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RESEARCH

# The effect of different live feed organisms and formulated diet on lumpfish (*Cyclopterus lumpus*) larval development: part 1—effects on growth, liver, bone development, lipids, and microbiology

**Elin Kjørsvik[1] · Tu Anh Vo[1] · Frank Thomas Mlingi[1] · Luciana Alves Musialak[1] · Tora Bardal[1] · Sunniva Brevik Kværnø[1] · Marte Solli Lindskog[1] · Saba Akbar[1] · Ibon García Gallego[2] · Arne Malzahn[3] · Dag Altin[1] · Bjørn Henrik Hansen[3] · Deni Ribičić[3] · Andreas Hagemann[3]**

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## Abstract

This study assessed the impact of different start-feeding regimes on lumpfish (*Cyclopterus lumpus*) larvae until 35 days post-hatch. Diets included enriched *Artemia* nauplii, cryopreserved cirriped nauplii (*Semibalanus balanoides*), cultivated copepodites (*Acartia tonsa*), formulated feed, and combinations thereof. Enriched *Artemia* yielded the best outcomes—fast early growth, with the largest larvae, most normal ossification, and the highest survival at the end of the experiment. Cryopreserved cirripeds from the start led to poor initial growth, but growth improved during later stages, and larval size and normal ossification were significantly better in the end than for larvae fed copepodites and formulated feed from the start. Copepodites and formulated feed resulted in poor nutritional status (starvation) from the start and with smaller size and frequent bone anomalies at the end. The largest live prey (*Artemia*, cirripeds) contained high levels of dietary PUFA n-3 fatty acids and supported the most normal development. They were also easiest to capture, and prey size and easy capture seemed important for the substrate-dwelling early start-feeding lumpfish larvae. Liver vacuolization served as a reliable diet-quality biomarker. Microbial patterns varied significantly across treatments, indicating active regulation of the larval microbiome by feed and water interactions. Our recommendation based on the results is the start-feeding of lumpfish larvae with enriched *Artemia* nauplii and optional co-feeding with cirripeds.

**Keywords** Lumpsucker · Larviculture · Formulated diet · *Artemia* · Copepods · Cirripeds · Liver biomarker

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Extended author information available on the last page of the article

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## Introduction

The use of lice-eating cleaner fish is considered one of the most sustainable methods for controlling sea lice infestations in salmon aquaculture. Norway is the world's leading producer of Atlantic salmon (*Salmo salar*), with an annual production of around 1.5 million tonnes (Directorate of Fisheries 2025). However, parasitic sea lice such as *Lepeophtheirus salmonis* and *Caligus elongatus* remain a major obstacle to increasing production in salmon farms (Costello 2006; Svåsand et al. 2016). Historically, chemical therapeutants have been widely used to combat sea lice, but their application has led to increased tolerance of sea lice populations and caused harmful effects on non-targeted species (Egidius & Møster 1987; Salte et al. 1987; Bjordal 1991; Åen et al. 2015). These issues have raised production costs and negatively impacted fish health and welfare in both farmed and wild salmon populations (Pike 1989; Costello 2009a,b).

To address these challenges, marine species such as lumpfish (*Cyclopterus lumpus*) and ballan wrasse (*Labrus bergylta*) are now mainly used as cleaner fish in sea-based salmon farming. The lumpfish, a cold-water species native to the North Atlantic Ocean (Davenport 1985), remains active and feeds at cold temperatures common along the Norwegian coast (Nytrø et al. 2014). In contrast, ballan wrasse are not effective delousers below 6 °C (Sayer and Reader 1996; Kelly et al. 2014). The commercial production of lumpfish has grown significantly, from fewer than 1 million juveniles in 2012 to 15–32 million juveniles per year between 2020 and 2024 (Directorate of Fisheries 2025). Lumpfish are ready for deployment after about 4 months, compared to 1.5 years for wrasses (Helland et al., 2014; Powell et al. 2018).

One of the main bottlenecks in lumpfish production is the start-feeding of larvae (Powell et al. 2018). Lumpfish hatch from demersal eggs (2.0–2.6 mm in diameter) and are relatively large and more developed at hatching compared to pelagic fish larvae such as ballan wrasse or the Atlantic cod (*Gadus morhua*) (Brown 1986; Kjørsvik et al. 2004). Newly hatched larvae attach to substrates and can ingest formulated diets from the start (Benfey and Methven 1986). In commercial settings, larvae are typically fed formulated diets from 2 to 5 days post hatching (dph) (Dahle et al. 2017). However, their nutritional requirements remain poorly understood, with production often resulting in variable growth, survival, and delousing efficiency (Treasurer 2018).

Developing suitable diets for marine fish larvae is challenging due to their lack of a functional stomach (Kjørsvik et al., 2011). Fish larvae have a very high growth potential, and the functional development of fish larvae is closely related to larval size (Pittman et al. 2013). Rapid growth during the early larval phase is critical for survival, access to a wider size range of prey, improved swimming ability, and reducing the predation risk (Houde 1987; Leggett and Deblois 1994). This period also involves key developmental processes such as maturation of the digestive system and ossification of the skeleton (Blaxter 1988; Wold et al. 2008; Pittman et al. 2013). In nature, lumpfish larvae primarily feed on crustaceans, selecting larger prey as they grow (Ingólfsson and Kristjánsson 2002). Natural live plankton is not an option for most hatcheries, and frozen plankton often fails to trigger feeding behavior (Yúfera 2011). While *Artemia* improved growth compared to formulated diets (Brown 1986; Nytrø et al. 2014), high mortalities may occur during weaning from *Artemia* to formulated diets around 25–30 dph (Powell et al. 2018).

The nutritional composition of marine larval diets strongly influences survival, growth, and skeletal development, and especially the initial live prey period seems critical (Hou and Fuiman 2020). Natural zooplankton or copepods generally outperform traditionally

live feeds such as rotifers and *Artemia* (Sargent et al. 1999a, b a,b; Shields et al. 1999; Rajkumar, 2006; Karlsen et al. 2015; Øie et al. 2017). Suboptimal diets may cause deformities (Barahona-Fernandes, 1982; Cobcroft and Battaglene 2013; Kitajima et al. 1994; Cahu et al. 2003a,b; Sæle et al. 2003; Boglione et al. 2013; Mazurais et al. 2008; Fontagné et al. 2009; Kjørsvik et al. 2009), impaired muscle development (Vo et al. 2016; 2022), and altered behavior and metabolism (Rønnestad et al. 2013), with long-term effects on growth, health, and welfare (Imsland et al. 2006; Koedijk et al. 2010; Vo et al. 2022).

Marine fish larvae require dietary marine phospholipids and highly unsaturated fatty acids (HUFAs), particularly DHA and EPA (Watanabe & Kiron 1994; Rainuzzo et al. 1992; 1997; Izquierdo et al. 2000; Tocher et al. 2008; Olsen et al. 2014) incorporated in the dietary polar lipids (Cahu et al. 2003a; Gisbert et al. 2005; Kjørsvik et al. 2009; Wold et al. 2009). These requirements seem especially pronounced in cold-water species, which show higher deformity rates when fed suboptimal diets (Mejri et al. 2021).

Marine copepods contain relatively much higher levels of protein and of n-3 highly unsaturated fatty acids (HUFAs) in their phospholipid fraction than enriched *Artemia* (Evjemo et al. 2003; Hamre 2006; Van der Meeren et al. 2008), suggesting potential benefits for lumpfish larvae. However, results are mixed; some studies report improved growth with copepods (Dahle et al. 2017), while others found *Artemia* superior (Marthinsen et al., NTNU, unpublished). Cirripede larvae (*Semibalanus balanoides*), which are naturally available to marine fish larvae (López 2012), have recently been tested as a new processed prey type. They are larger than copepod nauplii and rich in essential fatty acids and protein (Malzahn et al. 2022).

This study aimed to evaluate the effects of different start-feeding strategies—including formulated diets, enriched *Artemia* nauplii, cryopreserved cirripede nauplii (*Semibalanus balanoides*), cultivated copepods (*Acartia tonsa*), and mixed feeding regimes and co-feeding feeds—on the growth and survival of lumpfish larvae. We also assessed biological markers such as skeleton and liver development, fatty acid composition, and microbial parameters.

## Materials and methods

To evaluate the effect of a formulated diet and different live feed species on growth, survival, biochemistry, and microbiology of lumpfish larvae, we conducted a 35-day experiment from start-feeding to successful weaning.

### Larval rearing conditions

Egg incubation and larval rearing were carried out at NTNU Centre of Fisheries and Aquaculture (SeaLab) in Trondheim, Norway. Unfertilized lumpfish eggs from eight females and milt from one male were provided by MoreFish AS (Tjeldbergodden, Norway). The eggs were mixed and fertilized and then distributed equally to 15 incubators for demersal eggs (FT family hatcher, FishTech AS, Norway) with a continuous flow of natural seawater (34 ppt salinity and 10 °C) and complete darkness (0:24 light/dark). The egg incubators were designed with an upwelling current and an outlet on top, allowing newly hatched larvae at approximately 300 day-degrees (d°) to go with the flow directly into the rearing tanks (flat-bottomed cylindrical tanks), aiming for a density of 100 larvae $L^{-1}$. The mean number of

larvae per tank was around 7000 + 2000 at the start of the experiment. Larval survival was estimated from counts of dead and surviving larvae throughout the experiment.

Each larval tank contained 100 L of seawater (34 ppt salinity) with a water depth of 35 cm. The seawater was pumped from 70 m depth in Trondheimsfjorden and filtered through a sand filter, stored in a reservoir with a moving bed biofilter for about 24 h, and filtered through a 1 µm filter. Water exchange rate and outlet mesh size were adjusted according to feed types: for copepods, 3–6 tank volumes $day^{-1}$ and 100 µm; for cirripeds, 12 tank volumes $day^{-1}$ and 350 µm; for *Artemia* and formulated diet, 24 tank volumes $day^{-1}$ and 750 µm. The tanks were aerated from the bottom through an air hose attached to the water outlet in each tank. Oxygen saturation was measured every day (ProODO Optical Dissolved Oxygen Instrument, YSI, USA) and kept above 80%, water temperature was about 10 °C throughout the experiment and was measured daily (2000 T Digital Thermometer, Digitron, England). To provide additional attaching surface area for the larvae, two or three hanging grey silicon mats (ca. 15 × 20 cm) were added in the tanks from 2 to 24 dph. Continuous lighting was provided by fluorescent LED ("cool white") tubes above the tanks for the whole experimental period. The maximum light intensity experienced by the larvae in the tanks was in the range of 1.8–2.0 µmol photons $m^{-2}$ $s^{-1}$ (400–700 nm, measured at the surface; QSL100, Biospherical Instruments, San Diego, CA, USA). This corresponds to 130 to 150 lx applying a conversion factor of 74 for "cool white" fluorescent tubes (https://www.apogeeinstruments.com/conversion-ppfd-to-lux/).

Dead fish, excess feed, and floc were removed once per day when larvae were fed live preys, and twice per day when larvae were fed formulated diet from 4 to 35 dph. A mechanical motorized inbuilt cleaning arm was used to rotate and collect dirt from the tank bottom and along the walls from 8 to 35 dph, which was then siphoned off.

## Diets and larval feeding

*Artemia*: *Artemia* cysts (EG ® INVE Aquaculture, Belgium) were incubated in 60 L cylindro-conical tanks, with heavily aerated seawater and a temperature of 25–28 °C (Dhont and van Stappen, 2003). After 24 h, newly hatched *Artemia* were separated from unhatched cysts and hatching debris, following INVE's protocols and transferred to an enrichment tank at a maximum density of 300 nauplii $mL^{-1}$. The culture was enriched with 10 g Multigain (BioMar AS, Norway) per 60 L of seawater. After 18 h, another dose of the same concentration was added, and *Artemia* was rinsed and transferred to the larval feed reservoirs after 24 h of enrichment.

Cirripeds: Frozen cubes of "CryoPlankton Large," containing cirripede nauplii (*Semibalanus balanoides*) and cryoprotectant agent (CPA), were stored in a Dewar flask with liquid nitrogen (at − 196 °C). Following the producer's protocol (Planktonic AS, Trondheim, Norway), the frozen cubes were thawed in seawater and then transferred to a plankton net (mesh 100 µm) to rinse off the CPA. The cirripede nauplii were then transferred to a cylindro-conical revitalization tank with 50–55 L heavily aerated seawater in a temperature regulated room at 5 °C. The cirripede nauplii were left in the revitalization tank for a minimum of 6 h before transfer to the feed reservoirs, according to the CryoPlankton user's manual (https://planktonic.no/wp-content/uploads/2022/10/Planktonic_brosjyre_6s_A4_2022.pdf).

Copepods: Cultures of *Acartia tonsa* (clone DFH.AT1) copepodites (stages CIV–CVI) and microalgae *Rhodomonas baltica* (clone NIVA 5/91) were delivered four times, in 1000 L tanks and in 20 L plastic flasks, respectively, from C-Feed AS (Vanvikan, Norway)

to NTNU Centre of Fisheries and Aquaculture (SeaLab) in Trondheim, Norway. Copepodites were stored in the delivered tanks in a cold room (5 °C) in 3 to 5 days. The density of copepods in the tank was counted every day to estimate the total volumes needed for harvest using a sieve fitted with plankton net mesh (53 µm) according to the feeding requirement. The harvested copepods were fed *R. baltica* before being added to the feed reservoirs for the fish larval tanks. The copepod storage tanks were also replenished with seawater and fed *R. baltica* after each harvest to sustain the culture.

