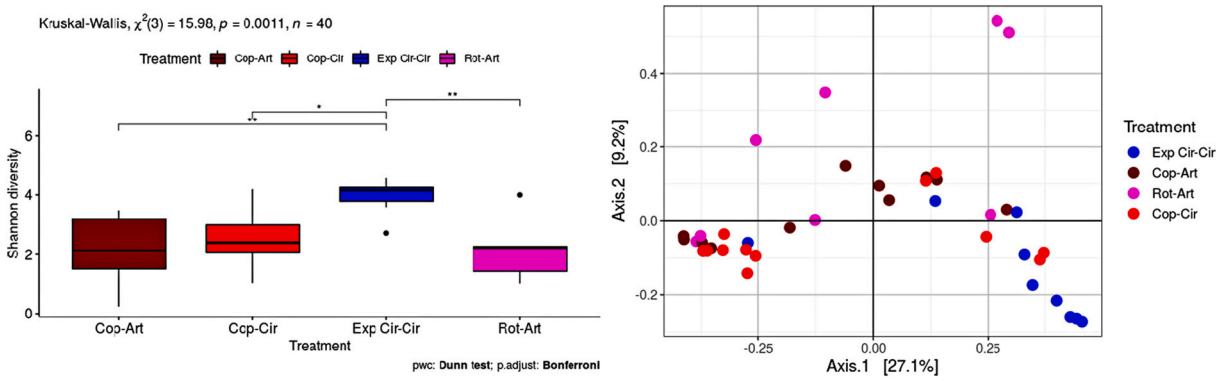

Fig. 11. Microbial alpha- (A) and beta- (B) diversity between treatments of ballan wrasse larvae samples with all replicates combined regardless of DPH. Alpha-diversity is represented by Shannon diversity index, while beta-diversity is based on Bray-Curtis dissimilarities. Treatment abbreviations: Exp. Cir - Cir: Experimental Cirriped/Cirriped. Cop – Art: Copepod/Artemia. Rot – Art: Rotifer/Artemia. Cop – Cir: Copepod/Cirripeds.

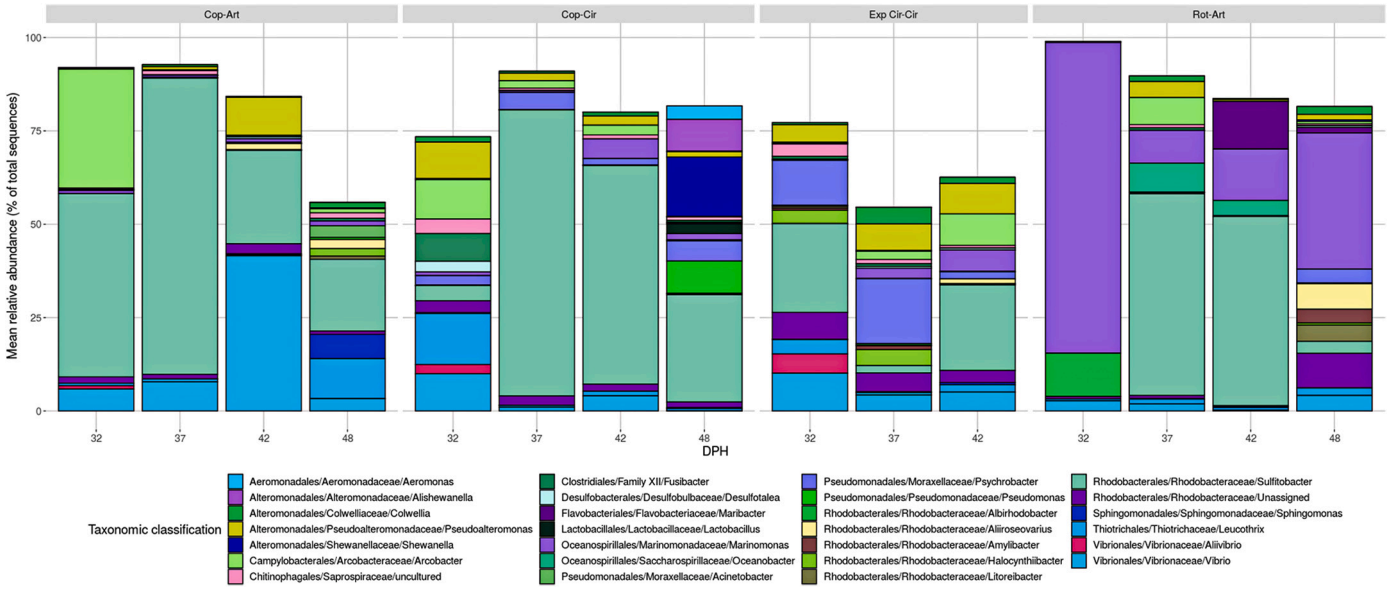

Fig. 12. Microbial community composition of larvae at cut-off of 7% in relative abundance. Stacked bars represent mean value of each taxon between replicates of specific treatment and DPH. Each treatment is represented by triplicates at corresponding DPH, except for Copepod/Artemia at DPH 31 represented by duplicates, and Rotifer/Artemia at DPH 31 and DPH 47 represented by a single sample and duplicates, respectively.

