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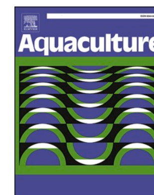

Ova lipid profiling and egg quality in wild and captive lumpfish, *Cyclopterus lumpus* (Linnaeus, 1758)

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ABSTRACT

Lumpfish (*Cyclopterus lumpus*) serves an important role as cleaner fish for removing salmon lice in salmon aquaculture. For this use, cultivated lumpfish are preferred over wild-caught fish to avoid overexploitation of natural stocks and provide a stable production of good quality eggs and larvae. To support this, high quality eggs containing the necessary building blocks to support high fertilization success, development into a normal embryo and high hatching success is needed. Ova from wild-caught and captive lumpfish females were collected and assessed to identify how wild and domesticated lumpfish differ in terms of ova lipid profiles, egg and larvae quality. Wild-caught lumpfish displayed higher fertilization and hatching success compared to captive lumpfish, whereas other biological variables like egg size, egg/larvae dry weight and respiration, and larval morphometry, were not significantly different between wild and captive lumpfish. Ova lipid profiles (total lipid, lipid classes, fatty acid composition and lipidomics) can separate egg batches based on their geographic origin of wild-caught females and composition of diets used for captive broodstock (lipid and carbohydrate content). Multivariate statistics were applied to identify lipid markers that can be used to predict the quality of lumpfish ova. Lumpfish ova lipidomic profiles covaried with several biological parameters, indicating that the former can be used to predict the latter. Lumpfish eggs were mostly composed of triacylglycerides (TAGs) with some phosphatidylethanolamines (PEs) and phosphatidylcholines (PCs), and their lipid profiles were more sensitive to the different origins, mother weight/size and fertilization success than variables such as eggs size or larvae morphometric measures. PC and PE content of lumpfish eggs were positively correlated to both hatching success and fertilization rates, thus the lipidomic profiles, with some key highlighted lipid species were putative predictors of egg quality.

1. Introduction

Norway is the largest Atlantic salmon (*Salmo salar*) producing country with a production share of 55.3% of the global production (Iversen et al., 2020). The ectoparasitic salmon louse (*Lepeophtheirus salmonis*, Krøyer 1837) remains a major cost driver for the salmon aquaculture industry (Iversen et al., 2017), and lice infestation levels sets premises for further growth in production areas along the Norwegian coast through regulations imposed by a so-called ‘Traffic Light system’ (Myksvoll et al., 2018; Sandvik et al., 2016).

Reduction in use of chemical delousing agents has resulted in development of a variety of new delousing methods (Greaker et al., 2020). Stocking cleaner fish in salmon cages to control lice infestation levels is becoming increasingly widespread (Erkinharju et al., 2021), and annually >60 million cleaner fish are stocked in salmon sea cages worldwide (Overton et al., 2020). One of the most widely used cleaner fish is the Atlantic lumpfish (*Cyclopterus lumpus* L. Linnaeus, 1758), which have been used to biologically control sea lice infestation for decades (Bjordal, 1990; Brooker et al., 2018). The expanding fisheries for mature lumpfish to obtain eggs for lumpfish farming can have

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negative effects on natural populations (Kennedy et al., 2018), and the industry needs to generate its own broodstocks in order to mitigate overexploitation of natural stocks, and to provide a stable production of good quality eggs and larvae.

High quality eggs contain the necessary material to support high fertilization success, development into a normal embryo, high hatch rates and viable offspring (Kjørsvik et al., 1990; Kjørsvik et al., 2003; Pavlov et al., 2004). Egg size (Barneche et al., 2018; Hixon et al., 2013; Lim et al., 2014) and female age (Lasne et al., 2018) have historically been considered as indicators for egg quality. Eggs from wild fish are usually considered of better quality than eggs from cultivated broodstocks (Srivastava and Brown, 2011) as they display higher fertilization rates, hatch rates, survival and size at hatch than eggs produced in captivity. One of the main drivers of egg quality is mothers nutrition (Brooks et al., 1997), however, much of the lumpfish's nutritional requirements are unknown. Knowledge on the dietary requirements of sexually maturing lumpfish is required to improve quality and robustness of farmed eggs and larvae, which is a prerequisite to ultimately meet the demand from the salmon industry and preserve wild stocks (Brooker et al., 2018).

Lipids are the most discussed biochemical group in relation to mothers' nutrition and egg quality. The dietary composition of the maturing mother fish is reflected in thelipid composition of their ova, which is the only energy source for the developing embryo and thus a critical factor for the viability and development of the embryos (Izquierdo et al., 2000). Especially the content of essential polyunsaturated fatty acids such as docosahexaenoic- (DHA) and eicosapentaenoic- (EPA) acid are important for the egg and larval quality in many marine fishes (Fuiman and Ojanguren, 2011; Furuita et al., 2000; Pickova et al., 1997). Lipid composition affects physiological properties of the developing embryonic membranes, as well as the membrane's ability to deal with stress, such as changes in temperature, pressure, and peroxidation (Parrish, 2013). Studies have shown that different lipid levels and/or lipid composition in fish broodstock diets changes egg quality (reviewed by Fernández-Palacios et al., 2011). Thus, nutritional requirements and protocols for cultivated lumpfish broodstock could be optimized by understanding how ova lipid composition influences egg- and larval quality. The heterogeneity of lipids and their relation to fertilization, physiological mechanisms and defence against environmental stress, make them attractive biomarkers to assess egg quality. In fact, lipidomics have successfully been implemented in ichthyology to detect environmental stress (Yan et al., 2012).

The main objectives of this study were *i*) to identify how wild and domesticated lumpfish differ in terms of ova lipid profiles, egg and larval quality, and *ii*) assess if ova lipid profiles are related to variations in the egg and larval quality in lumpfish. To do this, we collected ova from wild and domesticated lumpfish fed with three different diets. Sub-samples were taken for lipid profiling, and the remaining ova were fertilized with milt from one male. Eggs were incubated until 1 day post hatch, and during incubation we recorded fertilization and hatching success, physiological and morphometrical variables in developing lumpfish embryos and larvae. Multivariate statistics was applied as a step towards identifying lipid markers that can be used to predict the quality of lumpfish ova, which, to our knowledge, is the first attempt to apply lipid profiling for this purpose.

2. Materials and methods

2.1. Delivery of ova and cryopreserved milt

Ova were obtained from three different sources; Wild caught females from Namdal Rensefisk AS (3 females, named NR) and Skjerneset Fisk AS (3 females, named RK2), and eggs obtained from a first generation of captive female broodstock fish from Nofima (Sunndalsøra, Norway). The latter were procured from three separate groups of captive females fed different experimental diets varying in lipid and carbohydrate content named LS-BS-1 (fed 18.7% lipid, 7.6% carbohydrates), LS-BS-2 (13.9% lipid, 13.5% carbohydrates) and LS-BS-3 (7.3% lipid, 18.0% carbohydrates). Eggs were strip-spawned onsite and fertilized within 12 h. To exclude paternal effects in this study, cryopreserved milt from a single male lumpfish was supplied by Cryogenetics AS. All females were weighed and measured (Supplementary Material 1, Table S1.1).

2.2. Lipid extraction and total lipid analyses

Lipids were extracted from ova with the Folch method (Folch, Lees, Stanley, 1957). Roughly 100 mg (wet weight) of eggs were suspended in 3 mL 2:1 chloroform:methanol and homogenized using a mechanical dispenser with a stainless steel tip (IKA T 10 basic ULTRA-TURRAX). The samples were centrifuged at 2000 rpm for 5 min at 4 °C to remove particulates, and the subsequent supernatant was filtered through GF/C-grade glass microfibre filters. Next, samples were spiked with 10 μL of a heavy isotope-labelled internal standard mixture (SPLASH® II LIPIDOMIX® Mass Spec Standard from Avanti Polar Lipids). Lipophobic phase separation was achieved by adding 1.25 mL 0.9% NaCl followed by centrifugation at 2000 rpm for 15 min at 4 °C. The lower (organic) phase was recovered and dried under a gentle nitrogen stream. Finally, samples were reconstituted in 1 mL chloroform and stored at −80 °C. Total lipid content was determined gravimetrically by weighing the lipid extract after drying.