Formulated diet (FD): The commercial formulated startfeed for marine larvae GEMMA micro 150 and 300 was used, with two different particle size ranges, 100–200 µm and 200–500 µm, respectively (Skretting, Norway). A feeding automat (Sterner 905, FishTech AS, Norway) above each individual rearing tank was programmed with an online management system (Normatic WebServer, Normatic AS, Norway) to deliver predetermined doses of formulated diet based on feeding tables (Table 1). The formulated diet was distributed to the tanks from 4 to 24 times day$^{-1}$, depending on the feeding regime (Table 1). Before entering the tanks, the feed was mixed with intake water in a funnel located beneath each feeding automat.

Live feeds were transferred to 20 L PET-plastic beer kegs (KeyKeg 20L, OneCircle, the Netherlands), which were used as feed reservoirs and filled with seawater until the 20 L mark. The live feeds were transported from the reservoirs into the larval tanks through transparent silicone hose tubing using peristaltic tubing pumps (Kronos 50, Seko, Italy). The amount of live prey day$^{-1}$ were counted and estimated based on the required live prey densities in different treatments during the experiments (Table 1).

## Experimental treatments

Five feeding regimes were different in the type of feed given from 2 to 21 dph (Table 1), and three replicate tanks were used for each treatment. All larval groups were weaned to Gemma Micro 300 from 21 dph.

**Table 1** The different feeding regimes (treatments) that were used for the start-feeding experiment with Atlantic lumpfish (*Cyclopterus lumpus*), showing the periods during which different feed items were used. The weaning period for new diets is described in the text

| Treatment | Type of feeds and density of live prey ($\times 10^3$ prey tank$^{-1}$ day$^{-1}$ ) | | |
|---|---|---|---|
| | Period 1: 2–9 dph | Period 2: 9–21 dph | Period 3: 21–35 dph |
| *Artemia* (Art) | *Artemia* (600–5400) | | GEMMA 300 (12–24 g) (*Artemia* 21–25 dph) |
| Cirripeds (Cir) | Cirripeds (800–7800) | | GEMMA 300 (Cirripeds 21–25 dph) |
| Formulated diet (FD) | GEMMA 150 (4–12 g) | GEMMA 150:300 (1:1) | GEMMA 300 |
| Copepods/FD (Cop/FD) | Copepodites (3000–9000) | GEMMA 150:300 (1:1) (Copepodites 10–16 dph) | GEMMA 300 |
| Copepods/Cir (Cop/Cir) | Copepodites (3000–9000) | Cirripeds (5400–7800) (Copepodites 10–16 dph) | GEMMA 300 (Cirripeds 21–25 dph) |

***Artemia* (Art)** Lumpfish larvae were fed enriched *Artemia franciscana* instar III meta nauplii (800 µm length) from 2 to 21 dph, weaned with a mixed Gemma Micro 150 and 300 (1:1) from 21 to 25 dph and fed only GEMMA Micro 300 from 26 to 35 dph.

**Cirripeds (Cir)** Lumpfish larvae were fed cryopreserved, live cirripeds (*Semibalanus balanoides* nauplii (350 µm long, 150 µm wide) for 18 days (2–21 dph), weaned with a mixed Gemma Micro 150 and 300 (1:1) from 21 to 25 dph, and fed only GEMMA Micro 300 from 26 to 35 dph.

**Formulated diet (FD)** Lumpfish larvae were fed with GEMMA Micro 150 (100–200 µm) from 2 to 9 dph, then a mixed Gemma Micro 150 and 300 (1:1) from 10 to 21 dph, and fed only GEMMA Micro 300 (200–500 µm) from 21 to 35 dph. The formulated diet is a commercial product for marine fish larvae by Skretting (Norway).

**Copepod and formulated diet (Cop/FD)** Lumpfish larvae were fed live copepods (*Acartia tonsa* copepodites (stages N3–N6), 185–394 µm long) from 2 to 9 dph, weaned with a mixed Gemma Micro 150 and 300 (1:1) from 10 to 25 dph, and fed only GEMMA Micro 300 from 26 to 35 dph.

**Copepod and cirripeds(Cop/Cir)** Lumpfish larvae were fed copepods from 2 to 9 dph, and cirripeds from 10 to 21 dph, weaned with a mixed Gemma Micro 150 and 300 (1:1) from 21 to 25 dph, and fed only GEMMA micro 300 from 26 to 35 dph.

### Larval sampling and analyses

All larvae were randomly sampled from the fish tanks and euthanized by an overdose of tricaine methane-sulfonate (MS-222 Finquel ®, Argent Chemical Laboratories Inc., USA) mixed with seawater and briefly rinsed in freshwater.

### Standard length (SL) and dry weight (DW)

Fifteen yolk-sac larvae from three random tanks were sampled at 2 dph, just before feeding. Larvae were sampled at 9 and 15 dph ($n = 5$ per tank), 21 dph ($n = 10$ per tank), and 29 and 34 dph ($n = 15$ per tank). Pictures of all larvae were taken in a stereo microscope (Leica MZ75, Leica Microsystems, Germany) equipped with a CMOS camera (Zeiss Axiocam ERc 5 s, Zeiss Inc., Germany). Larval standard length (SL) was measured from the tip of the snout to the end of the notochord and from post-flexion to the base of the tail fin by using the image processing software ImageJ (Schneider et al. 2012) on calibrated images.

For dry weight, the larvae were rinsed in de-ionized water and placed individually in pre-weighed tin capsules. Their wet weight (WW) was determined using a micro balance (UMX2 Ultra-microbalance, Mettler-Toledo, USA). The samples were then dried at 60 °C for a minimum of 24 h before weighing again to obtain the dry weight (DW). Specific growth rates (SGR) were calculated for specific sampling intervals according to the following equation (Houde & Schekter 1981):

$$SDR = (In\ W2 - In\ W1)/(t2 - t1)$$

where W2 and W1 are the individual larval dry weights at times t2 and t1, respectively.

The SGR values were used to calculate the percentage daily weight increase (DWI) (Houde & Schekter 1981):

$$DWI = (e^{SGR} - 1) \bullet 100\%$$

As larvae were more difficult to catch at 34 dph, sampling to check for possible sampling bias was done at the end of the experiment (35 dph). At least 250 fish per tank were sampled at the end of the trial when the tanks were emptied and all fish larvae were collected and euthanized. The larvae were fixed in 4% paraformaldehyde (PFA) in phosphate buffered saline (pH 7.4, Apotekproduksjon AS; Oslo, Norway) and stored cold (4 °C) for later measurement of SL as described above.

## Liver histology

Fifteen yolk-sac larvae from three random tanks were sampled at 2 dph, the common starting point. Larvae were sampled at 9, 21, and 34 dph ($n = 5$ per tank); they were fixed in 4% paraformaldehyde (PFA) in phosphate buffered saline and stored cold (4 °C) until further processing. For liver histology analysis, five random larvae per treatment were rinsed in phosphate buffer, and a transverse cut was made through the larvae anteriorly to the anus. The anterior part was embedded separately in plastic (Technovit® 7100, Kulzer, Germany), a plastic embedding system based on HEMA* (2-hydroxyethyl methacrylate). The samples were dehydrated and polymerized through a graded series of water and resins, all according to the procedure from the manufacturer. Longitudinal Sects. (2 µm) in the sagittal plane were cut with 10 µm intervals to avoid measuring the same nuclei/hepatocyte twice for liver analysis (Fig. 1). The sections were stained with 0.05% toluidine blue (Honeywell Riedel-de-Haën™, Germany) and scanned at × 40 magnification with a digital slide scanner (Hamamatsu NanoZoomer). Lipid vacuoles appear empty in these histology sections, as fat is extracted from the tissues through the embedding process. Some sections were also stained with Alcian Blue-Periodic Acid Schiff (ABPAS) to validate glycogen deposits in vacuoles with visible content.

The quantitative analysis of liver was done by using the image analysis programs QuPath v.0.2.3 (University of Edinburgh, Scotland) and ImageJ v.1.53 k (Schneider et al. 2012). The polygon tool in QuPath was used to measure hepatocyte nucleus area size, and the number of nuclei in a counted grid area was used to estimate the mean hepatocyte cell area size ($A_{liver}/N_{nuclei}$) (Fig. 1). An average of 264 nuclei were measured per fish. Nuclei touching the edges of the grid, or missing one or more distinct nucleoli, were not measured. Each grid was also exported to ImageJ, and a point-grid of crosses was applied to estimate the vacuole fraction in the hepatocytes ($A_{vacuoles}/A_{liver}$) using the multi-point tool for counting.

## Bone development

At the end of the experiment (35 dph), 30 larvae from each tank were anesthetized (MS222) and fixed in 4% paraformaldehyde (PFA) in phosphate buffered saline (pH 7.4). For bone staining, they were all combined, and 90 fixed larvae from each treatment were stained with Alizarin red using a procedure modified after Balon (1985) and Kjørsvik et al. (2009). The samples were rinsed 2 × 15 min in distilled water, rehydrated in ethanol (2 × 96%, 50%, and 15%, 90 min each), and rinsed again for 90 min. Pigments were

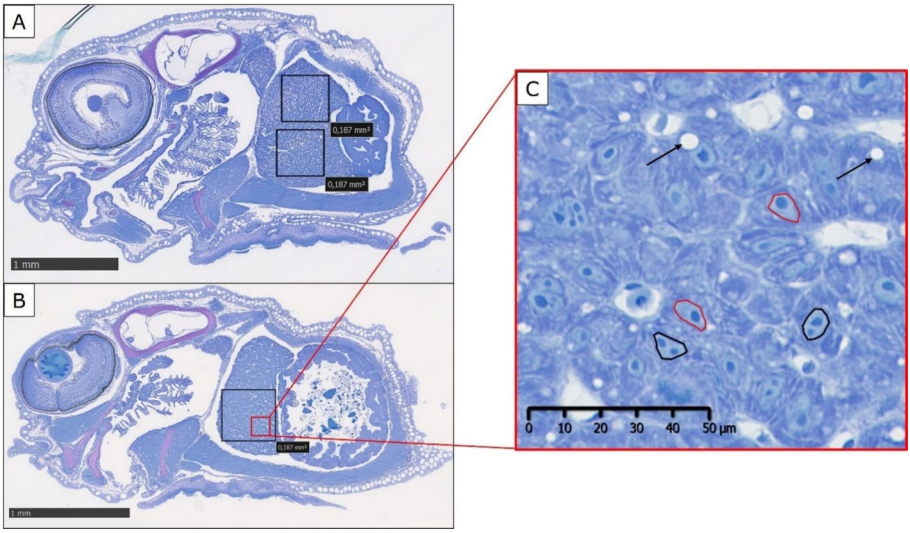



**Fig. 1** Liver histology analysis of lumpfish larvae was done on longitudinal sections during development (2–35 dph). For each larva, up to three grids á 0.1875 $mm^2$ were used. **A** An Art-larva at 35 dph, where two grids could be placed, thus only needing one more section to place the third grid. Scale bar: 1 mm. **B** The liver of a Cop/FD-larva from 21 dph, where only one grid could be placed and thus several sections were used to place all three grids. Scale bar: 1 mm. **C** Close-up of the liver of the Cop/FD-larva from 21 dph. In the analysis, nuclei with one prominent nucleolus (circled in red) and nuclei with two or more nucleoli (circled in black) were observed. The black arrows point to vacuoles in the hepatocytes. Scale bar: 50 µm

bleached with 3% $H_2O_2$ in 1% KOH (1:9, v/v) under strong light for about 3.5 h, and soft tissues were digested using 0.5–0.8% trypsin (T4799, Sigma-Aldrich, USA) in 0.3% borate buffer (w/v) for 24–46 h. Ossified structures were stained for 1–2 days with a 0.25% Alizarin Red S (A5533, Sigma-Aldrich, USA) solution prepared in 96% ethanol (w/v) and diluted 1:10 (v/v) with 1% KOH. After rinsing, the larvae were transferred to glycerol in 1% KOH (4:10, v/v) for examination. Photographs were taken through a dissecting microscope (Leica M205, Leica Microsystems, Germany) equipped with a CMOS-camera (AxioCam ERc 5 s, Zeiss Inc., Germany), and the images were visually analyzed for the number of partly and fully ossified vertebrae and fins. The ossification of the spinal column (Table 2, Fig. 2) and fins (Table 2, Fig. 3) was classified according to the degree of staining of the different elements, and nomenclature of ossified elements is according to Boglione et al. (2013).