approximately 100 degree-days more during a 48-day experimental phase of the Hansen et al. experiment. The poor growth performance of the Exp. Cirriped started group might be explained by the same argument as for the high mortality rates; larvae not being able to ingest approximately 50% of the diets offered due to gape limitation.

We found no differences in body area or length-weight relationships between the treatments. This resembles the description of growth patterns by Gagnat et al. (2016), who studied ballan wrasse larval growth in relation to diet in detail. In that study, all fish followed the same growth pattern, irrespectively of feeding history, with a slow increase of body

mass (expressed in Gagnat et al. (2016) as volume) followed by a steeper increase in body mass after flexion at around 6 mm SL.

Vertebrae ossification was dependent on the length of the fish with only a minor influence from the feeding regimes. Ossification started at around 6 mm and was completed at a length of around 10 mm SL. This was comparable to other studies on ballan wrasse (Sørøy, 2012); however, the authors report no differences in ossification relating to diets. We did find a delay in vertebrae ossification, especially in the largest larvae from the groups started with copepods. On average, fish reached 6 mm at around day 25 post-hatch, which was at the time when dry diets were introduced. Obviously, faster growing larvae reached the 6 mm threshold for ossification earlier, during the phase of the second live feed (*Artemia* or cirripeds). The main minerals necessary for ossification are phosphorus, magnesium, and calcium (Toppe et al., 2007), and while magnesium and calcium can be taken up from the surrounding seawater, phosphorus needs to be taken up with the diet. Phosphorus levels in formulated diets are usually rather high (e.g. the Gemma micro we used contains 1.3% P), while enriched *Artemia* might contain an order of magnitude less phosphorus (0.08–0.15% of body mass using several different enrichments products reported by de Azevedo et al., 2021). It might well be possible that the ossification of the fastest growing larvae was limited by phosphorus availability when feeding on copepods, cirripeds or *Artemia*, while slower growing larvae reached the onset of ossification when high phosphorus diets in the form of formulated diets were abundant. Indeed, it has been shown that growth in larval fish (Boersma et al., 2008; Malzahn et al., 2007) and lobster larvae (Schoo et al., 2012; Schoo et al., 2014) can be limited by phosphorus supplied through copepods. This would also explain the higher incidence of bone deformations recorded in the copepod groups (data not shown), as these are often explained by a lack of phosphorus at critical periods (Kousoulaki et al., 2021).

At the end of the experiment, larvae from the Copepod/Cirriped treatment excelled in both gut and liver histology, followed by the Copepod/*Artemia* group. The higher surface area found in the gut might have resulted in a higher nutrient absorption and higher energy availability, ultimately enabling the larvae to store energy as glycogen in their livers, while the other groups probably may not have had the resources to store energy. Feed dependent differences in energy storage was also reported for larval cod. When fatty acids were supplied as polar lipids, cod showed a higher nutrient utilization and a higher energy storage compared to larvae which were supplied with the same fatty acids as neutral lipids (Wold et al., 2009). Such results indicate that minute differences in nutrition early in the life of a fish can set the direction for later performance, and such an advantage can further be cemented by the second diet.