2.3. Fatty acid composition

Fatty acid (FA) composition was determined by fatty acid transmethylation to fatty acid methyl esters (FAMEs) and GC-FID analysis. Lipid extracts were dissolved in 1 mL 0.1 NaOH in methanol and heated to 100 °C for 15 min. Next, 2 mL 50% boron trifluoride in methanol were added and the reactions heated to 100 °C for 5 min. The reactions were cooled to room temperature, and 1 mL hexane was added before heating to 100 °C for 1 min. FAMEs were extracted by adding 1 mL hexane and 2 mL of a saturated NaCl solution. The phases were separated by centrifugation at 2000 rpm for 5 min and the upper phase was collected in a new tube. The extraction was repeated twice with 1 mL and 2 mL hexane and mixed. The hexane-extracted FAMEs were finally subjected to analysis by GC-FID.

The FAMEs were analysed according to Daukšas et al. (2005) with the following modifications: an Agilent Technologies 7890A gas chromatograph with flame ionization detection (GC–FID) equipped with a 7693 autosampler was used. The detector temperature was held at 270 °C, and the flame was maintained with 25 mL/min H2 gas and 400 mL/min filtered air. Chromatography was carried out using a Cp-wax 52CB, 25 m, 0.25 mm with i.d. 0.2 mm column (Agilent Technologies). Helium was used as the carrier gas at a flow rate 1.5 mL/min. GC inlets were held at 250 °C. The initial oven temperature was held at 80 °C and increased to 180 °C at 25 °C/min, followed by a 2 min hold, after which the temperature was increased to 205 °Cat 2.5 °C/min, followed by a 6 min hold, after which the temperature was increased to 215 °C at 2.5 °C/min, followed by a 4 min final hold. Fatty acids were characterized by comparison to the retention times of commercial standards and quantified by internal standard. The accuracy of the method was verified by comparison of FA profiles of selected marine oils against profiles assessed by accredited laboratories.

2.4. Lipid class determination

Lipid classes were analysed using liquid chromatography coupled to charged aerosol detection (LC-CAD) as previously described by Khoomrung et al. (2013). Lipid extracts were redissolved chloroform and analysed on an Agilent 1200 HPLC equipped with a CAD detector. The DAD was a Dionex Corona Ultra RS (ThermoFisher Scientific) and was supplied with N2 at 4 bar and recorder at 10 Hz with 100 pA gain. The injection volume was 2 μL on a Luna HILIC 200 A column (250 ×

4.6 mm, 5 μm article size) at 35 °C with a flow rate of 0.8 mL/min. The mobile phase was comprised of (A) hexane-acetic acid (99:1), (B) acetone-isopropanol-acetic acid (29:70:1), and (C) water-acetone-isopropanol-acetic acid (9:20:70:1). The mobile phases B and C contained 0.08% triethylamine. The mobile phase gradient is given in the Supplementary Material 2 (Table S2.1). Standards with known concentrations were used for peak identification and quantification.

2.5. Lipidomics analyses

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 UPLC coupled to a 4670 triple quadrupole mass spectrometer with an electrospray ion source. The ion source parameters were as follows: drying gas temperature 320 °C, drying gas flow 12 L/min, nebulizer pressure 30 psi, sheath gas temperature 400 °C, sheath gas glow 12 L/min, capillary voltage 5000 V, and nozzle voltage 2000 V. 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) acetonitrile-water (40:60) and (B) isopropanol-acetonitrile (90:10), both containing 10 mM ammonium formate and 0.1% formic acid. The mobile phase gradient is given in the Supplementary Material 2 (Table S2.2).

Each sample was injected three times with each injection analysing a different set of lipids to ensure a dwell time of >5 ms per mass transition. 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). However, it must be noted that the mass transitions were not confirmed with high resolution mass spectrometry, so there is a degree of uncertainty to each individual lipid species. Furthermore, we could not distinguish between isobaric species of the same lipid class with different branch chain configurations, e.g. a diacylglyceride with 18:0 and 16:1 and one with 18:1 and 16:0 would both be detected as 34:1.

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 an 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.

2.6. Fertilization, incubation and hatching

Lumpfish ova (50 mL) were transferred to glass beakers (100 mL); excess liquid was poured off and 1 mL cryopreserved milt was added. The eggs were carefully stirred using a glass rod for 3 min before filtered sea water (0.1 μm, 50 mL, 10 °C, 34 ppt salinity) was added followed by another 2 min of stirring. Fertilized eggs were moulded into circular monolayers (2 cm diameter) of tightly packed eggs ($n = 80$–90) using a custom-made mould, and thereafter allowed to harden for 15–20 min. We incubated 6 to 13 replicate egg-monolayers per female (n for each female can be found in Table 2) before they were transferred into incubator tubes (50 mL) with plankton mesh (SEFAR NITEX, 300 μm light opening) fixed at the top and bottom of the tubes. Incubator tubes were mounted in a flow-through incubator (approx. 15 mL) and seawater (10 °C, pH 7.8, 34 ppt salinity) with a flow-through rate of approx. 50 mL/min for each tube with a 12-h light:12-h dark regime where the eggs developed until they hatched (29–30 days after fertilization). Fertilization was confirmed by visual inspection 7 days post fertilization (dpf). Fertilization success was estimated as the percentage of fertilized eggs in relation to the total amount of eggs in the sample, and hatching success was estimated as the percentage of all fertilized eggs in the sample which hatched.

2.7. Respiration, dry weight and elemental analyses

Respiration rates of groups of 5 eggs or individual larvae were performed as described in Hansen et al. (Hansen et al., 2019), using a MicroRespiration System (Unisense, Denmark). After measurement, eggs were pipetted from the respiration chamber into distilled water to rinse off seawater. Eggs were then placed into pre-weighed tin capsules for later weighing and elemental analysis. Larvae were flushed into a pH buffered, overdosed MS-222 /seawater solution (500 mg/L). Euthanized larvae where then rinsed in distilled water and put individually into pre-weighed tin capsules. Both eggs and larvae were dried at 60 °C. Once dried, eggs and larvae were weighed and shipped to NC Technologies (Milano, Italy) to be analysed for carbon and nitrogen content.

2.8. Larvae morphometry

ImageJ software (Schneider et al., 2012) was used to measure the total egg diameter and standard length on images. Standardised images of larvae at 1 dph were used for morphometric analyses of standard length, body area, lateral yolk-sac area, myotome height, eye area, number of lipid droplets, ventral yolk sac area and ventral body width. We used Mask R-CNN (He et al., 2017), trained on 373 manually annotated images of lumpfish larvae, to automatically outline the eye-, yolk-, lipid- and body area for morphometric analysis. The morphometric measurements such as area and length were calculated using automated image processing techniques on the outlined parts such as Topological Structural Analysis (TSA, (Suzuki, 1985)), ellipse fitting (Fitzgibbon and Fissher, 1996; Zhang and Suen, 1984) and skeletonize (Zhang and Suen, 1984). A complete description of the automated morphometric analysis flow (AutoMOMI) is described in Kvæstad et al. (Kvæstad et al., 2022), and an example of AutoMOMI output is given in Supplementary Material 3 (Fig. S3.1).