## Lipid content and fatty acid (FA) composition

For lipid content and fatty acid analysis, larvae were randomly sampled at 9, 15, 21, 29, and 34 dph ($n = 45$ per tank). Diets were the same as used in a similar experiment with ballan wrasse larvae (Malzahn et al. 2022), and values of diets used in this experiment for lumpfish will be used here (Table S1). The larval samples were immediately flushed with $N_2$, snap frozen in liquid $N_2$, and stored at − 80 °C until analysis. Samples were freeze-dried and weighed in a UMX2 ultra-microbalance (Model UMX2, Mettler-Toledo, USA). Lipids

**Table 2** Ossification categories of the vertebral column and dorsal and anal fins in lumpfish

| **Spinal column was classified according to the degree of staining of the vertebral bodies** | |
|---|---|
| Transparent | No staining in the vertebral bodies |
| Partly | Staining did not cover the whole vertebral body and was less intense |
| Compact | Staining covered the entire circumference of the vertebral body, with less saturated color than in fully ossified vertebrae and larger visible non-stained distance between the vertebrae |
| Fully ossified | Saturated color which covered the entire vertebrae |
| **Dorsal and anal fins were evaluated according to this score** | |
| 1 | Minor ossification of fin rays |
| 2 | Medium ossification; staining of more than half of the fin ray lengths |
| 3 | Full ossification of all fin rays and pterygiophores |

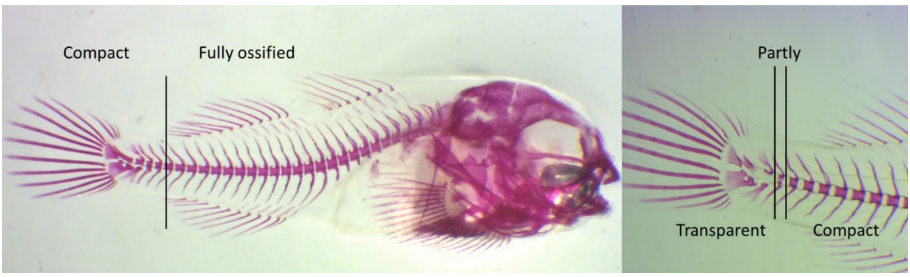



**Fig. 2** Classification for degree of ossification of vertebrae in lumpfish larvae; see Table 2 for a more detailed description

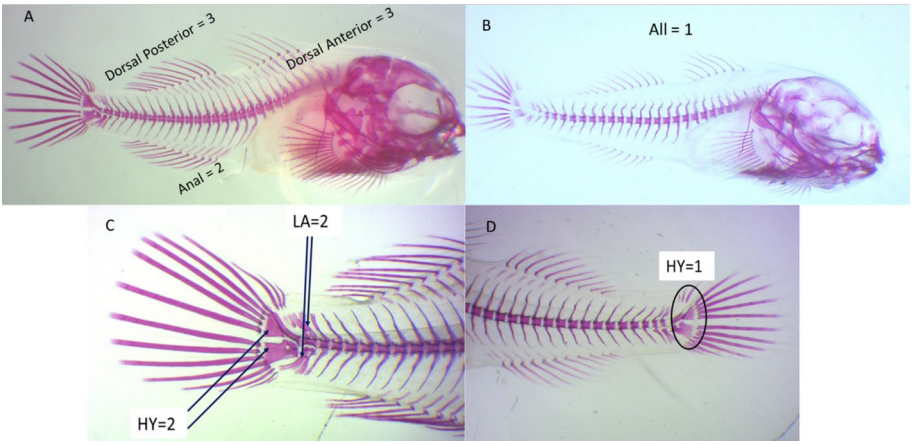



**Fig. 3** Classification of fin scores in 35-day old lumpfish larvae for evaluation of dorsal and anal fin ossification (A, B) and for ossification of the hypural and last arches and spines (C, D); see Table 2 for a more detailed description. HY = hypural; LA = last haemal and neural arches and spines

were extracted after Folch et al. (1957), with further details in Malzahn et al. (2022). Total lipid content was determined gravimetrically by weighing the lipid extract after drying.

FA composition was determined by fatty acid transmethylation to fatty acid methyl esters (FAMEs) and gas chromatography with flame ionization detection (GC-FID) analysis. The analysis of FAMEs was performed according to the protocol described by Daukšas et al. (2005) with the following modifications. An Agilent Technologies 7890B GC–FID equipped with a 7693 A autosampler was used. The detector temperature was held at 280 °C, and the flame was maintained with 30 mL/min H2 gas and 400 mL/min filtered air. The chromatographic separation was performed 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 of 3 mL/min. GC inlets were held at 250 °C. The initial oven temperature was maintained at 50 °C for 2 min and increased to 150 °C at 30 °C/min, after which the temperature rose to 230 °C at 2.5 °C/min, followed by an increase to 240 °C at 10 °C/min, and a 23 min final hold. Free FAMEs were identified through comparing their RTs with those of a commercial standard mix submitted to identical chromatography conditions. Quantification was based on a commercial standard mix (68D mix from NuChekPrep, Chiron) using tricosanoic acid (C23:0, NuChekPrep, Chiron) as an internal standard.

## Microbiology

Samples for the microbial community of the water and larvae were taken at 10, 17, 21, 26, and 33 dph. Tank water was sampled by pre-filtering 1 L of tank water through a 64 µm sieve, followed by filtration on a 0.22 µm MF-Millipore membrane (Merck, Germany). For assessing the microbial community of larvae, five larvae were collected from each tank and euthanized in tricaine methanesulfonate, MS-222 (Merck, Germany). Both membranes and euthanized larvae were placed in extraction tubes containing beads and nucleic acid preservation liquid, DNA/RNA shield (ZymoBiomics, USA). All samples were stored at − 20 °C until extraction. The DNA of the larvae and water was extracted using a 96 MagBead DNA kit (ZymoBIomics, USA). DNA was then eluted in DNase/RNase-free water, and concentrations and quality were assessed using a NanoDrop (Thermo Fisher Scientific, USA) and a Qubit 2.0 (Thermo Fisher Scientific, USA). Sequencing was conducted by a commercial provider on a MiSeq platform (Illumina, CA, USA) on previously generated amplicons using 338 F (5′-ACTCCTACGGGAGGCAGCAG-3′) and 806R (5′-GGACTACHVGGG TWTCTAAT-3′) primer set covering the V3–V4 hypervariable region of the 16S rRNA gene; 300 bp paired-end chemistry was utilized for sequencing purposes.

Obtained sequences were treated using Quantitative Insights Into Microbial Ecology 2 (QIIME2) pipeline (Bolyen et al. 2019). Initially, forward and reverse reads of each sample were loaded in the appropriate QIIME2 artifact. Subsequently, quality control (based on median Phred value > 20), denoizing, and Amplicon Sequence Variants (ASV) were conducted using the DADA2 algorithm (Callahan et al. 2016). DADA2 was used subsequently to merge complementary ASV forward and reverse reads, and a table containing ASVs and counts was generated for each sample. Additionally, chimera sequences were screened and filtered based on the DADA2 de novo approach. Next, representative sequences were aligned using MAFFT (de novo multiple sequence aligner) (Katoh and Standley 2013), masked, and a phylogenetic tree was generated using FastTree (Price et al. 2009). Taxonomic assignment to each of the representative sequences was conducted using a classifier based on the Silva database (v.138) (Quast et al. 2012) and specifically pretrained for the

primer set region used to amplify sequenced amplicons. Finally, ASVs annotated to chloroplast and mitochondria were removed from the dataset. Furthermore, features with < 10 hits were removed as well. Subsequently, the dataset was rarefied to 35,000 randomly selected sequences for each sample in order to obtain an even sampling depth for the purpose of downstream statistical analysis. The chosen threshold was sufficient to retain all 89 samples for further analysis.

## Statistical analysis

Statistical analyses and visualization were conducted using SigmaPlot v.14 for Windows (Systat Software Inc., USA), at a significance level of $\alpha = 0.05$. Larval growth and survival data were tested for normality with Shapiro–Wilk's test and for homogeneity of variance with the Brown-Forsythe test. Differences either between treatment groups at the different time points or within groups were compared using a one-way ANOVA (analysis of variance) for normally distributed data. Student–Newman–Keuls post hoc test was used for homogenous data, while a Dunnett T3 post hoc test was used for non-homogenous data. If data were non-normal and of unequal variance, the non-parametric Kruskal–Wallis was used. Arcsine transformation was applied to percentage data before statistical testing. For comparison of more than two groups, an independent-sample Kruskal–Wallis test (two-sided, asymptotic significances) with inbuilt pairwise comparison and adjustment of the significance value was applied. 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 the Holm-Sidak post hoc test.

For the liver analyses, linear regressions were made of data from the liver histology and the corresponding larval standard length (SL). A one-way analysis of covariance (ANCOVA) was used to investigate if the linear equations differed significantly between the treatments and whether the data were significantly affected by the treatment, SL, or the combination thereof. Further, the Holm-Sidak method was used for pairwise multiple comparisons to investigate whether the linear equations of the different treatments were significantly different.

For larval vertebral ossification, a Shapiro–Wilk test was used to test for normality on data from larvae after different dietary treatments. An *F*-test of equality of variances and ANOVA was used to test for differences in ossification between the larval groups. A *t*-test was used to evaluate whether the binomial data of the caudal region differed between pairs of treatments. For the multinomial data with three categories (fin ossification), the number of significant *t*-tests necessary for a pair of treatment groups to differ was determined by simulation under H0. For multinomial data with four categories (deformities), a likelihood ratio test was performed.

The microbial community's data treatment and statistical analysis were carried out using various R packages including phyloseq (McMurdie and Holmes 2013), microbiome (Lahti and Shetty, 2018), rstatix (Kassambara 2023), and vegan (Oksanen et al. 2025). To evaluate per sample diversity, the *chao1* and *Shannon* diversity indices were employed, and their statistical significance was assessed by a non-parametric Kruskal–Wallis test, followed by pairwise Dunn's test. A significance level of $p < 0.05$ was employed, taking into consideration the Bonferroni correction.

For multivariate analysis, the plot_ordination function from the phyloseq package was employed to visualize similarities and dissimilarities between samples using the Bray–Curtis dissimilarity index. To assess the statistical significance of the dissimilarity index results, PERMANOVA, a non-parametric multivariate analysis of variance, was employed to compare groups and test the null hypothesis of equivalence in centroids and dispersion. Subsequently, a permutation test using the *betadisper* function in R was performed to assess the homogeneity of multivariate dispersions among sample groups.

## Results

### Growth and survival

Larvae fed *Artemia* were significantly larger (DW and SL) than larvae from all other groups during the whole experiment, and larvae fed copepods and the formulated diet had the slowest growth (Figs. 4A, B). Only larvae fed *Artemia* (Art) had a positive increase in DW up to 15 dph, and these larvae were about twice the size of other larvae at the end of the live feed period (21 dph). Between 15 and 21 dph, the larvae fed cirripeds (Cir- and Cop/Cir-groups) grew significantly more (DW and SL) than those fed copepods (Cop/FD) and formulated diet (FD), and the differences in size were maintained throughout the experiment to 35 dph.

After weaning all larvae to the formulated diet (from 21 to 29 dph), the *Artemia*-larvae were still significantly larger than those in the other larval groups (Fig. 4A, B). Cirripede-larvae attained a higher growth than during the live feed phase and were significantly larger than larvae fed copepods and formulated diet (FD, Cop/FD, Cop/Cir) at 29 dph.

At the end of the experiment (34 dph), no difference in DW or SL was found between larvae fed *Artemia* and cirripeds, whereas larvae from the three other groups were significantly smaller ($n = 15$ per tank). At 34 dph, the mean DW of the *Artemia*- and cirripede-fed larvae was 4.9 × and 4.8 × heavier than the 2-day-old larvae. The mean FD- and Cop/Cir-larval DW was 3.7 × larger, and the DW of the Cop/FD-larvae was only 3.3 × larger than the initial mean DW at 2 dph. However, after emptying the tanks on 35 dph, the more random and much larger larval sample ($n = 250$ from each tank) demonstrated that the mean SL in the *Artemia* larval group was significantly larger than in the Cirripede larval group (Fig. 4B). The lumpfish larvae fed *Artemia* thus had significantly higher somatic growth than the other groups throughout the whole experiment.

The increase in larval DW versus SL did not differ between treatments, suggesting that diet had no effect on the growth pattern (Fig. 4C). The pooled data demonstrated a strong correlation between DW and SL for all larvae regardless of treatment (Pearson-correlation, $r^2 = 0.97$).