An animal's fatty acid composition usually reflect that of its diet, and in ecological studies fatty acids are a widely used tool to reconstruct feeding history (Perga et al., 2006) and habitat use (Hielscher et al., 2015). In the present study, the fish samples grouped well considering the diets the fish received during the last weeks, demonstrating the high rates of changes in body composition in larval fish. However, the main role of fatty acids is not their usefulness as biomarkers. Fatty acids are functional biochemicals, which have been linked to many different traits. The role of essential long chained highly unsaturated fatty acids such as EPA and DHA during early nutrition of fishes has been studied for a long time (Watanabe, 1982). Bell et al. (1995) e.g. reported the importance of DHA supply, as DHA is a prominent fatty acid in the rods of the fish eye, and hence important in vision, relating to the ability of herring to successfully hunt in twilight. Further, high concentrations in DHA have been shown to counteract bone deformities. In turn, EPA is important for growth in larval Pacific cod (*Gadus macrocephalus*) (Cahu et al., 2003). Cahu et al. highlighted the importance of not only considering individual fatty acids but considering them in concert. A ratio of PUFAs matching the demand of an organism is very important, due to the competitive nature of fatty acid uptake (Sargent et al., 1999), and the ratio between ω6 and ω3 fatty acids is important for robustness, immune response, skeletal development and disease resistance in fish (Bou et al., 2017; Bou et al., 2020; Martinez-Rubio et al., 2012; Martinez-Rubio et al., 2014; Ytteborg et al., 2010). Several polyunsaturated or monounsaturated fatty acids regulate different immunological processes, including eicosanoid synthesis, formation of lipid peroxides, regulation of gene expression, apoptosis, alteration of antigen presentation, or modulation of intestinal microbiota (Puertollano et al., 2008). DHA/EPA ratios above 4 have been reported to increase growth, survival and stress resistance in Pacific cod (*Gadus macrocephalus* (Choi et al., 2021). In our experiment, we only found such high ratios in the enriched rotifers (4) while copepod nauplii were around 2 and cirripeds around 0.5, which was due to their richness in EPA. Sargent et al. (1999) reported DHA/EPA around 2 being optimal for seabass and turbot, hence the high ratio suggested by Choi et al. (2021) might be either species specific, or simply be reported too high, especially as El-Sabaawi et al. (2009) suggested lower values for Pacific cod than for Atlantic cod.

Fatty acids such as EPA and ARA form the base of eicosanoids lipid mediators, which have been of significant interest in the study of immunity-lipid interactions. Eicosanoids (i.e., prostaglandins, tromboxane, prostacyclins and leukotrienes) have multiple effects on immune cells, most notably in the regulation of the onset and resolution of inflammation, immune responses, and tissue repair (Joshua, 2008; Serhan et al., 2000). Hence, it is useful to not only focus on fatty acids, but on the entirety of lipid species.

Though the importance of dietary fatty acids on immunity and health in general is largely known, there is little evidence on how the entirety of lipid species affect early life stages of fish. The lipidomics approach revealed that certain sets of lipid species correlated with growth and survival of ballan wrasse. Several diacylglycerides correlated positively with growth rates, which can be explained as the largest larvae in the experiments, which are those with the highest growth rates, were able to store energy as TAG. Unfortunately, the method we applied does not identify all different lipids, but often results in finding e.g., a TAG consisting of 48C-atoms with 2 double bounds. A more refined instrumentation and method can give deeper insights in individual lipid species. To our knowledge, this is the first time that lipidomics have been applied to larval ballan wrasse. Two recent studies were able to link stress response of cod to lipidomics (Dale et al., 2020; Khan et al., 2020), and Yan and *co*-workers have been able to show a response of the lipid profiles of yellow croaker (*Larimichthys polyactis*) and Japanese sea bass (*Lateolabrax japonicus*) to a tropical storm (Yan et al., 2012). It seems that there is a high potential in lipidomics approaches, and we just made a first step in unravelling the role of the lipidome in shaping the condition of larval fish. Such knowledge can then be considered in greater detail to gain a more mechanistic understanding on larval fish nutrition.

In relation to lipids, the gene expression results of this study might take us a step further. The main differences in gene expression could be found between the groups started on rotifers, which were enriched with a commercial enrichment product, and larvae which received natural diets (copepods and experimental cirripeds). Within the latter group we found considerably less differences in gene expression. Interestingly, there was a pronounced difference in gene expression of genes involved in fatty acid elongation, which were upregulated in the Rotifer/*Artemia* group. Such differences in gene expression reflect the differences of the lipids the larvae were supplied with. Natural diets such as copepod or cirriped nauplii contain large amounts of fatty acids in the polar lipid fraction (Bell and Sargent, 2003; Tocher et al., 2008), while commercial enrichments usually supply large amounts of neutral lipids, and this is even worse as e.g. *Artemia* tend to convert polar lipids supplied with enrichments into neutral lipids (Guinot et al., 2013). Hence, the challenge of handling lipids from different sources (enrichments vs. natural zooplankton) was still visible in the metabolic fingerprint of the larvae. Maybe even more interesting was that we found no effect on gene expression patterns from the second diet the larvae received, namely enriched *Artemia* or natural cirripeds. This indicates the very important role of the first diet a fish receives.