2.9. Data treatment and statistical analyses

GraphPad ver 9.0 was used to visualize the data and to show the range of the individual data sets, including analysis of variance (ANOVA). Data analysis and multivariate statistics were performed using the mixOmics R package (Lê Cao et al., 2009; Rohart et al., 2017). Multivariate statistics were used to categorize the ova batches based on their lipid compositions (fatty acids and other lipid species). Clustering of groups based on lipid composition was visualized using plots from principal component analyses (PCA) and sparse partial least squares discriminant (sPLS-DA) analyses. The data was further visualized using a clustered image map (CIM) to illustrate correlation strength (using a color gradient) between lipid profiles and traditional egg quality variables (mother weight, mother size, fertilization success, hatching success).

3. Results and discussion

3.1. Ova lipid profiling

Ova from wild-caught (NR and RK2) had a slightly higher lipid content than captive lumpfish (LS-BS-1-3) (*t*-test, $p = 0.03$), but when

comparing all groups, significant differences were only found between RK2 and LS-BS-2-3 ($p < 0.05$). For the ova stripped from captive females, an apparent trend towards a lower lipid content corresponding with reduced lipid content in the broodstock diets (from LS-BS-1 (highest) to LS-BS-3 (lowest)) (Fig. 1A) was tested statistically, but no significant correlation (Spearman correlation, $p = 0.33$) was found. There were also no significant differences in lipid content in ova from females fed different diets ($p > 0.05$).

The lipid classes detected in the ova were steryl esters (SE), triacylglycerides (TAG), cholesterol (CH), phosphatidylethanolamines (PE), and phosphatidylcholines (PC) (Fig. 1 B). Other lipid classes were not present in our samples at high enough quantities to be detected. The analyses showed that the lumpfish ova were mostly composed of TAGs with some phospholipids (PEs and PCs), which is in line with lipid composition of other fish eggs (Wiegand, 1996). PCs serve as both an energy reserve and are, together with PEs, the main source for structural lipids in fish eggs (Cejas et al., 2004; Rainuzzo et al., 1997; Wiegand, 1996). Ova from wild-caught females trended towards having higher TAG content, being significantly higher in NR compared to LS-BS-2 ($p > 0.0001$) and LS-BS-3 ($p = 0.0258$). There was no significant correlation (Spearman $r = 0.36$, $p > 0.05$) between TAG content and lipid content in the mothers' diets, even though TAG was significantly higher in LS-BS-1 (high lipid feed) compared to LS-BS-2 (medium lipid feed) ($p = 0.0390$).

The fatty acid profiles of all ova batches were dominated by polyunsaturated fatty acids (41.0%), followed by saturated (25.2%) and monounsaturated (30.2%) fatty acids. The average omega-3 content was 34.6%. The most abundant fatty acid type was oleic acid (C18:1n9, 19.7%), followed by palmitic acid (C16:0, 18.0%), docosahexaenoic acid (DHA, C22:6n3, 17.9%), and eicosapentaenoic acid (EPA, C20:5n3, 13.0%) (Table 1).

There were significant differences between ova from wild-caught and cultivated females, with DHA (C22:6n3) and EPA (C20:5n3) displaying higher levels, and linoleic acid (C18:2n6) being at lower levels in the "wild" ova. Significantly higher levels of PUFAs were found for one of the groups of wild caught fish (RK2) compared to the captive groups. This coheres with observations done for wild and domesticated beluga sturgeon which suggests that elevated levels of linolenic and linoleic acids in captive beluga reflect diet just as much as a physiological response to a high temperature environment (Ovissipour and Rasco, 2011), observations also reported from cultured turbot (*Scophthalmus maximus*) (Silversand et al., 1996). In eggs of farmed Atlantic cod, higher levels of C16:0, C18:0, C18:2n6, C18:3n3, C18:3n6, C20:4n6, C20n:5n3, C22:5n3 and C22:5n6 and lower levels of C14:0, C16:1n7, C18:4n3, C20:4n3, C22:6n3 and C24:1n9 were found compared to wild cod eggs (Lanes et al., 2012), which to a large extent is in contrast to our observations for lumpfish (Table 1). Oleic acid (C18:1n9) content was significantly higher in ova from the captive groups LS-BS-2 and LS-BS-3, and this is possibly due to high content of this fatty acid in the feed as previously observed for cultured chinook salmon (Ashton et al., 1993), beluga, salmon (*Salmo trutta labrax*), grey flathead mullet (*Mugil cephalus*) (Mol and Turan, 2008) and turbot (*S. maximus*) (Silversand et al., 1996). Sparse partial least squares discriminant analysis (sPLS-DA) identified the key fatty acids responsible for the differences between groups (Fig. 2A). The unsaturated fatty acids linoleic acid (C18:2n6), stearidonic acid (C18:4n3), and eruric acid (C22:1n9) were the top three fatty acids accounting for the variance between ova from wild-caught and captive female. Linoleic acid was lower in ova from wild-caught fish compared to ova from captive females, whereas stearidonic- and eruric acids were higher. The EPA/arachidonic acid (ARA, 20:4n6) ratio was also higher in wild-caught fish compared to captive females. Adrenic acid (C22:4), cetoleic acid (C22:1n11) and arachidonic acid (ARA, C20:4n6) explained the covariance between feeding regimes. All three fatty acids increased when the mothers were fed diets with increasing lipid content. We also observed that oleic acid (C18:1n9) and linoleic acid (C18:2n6) were lower and docosahexaenoic acid (DHA, C22:6n3), eicosapentaenoic acid (EPA, C20:5n3) and EPA/ARA ratios were higher in ova from wild-caught fish than in captive females. Lipid profiles of feed of the wild-caught females can obviously not be assessed and compared to the feed from the domestic lumpfish, but differences in lipid profile of the feed may certainly be a factor separating these groups.

Comparable to the fatty acid analysis (Fig. 2A), the egg batches can be separated based on lipidomics data into captive vs. wild-caught mothers, as well as the mothers' diets. Unsupervised PCA of lipidomics data (Fig. 2) grouped the ova batches by wild-caught vs captive females (principal component 1 (PC1)) and by mothers' feed (principal component 2 (PC2)). The PCA plot based on lipidomics profile shows that 38% of the variance in the data results from PC1 and 30% from PC2 (Fig. 2B).

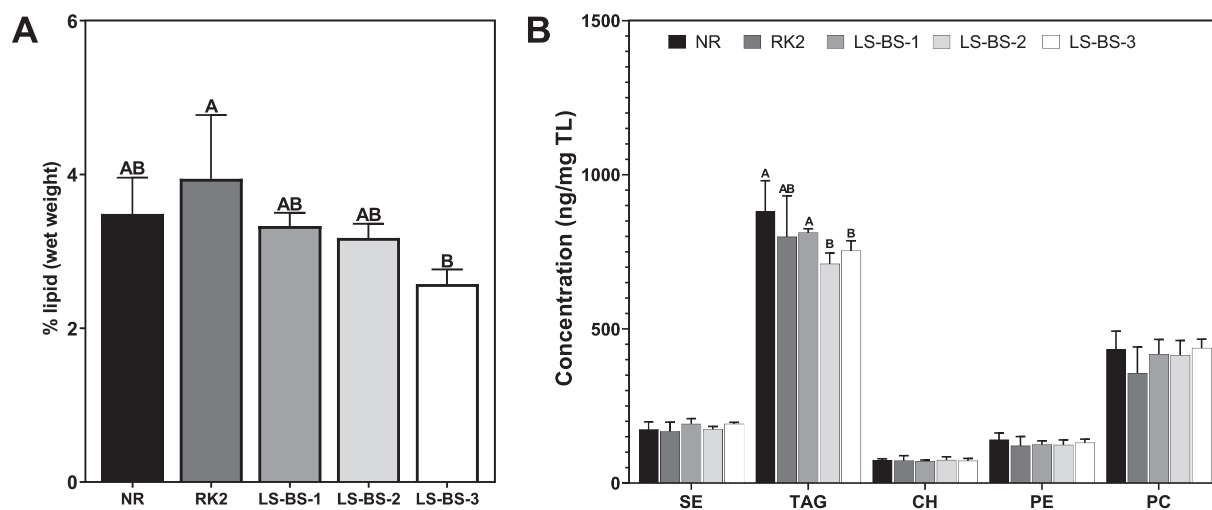

Fig. 1. A: Total lipid content (wet weight) in oocytes of wild-caught lumpfish from Namdalen Rensefisk (NR, $N = 3$) and Skjerneset Rensefisk (RK2, $N = 3$), and captive females fed different diets LS-BS-1 ($N = 3$), LS-BS-2 ($N = 4$) and LS-BS-3 ($N = 3$) determined gravimetrically. B: Lipid class composition of lipid extracts determined by LC-CAD. SE = steryl esters, TAG = triacylglyserols, CH = cholesterol, PE = phosphatidylethanolamines, and PC = phosphatidylcholines. Significant differences ($p < 0.05$) between groups are denoted with different letters.