The mean daily weight increase (% DWI) of larvae for the whole experimental period was significantly higher for the *Artemia* and Cirripede larvae than for the Cop/FD, Cop/Cir, and FD larval groups (2–34 dph) (Fig. 4D). Although the Cirripede and FD larvae at first had negative mean % DWI, no significant differences in mean % DWI were found between the five larval groups in the first feeding phase (2–9 dph). In the second feeding period (9–21), the mean % DWI of the *Artemia* and Cirripede larvae was the highest, and after weaning to FD/300 µm in the third feeding period (21–34 dph), the *Artemia* larval mean % DWI decreased, whereas the growth rate for the Cirripede larvae stayed high. For FD larvae, mean % DWI was much lower than for *Artemia* larvae during the first two feeding

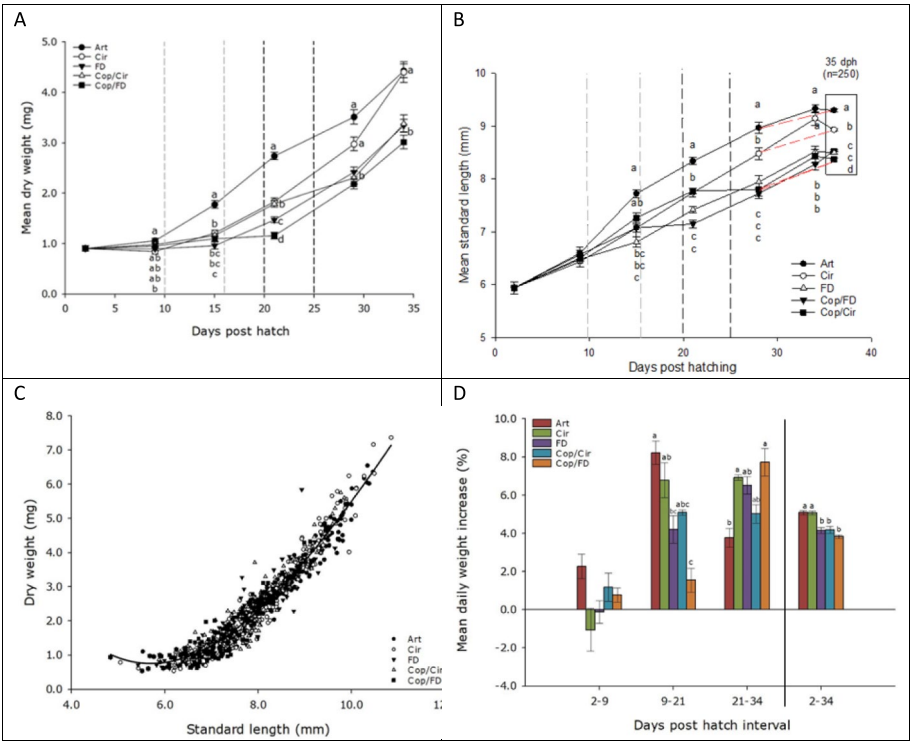



**Fig. 4** Lumpfish larval growth in relation to start-feeding regimes. Mean values are based upon the average of the three replicate tanks of each treatment. **A** Mean dry weight of lumpfish 2–34 dph. **B** Mean standard length of lumpfish at 2–34 dph and of the extra (unbiased) sample at 35 dph. **C** Correlation between standard length and dry weight per individual during the start-feeding experiment (2–34 dph). Pearson correlation: $r = 0.970$ (all treatments). **D** Mean daily weight increase after each feeding type in lumpfish 2–34 dph. Mean daily weight increase (% day$^{-1}$) in each treatment between specific time intervals. The solid vertical line separates consecutive intervals in the treatment period and the whole experimental period (2–34 dph). Significant differences ($p < 0.05$) between treatments at sampling days are indicated by different letters. Error bars indicate ± standard error (SE) at sampling days

periods. However, the FD larval % DWI increased during the whole experiment, and after the shift to larger feed particles in the third feeding period (to FD/300 µm), the FD larval growth rate was among the highest. Especially, the Cop/FD larvae had a very low growth rate through the first two feeding periods with copepods and FD/150 µm, but mean % DWI was among the highest after the switch to FD/300 µm. The Cop/Cir larvae had an increasing but relatively low mean % DWI after the switch from copepods to cirripeds in the second feeding period, and it remained at that low level after weaning to FD/300 µm.

All larval groups had a relatively high mean survival at the end of the experiment (Fig. 5). The *Artemia* larval group had the highest mean survival rate (about 95%), closely followed by the cirripeds, Cop/Cir, and FD groups (about 92–94%). The lowest mean survival was in the Cop/FD larval group (86%), but a significant difference in survival was only found between the Art and Cop/DF larval groups. Most of the larval mortality occurred after all larvae were weaned to the formulated diet from 21 dph.

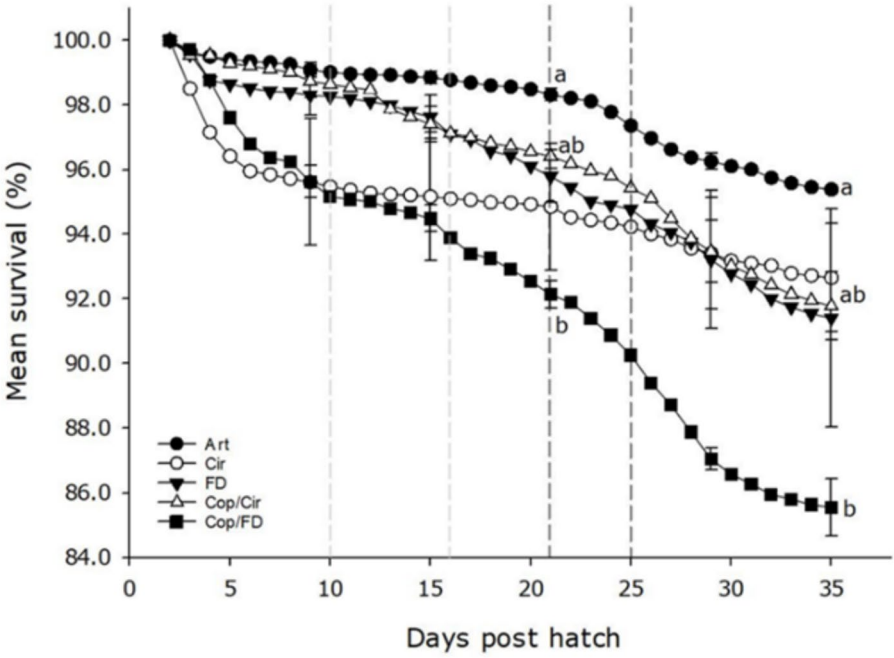



**Fig. 5** Mean survival of lumpfish larvae in the start-feeding experiment (0–35 dph). Mean survival (%) is based upon the average of the three replicate tanks of each treatment. Significant differences ($p < 0.05$) between treatments at sampling days are indicated by different letters. Error bars indicate ± standard error (SE) at sampling days

## Liver histology

The liver parenchyma seemed well differentiated at 2 dph (Fig. 6), and hepatocytes were arranged around central veins with sinusoids and bile canaliculi scattered in between. The nuclei were almost spherical, and the sinusoids appeared tubular and irregularly shaped in between the hepatocytes. In most larvae, the cytoplasm was filled with large irregularly shaped vacuoles, which displaced the nucleus to the periphery of the cell (Fig. 6A). A general pattern was observed during development for the hepatocyte vacuolization: Glycogen-like vacuoles were observed in all the youngest larvae (2 and 9 dph; Figs. 6 and 7), whereas increasingly more lipid-like vacuoles (droplets) were observed at 21 and 35 dph, see Figs. 8 and 9).

Mean hepatocyte vacuolization was around 15% at 2 dph (Fig. 10A). At 9 dph, the *Artemia* and the cirripeds larvae had the highest mean vacuolization (10–15%). The other larval groups only had around 3% mean hepatic vacuolization, with a central positioning of the nuclei, and the FD larvae had signs of sinusoidal dilatation (Figs. 7B and 10A).

The main difference in hepatic vacuolization between the larval groups was observed at the end of the live feed period in 21 dph larvae. The 21 dph *Artemia* larvae had the highest vacuole fraction of all larvae in the experiment (Fig. 10 A, 32.2 ± 1.6%), and only glycogen-like vacuoles were observed (Figs. 8A, B). The FD-larvae also had quite high vacuolization with similar glycogen-type vacuoles (Fig. 8C), although not as large and abundant as in the *Artemia* larvae. In contrast to larvae fed *Artemia*, the Cop/FD larvae group

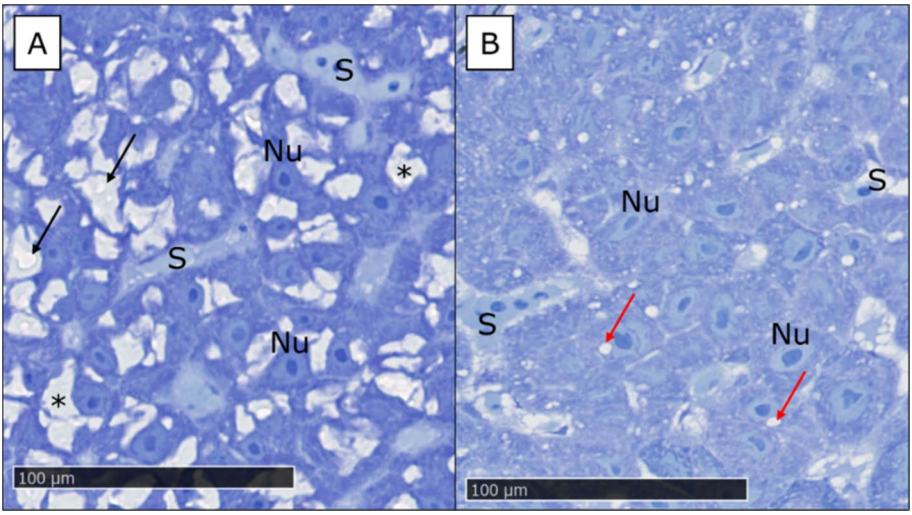



**Fig. 6** Liver structure of lumpfish larvae at 2 dph. **A** In most larvae, the liver parenchyma appeared fully differentiated, with hepatocytes containing irregularly shaped glycogen-like vacuoles (* and black arrows). **B** In some larvae, there were only very small vacuoles (red arrow) present. Scale bars: 100 µm. The sections were stained with TB 0.05%. Abbreviations: Nu, nucleus; S, sinusoid

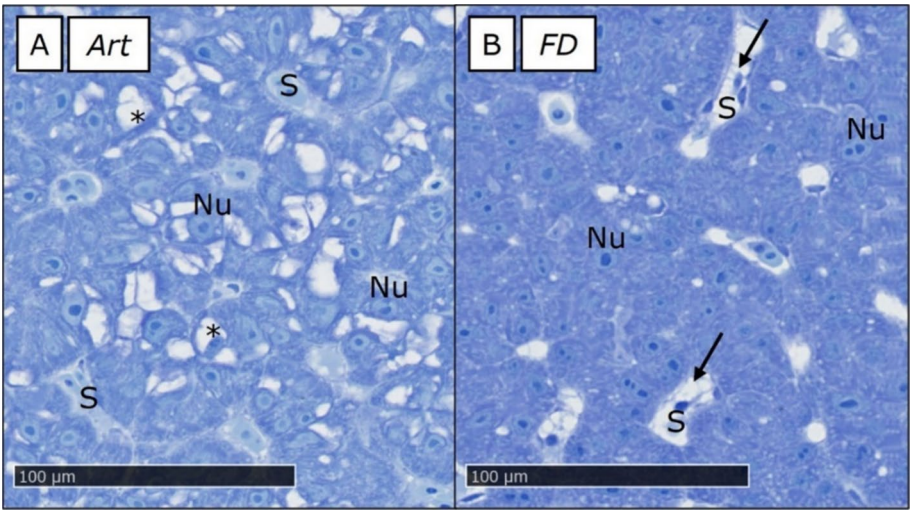
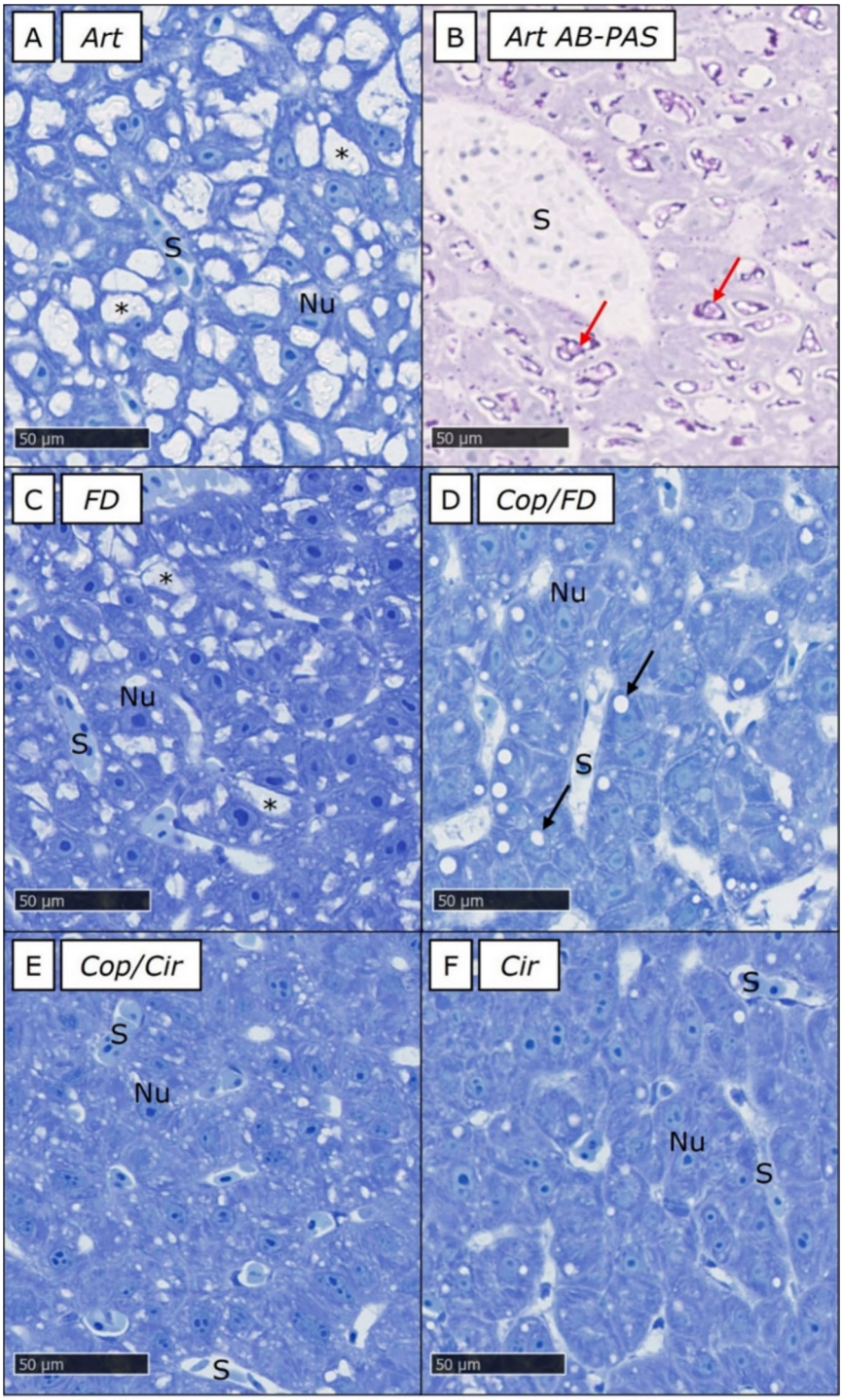