The microbial fingerprint of water and larvae differed significantly, the feed being the connecting element in this study. Within the water samples, the influence of the sampling date was as pronounced as the influence of the treatment (i.e., feed added several times per day). Additionally, the use of flow through water supply interfered with the establishment of a specific microbiota community. This usually results in a less stable microbial community, as new bacteria are introduced to the tank at all times, while the tank itself hosts a rich environment for the new arriving bacteria to thrive (Attramadal et al., 2014). However, it could also be argued that the different feed types added to the water might have favoured different microbial communities. Vadstein et al. (2018) reasoned that each introduction of feed is an introduction of microbiota itself, but also a perturbation in nutrient availability. We did enter different feed types during the first month of the experiment; however, the influence of the bacteria coming into the experiment with the water partially overruled this influence. An example of how constant water inflow into nutrient rich tank influenced larval microbiota can be observed through genus *Sulfitobacter*, which was very abundant initially in the inflow water (>30% in relative abundance, data not shown), but less in feed (<12%). *Sulfitobacter* became the most abundant representative of larval microbiota later in the experiment (DPH > 32), regardless of the treatment, reaching abundances as high as 79% of the whole community. Microbial colonization of newly hatched larvae seems to be driven by the availability of bacteria rather than a selective force regulating the community structure (Vadstein et al., 2013) hence, the first feed in our experiment should have provided the initial inoculum, as observed in the case of *Sulfitobacter*. On the other hand, shifts in community composition in tank water could be observed after every feed change (data not shown), supporting the hypothesis that the feed is influencing community composition of closed systems. Members of the genus *Nautella* was an example of microbial transfer from feed to the closed system. *Nautella* was very abundant in *Artemia* feed (>45% of relative abundance, data not shown), and less so in the Copepod/*Artemia* and the Rotifer/*Artemia* treatment, before the addition of sole *Artemia* feed to the tanks (DPH 23). Although, the late introduction of *Nautella* through *Artemia* significantly influenced the tank water microbiota, it seemed not to influence larvae microbiota. The larvae indeed differed significantly in their microbiota, with the Exp. Cirriped/Cirriped group having a more diverse microbial community. This is in such a way remarkable as we only have microbiology data on fish from day 32 post hatch on, so we can assume that the effect of first feed microbiota can still be seen 10 days after the last dose of the Exp. Cirriped diet. This means that 10 days of feeding a common diet to the larvae in the Exp. Cirripeds/Cirriped and in the Copepod/Cirriped treatment did not result in a common microbial community. Indeed, there is good indication that the microbiome of fish can be stable over time, even if strong perturbations such as oil contaminations are applied (Bagi et al., 2018) or toxic algal blooms surround the fish (Duan et al., 2020). However, our results suggest that with the ongoing experiment, the microbial fingerprints of the four different treatments are getting more similar, suggesting that the use of dry diets had a pronounced effect on the microbial community of the larvae.

5. Conclusion & recommendation

Based on our results, a feeding regime for ballan wrasse should replace rotifers as the first live feed type with copepods, as the copepod started groups showed the highest growth and survival rates. The second diet could be cirripeds or *Artemia*. Cirripeds showed comparable growth to *Artemia*, but the gut and liver histology suggests better digestive capacity and energy storage. Further, the gene expression patterns suggest that cirripeds as the second diet resemble copepods, so no major reworking of genes expressed seemed necessary. Weaning should be handled more dynamically in ballan wrasse than we did in this experiment (initiated weaning at day 32 post-hatch, irrespective of larval growth). As the size variation can be great within a population of larvae in general, and ballan wrasse in particular, larvae might reach the flexion stage earlier than expected. Larvae reaching 6 mm standard length the earliest in our experiment were less ossified, which might be an effect of phosphorus or other mineral deficiencies.

CRediT authorship contribution statement

Arne M. Malzahn: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision. **Deni Ribičić:** Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing. **Bjørn Henrik Hansen:** Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Antonio Sarno:** Data curation, Visualization. **Elin Kjørsvik:** Conceptualization, Supervision, Writing – review & editing. **Anna Sigrid Norberg Aase:** Investigation, Writing – review & editing. **Luciana Alves Musialak:** Investigation, Writing – review & editing. **Laura García-Calvo:** Investigation, Writing – review & editing. **Andreas Hagemann:** Project administration, Funding acquisition, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

Arne M. Malzahn reports financial support was provided by Norwegian Seafood Research Fund.

Acknowledgements

We thank FHF (Norwegian Seafood Research Fund, Norway) for funding of the work (FHF #901561). The project was carried out within the framework of the national research infrastructure "Norwegian Center for Plankton Technology" funded by the Norwegian Research Council (#245937/F50). We thank Ann Isabell Rosvoll for DNA and RNA extraction. We also thank the two anonymous reviewers for their constructive criticism.

The study complies with the Norwegian Animal Welfare Act and was approved by the Norwegian Animal Research Authority (#23020).

References

Abbott, S.K., Jenner, A.M., Mitchell, T.W., Brown, S.H.J., Halliday, G.M., Garner, B., 2013. An improved high-throughput lipid extraction method for the analysis of human brain lipids. Lipids. 48, 307–318.