Table 1
Fatty acid profile of ova of lumpfish. Results are presented as percent of total FAME content (%). Data are given as mean ± SD. N = 3 throughout.

		NR	RK2	LS-BS-1	LS-BS-2	LS-BS-3
C14:0	Myristic acid	1.97 ± 0.13	1.61 ± 0.45	1.29 ± 0.07	1.26 ± 0.07	0.92 ± 0.02
C14:1	Myristoleic acid	0.12 ± 0.03	0.11 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.06 ± 0.00
C15:0	Pentadecanoic acid	0.42 ± 0.08	0.36 ± 0.08	0.29 ± 0.01	0.26 ± 0.01	0.20 ± 0.00
C16:0	Palmitic acid	18.07 ± 2.65^{AB}	16.97 ± 0.44^{A}	18.49 ± 0.42^{B}	18.24 ± 0.40^{B}	18.30 ± 0.19^{B}
C16:1	Palmitoleic acid	2.14 ± 0.09 ^{AB}	1.80 ± 0.07^{A}	2.88 ± 0.25 ^{AB}	2.91 ± 0.09^{B}	2.80 ± 0.23 ^{AB}
C17:0	Heptadecanoic acid	0.76 ± 0.07	0.70 ± 0.18	0.41 ± 0.04	0.38 ± 0.03	0.27 ± 0.00
C17:1	Heptadecanoic acid	0.38 ± 0.06	0.35 ± 0.04	0.37 ± 0.02	0.37 ± 0.02	0.34 ± 0.02
C18:0	Stearic acid	4.79 ± 0.52	4.66 ± 0.31	4.76 ± 0.14	4.77 ± 0.16	5.14 ± 0.34
C18:1n9	Oleic acid	18.81 ± 2.31^{B}	17.63 ± 0.12^{A}	19.12 ± 0.91^{B}	20.60 ± 0.64^{C}	23.25 ± 0.84^{D}
C18:1n7	Vaccenic acid	3.18 ± 0.23^{AB}	3.06 ± 0.77^{A}	4.32 ± 0.22^{C}	4.18 ± 0.38^{BC}	4.55 ± 0.32^{C}
C18:2n6	Linoleic acid	1.20 ± 0.05^{A}	1.07 ± 0.11^{A}	5.80 ± 0.21^{B}	6.45 ± 0.35^{B}	10.69 ± 0.76^{C}
C18:3n6	`Y-Linoleic acid	0.15 ± 0.05	0.12 ± 0.03	0.06 ± 0.02	0.07 ± 0.01	0.07 ± 0.01
C18:3n3	α-linoleic acid	0.66 ± 0.17	0.60 ± 0.22	0.61 ± 0.01	0.63 ± 0.03	0.80 ± 0.05
C18:4n3	Stearidonic acid	0.87 ± 0.14	0.88 ± 0.12	0.56 ± 0.03	0.53 ± 0.03	0.41 ± 0.02
C20:0	Arachidic acid	0.06 ± 0.02	0.05 ± 0.00	0.07 ± 0.01	0.08 ± 0.01	0.09 ± 0.02
C20:1	Gondoic acid	3.67 ± 0.68^{A}	2.89 ± 0.39^{AB}	2.76 ± 0.15^{AB}	2.47 ± 0.22^{BC}	1.45 ± 0.15^{C}
C20:2n6		0.24 ± 0.03	0.20 ± 0.03	0.21 ± 0.01	0.21 ± 0.01	0.27 ± 0.06
c20:3n6	Dihomo-`Y-linoleic acid	0.10 ± 0.03	0.08 ± 0.01	0.12 ± 0.01	0.11 ± 0.02	0.11 ± 0.02
C20:4n6	Arachidonic acid (ARA)	0.64 ± 0.08	0.67 ± 0.08	0.79 ± 0.05	0.77 ± 0.02	0.68 ± 0.01
C20:3n3	Eicosatrienoic acid	0.13 ± 0.01	0.11 ± 0.03	0.11 ± 0.02	0.10 ± 0.00	0.09 ± 0.01
C20:4n3		0.93 ± 0.21	0.97 ± 0.20	0.91 ± 0.04	0.90 ± 0.09	0.84 ± 0.11
C20:5n3	Eicosapentaenoic acid (EPA)	14.73 ± 2.79^{B}	16.13 ± 0.96^{A}	12.25 ± 0.52^{C}	11.61 ± 0.34^{C}	9.85 ± 0.02^{D}
C22:0	Behenic acid	0.03 ± 0.01	0.07 ± 0.06	0.04 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
c22:1n11	Cetoleic acid	0.67 ± 0.12	0.46 ± 0.13	0.65 ± 0.04	0.54 ± 0.08	0.23 ± 0.00
C22:1n9	Eruric acid	0.39 ± 0.06	0.29 ± 0.06	0.15 ± 0.01	0.14 ± 0.01	0.10 ± 0.01
C22:2	Docosaenoic acid	0.25 ± 0.04	0.26 ± 0.02	0.26 ± 0.01	0.26 ± 0.02	0.24 ± 0.01
C22:3		0.12 ± 0.10	0.12 ± 0.03	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
C22:4	Adrenic acid	0.19 ± 0.03	0.20 ± 0.02	0.25 ± 0.01	0.23 ± 0.01	0.17 ± 0.01
C22:5n3	Docosapentaenoic acid (DPA)	1.13 ± 0.27	1.17 ± 0.12	1.70 ± 0.15	1.53 ± 0.11	1.41 ± 0.17
C24:0	Lignoceric acid	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
C22:6n3	Docosahexaenoic acid (DHA)	18.61 ± 3.36^{B}	22.07 ± 1.42^{A}	16.98 ± 0.46^{C}	16.89 ± 0.25^{C}	13.73 ± 0.64^{D}
C24:1n9	Nervonic acid	0.20 ± 0.03	0.19 ± 0.10	0.21 ± 0.03	0.22 ± 0.02	0.23 ± 0.01
EPA/ARA		22.95 ± 2.01^{A}	24.53 ± 4.48^{A}	15.45 ± 0.34^{B}	15.18 ± 0.46^{B}	14.60 ± 0.28^{B}
EPA/DHA		0.79 ± 0.03	0.73 ± 0.09	0.72 ± 0.01	0.69 ± 0.02	0.72 ± 0.03
Sum sat		26.11 ± 3.39	24.41 ± 0.14	25.34 ± 0.36	25.02 ± 0.33	24.97 ± 0.53
Sum MUFA		29.55 ± 3.24^{B}	26.78 ± 0.59^{A}	30.54 ± 1.14^{B}	31.51 ± 0.63^{BC}	33.00 ± 1.21^{C}
Sum PUFA		39.94 ± 6.91^{A}	44.62 ± 0.19^{C}	40.62 ± 0.98^{AB}	40.33 ± 0.55^{AB}	39.36 ± 0.58^{A}
Sum omega 3		37.06 ± 6.61^{B}	41.92 ± 0.08^{A}	33.10 ± 1.05^{C}	32.19 ± 0.46^{C}	27.12 ± 0.30^{D}