**Fig. 7** Liver structure of lumpfish at 9 dph. **A** The Art-larvae as well as the Cir-larvae showed a high degree of vacuolization (*), similar to larvae at 2 dph. **B** The liver structure of FD-larvae was similar to those fed copepods, showing an absence of vacuoles. Unique for the FD-larvae was that they also showed signs of sinusoidal dilation (black arrow). Scale bars: 100 µm. The sections were stained with TB 0.05%. Abbreviations: Nu, nucleus; S, sinusoid

now had increasing mean vacuolization with lipid-like vacuoles, and dilated sinusoids were observed (Fig. 8D). Liver vacuoles in the Cop/Cir- and Cir-larvae were much depleted at 21 dph (Figs. 8E, F and 10A), and those observed seemed to be of lipid origin.


A
Art
S
Nu
50 µm
B
Art AB-PAS
S
50 µm
C
FD
Nu
S
50 µm
D
Cop/FD
Nu
S
50 µm
E
Cop/Cir
S
Nu
S
50 µm
F
Cir
S
Nu
S
50 µm

◀ **Fig. 8** Liver structure in larvae from all treatments at 21 dph in lumpfish larvae, the sections were stained with TB 0.05%. **A** Art larva, **B** Art larva, with additional staining with AB-PAS to reveal glycogen deposits in the vacuoles. **C** FD larva, **D** Cop/FD larva, **E**Cop/Cir larva, **F** Cir larva. Scale bar: 50 µm. Abbreviations and indicators: Nu, nucleus; S, sinusoid; * = vacuoles, black arrow = lipid vacuoles, red arrows = glycogen deposits

At 35 dph, after all larvae were weaned to the formulated diet (FD/300 µm) from 21 dph, no major differences in hepatic vacuolisation between the larval groups were observed (Fig. 10A). All larvae had similar levels of lipid-like vacuolization (Fig. 9A), as confirmed by the positive AB-PAS staining of the cytoplasm and not in the vacuoles in the *Artemia* larvae (Fig. 9B).

Mean hepatocyte size at 2 dph was $750 \pm 63$ µm$^2$ (Fig. 10B). The mean hepatocyte sizes were stable for all larvae from 0 to 9 dph, except for FD-larvae, which exhibited a significant decrease in size to $535 \pm 15$ µm$^2$. After Cop/FD-larvae were weaned to a formulated diet (10–15 dph), the mean hepatocyte size had decreased at 21 dph. However, after the weaning of the Cop/Cir-larvae to cirripeds, the hepatocyte size remained stable. The *Artemia* larvae exhibited hypertrophy at 21 dph and had a significantly larger hepatocyte size than both cirripeds and Cop/FD-larvae. After weaning to the formulated diet from 21 dph, all larval hepatocyte sizes had increased to the same range as the Art-larvae at 35 dph, and there was no significant difference between the treatment groups.

Mean hepatocyte nucleus size was largest in young larvae, with mean size $52.4 \pm 3.9$ µm$^2$ at 2 dph (Fig. 10C). A decrease (n.s.) in nucleus size was observed in all larvae at 9 dph, especially in FD larvae, which had the smallest nucleus size of all larvae (n.s.). By 21 dph, the mean nucleus size in FD larvae had increased to be the significantly largest of the larval groups, whereas all other larvae showed a decreased hepatocyte size. After all larvae had been weaned to the formulated diet, there were no longer any significant differences between the larvae at 35 dph.

The hepatocyte size showed a significant positive correlation with standard length ($r = 0.37$, $r^2 = 0.13$, Fig. 11A). However, no significant differences between treatments were

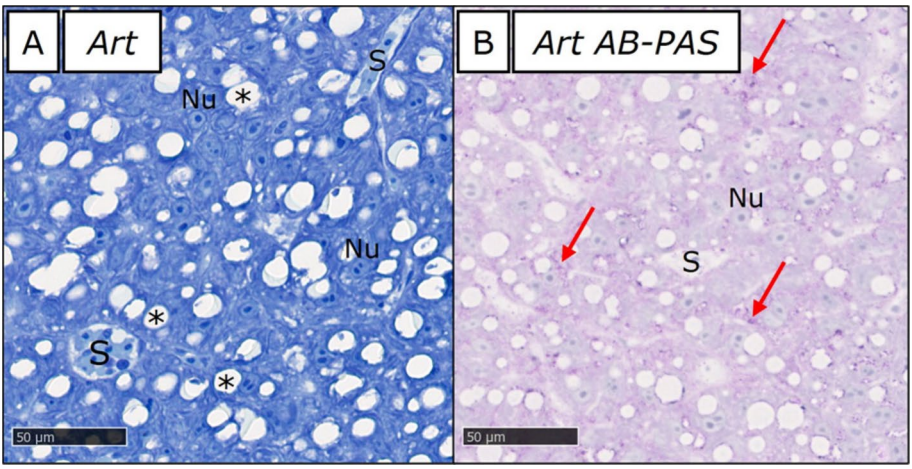
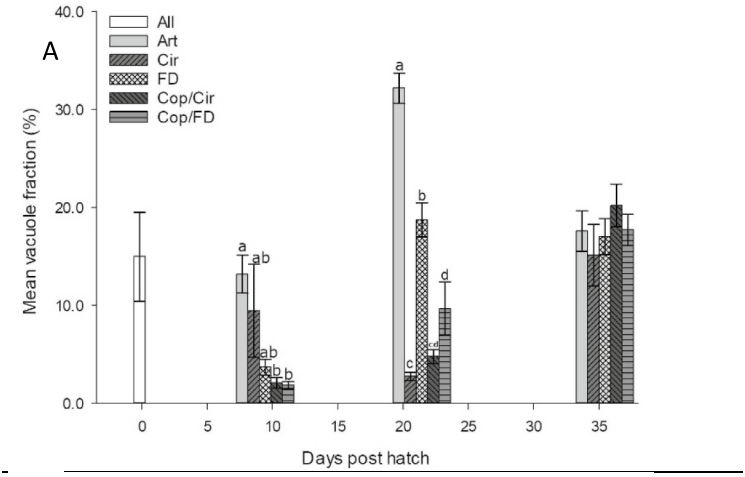
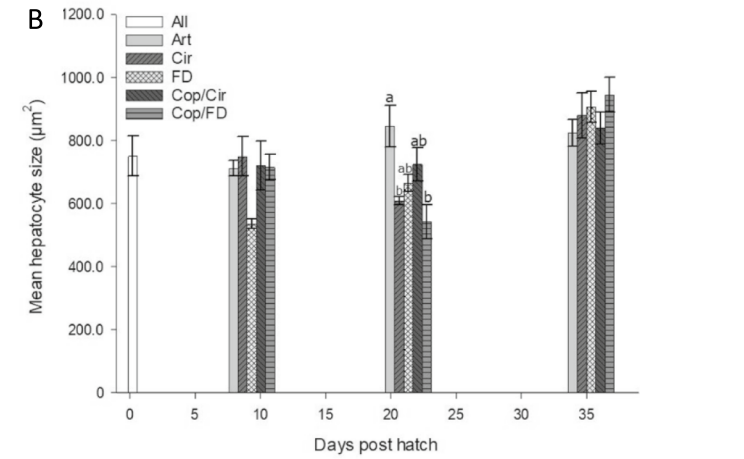
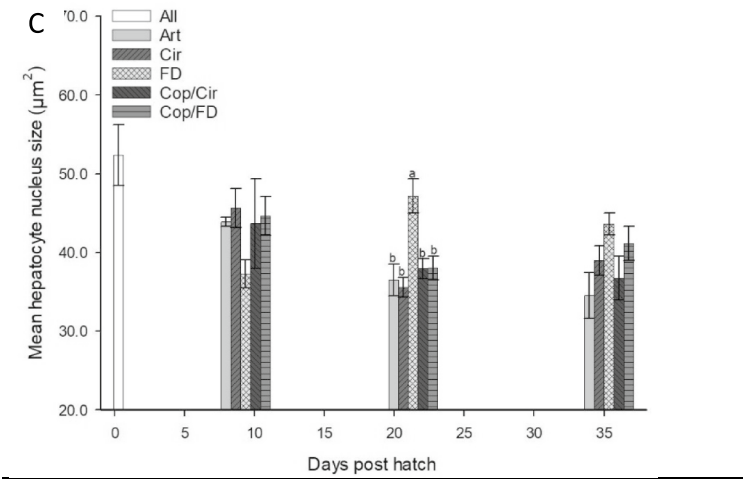



**Fig. 9** Typical liver structure of lumpfish larvae from all treatments at 35 dph, as demonstrated from the liver of an Art-larva. **A** All observed liver vacuoles (*) resembled lipid droplets and no longer seemed to store glycogen. The section was stained with TB 0.05%. **B** The liver section stained positive for AB-PAS staining in the hepatocyte cytoplasm (red arrow), not within the vacuoles but in the cytoplasm, further supporting that the observed vacuoles are likely of lipid origin. Scale bars: 50 µm. Abbreviations: Nu, nucleus; S, sinusoid


A
All
Art
Cir
FD
Cop/Cir
Cop/FD
Mean vacuole fraction (%)
Days post hatch


B
All
Art
Cir
FD
Cop/Cir
Cop/FD
Mean hepatocyte size (μm²)
Days post hatch


C
All
Art
Cir
FD
Cop/Cir
Cop/FD
Mean hepatocyte nucleus size (μm²)
Days post hatch

◂ **Fig. 10** Liver histological analysis in lumpfish larvae 2–35 dph from all treatments ($n=5$ per treatment). **A** Mean liver vacuolization (% of vacuoles total area), **B** mean hepatocyte size, and **C** mean hepatocyte nucleus size. The cell size was estimated in each larva by dividing the analyzed liver area by the number of nuclei (assuming mononuclear hepatocytes). Significant differences ($p<0.05$) between treatments at a given time (dph) or length (mm) are marked by different letters. Error bars are ± standard error (SE)

found for this relationship. Negative correlations between the mean hepatocyte nuclear size and the standard length were found in all larval groups (Fig. 11B), and the largest decline occurred between 6 and 7 mm SL. The largest decline in nucleus size with SL was found in the *Artemia* and cirripeds larvae, and the Cop/Cir larval group was also very similar to these. The least decline in nucleus size was found in the FD larvae; these larvae were also

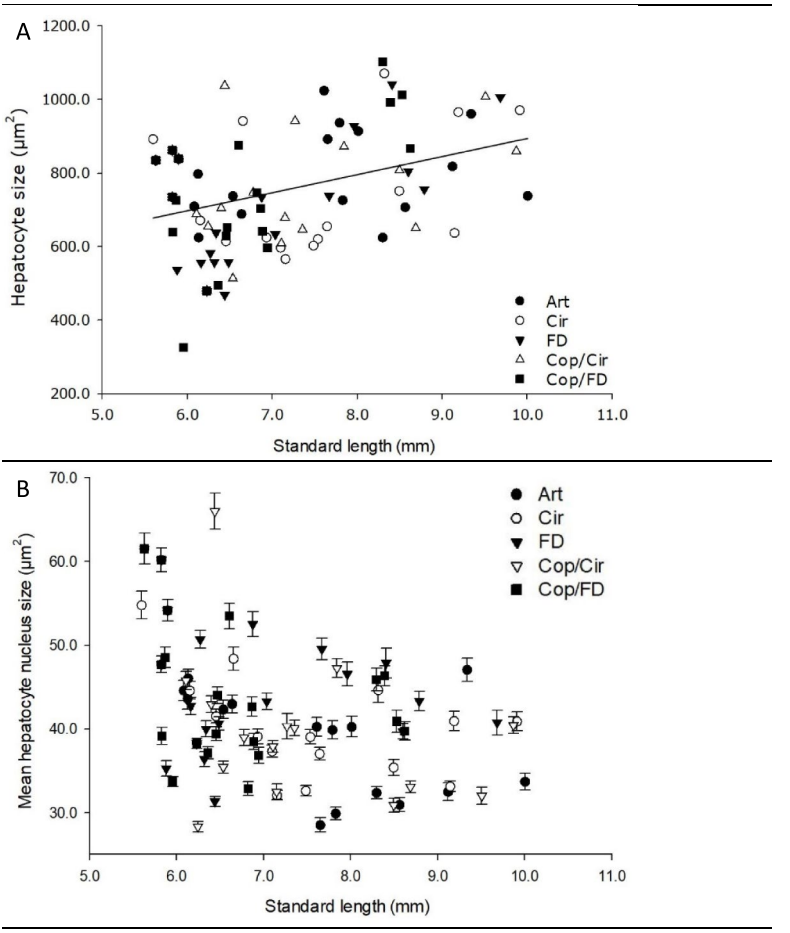



**Fig. 11** Correlation between larval standard length and histological liver parameters in lumpfish larvae 2–35 dph. No significant differences between the treatment groups were found, and the linear relationship for the pooled data is shown. **A** Changes in hepatocyte size and standard length showed a linear positive correlation. Pearson correlation and $r^2$ for the pooled data: $r=0.37$, $r^2=0.13$. Note that the horizontal axis starts at 5.0 mm and the vertical axis at 200.0 µm$^2$. **B** Changes in mean hepatocyte nucleus size and standard length. Each point represents the mean hepatocyte nucleus size ± standard error (SE) and corresponding SL of an individual larva, with a total of $n=20$ larvae per treatment group. Note that the horizontal axis starts at 5.0 mm and the vertical axis at 30.0 µm.$^2$

significantly different from all other larval groups. The slope for the Cop/FD larvae was in between the FD and the Art larval group, being significantly different from all other groups.