Attramadal, K.J.K., Truong, T.M.H., Bakke, I., Skjermo, J., Olsen, Y., Vadstein, O., 2014. RAS and microbial maturation as tools for K-selection of microbial communities improve survival in cod larvae. Aquaculture. 432, 483–490.

Bagi, A., Riiser, E.S., Molland, H.S., Star, B., Haverkamp, T.H.A., Sydnes, M.O., Pampanin, D.M., 2018. Gastrointestinal microbial community changes in Atlantic cod (*Gadus morhua)* exposed to crude oil. BMC Microbiol. 18, 25.

Balon, E.K., 1985. Microscopic techniques for studies of early ontogeny in fishes: Problems and methods of composite descriptions. In: Balon, E.K. (Ed.), Early Life Histories of Fishes: New Developmental, Ecological and Evolutionary Perspectives. Dr. W. Junk Publishers, Lancaster, pp. 33–35.

Balon, E.K., 1999. Alternative ways to become a juvenile or a definitive phenotype (and on some persisting linguistic offenses). Environ. Biol. Fish 56, 17–38.

Bell, J.G., Sargent, J.R., 2003. Arachidonic acid in aquaculture feeds: current status and future opportunities. Aquaculture. 218, 491.

Bell, M., Batty, R., Dick, J., Fretwell, K., Navarro, J., Sargent, J., 1995. Dietary deficiency of docosahexaenoic acid impairs vision at low light intensities in juvenile herring (*Clupea harengus* L.). Lipids. 30, 443.

Boersma, M., Aberle, N., Hantzsche, F.M., Schoo, K.L., Wiltshire, K., Malzahn, A.M., 2008. Nutritional limitation travels up the food chain. Int. Rev. Hydrobiol. 93, 479–488.

Bou, M., Berge, G.M., Baeverfjord, G., Sigholt, T., Østbye, T.K., Ruyter, B., 2017. Low levels of very-long-chain n-3 PUFA in Atlantic salmon (*Salmo salar*) diet reduce fish robustness under challenging conditions in sea cages. J. Nutr. Sci. 6, e32.