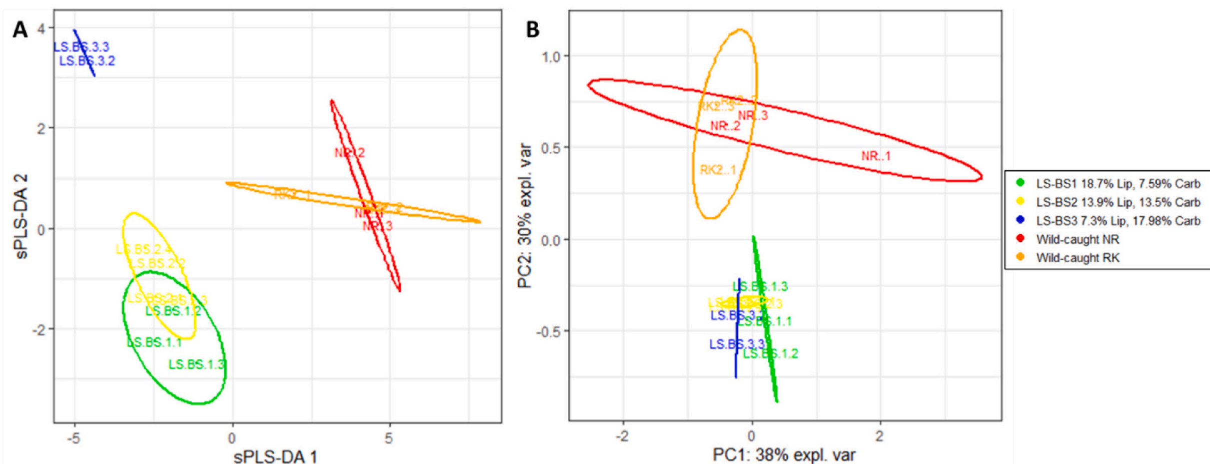

Fig. 2. A: sPLS-DA of lumpfish samples based on fatty acid composition determined by GC-FID analysis of FAMEs. B: Unsupervised PCA of lumpfish egg batches based on the total lipidomics profile measured by LC-MS/MS.

3.2. Quality indicators measured in eggs and larvae

Eggs from wild-caught lumpfish (NR and RK2-groups) exhibited high fertilization rates ranging from 67.2 ± 7.0% (batch RK2-#3) to 98.5 ± 1.2% (batch NR-#1) (Table 2).

Compared to the captive females, the two wild-caught groups (NR and RK2) displayed higher fertilization success than captive females, being significantly higher ($p < 0.05$) than LS-BS-2. For the captive females, fertilization rates varied from 0% (batches LS-BS-2-4 and 2–6) to 85.2 ± 7.1% (LS-BS-3-4). Eggs from females fed the medium and low lipid/carbohydrate diets (LS-BS-2 and -3) displayed low and highly variable fertilization rates; <10% was observed for all LS-BS-2 batches, except LS-BS-2-5 (70.9 ± 8.3%). The batches LS-BS-2-4, 2–5, 2–6 and 3–4 were the last egg batches to be delivered, and for two of them, 2–5 and 3–4, fertilization success was above 70%.

Lumpfish eggs harden after fertilization, and this process changes

Table 2
Fertilization success (%), egg diameter (mm) and hatching success (%) for all groups of egg batches (7–13 circular egg batches were prepared from each group). Data presented are mean, standard deviation (SD), N and range (with minimum and maximum values) for all egg batches.

Group	Fertilization success (%)		Egg diameter (mm)		Hatching success (%)	
	Mean ± SD (N)	Range (min-max)	Mean ± SD (N)	Range (min-max)	Mean ± SD (N)	Range (min-max)
NR-#1	98.5 ± 1.2 (13)	96.2–100	2.28 ± 0.06 (12)	2.21–2.39	90.9 ± 4.1 (13)	80.9–98.7
NR-#2	91.9 ± 2.3 (13)	88.8–96.3	2.19 ± 0.06 (12)	2.11–2.30	95.1 ± 3.6 (13)	86.5–100
NR-#3	91.8 ± 2.9 (12)	85.7–95.8	2.27 ± 0.07 (12)	2.17–2.44	87.8 ± 4.9 (12)	82.2–97
RK2-#1	83.6 ± 9.4 (9)	61.5–92.7	2.23 ± 0.05 (12)	2.15–2.30	90.6 ± 4.4 (8)	83.5–96.7
RK2-#2	84.4 ± 4.1 (9)	79.7–91.3	2.36 ± 0.07 (12)	2.27–2.46	78.8 ± 3.8 (9)	71–84.5
RK2-#3	67.2 ± 7.0 (8)	57.1–77.1	2.50 ± 0.05 (12)	2.40–2.58	44.1 ± 10.9 (8)	27.5–60
LS-BS-1-1	74.2 ± 5.4 (8)	68.0–85.1	2.11 ± 0.06 (12)	2.01–2.19	90.7 ± 12.8 (7)	70.2–100
LS-BS-1-2	71.0 ± 4.8 (8)	64.3–78.5	2.20 ± 2.20 (12)	2.04–2.27	79.8 ± 28.2 (7)	25–100
LS-BS-1-3	46.2 ± 6.7 (8)	39.5–57.1	2.16 ± 0.10 (12)	2.03–2.31	57.6 ± 48.7 (8)	0–100
LS-BS-2-1	6.5 ± 6.0 (9)	0.0–19.2	2.25 ± 0.08 (12)	2.11–2.42	0.0 ± 0.0 (7)	0–0
LS-BS-2-2	3.7 ± 2.9 (9)	0.0–7.9	2.21 ± 0.07 (12)	2.07–2.32	47.6 ± 38.7 (7)	0–100
LS-BS-2-3	2.4 ± 2.6 (9)	0.0–7.1	2.21 ± 0.07 (12)	2.11–2.42	0.0 ± 0.0 (9)	0–0
LS-BS-2-4	0.0 ± 0.0 (8)	0.0–0.0	2.20 ± 0.08 (12)	2.11–2.36	0.0 ± 0.0 (8)	0–0
LS-BS-2-5	70.9 ± 8.3 (13)	57.6–90.0	2.27 ± 0.09 (12)	2.12–2.42	66.7 ± 7.7 (13)	53.7–80
LS-BS-2-6	0.0 ± 0.0 (9)	0–0	2.04 ± 0.16 (12)	1.73–2.31	0.0 ± 0.0 (9)	0–0
LS-BS-3-1	24.4 ± 4.8 (6)	19.2–32.0	2.25 ± 0.06 (12)	2.15–2.34	89.4 ± 14.8 (6)	66.7–100
LS-BS-3-2	16.3 ± 7.6 (9)	6.6–30.0	2.33 ± 0.07 (12)	2.19–2.45	71.5 ± 28.7 (9)	13.3–100
LS-BS-3-3	2.1 ± 1.5 (7)	0.0–3.7	2.20 ± 0.08 (12)	2.09–2.33	40.0 ± 41.8 (5)	0–100
LS-BS-3-4	85.2 ± 7.1 (13)	74.1–100	2.22 ± 0.08 (12)	2.12–2.35	83.8 ± 6.2 (13)	66.7–90.8

both the thickness and surface appearance of the chorion, and increases the size and diameter of the lumpfish egg (Lönning et al., 1984). Therefore, egg diameter was measured 1–2 days after fertilization to ensure the hardening process was finished upon measurements (see Table 2 for values for individual batches and Table 3 for average values for each group). The egg diameter was larger for some of the wild-caught groups compared to eggs from captive females, but there were some

Table 3
Biological variables measured in mother (weight and total length), eggs (1 dpf; fertilization success, diameter, carbon and nitrogen content, C/N and respiration rate, hatching success) and larvae (1 dph; standard length, weight, carbon and nitrogen content, C/N, respiration rate, side yolk fraction, ventral yolk fraction and lipid droplet fraction) lumpfish collected from wild-caught fish (Namdalen Rensefisk (NR) and Skjerneset (RK2)) and captive females from Nofima fed different experimental diets varying in lipid and carbohydrate content named LS-BS-1 (fed 18.7% lipid, 7.6% carbohydrates), LS-BS-2 (13.9% lipid, 13.5% carbohydrates) and LS-BS-3 (7.3% lipid, 18.00% carbohydrates). Significant differences ($p < 0.05$) between groups are noted with different capital letters (A, B, C).