## Bone development

At the end of the experiment (35 dph), the stained larvae fed *Artemia* were significantly larger than the other larvae; their vertebrae were much better ossified, and these larvae had no transparent vertebrae (Table 3). Both groups fed cirripeds at one point were quite similar to each other in ossification of vertebrae. Although the Cop/Cir group were among the smaller larvae, they had significantly more fully ossified vertebrae than those fed copepods and/or formulated diet. These latter two groups had the lowest vertebral ossification of all groups.

In the size range between 8.5 and 9.5 mm SL, most of the vertebrae in *Artemia-fed* larvae were fully ossified, whereas in the other groups, the ossification was highly variable in this size range (Fig. 12). The *Artemia* larvae also had significantly better fin ossification scores than larvae from the other groups, followed by the cirripeds and Cop/Cir larvae (Table 4).

**Table 3** Observed vertebrae ossification in the lumpfish stained with Alizarin Red at the end of the experiment (35 dph, $n = 90$ per treatment). Results are given as the mean number of vertebrae in the different categories + SD of the different larval groups. Significant differences between the different larval groups are given by the letters a, b, c

| Experimental treatment | *n* | SL (mm) | Fully ossified | Compact | Partly | Transparent | No. of vertebrae |
|---|---|---|---|---|---|---|---|
| *Artemia* | 90 | 9.31 ± 0.56a | 26.5 ± 2.8a | 1.46 ± 2.7 | 0.02 ± 0.21 | 0.00 ± 0.0 | 28 ± 0.62a |
| Cirripeds | 89 | 9.09 ± 0.54b | 22.8 ± 7.2b | 5.06 ± 7.0 | 0.124 ± 0.5 | 0.00 ± 0.0 | 28 ± 0.55a |
| Dry feed | 90 | 8.47 ± 0.73c | 13.8 ± 8.4c | 11.8 ± 7.4 | 2.07 ± 1.8 | 0.42 ± 0.69 | 28.1 ± 0.57a |
| Copepods | 90 | 8.40 ± 0.75c | 13.6 ± 9.6c | 12.8 ± 8.6 | 1.38 ± 2.7 | 0.267 ± 0.6 | 28.1 ± 0.47 a |
| Cop/Cir | 90 | 8.54 ± 0.69c | 22.1 ± 6.7b | 5.7 ± 6.2 | 0.189 ± 0.7 | 0.03 ± 0.24 | 28 ± 0.66a |

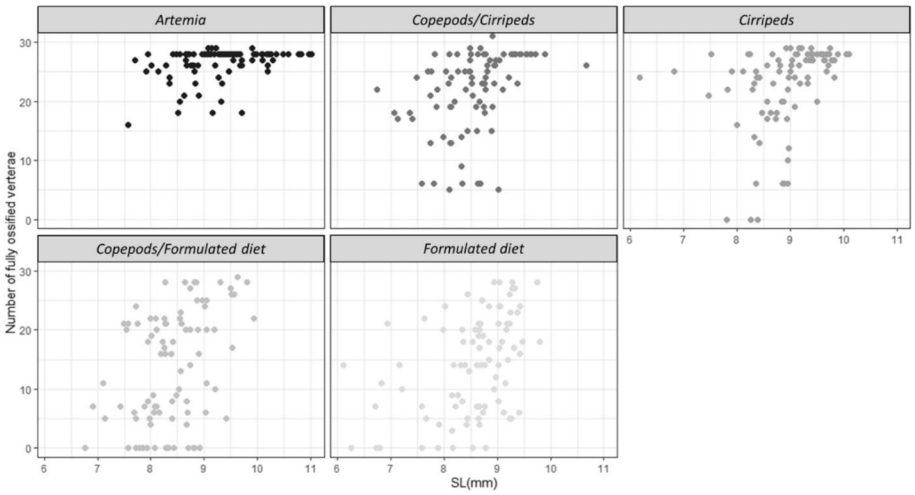



**Fig. 12** The number of fully ossified vertebrae in lumpfish larvae at 35 dph in relation to standard length and different start-feeding diets demonstrated the size-related difference between treatments in larval ossification. SL = larval standard length

**Table 4** The upper part of the table shows the percentage of fish with the different ossification scores of caudal, dorsal, and anal fins from each treatment in 35 dph lumpfish larvae stained with Alizarin Red. The lower part of the table shows significant differences between the larval groups in ossification of the fins, based on the combination of individual ossification scores in each group (full dataset is not shown). HY = hypural, LA = last haemal and neural arches/spines, AT = anterior, and PT = posterior

| | Caudal fin score | | | | Dorsal fin scores | | | | | | Anal fin score | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | HY | | LA | | Anterior (AT) | | | Posterior (PT) | | | AN | | |
| | 1 | 2 | 1 | 2 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| Artemia ($n=90$) | 1.1 | 98.9 | 51.1 | 48.9 | 0 | 64.4 | 35.6 | 0 | 78.9 | 21.1 | 0 | 88.9 | 11.1 |
| Cirripeds (Cir) ($n=89$) | 9.0 | 91.0 | 70.8 | 29.2 | 1.1 | 84.3 | 14.6 | 1.1 | 89.9 | 9.0 | 2.3 | 93.3 | 4.5 |
| Formulated diet (FD) ($n=90$) | 68.9 | 31.1 | 100 | 0 | 5.6 | 94.4 | 0 | 15.6 | 83.3 | 1.1 | 28.9 | 71.1 | 0 |
| Copepods/FD ($n=90$) | 44.4 | 55.6 | 94.4 | 5.6 | 10 | 87.8 | 2.2 | 15.6 | 81.1 | 3.3 | 24.4 | 74.4 | 1.1 |
| Cop/Cir ($n=90$) | 14.4 | 85.6 | 81.1 | 18.9 | 1.1 | 95.6 | 4.4 | 0 | 94.4 | 5.6 | 2.2 | 96.7 | 1.1 |
| **Statistical differences in ossification scores between the larval groups** | | | | | | | | | | | | | |
| Artemia | a | | a | | a | | | a | | | a | | |
| Cirripeds (Cir) | b | | b | | a | | | b | | | a | | |
| Formulated diet (FD) | c | | c | | b | | | c | | | b | | |
| Copepods/FD | d | | d | | b | | | bc | | | b | | |
| Copepods/Cir | b | | b | | b | | | b | | | a | | |

**Table 5** Developmental assessment of the spinal ossification in lumpfish larvae 35 dph, presented as mean % of normal fish and fish with deviating vertebral development, after feeding with different start-feeding diets ($n = 90$ per treatment)

| Experimental treatment | Normal larvae | Twisted/bent spines | Axis abnormal vertebrae/deviation | Abnormal skull |
|---|---|---|---|---|
| | Percentage (%) of larvae | | | |
| ***Artemia*** | 76.7[a] | 20.0 | 0 | 3.3 |
| Cirripeds | 31.5[b] | 64.0 | 4.5 | 1.2 |
| Dry feed | 1.1[c] | 96.7 | 2.2 | 0 |
| Copepods | 1.1[c] | 94.4 | 3.3 | 1.1 |
| Cop/Cir | 24.4[b] | 73.3 | 2.2 | 0 |

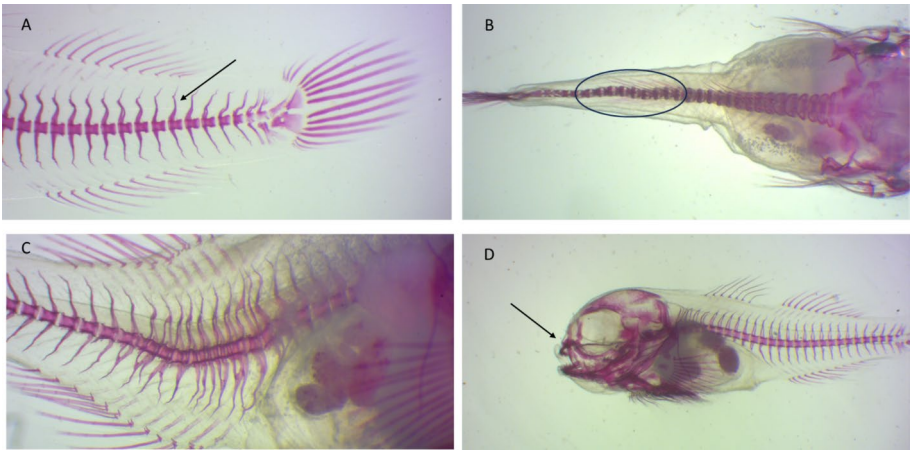



**Fig. 13** Observed skeletal anomalies in bone-stained 35 dph lumpfish larvae. The most common anomaly was twisted/bent neural and haemal arches and spines **A**. Very few serious anomalies such as axis deviations (picture shows scoliosis) **B**, abnormal vertebrae deviations **C**, or abnormal skulls **D** shows "pughead") were observed in the larvae, see also Table 5 for more detailed information

Skeletal anomalies/deformities—Very few serious anomalies such as axis deviation or abnormal skull development were observed in any of the larval groups (Table 5, see also Fig. 13). Most of the observed anomalies were twisted or bent neural and haemal arches/spines, ranging from 20% in *Artemia* larvae to more than 90% in larvae fed copepods and formulated diet. The *Artemia* larvae had a much higher incidence of normally ossified larvae, with about 77% of the larvae without any observed anomalies, followed by larvae fed cirripeds with between 24 and 32% of larvae without anomalies (Table 5). Almost no larvae fed copepods and/or formulated diet developed without showing any bone anomalies.

## Lipids and fatty acid composition

**Feeds** Mean lipid content and fatty acid profiles in the different feeds showed pronounced differences between the feed types (Table S1). The *Artemia* diet contained a mean of 21.3% lipids, significantly more total lipid than the two marine prey diets (mean 9.1–9.6% lipids), with the formulated diet in between (mean 16.4%). No difference in lipid content was found

between the cirripeds and copepods (ANOVA $p < 0.05$, followed by Holm Sidak post hoc test). Cirripeds contained relatively significantly more EPA (C20:6n3) and copepods significantly more DHA (22:6n3) than the other diets (Table S1). These two diets had the highest relative content of n3 fatty acids (mean between 53 and 59% of all fatty acids), although with different EPA/DHA ratios of 1.95 for cirripeds and 0.9 for copepods. The formulated diet contained a significantly higher % of linoleic acid (C18:2n6) than the other diets, reflecting the vegetable oil component.Larvae:

**Larvae** As shown in Fig. 14 (and in Tables S2–S6), mean total larval lipid content was relatively similar between the larval groups at 9 dph, although the FD larvae had an overall lower lipid content than the other groups. There was a tendency for a decline in larval lipid content between 9 and 21 dph, but variation was high, and only the Cop/Cir larvae showed a significant decline in total lipid content. After weaning to FD for all groups from 21 dph, there was a tendency towards an increased lipid content in all groups, except the FD larvae, which stayed at a very low level throughout the experiment.