Bou, M., Torgersen, J.S., Østbye, T.-K.K., Ruyter, B., Wang, X., Škugor, S., Todorčević, M., 2020. DHA modulates immune response and mitochondrial function of Atlantic salmon adipocytes after LPS treatment. Int. J. Mol. Sci. 21, 4101.
Bromke, M.A., Hochmuth, A., Tohge, T., Fernie, A.R., Giavalisco, P., Burgos, A., Brotman, Y., 2015. Liquid chromatography high-resolution mass spectrometry for fatty acid profiling. Plant J. 81, 529–536.
Cahu, C., Infante, J.Z., Takeuchi, T., 2003. Nutritional components affecting skeletal development in fish larvae. Aquaculture. 227, 245–258.
Choi, J., Han, G.S., Lee, K.W., Byun, S.-G., Lim, H.J., Lee, C.-H., Kim, H.S., 2021. Effects of feeding differentially enriched *Artemia nauplii* on the survival, growth, fatty acid composition, and air exposure stress response of Pacific cod (*Gadus macrocephalus*) larvae. Aquacult. Rep. 21, 100829.
Cury, P., Roy, C., 1989. Optimal environmental window and pelagic fish recruitment success in upwelling areas. Can. J. Fish. Aquat. Sci. 46, 670–680.
Cushing, D.H., 1974. The natural regulation of fish populations. In: Harden Jones, F.R. (Ed.), Sea Fisheries Research. Paul Elek, London, pp. 399–412.
Dale, K., Yadetie, F., Müller, M.B., Pampanin, D.M., Gilabert, A., Zhang, X., Lyche, J.L., 2020. Proteomics and lipidomics analyses reveal modulation of lipid metabolism by perfluoroalkyl substances in liver of Atlantic cod (*Gadus morhua*). Aquat. Toxicol. 227, 105590.
Daukšas, E., Falch, E., Šližytė, R., Rustad, T., 2005. Composition of fatty acids and lipid classes in bulk products generated during enzymic hydrolysis of cod (*Gadus morhua*) by-products. Process Biochem. 40, 2659–2670.
de Azevedo, A.M., Losada, A.P., Ferreiro, I., Riaza, A., Losada, V., Russo, T., Quiroga, M. I., 2021. Skeletal anomalies in Senegalese sole (*Solea senegalensis*, Kaup) fed with different commercial enriched artemia: a study in postlarvae and juveniles. Animals. 11, 22.
Duan, Y., Xiong, D., Li, Y., Dong, H., Wang, W., Zhang, J., 2020. Changes in the gastrointestinal microbial community of *Lateolabrax maculatus* in a naturally occurring *Microcystis aeruginosa* bloom environment. Aquaculture. 528, 735444.
El-Sabaawi, R., Dower, J.F., Kainz, M., Mazumder, A., 2009. Interannual variability in fatty acid composition of the copepod *Neocalanus plumchrus* in the Strait of Georgia, British Columbia. Mar. Ecol. Prog. Ser. 382, 151–161.
Fall, C.H.D., Kumaran, K., 2019. Metabolic programming in early life in humans. Philos. Trans. R. Soc. Lond. B Biol. Sci. 374, 20180123.
Fitzgibbon, A.W., Fisher, R.B., 1995. A buyer's guide to conic fitting. In: Pycock, D. (Ed.), BMVC '95: Proceedings of the 6th British Conference on Machine Vision, Birmingham, U.K, pp. 513–522.
Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.
François, N.R.L., Jobling, M., Carter, C., 2010. Finfish Aquaculture Diversification. CABI, Oxfordshire, U.K.
Gagnat, M.R., Wold, P.-A., Bardal, T., Øie, G., Kjørsvik, E., 2016. Allometric growth and development of organs in ballan wrasse (*Labrus bergylta*, Ascanius, 1767) larvae in relation to different live prey diets and growth rates. Biol. Open. 5, 1241–1251.
Giavalisco, P., Li, Y., Matthes, A., Eckhardt, A., Hubberten, H.M., Hesse, H., Willmitzer, L., 2011. Elemental formula annotation of polar and lipophilic metabolites using ^{13}C, ^{15}N and ^{34}S isotope labelling, in combination with high-resolution mass spectrometry. Plant J. 68, 364–376.
Guinot, D., Monroig, Ó., Hontoria, F., Amat, F., Varó, I., Navarro, J.C., 2013. Enriched on-grown *Artemia metanauplii* actively metabolise highly unsaturated fatty acid-rich phospholipids. Aquaculture. 412-413, 173–178.
Hansen, T.W., Folkvord, A., Grøtan, E., Sæle, Ø., 2013. Genetic ontogeny of pancreatic enzymes in Labrus bergylta larvae and the effect of feed type on enzyme activities and gene expression. Comp. Biochem. Physiol. B. 164, 176–184.
He, K., Gkioxari, G., Dollár, P., Girshick, R., 2017. Mask r-cnn. In: Proceedings of the IEEE International Conference on Computer Vision, pp. 2961–2969.
Hielscher, N.N., Malzahn, A.M., Diekmann, R., Aberle, N., 2015. Trophic niche partitioning of littoral fish species from the rocky intertidal of Helgoland, Germany. Helgol. Mar. Res. 69, 385–399.
Hou, Z., Fuiman, L.A., 2019. Nutritional programming in fishes: insights from mammalian studies. Rev. Fish Biol. Fish. 30, 67–92.
Houde, E.D., 1987. Fish early life dynamics and recruitment variability. Am. Fish. Soc. Symp. 2, 17–19.
Houde, E.D., 2008. Emerging from Hjort's shadow. J. Northwest Atl. Fish. Sci. 41, 53–70.
Imsland, A.K., Foss, A., Koedijk, R., Folkvord, A., Stefansson, S.O., Jonassen, T.M., 2006. Short- and long-term differences in growth, feed conversion efficiency and deformities in juvenile Atlantic cod (*Gadus morhua*) startfed on rotifers or zooplankton. Aquac. Res. 37, 1015–1027.
Joshua, A.B., 2008. Eicosanoids in asthma, allergic inflammation, and host defense. Curr. Mol. Med. 8, 335–349.
Jouhet, J., Lupette, J., Clerc, O., Magneschi, L., Bedhomme, M., Collin, S., Rebeille, F., 2017. LC-MS/MS versus TLC plus GC methods: consistency of glycerolipid and fatty acid profiles in microalgae and higher plant cells and effect of a nitrogen starvation. PLoS One 12, e0182423.
Karlsen, Ø., van der Meeren, T., Rønnestad, I., Mangor-Jensen, A., Galloway, T.F., Kjørsvik, 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, 27 pages.
Khan, E.A., Zhang, X., Hanna, E.M., Bartosova, Z., Yadetie, F., Jonassen, I., Arukwe, A., 2020. Quantitative transcriptomics, and lipidomics in evaluating ovarian developmental effects in Atlantic cod (*Gadus morhua*) caged at a capped marine waste disposal site. Environ. Res. 189, 109906.
Kjørsvik, E., Olsen, C., Wold, P.-A., Hoehne-Reitan, K., Cahu, C.L., Rainuzzo, J., Olsen, Y., 2009. Comparison of dietary phospholipids and neutral lipids on skeletal development and fatty acid composition in Atlantic cod (*Gadus morhua*). Aquaculture. 294, 246–255.