		NR	RK2	LS-BS-1	LS-BS-2	LS-BS-3
Mother - weight	gram	3743 ± 1086 (3)	2940 ± 922 (3)	2421 ± 201 (3)	2736 ± 416 (6)	3090 ± 259 (4)
Mother - length	cm	43 ± 3 (3)	35 ± 3 (3)	36 ± 2 (3)	35 ± 2 (6)	37 ± 1 (4)
Fertilization success	%	94.1 ± 3.9 (3) **A**	78.9 ± 10.6 (3) **A**	63.8 ± 13.7 (3) **AB**	18.2 ± 29.2 (6) **B**	40.2 ± 35.7 (4) **AB**
Egg - Diameter	mm	2.24 ± 0.08 (3)	2.36 ± 0.12 (3)	2.16 ± 0.08 (3)	2.20 ± 0.12 (6)	2.25 ± 0.09 (4)
Egg - Weight	mg dw	1.73 ± 0.17 (3)	1.97 ± 0.40 (3)	1.29 ± 0.25 (3)	1.24 ± 0.21 (6)	1.36 ± 0.11 (4)
Egg - Carbon content	µg	885.8 ± 90.9 (3) **A**	1003.2 ± 195.0 (3) **A**	715.8 ± 137.4 (3)**B**	685.3 ± 121.1 (6) **B**	715.5 ± 70.6 (4) **B**
Egg - Nitrogen content	µg	179.1 ± 21.8 (3)	215.0 ± 45.8 (3)	140.8 ± 22.5 (3)	134.8 ± 23.0 (6)	146.7 ± 9.8 (4)
Egg C/N	–	5.0 ± 0.1 (3)	4.7 ± 0.1 (3)	5.1 ± 0.2 (3)	5.1 ± 0.1 (6)	4.9 ± 0.2 (4)
Egg - Respiration	pmol O2 • ind-1 • h-1	1.07 ± 0.39 (3)	2.56 ± 0.61 (3)	0.78 ± 0.07 (3)	0.92 ± 0.55 (6)	0.83 ± 0.32 (4)
Hatching success	%	91.3 ± 5.1 (3)	71.5 ± 20.6 (3)	75.2 ± 36.9 (3)	60.0 ± 25.4 (2)	74.8 ± 28.3 (4)
Larvae - standard length	mm	5.78 ± 0.22 (3)	5.94 ± 0.18 (3)	5.76 ± 0.22 (3)	5.94 ± 0.21 (2)	5.85 ± 0.29 (4)
Larvae - Weight	mg dw	1.18 ± 0.20 (3)	1.38 ± 0.29 (3)	0.82 ± 0.20 (3)	0.89 ± 0.11 (2)	0.88 ± 0.11 (3)
Larvae - Carbon content	µg	543.3 ± 54.1 (3) **B**	669.4 ± 87.3 (3) **A**	439.4 ± 114.7 (3) **C**	448.0 ± 37.0 (2) **C**	441.1 ± 55.0 (3) **C**
Larvae - Nitrogen content	µg	93.5 ± 14.3 (3)	118.3 ± 15.2 (3)	73.0 ± 17.5 (3)	82.1 ± 8.8 (2)	79.0 ± 6.3 (3)
Larvae - C/N	–	5.9 ± 0.5 (3)	5.7 ± 0.2 (3)	6.1 ± 0.4 (3)	5.5 ± 0.2 (2)	5.6 ± 0.3 (3)
Larvae - Respiration	pmol O2 • ind-1 • h-1	81.56 ± 37.51 (3)	57.31 ± 14.93 (3)	43.38 ± 10.97 (3)	75.08 ± 23.98 (2)	52.63 ± 9.93 (3)
Larvae - Side yolk fraction	–	0.20 ± 0.02 (3)	0.21 ± 0.02 (3)	0.18 ± 0.03 (3)	0.20 ± 0.04 (2)	0.18 ± 0.02 (3)
Larvae - Ventral yolk fraction	–	0.47 ± 0.03 (3)	0.48 ± 0.03 (3)	0.42 ± 0.05 (3)	0.44 ± 0.04 (2)	0.42 ± 0.03 (3)
Larvae - Lipid droplet fraction	–	0.17 ± 0.02 (3)	0.19 ± 0.03 (3)	0.15 ± 0.03 (3)	0.12 ± 0.01 (2)	0.12 ± 0.02 (3)

variations between batches. Wild-caught batches ranged from 2.19 ± 0.06 mm (NR-#2) to 2.50 ± 0.05 mm (RK2-#3), whereas eggs from captive females ranged 2.04 ± 0.16 mm (LS-BS-2-6) to 2.33 ± 0.08 mm (LS-BS-3-4). However, when comparing group averages, no significant differences ($p > 0.05$) were observed between any egg batches. A positive correlation was found between mothers' weight and egg diameter for domesticated lumpfish ($R^2 = 0.07$, $p = 0.0009$), but not for the wild-caught lumpfish (Supplementary Material 4, Fig. S4.1).

As for fertilization success, hatching success was generally higher for eggs obtained from wild-caught females, whereas the broodstock groups exhibited highly variable hatching success (.

Table 2). The NR group displayed over 85% hatching success, with the highest being batch NR-#2 (95.1 ± 3.6%). The lowest wild-caught batch was RK2-#3 with 44.1 ± 11% hatching success. For the captive females, the three LS-BS-1 batches displayed high hatching success with 90.7 ± 12.8, 79.8 ± 28.2 and 57.6 ± 48.7%. We observed that the low fertilization rate that was found for the LS-BS-2 group was reflected in a low and variable hatching success as well. None of the eggs hatched for the BS-2 except from-BS-2-2, were almost 50% of the eggs that were fertilized (3.7%), did hatch. Comparing average values for the 5 groups, significantly lower hatching success was observed in LS-BS-2 compared to NR ($p < 0.05$) and LS-BS-3 ($p < 0.05$).

In line with our results, eggs from wild caught females have been shown to be of higher quality than eggs from broodstock in other studies, e.g. in Atlantic cod (Salze et al., 2005) and wild beluga (*Hugo hugo*) (Ovissipour and Rasco, 2011) where both fertilization rates and hatching success were lower for eggs obtained from farmed compared to wild fish. Eggs from wild fish are usually considered of better quality (Srivastava and Brown, 2011), and fertilization rates, hatch rates, survival and size at hatch are usually higher than in eggs produced in captivity.