For larval relative composition of fatty acids (Fig. 15 and Tables S2–S6), there were no large differences between 2 and 9 dph, although larvae fed formulated diet or copepods had the highest relative content of DHA (ca 26%) and consistently the lowest lipid content. By 15 dph, the mean DHA content increased to 30% of total fatty acids for Cop/FD larvae, resulting in the significantly highest relative n3 PUFAs content for this group. Larvae fed *Artemia* and the Cop/Cir diet also contained high and very similar relative n3 PUFA content (37–38%). Larvae fed *Artemia* received a more lipid-rich diet than the other groups, and the *Artemia* contained relatively more monounsaturated fatty acids and less n3 PUFAs. Not surprisingly, larvae fed *Artemia* had higher proportions of monounsaturated fatty acids at 15 dph, as well as lower n3 PUFAs at 21 dph. At the end of the second feeding period (21 dph), larvae fed cirripeds and the Cop/Cir diet had the highest relative content of n3 PUFAs (ca 40%), mainly due to a relative increase in both EPA and DHA in the larvae. The *Artemia*-fed larvae had an increasing level of arachidonic acid (ARA) up to 21 dph, and they kept a higher ARA level than the other larval groups throughout the experiment. Larvae fed the formulated diet (FD) from the start showed a significant increase in linoleic acid for the first time at 21 dph. Unfortunately, the lipid sample for Cop/FD larvae from 21 dph was lost. After weaning all larvae to the formulated diet (from 21 dph), there was no difference in fatty acid composition in any of the larval groups at 29 and 34 dph. Compared to the larvae on 2 and 9 dph, the relative content of n3 PUFAs had decreased and the n6 PUFAs had increased significantly, reflecting the last diet composition in the older larvae.

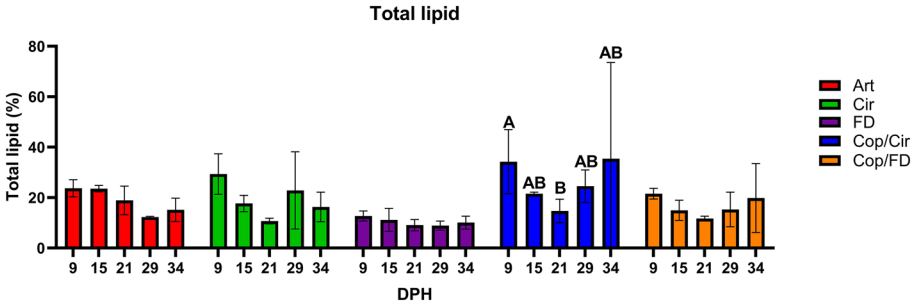



**Fig. 14** Mean total lipid content in lumpfish larvae during the experiment (as % of dry weight). Significant differences ($p < 0.05$) within treatments at a given time (dph) are marked by different letters. Error bars are ± standard error (SE)

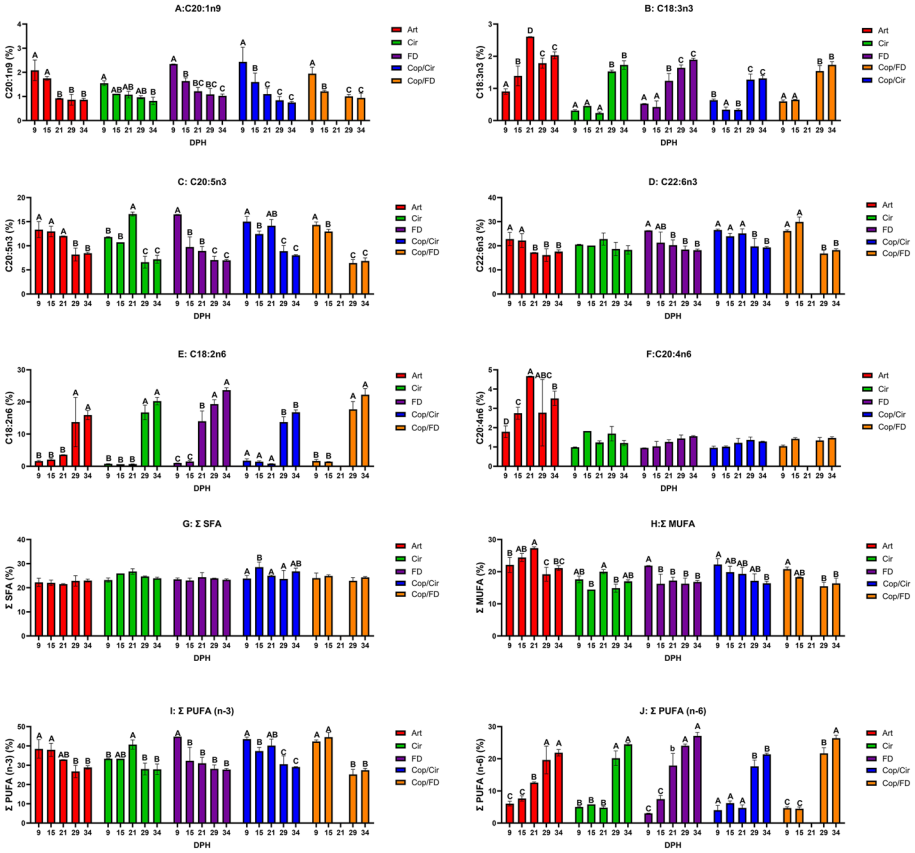



**Fig. 15** Mean relative fatty acid composition (% FA of total fatty acids, $n = 1$ to 3) during development of lumpfish larvae fed different start-feeding diets. Bar values are for the different sampling days after hatching. The samples for "Cop/FD" larvae on 21 dph were lost before analysis. Feeding groups: Art = *Artemia*, Cir = cirripeds, FD = formulated diet, Cop/FD = copepods/formulated diet, Cop/Cir = copepods/cirripeds. Significant differences ($p < 0.05$) within treatments at a given time (dph) are marked by different letters. Error bars are ± standard error (SE). NB! The *y*-axis is not the same scale for all graphs

## Microbiology

### Microbial diversity

Per sample diversity (or alpha diversity) measure of richness and evenness was evaluated based on *chao1* and *Shannon* diversity index, respectively. *Chao1* diversity index showed that there were no significant differences found between water (median = 322.2) and larvae (median = 295.3) samples, considering the complete dataset regardless of treatment or timeline (Table S7). On the contrary, when *Shannon* diversity index was assessed, the larvae samples (median = 4.1) showed significantly higher ($p = 0.03$) microbial diversity compared to water samples (median = 3.5). This indicates that water samples are dominated by fewer taxa compared to larval samples. A separate evaluation of *Shannon* index on water microbial diversity revealed no differences between different treatments, but there were significant differences when the *chao1* index was considered (Table S7). Pairwise comparisons

showed that the Art treatment (mean = 517.9) had significantly higher richness ($p = 0.03$) of microbial species compared to formulated diet (mean = 204.5), but not to other treatments (Table S7). Considering the diversity of the larval microbial community per sample, similarly to the water one, no differences between treatments were observed by *Shannon* diversity index (Table S7). However, exploring *chao1*, significant differences between microbial diversity were found. Cop/Cir and Cir treatments were most diverse, exhibiting mean values of 378.5 and 366.2, respectively (Table S7). Cop/Cir and Cir were statistically different compared to Art ($p < 0.01$) and FD ($p = 0.03$ and $p < 0.01$, respectively) (Table S7). The Art treatment exhibited the lowest median richness value (213.9) (Table S7).

Inter-sample diversity (or beta-diversity) exhibits no apparent differences in microbial community composition between larvae and water microbiota based on sample clustering in principal coordinate analysis (PCoA) (Fig. 16). Nevertheless, when statistically tested, significant differences were found ($p < 0.01$) (Table S8). However, more apparent differences driving the clustering of groups of samples were based on dph. Three clusters of samples can be observed here: cluster one consisting of 10 and 17 dph, cluster two of 17 and 21 dph, and cluster three of 26 and 33 dph (Fig. 16). Assessing water and larvae microbial community dynamics of different treatments separately revealed significant differences between all of them ($p < 0.05$), except for the Cop/FD and Cop/Cir treatments for water samples ($p = 0.23$) (Table S8). When broken down to each DPH (Figs. S1 and S2), more pronounced clustering based on treatments can be observed; however, significance is not possible to test due to only triplicates for each treatment at each dph.

The taxonomic composition of microbial communities in water and larval samples across different treatments and days post hatch is shown in Figs. S3A and B. The five most abundant taxa for each treatment group are listed in Table 6, along with their mean relative abundances and standard deviations, providing a summary of dominant community members.

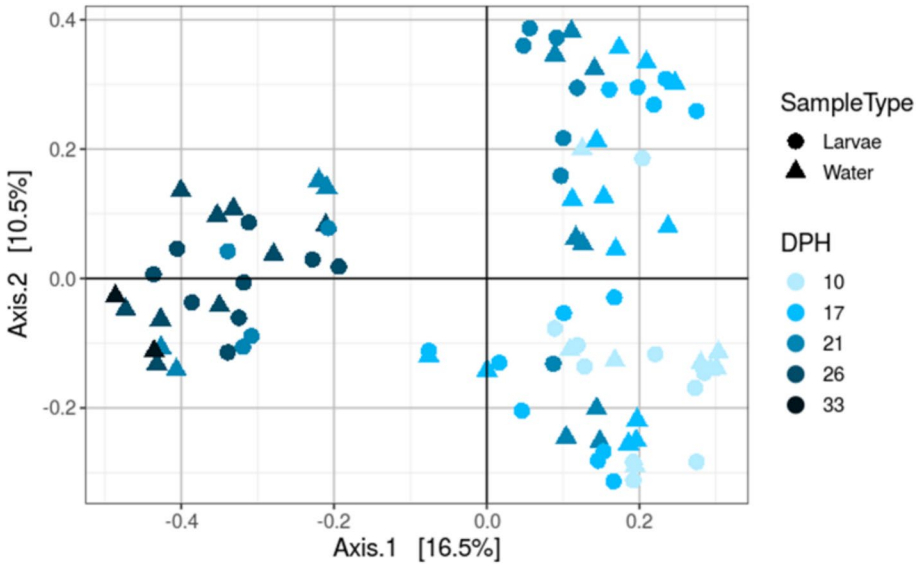



**Fig. 16** Principal coordinates analysis (PCoA) illustrating beta diversity of microbial communities in larval and water samples. Samples are differentiated by type (shape) and sampling date (color) based on the Bray–Curtis dissimilarity. The graph highlights variation in microbial community composition across sample types and developmental time points

**Table 6** The top five most abundant microbial taxa within each treatment group for water and larval sample types. For each taxon, mean relative abundance (mean) and standard deviation (SD) are provided

| Taxonomy | Feed | Sample type | Mean | SD |
|---|---|---|---|---|
| *Leucothrix* | Art | Water | 16.21 | 29.73 |
| *Nautella* | | | 9.35 | 13.13 |
| *Sulfitobacte* | | | 8.25 | 6.34 |
| *Nonlabens* | | | 4.31 | 6.10 |
| *Vibrio* | | | 3.59 | 3.60 |
| *Sulfitobacter* | Cir | | 17.13 | 19.26 |
| *Polaribacter* | | | 16.49 | 20.17 |
| *Leucothrix* | | | 13.11 | 22.32 |
| *Tenacibaculum* | | | 9.77 | 17.38 |
| *Lacinutrix* | | | 6.07 | 6.60 |
| *Polaribacter* | Cop/Cir | | 28.10 | 33.04 |
| *Leucothrix* | | | 8.77 | 19.44 |
| Rhodobacteracea | | | 7.57 | 8.21 |
| *Tenacibaculum* | | | 5.33 | 6.90 |
| *Sulfitobacter* | | | 5.33 | 4.73 |
| *Leucothrix* | Cop/FD | | 25.64 | 30.91 |
| *Polaribacter* | | | 16.05 | 20.71 |
| Rhodobacteracea | | | 7.15 | 6.04 |
| *Sulfitobacter* | | | 4.63 | 3.24 |
| *Pseudiphaeobacter* | | | 3.76 | 6.61 |
| *Leucothrix* | FD | | 51.42 | 33.54 |
| *Sulfitobacter* | | | 5.54 | 6.14 |
| *Polaribacter* | | | 5.52 | 6.03 |
| *Ulvibacter* | | | 3.10 | 4.47 |
| *Sedimentitalea* | | | 2.12 | 3.96 |
| *Vibrio* | Art | Larvae | 48.19 | 26.91 |
| Vibrionaceae | | | 4.90 | 7.50 |
| *Leucothrix* | | | 3.76 | 9.25 |
| *Pseudoalteromonas* | | | 2.58 | 2.07 |
| Arcobacteracea | | | 2.44 | 3.33 |
| *Leucothrix* | Cir | | 9.76 | 9.66 |
| *Psychromonas* | | | 7.43 | 5.62 |
| *Vibrio* | | | 6.23 | 5.30 |
| *Fluviicola* | | | 5.62 | 8.15 |
| *Sulfitobacter* | | | 5.42 | 3.94 |

**Table 6** (continued)

| Taxonomy | Feed | Sample type | Mean | SD |
|---|---|---|---|---|
| *Leucothrix* | Cop/Cir | | 11.64 | 15.41 |
| *Polaribacter* | | | 5.14 | 4.25 |
| *Psychrobacter* | | | 4.87 | 4.99 |
| *Vibrio* | | | 4.41 | 3.43 |
| *Colwellia* | | | 4.35 | 1.81 |
| *Leucotrhix* | Cop/FD | | 28.61 | 24.37 |
| *Vibrio* | | | 7.48 | 12.59 |
| Thodobacteracea | | | 5.09 | 3.05 |
| *Amylibacter* | | | 3.48 | 3.89 |

## Discussion

The results from this experiment clearly demonstrated that lumpfish larvae fed the enriched *Artemia* nauplii showed better early growth, had higher ossification rates at the end of the experiment, and had a much lower incidence of bone anomalies than larvae fed the other diets. This is also in line with recent work that showed lower growth and survival, poorer bone development, and a higher occurrence of bone anomalies in lumpfish larvae fed copepods and a formulated diet compared to *Artemia* (Marthinsen et al., NTNU, unpublished). Feeding with cirripeds showed improved growth during the later larval stages during weaning to a formulated diet. Larvae fed copepods or the formulated diet from the beginning had much slower growth and less normal development than the larvae fed the larger *Artemia* and cirripeds. Lumpfish fed *Artemia* had the highest glycogen stores in the liver during the live prey period. The better growth in the *Artemia* larvae was thus probably related to having more energy available for growth, rather than promoting glucose metabolism as in cod larvae (Vo et al. 2022). In addition, the largest biomass of *Artemia* was probably more energetically favorable due to a higher nutrient content (Pastorok 1981; Olsen 2004), which might explain the highest growth and highest glycogen store in *Artemia* larvae. Combined with our histological studies of liver and the larval ossification status at the end, it seemed like the larvae from the other treatments did not ingest the energy required for growth with these diets and that the reduced early nutritional status from the start resulted in early starvation and a lasting effect of poorer growth and development throughout the experiment (Kjørsvik et al. 2011, Gistelinck et al. 2016). Marthinsen et al. (NTNU, unpublished) found typical signs of starvation in the gut epithelium of lumpfish larvae fed copepods or formulated diet from the start, and transcriptomic analysis showed a significant starvation response in larvae fed copepods (Volpe et al. 2025). Start-feeding with copepods or the formulated diet from 2 dph would thus not be optimal from the start for lumpfish larvae, as we observed in this study.