Kotani, T., Yokota, M., Fushimi, H., Watanabe, S., 2011. How to determine the appropriate mortality in experimental larval rearing? Fish. Sci. 77, 255–261.
Kousoulaki, K., Grøtan, E., Kortner, T.M., Berge, G.M., Haustveit, G., Krogdahl, Å., Lein, I., 2021. Technical feed quality influences health, digestion patterns, body mineralization and bone development in farming of the stomachless cleaner fish ballan wrasse (*Labrus bergylta*). Anim. Feed Sci. Technol. 274, 114830.
Kvæstad, B., Hansen, B.H., Davies, E.J., 2022. Automated morphometrics on microscopy images of ichthyio- and zooplankton using Mask R-CNN and classical machine vision techniques. MethodsX. 9, 101598.
Lasker, R., 1981. The role of a stable ocean in larval fish survival and subsequent recruitment. In: Lasker, R. (Ed.), Marine Fish Larvae, Morphology, Ecology and Relation to Fisheries. Washington Sea Grant Program, Seattle, WA, pp. 80–87.
Leggett, W.C., Deblois, E., 1994. Recruitment in marine fishes: is it regulated by starvation and predation in the egg and larval stages? Netherl. J. Sea Res. 32, 119.
Lund, I., Skov, P.V., Hansen, B.W., 2012. Dietary supplementation of essential fatty acids in larval pikeperch (*Sander lucioperca*); short and long term effects on stress tolerance and metabolic physiology. Comp. Biochem. Physiol. A Mol. Integr. Physiol. 162, 340–348.
Malzahn, A.M., Aberle, N., Clemmesen, C., Boersma, M., 2007. Nutrient limitation of primary producers affects planktivorous fish condition. Limnol. Oceanogr. 52, 2062–2071.
Martinez-Rubio, L., Morais, S., Evensen, Ø., Wadsworth, S., Ruohonen, K., Vecino, J.L.G., Tocher, D.R., 2012. Functional feeds reduce heart inflammation and pathology in Atlantic salmon (*Salmo salar* L.) following experimental challenge with Atlantic salmon reovirus (ASRV). PLoS One 7, e40266.
Martinez-Rubio, L., Evensen, Ø., Krasnov, A., Jørgensen, S.M., Wadsworth, S., Ruohonen, K., Tocher, D.R., 2014. Effects of functional feeds on the lipid composition, transcriptomic responses and pathology in heart of Atlantic salmon (*Salmo salar* L.) before and after experimental challenge with Piscine Myocarditis Virus (PMCV). BMC Genomics 15, 462.
Metian, M., Troell, M., Christensen, V., Steenbeek, J., Pouil, S., 2020. Mapping diversity of species in global aquaculture. Rev. Aquacult. 12, 1090–1100.
Moser, H.G., 1984. Ontogeny and systematics of fishes. Am. Soc. Ichthyol. Herpetol. Spec. Publ 760.
Norberg Aase, A.S., 2022. Nutritional condition of Ballan wrasse (*Labrus bergylta*) larvae reared on different live feed diets. Biology Department, NTNU, Trondheim, Norway.
Øie, G., Galloway, T., Soroy, M., Hansen, M.H., Norheim, I.A., Halseth, C.K., Kjørsvik, E., 2017. Effect of cultivated copepods (*Acartia tonsa*) in first-feeding of Atlantic cod (*Gadus morhua*) and ballan wrasse (*Labrus bergylta*) larvae. Aquac. Nutr. 23, 3–17.
Perga, M.E., Kainz, M., Matthews, B., Mazumder, A., 2006. Carbon pathways to zooplankton: insights from the combined use of stable isotope and fatty acid biomarkers. Freshwat. Biol. 51, 2041–2051.
Puertollano, M.A., Puertollano, E., Alvarez de Cienfuegos, G., Pablo, M., 2008. Dietary lipids, modulation of immune functions, and susceptibility to infection. Nutr. Ther. Metabol 26.
Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture. 177, 191–199.
Schoo, K.L., Aberle, N., Malzahn, A.M., Boersma, M., 2012. Food quality affects secondary consumers even at low quantities: an experimental test with larval European lobster. PLoS One 7, e33550.
Schoo, K.L., Aberle, N., Malzahn, A.M., Schmalenbach, I., Boersma, M., 2014. The reaction of European lobster larvae (*Homarus gammarus*) to different quality food: effects of ontogenetic shifts and pre-feeding history. Oecologia. 174, 581–594.
Serhan, C.N., Clish, C.B., Brannon, J., Colgan, S.P., Chiang, N., Gronert, K., 2000. Novel functional sets of lipid-derived mediators with antiinflammatory actions generated from omega-3 fatty acids via cyclooxygenase 2-nonsteroidal antiinflammatory drugs and transcellular processing. J. Exp. Med. 192, 1197–1204.
Sørøy, M.O., 2012. Dietary effects of different live prey on growth and functional development in ballan wrasse (*Labrus bergylta*) larvae and juveniles. Biology Institute, NTNU, Trondheim, Norway, p. 83.
Støttrup, J.G., Norsker, N.H., 1997. Production and use of copepods in marine fish larviculture. Aquaculture. 155, 231–247.
Suzuki, S., 1985. Topological structural analysis of digitized binary images by border following. Comput. Graph. 30, 32–46.
Takeda, H., Izumi, Y., Takahashi, M., Paxton, T., Tamura, S., Koike, T., Shiomi, M., 2018. Widely-targeted quantitative lipidomics method by supercritical fluid chromatography triple quadrupole mass spectrometry. J. Lipid Res. 59, 1283–1293.
Tocher, D.R., Bendiksen, E.A., Campbell, P.J., Bell, J.G., 2008. The role of phospholipids in nutrition and metabolism of teleost fish. Aquaculture. 280, 21–34.
Toppe, J., Albrektsen, S., Hope, B., Aksnes, A., 2007. Chemical composition, mineral content and amino acid and lipid profiles in bones from various fish species. Comp. Biochem. Physiol. B. 146, 395–401.
Tsugawa, H., Ohta, E., Izumi, Y., Ogiwara, A., Yukihira, D., Bamba, T., Arita, M., 2015. MRM-DIFF: data processing strategy for differential analysis in large scale MRM-based lipidomics studies. Front. Genet. 5, 471.
Vadstein, O., Bergh, Ø., Gatesoupe, F.-J., Galindo-Villegas, J., Mulero, V., Picchietti, S., Bossier, P., 2013. Microbiology and immunology of fish larvae. Rev. Aquacult. 5, S1–S25.
Vadstein, O., Attramadal, K.J.K., Bakke, I., Olsen, Y., 2018. K-selection as microbial community management strategy: a method for improved viability of larvae in aquaculture. Front. Microbiol. 9.
Vo, T.A., Galloway, T.F., Bardal, T., Halseth, C.K., Oie, G., Kjørsvik, E., 2016. Skeletal muscle growth dynamics and the influence of first-feeding diet in Atlantic cod larvae (*Gadus morhua* L.). Biology Open 5, 1575–1584.