We recorded significant differences in fertilization rates both within and between egg groups. Although we cannot be sure that these differences were not caused by differences in the degree of egg maturation at the time of strip spawning or time of the year in relation to both spawning season and experienced temperatures, the spread proved useful for the purpose of molecular targets of egg quality and conducting multivariate statistics (see below). For all groups, the egg diameter (Table 3) were within the size range previously reported (Davenport, 1985; Imsland et al., 2019), and no significant differences were observed comparing wild-caught and captive fish. Dry weights, carbon and nitrogen content and respiration of eggs at 1 dpf were generally higher in eggs from wild-caught females compared to eggs from captive females (Table 3). Carbon content was significantly lower in eggs from captive females compared to wild-caught females ($p < 0.05$), which probably reflects the differences in total lipid content. Accordingly, newly hatched larvae from the wild-caught female groups also displayed a higher dry weight, carbon and nitrogen content, but also here only carbon content was significantly higher ($p < 0.05$). Carbon:nitrogen ratios were varying slightly both between and within groups, but there were no systematic differences between larvae hatched from eggs from wild-caught and captive females. Respiration rates steadily increased from 0.001 to 0.003 to about 0.04 nmol O_2/egg/h just prior to hatch and increased further after hatch (Table 3). The large within-batch variations in larval respiration rates (0.04–0.14 nmol/O_2/egg/h) were caused by different swimming activity: some larvae settled in quickly in the respiration chambers by attaching to the walls using their suction disc, while others were constantly swimming during the measurements.

Measurements of biological variables in larvae was limited for some of the batches (LS-BS-2-1, LS-BS-2-3, LS-BS-2-4, LS-BS-2-6 and LS-BS-3-3) due to low fertilization success and subsequent lack of hatched larvae (Table 2). Morphometric analyses were performed on 2D images of larvae (1 dph). Although some indications of differences in standard length (SL), lateral yolk fraction, ventral yolk fraction and lipid droplet fraction were observed (Table 3), these were not significant ($p > 0.05$). All data are given in the Supplementary Material 2 (Table S2.1) Standard length (SL) of the larvae at 1 dph ranged between 4.85 and 6.58 mm, comparable to the size range reported in other studies (Davenport, 1985; Imsland et al., 2019), and we did not find any significant differences in SL between wild-caught and captive fish. Several studies have pointed out the advantage of a larger size at hatching which could be an advantage for increasing survival (Garrido et al., 2015; Hare and Cowen, 1997; Litvak and Leggett, 1992; Sogard, 1997; Suthers, 1998). The relative areas of the yolk sac and the lipid droplet(s) compared to body area were also calculated from images of larvae (1 dph). The lipid droplet fractions were somewhat lower for the LS-BS-2 and -3 groups compared to the wild-caught fish, corresponding well to their low lipid-diet. Furthermore, the TL content of the ova significantly correlated to the lipid droplet size of larvae (Pearson $r = 0.5907$, $p < 0.05$) (Supplementary Information 4, Fig. S4.2).

3.3. Can ova lipid profiles predict egg and larvae quality?

In a recent study by Malzahn et al. (2022), lipid profiles of fertilized ballan wrasse (*Labrus bergylta*) eggs were analysed to assess if they could be used as predictors for egg quality. Similarly, we assessed if distinct lipid species were predictive for egg quality in lumpfish ova by combining fatty acid- and lipidomics data with different biological data measured in mature female lumpfish, their eggs and the resulting larvae post hatching. Partial least square (PLS) regression analyses were performed to see if the data grouped the different egg batches and to identify key lipid species that explained the grouping (Fig. 3A, C and E). The key lipid species were visualized using clustered image maps (CIM) which displays correlations between variables using color gradients (Fig. 3B, D and F).

Fig. 3A–B displays analyses of fatty acid profiles of the ova combined with the data on fertilization success, hatching success, mother weight, and mother length. The partial least square (PLS) regression analysis related the two data matrices, in this case lipidomics data with endpoint analyses values. Here, comparable to observations of fatty acid profiles alone (Fig. 2A), the eggs from captive females were clearly separated from eggs from wild-caught fish. In addition, the batches were separated based on the mothers' feeding regimes. These groupings therefore indicate that the fatty acid profiles together with diet may be used to predict biological variables like fertilization and hatching success. The data is further visualized using a clustered image map (CIM), which visualizes the correlation between variables using a color gradient (Fig. 3B), and fertilization success was the variable most strongly correlated with the fatty acid profile, suggesting that certain fatty acids may be used as predictive markers for fertilization success (Supplementary Information, Fig. S4.3). Fertilization success was negatively correlated (Pearson $r = -0.8158$, $p < 0.001$) with linoleic acid (C18:2n6) and positively correlated (Pearson $r > 0.7$, $p < 0.01$) with stearidonic acid (C18:4n3), erucic acid (C22:1n9) and eicosapentaenoic acid (EPA, C20:5n3). No significant correlation was observed for arachidonic acid (ARA, C20:4n6, Pearson $r = -0.4367$, $p > 0.05$), and the correlation with docosahexaenoic acid (DHA, C22:6n3), a presumably important fatty acid, was weak (Pearson $r = 0.5715$, $p < 0.05$). Causalities behind these correlations is debatable as some of the fatty acids may be enriched in ova from captive females due to the fatty acid composition of the diets, as have been shown for the beluga (*Huso huso*) where elevated levels of linoleic acid were presumably diet-correlated (Ovissipour and Rasco, 2011). However, low levels of PUFAs in cultivated sturgeon negatively affected fertilization and hatching ratios which were significantly lower than for the wild fish (Ovissipour and Rasco, 2011). Salze et al. (2005) suggested that higher fertilization and hatching success in wild compared to farmed cod were caused by higher levels of arachidonic acid (ARA), phosphatidylinositol, and astaxanthin present in eggs from wild fish. Differences in lipid profiles between eggs from farmed and wild broodstock, especially for ARA, eicosapentaenoic acid (EPA) and EPA/ARA-ratios, are believed to influence egg quality in many fish species such as European eel (*Anguilla anguilla*) (Støttrup et al., 2016), Japanese eel (*Anguilla japonica*) (Ozaki et al., 2008) and striped