The small pelagic yolk-sac larvae such as cod, halibut, and ballan wrasse have shown a remarkable improvement in growth and normal development from feeding with copepods/zooplankton rather than with rotifers and *Artemia*, which is correlated to the more optimal nutritional composition of copepods (Næss et al. 1995; Shields et al. 1999; Evjemo et al. 2003; Imsland et al. 2006; Koedijk et al. 2010; Busch et al. 2011; Øie et al. 2017). Copepods contain relatively much more proteins and a more suitable lipid class composition,

which seem to be the main reasons for the observed differences in growth (Van der Meeren et al. 2008; Evjemo et al. 2003; Karlsen et al. 2015; Øie et al. 2017). Cirripeds (also a crustacean) seem to have similar lipid and protein composition as copepods. Although initial larval growth in Cir-larvae was poor, the cirripede nutrients obviously provided a foundation for the major organogenesis taking place during the first feeding period (Wold et al. 2008; Pittman et al. 2013), resulting in the observed improved growth and development in the later larval stages. A similar phenomenon was also observed by Malzahn et al. (2022) for ballan wrasse, where larvae fed copepods first and cirripeds as the second diet showed more developed intestinal tissue compared to the other larval groups at the end of the experiment (after weaning to formulated diets, 48 days after hatching). Cirripeds should thus be a potentially good nutrition source for marine fish larvae, although more studies would be necessary related to dietary composition and feeding regimes.

All live prey contained relatively high levels of n-3 PUFA, and a high content of dietary PUFA n-3 fatty acids resulted in the best growth and development. Compared to the small cold-water pelagic larvae, the demersal lumpfish larval growth appeared less dependent on the essential n-3 fatty acids being in the phospholipid fraction of the lipids (Øie et al. 2017). The relative DHA content was not much reduced in any larval groups, suggesting that these fatty acids are preserved even though some larval groups did not feed much. The lumpfish larvae did not grow well on copepods, and feeding with cirripeds and copepods showed very slow growth during the first and second feeding phases, indicating a rather low feed uptake. The larval fatty acid composition reflected the composition of the present diet. However, the n-6 fatty acids from the formulated diet did not show up in the FD larvae before 21 dhp, suggesting that the larvae fed formulated diet did not ingest much food at all during the first feeding period.

Larvae in all treatments in this experiment displayed relatively low growth during the first week of feeding, although *Artemia-fed* larvae had a better growth rate than larvae in the other treatments. In contrast to the smaller cod larvae (Kjørsvik et al. 1991), the 2-day-old lumpfish larvae had an endogenous energy store of glycogen vacuoles in the liver, which probably made them more tolerant to suboptimal nutritional conditions during this mixed-feeding period, as reviewed by Hoehne-Reitan and Kjørsvik (2004). The nutritional status of the liver (% vacuolization) observed at 9 dph clearly demonstrated that the glycogen energy stores were maintained high in *Artemia-fed* larvae. However, glycogen was rapidly exhausted in larvae fed formulated diet and copepods, indicating that these larvae had either ingested much less feed or that they had a lower capacity to extract energy from the ingested feed. Larvae fed cirripeds had less decline in hepatic vacuoles at 9 dph. Only very few glycogen vacuoles and some lipid vacuoles were observed in the liver of cirripeds-fed larvae at 21 dph, demonstrating that the feed was supporting some growth but not enough energy to deposit glycogen storage. At the end of the experiment (34 dph), all larvae had similar and high hepatic vacuolization mostly of lipid nature, indicating they all were in the juvenile stage. As the degree of vacuolization was closely connected to the larval size and growth rate in this experiment, we suggest that this histological parameter is a useful parameter for assessing the nutritional condition during the larval stage.

Likewise, normal bone development was found to be linked to diet. Most larvae fed copepods and formulated diet and more than half of the larvae fed cirripeds had anomalies such as altered vertebral arches and spines, which are considered less severe and will not affect the external shape of the fish (Witten et al. 2005). Very few larvae in this experiment had severe vertebrae anomalies such as fusion, compression, and modified shape, and these could not be linked to any specific diets. However, any observed skeletal defects originate mostly from the chondrogenic and osteogenic differentiation at early larval stages, and they

are often found to be defined by the larval nutritional and environmental conditions (Witten et al. 2005). This indicates that osteogenic processes were affected in these larvae, and such anomalies could possibly weaken the neural and haemal arches' protection of the spinal cord and of the major blood vessels.

During the weaning period (21–35 dph), the mean daily weight decreased from about 8% to 4% in the *Artemia* larvae, while it remained at a higher rate of about 7% in the cirripeds larvae, suggesting that feeding cirripeds were more suitable before the weaning phase in lumpfish larvae. At 21 dph, the *Artemia* larvae had the highest somatic (SL and DW) growth and highest glycogen storage in the liver, whereas the cirripeds larvae had a significantly lower somatic growth and nearly no glycogen stored in the liver. The better growth in lumpfish fed cirripeds at this stage was thus probably not related to energy stored in the previous stage. In nature, lumpfish larvae and juveniles are selecting larger prey sizes corresponding with their growing sizes (Ingólfsson and Kristjánsson 2002). The body size of cirripeds was about 350 µm, whereas the size of enriched *Artemia* was about 800 µm. Lumpfish larvae were weaned with a 300 µm (100–500 µm) formulated diet. The dry feed size in this experiment may thus be more favorable for the cirripeds larvae than for the *Artemia* larvae. Nevertheless, the present results show that cirripeds can be a good second live prey for lumpfish larvae, and further investigations of formulated diet sizes during weaning and/or cofeeding *Artemia* and cirripeds would be interesting.

Both larval prey size and activity can be important for ingestion and growth in lumpfish larvae. *Artemia* was by far the largest live prey organism, and the larger biomass of nutrients was probably more energetically suitable for lumpfish larvae than the smaller and leaner copepods and cirripeds (Pastorok 1981; Olsen 2004). In addition, lumpfish larvae are mostly attached to a substrate and do not seem to swim around much (Brown 1986; Davenport 1985). They may thus have problems catching the fast swimming copepods and even the cirripeds during the earliest feeding period compared to the very slow-swimming and larger *Artemia* nauplii.

Observations of lumpfish larval behavior also showed that compared to the other larval groups, *Artemia*-fed larvae were swimming and orienting more towards prey at 16 and 22 dph, whereas larvae fed cirripeds had the highest frequency of successful prey attacks at 28 dph (I. Garcia-Gallego, unpublished). This agrees well with higher growth with cirripeds from the second feeding period. Our combined results showed that cirripeds could be a relevant prey organism for lumpfish larvae, and further studies are needed for investigating possible use of combinations of *Artemia* and cirripeds as prey, and of how nutritional content and prey/particle size may affect larval ingestion.

The microbial community, of both water and larvae, showed dynamism over the experimental period, developing in a synchronized manner across all the treatments. The time component appeared to be an important factor in the community development, as the period of 26–30 dph led to a more unique "fingerprint" compared to the period 10–21 dph. This coincided with the weaning of all treatments to the same formulated feed.

As noted by Malzahn et al. (2022), it seems that the flow-through system (FTS) affected the establishment of a specific microflora in water at the onset of the experiment. This led to a less stable microbial environment as new microorganisms were supplied to the rearing tanks but were also washed out at the same time (Attramadal et al. 2014). Nevertheless, the feed itself appeared to play a critical role in the development of the microbial community. The community of the seawater samples was different between the treatments at the onset of the experiment, but not between those starting with the same feed type, e.g., copepods (Cop/FD and Cop/Cir) or between Cir and Cop/Cir. Additionally, after weaning, all treatments with FD, the microbiota seem to converge, with *Leucotrix* being indicative of that feed type. This

can be substantiated by the fact that the emergence of *Leucothrix* in the FD treatment was occurring earlier compared to other treatments. This is an indication of feed influencing water microbiota either by enriching it as a part of the feed or by providing favorable growth conditions for indigenous microbiota and, hence, shaping its community (Vadstein et al. 2018).

Although statistical differences between water and larval total microbial community could be established, some of the most abundant genera across treatments were shared between both sample types (Giatsis et al. 2015). It has been noted before that the tank environment was selecting for bacterial communities which were colonizing newly hatched larvae (Vadstein et al. 2013). Like the seawater microbiota, the larval microbial associations demonstrated significantly different patterns between the treatments. Nevertheless, there was just a weak correlation between treatments which started with the same feed type (Cop/FD and Cop/Cir), but a stronger one for all the treatments at the end of the experiment, showcasing the abundance of *Leucothrix*, which were again indicative after weaning larvae to formulated feed. Our results indicated that the bacterial colonization in the larvae was actively controlled by the interaction between microbial communities in water and/or in feed, and will, along with the other environmental factors, play an important role in the development of the larval microbiome.

## Conclusion

In our experiment, using enriched *Artemia* nauplii caused early growth and normal development, whereas feeding with cryopreserved cirripeds led to higher growth rates only in the later stages of the experiment and during weaning to formulated feeds. These two prey organisms also resulted in the highest survival, the largest larvae, and better ossification than the other diets by the end of the experiment. Start-feeding with copepod nauplii or formulated diet both resulted in the overall slowest growth, and the poor nutritional status during the earliest larval development probably had a long-lasting effect with poor growth and development throughout the whole experiment. Vacuolization of the liver was a good biomarker for evaluating the energy available for growth from different feeding types in lumpfish larvae. Ossification and bone anomalies at the end of the experiment had a strong correlation to both diet and lumpfish larval size. Microbial patterns varied significantly across treatments, suggesting active regulation of the larval microbiome by feed and water interactions. Our results demonstrated that nutritional quality during the first 20 days after hatching (dph) was crucial for good growth and development up to 35 dph in lumpfish. Based on the results, a recommended larval start-feeding regime for lumpfish would be with enriched *Artemia* nauplii, with possible secondary (co-)feeding with cirripede nauplii. More long-term effects of the start-feeding diets are recommended to elucidate the more long-lasting effects.

During the earliest start-feeding period, lumpfish larvae were mainly stationary and attached to the substrate. Both prey size as well as ease of capture should be considered in addition to the nutritional quality of the live prey to support good growth and development. A more detailed nutritional composition of the cryopreserved cirripeds should be available before a further evaluation of these organisms as commercial live feed for large-scale industrial production.

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**Author contributions** All authors contributed to the study conception, design, and experimental activity, funding acquisition was performed by Andreas Hagemann and Elin Kjørsvik; material preparation, data collection and analyses were performed by Tu Anh Vo, Luciana Alves Musialak, Tora Bardal, Sunniva Brevik Kvernø, Marte Solli Lindskog, Saba Akbar, Bjørn Henrik Hansen, and Deni Ribičić. The first draft of the manuscript was written by Elin Kjørsvik, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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**Data availability** No datasets were generated or analysed during the current study.

## Declarations

**Ethical approval** 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 (experiment approval FOTS ID 24906, reference number 20/155217).

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

**Open access** This is for you at Aquaculture International.



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## Authors and Affiliations

**Elin Kjørsvik[1] · Tu Anh Vo[1] · Frank Thomas Mlingi[1] · Luciana Alves Musialak[1] · Tora Bardal[1] · Sunniva Brevik Kværnø[1] · Marte Solli Lindskog[1] · Saba Akbar[1] · Ibon García Gallego[2] · Arne Malzahn[3] · Dag Altin[1] · Bjørn Henrik Hansen[3] · Deni Ribičić[3] · Andreas Hagemann[3]**

✉ Elin Kjørsvik
elin.kjorsvik@ntnu.no

[1] Department of Biology, Norwegian University of Science and Technology, Trondheim 7491, Norway

[2] Grupo de Investigación en Acuicultura (GIA), IU-ECOAQUA, University of Las Palmas Gran Canaria, Las Palmas, Spain

[3] SINTEF Ocean, Trondheim 7465, Norway