von Herbing, I.H., Boutilier, R.G., Miyake, T., Hall, B.K., 1996. Effects of temperature on morphological landmarks critical to growth and survival in larval Atlantic cod (*Gadus morhua*). Mar. Biol. 124, 593–606.
Walne, P.R., 1970. Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria, and Mytilus. Fish. Investig. 26, 162.
Watanabe, T., 1982. Lipid nutrition in fish. Comp. Biochem. Physiol. B. 73, 3–15.
Wold, P.-A., Hoehne-Reitan, K., Cahu, C.L., Infante, J.Z., Rainuzzo, J., Kjørsvik, E., 2009. Comparison of dietary phospholipids and neutral lipids: effects on gut, liver and pancreas histology in Atlantic cod (*Gadus morha* L.) larvae. Aquac. Nutr. 15, 73–84.
Woodfield, H.K., Cazenave-Gassiot, A., Haslam, R.P., Guschina, I.A., Wenk, M.R., Harwood, J.L., 2018. Using lipidomics to reveal details of lipid accumulation in developing seeds from oilseed rape (*Brassica napus* L.). Biochim. Biophys. Acta 1863, 339–348.
Xuan, Q., Hu, C., Yu, D., Wang, L., Zhou, Y., Zhao, X., Xu, G., 2018. Development of a high coverage pseudotargeted lipidomics method based on ultra-high performance liquid chromatography–mass spectrometry. Anal. Chem. 90, 7608–7616.
Yan, X., Xu, J., Chen, J., Chen, D., Xu, S., Luo, Q., Wang, Y., 2012. Lipidomics focusing on serum polar lipids reveals species dependent stress resistance of fish under tropical storm. Metabolomics. 8, 299–309.
Ytteborg, E., Vegusdal, A., Witten, P.E., Berge, G.M., Takle, H., Østbye, T.-K., Ruyter, B., 2010. Atlantic salmon (*Salmo salar*) muscle precursor cells differentiate into osteoblasts in vitro: polyunsaturated fatty acids and hyperthermia influence gene expression and differentiation. Biochim. Biophys. Acta 1801, 127–137.
Zambonino-Infante, J.-L., Cahu, C., 2010. Effect of nutrition on marine fish development and quality. Rec. Adv. Aquacul. Res. 103–124.
Zhang, T.Y., Suen, C.Y., 1984. A fast parallel algorithm for thinning digital patterns. Commun. ACM. 27, 236–239.