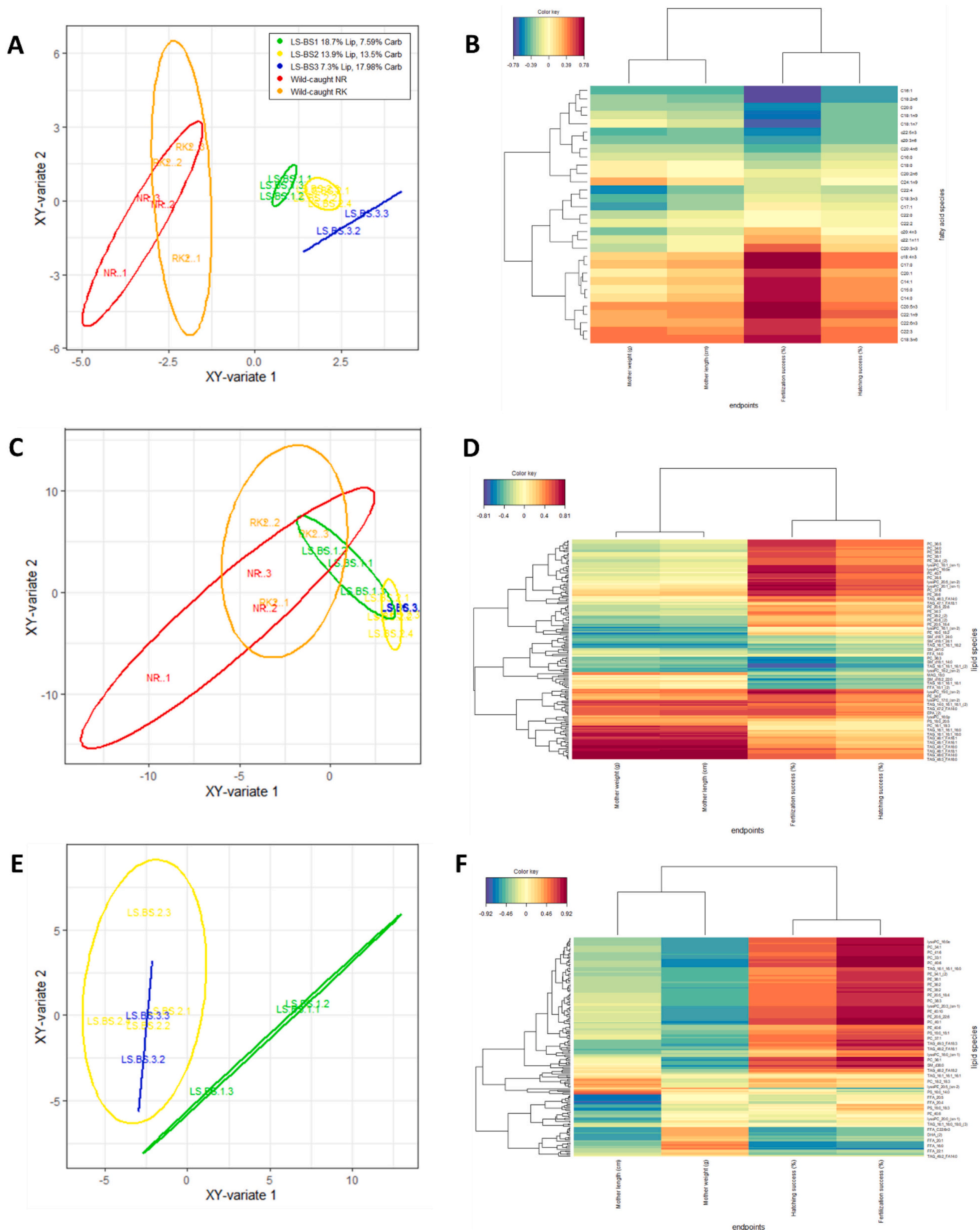

Fig. 3. sPLS-DA analyses of data on wild-caught (NR and RKs) and captive lumpfish egg batches fed different diets (LS-BS-1, LS-BS-2 and LS-BS-3). A–B: Comparison of fatty acid profile of lumpfish eggs with biological variables. (A) sPLS plot. (B) CIM plot of the top fatty acids that most contribute to the variance biological variables. C–D: Comparison of lipidomics of lumpfish eggs with biological variables (C) PLS plot. (D) CIM plot of the top lipid species that most contribute to the variance of the sPLS analysis. E–F: Comparison of lipidomics of captive lumpfish eggs only with biological variables (E) sPLS plot. (F) CIM plot of the top lipid species that most contribute to the variance of the sPLS analysis. Right panel heat maps: Colours represent correlation coefficients on a gradient scale ranging from blue = negative- to red = positive correlation.

bass (*Morone saxatilis*) (Gallagher et al., 1998). We measured a lower EPA/ARA ratio in fish from captive mothers compared to wild-caught (Table 3), and there was a moderate positive correlation between this ratio and fertilization success. We did not find any correlation between hatching success and fatty acids analysed as FAMEs.

From PLS analyses of lipidomics data combined with mother size/weight, fertilization and hatching success (Fig. 3C–D), the CIM plot revealed strong correlations between PC and fertilization success indicating that this class of phospholipids play an important role in embryonic development (Fig. 3D). Both mother's weight and length (which inherently are closely related) correlated strongly with ova triacylglyceride (TAG) content, demonstrating that larger mothers both impart a larger energy reserve of TAGs into their ova and produce larger eggs. To focus on the differences in mothers' feeding regimes, we repeated the analysis and excluded wild-caught fish (Fig. 3E–F). We again observed that the eggs from mothers fed with high lipid-containing diets were separated from the rest of the samples, and that fertilization success was strongly correlated to phospholipids (PC and PE) (Fig. 3F) indicating that these lipids have an important role in embryonic development. However, we did not observe the same correlation with TAGs and mothers' size, indicating that that correlation is a function of wild-caught vs captive broodstock and not the broodstock diet. The lipidomics data combined suggests that the lipid composition of lumpfish ova are closely tied to both the mothers' size and fertilization success. Our data suggests that the following lipid species play key roles in lumpfish ovum development and may serve as predictive biomarkers for fertilization success: PC 33:1, 34:1, 40:1, 40:6, and 41:6 and PE 34:1, 36:1, 38:2, and 40:1.

4. Conclusions

Wild-caught lumpfish generally displayed higher fertilization- and hatching success compared to captive lumpfish, whereas other biological variables like egg size, egg/larval dry weight, respiration rates and larval morphometry did not differ significantly.

Similar to Malzahn et al. (2022) we attempted to utilize a discovery-driven lipidomics-approach as a research tool to provide lipid profiles (lipidome) of ova from wild-caught and captive lumpfish. In combination with multivariate statistical analyses, a first step towards identifying lipid markers that can be used to explain variations and predict the quality of lumpfish ova was made, and this is, to our knowledge, the first attempt to apply lipidomics for this purpose in this species. Ova lipid profiles (total lipid, lipid classes, fatty acid composition and lipidome) was shown to separate egg batches based on their geographic origin (for wild-caught females) and composition of diets used for captive broodstock.

Lumpfish ova lipid profiles covaried with several biological parameters, indicating that the former may be used to predict the latter. Lumpfish ova were mostly comprised of TAGs and some phospholipids (PEs and PCs), and their lipid profiles were more dependent on differences in origin, mothers' weight/size and fertilization success than variables such as eggs size or larvae morphometric measures. PC and PE content were positively correlated to both hatching success and fertilization rates, thus the lipid profiles, with some key highlighted lipid species, were putative predictors of egg quality. The way forward would be to link lipid groups to function and achieve a better understanding of the underlying mechanistic processes relating to the role of different lipid species in oogenesis and embryonic development.

The utilized PLS analysis compared lipidome data with biological variables through linear regression models to identify covariance between the data sets. With larger sample material, more sophisticated machine learning techniques may be better options as they do not assume linearity, enabling them to discover more complex associations. Conversely, linear methods assume lipids are combined linearly and then impact biological variables. Follow-up studies should therefore focus on identifying the composition of these lipids, testing whether they indeed have a predictive value for egg and larvae health in a larger scale using nonlinear machine learning techniques and elucidate the molecular mechanism of their roles in embryonic development.

Such information is relevant for lumpfish farmers, which in time could utilize it to weed out sub-par egg batches to save time and money by rather investing in groups that have better prospects. The methods are also relevant for the research community which strive towards developing broodstock diets which ultimately produce eggs that are of similar quality as eggs obtained from wild caught females. If successful, lumpfish producers will in time be able to operate independently of wild populations and relieve natural stocks from overfishing.

Ethics statement

The manuscript contains original work that has not been previously presented or is currently submitted elsewhere. All listed authors have agreed to be listed and have approved the submitted version of the manuscript. All work has been done on ova, fertilized eggs or non-feeding larvae and the experiments cohere with national animal experimentation laws.

Data availability statement

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researchers.

CRediT authorship contribution statement

Bjørn Henrik Hansen: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Elin Kjørsvik:** Conceptualization, Supervision, Writing – review & editing. **Arne M. Malzahn:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Antonio Sarno:** Conceptualization, Data curation, Formal analysis, Investigation, Writing – original draft. **Ole Martin Kulild:** Investigation, Data curation, Formal analysis, Writing – review & editing. **Julia Farkas:** Investigation, Methodology, Writing – review & editing. **Trond Nordtug:** Conceptualization, Methodology, Writing – review & editing. **Rebecca Rye:** Investigation, Writing – review & editing. **Bjarne Kvæstad:** Methodology, Software, Formal analysis, Writing – review & editing. **Ingrid Lein:** Conceptualization, Writing – review & editing. **Andreas Hagemann:** Funding acquisition, Resources, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of Competing Interest

All authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Appendix: supplementary material

Supplementary material consists of sources and data of mother fish (SM1), details regarding mobile phases for lipid class and lipidomics analyses (SM2), details regarding automated analyses of larvae morphometry (SM3), and various correlation analyses (SM4). The supplementary material to this article can be found online.

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