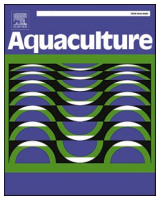
Aquaculture 552 (2022) 738018

Contents lists available at ScienceDirect

Aquaculture

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



Short communication

Can lipidomics help identifying egg quality in ballan wrasse?

Arne M. Malzahn [a,*], Antonio Sarno [a], Andreas Hagemann [a], Julia Farkas [b], Luciana Alves Musialak [c], Elin Kjørsvik [c], Bjørn Henrik Hansen [b]

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

ARTICLE INFO

Keywords:
Aquaculture
Cleaner fish
Sea lice
Salmon lice
labrus bergylta

ABSTRACT

Ballan wrasse (*Labrus bergylta*) is in the limelight of aquaculture research due to the species role as cleaner fish in salmon aquaculture. A major drawback is that the salmon industry still depends on wild caught fish as there are still many unsolved challenges in ballan wrasse aquaculture, amongst them the determination of egg quality. We measured a range of different egg quality markers on 6 different batches of eggs from ballan wrasse. Additionally, we performed lipidomics on these eggs to test whether their lipid profiles relate to, and can be thereby used as, a predictor for egg quality. We identified relationships between several lipid species and physiological and morphological egg quality markers, and the lipids grouped into two groups. The first group consisted of a diverse blend of 32 lipids, and was positively correlated with size related measures while it was negatively related to measures describing metabolic activity. It consisted of phosphatidylcholines (PC), lysophosphatidylcholine (lysoPC), and triacylglicerids (TAG) and three different sphingomyelins (SM). The second group consisted of 24 lipid species, and showed the inverse pattern, positive relationships with metabolic activity and negative relationships to size related measures. This group of lipid species contained several phosphatidylethanolamines (PE) and free fatty acids. These lipid species may be proposed as key egg quality markers which ultimately can be used by the aquaculture industry to select high quality egg batches in hatcheries.

1. Introduction

What makes an egg a good egg is a long-discussed topic in many biological disciplines (Kjørsvik et al., 1990), but especially in aquaculture, as the fish farmer is betting that the eggs will result in high value fish. Many different ideas have been postulated how to describe a good egg, and fertilization success, development into a normal embryo and high hatch rates are probably the most named descriptors (Kjørsvik et al., 2003; Pavlov et al., 2004). Brooks et al. (1997) elegantly described a good egg as one that, "… contains all the necessary materials (both genetic and nutritive) to support the development of the subsequent embryo." This definition is certainly true and gives a good hint what to consider but does not supply a testable tool to describe egg quality.

Various measures have been proposed to indicate a good egg. Egg size has been put forward numerous times (Barneche et al., 2018; Hixon et al., 2013; Lim et al., 2014) as *e.g.* egg size is prioritized over fecundity in Atlantic cod, indicating that larger egg size is more important than releasing more propagules (Kjesbu et al., 1996). Female age has also been discussed to be a predictor of egg quality. In many cases age and weight are used synonymously, and both are usually well related, but recent research shows that age is probably the more important determinant for egg size and quality (Lasne et al., 2018). However, egg size is often argued to vary with predicted environmental situations at hatch of the embryo (Malzahn and Boersma, 2007; Smith and Fretwell, 1974), and theoretically, offspring size should decrease with environmental quality (Morrongiello et al., 2012). Such patterns have been reported for various taxa, including fish. Johnston and Leggett (2002) showed that egg size in walleye decreased with increasing lake productivity, indicating that in a more favourable environment less per offspring invest is necessary. Environmental conditions are constant and predictable in aquaculture settings, so an egg size/egg number trade off should rather be skewed towards propagule number, as the advantage of producing large eggs in natural settings might not be present under controlled conditions and the production of numerous, smaller eggs should be sufficient. However, this argument only holds true if the environment provided by the fish farmer is actually good from a larval point of view.

* Corresponding author.
E-mail address: arne.malzahn@sintef.no (A.M. Malzahn).

https://doi.org/10.1016/j.aquaculture.2022.738018
Received 5 August 2021; Received in revised form 2 February 2022; Accepted 5 February 2022
Available online 8 February 2022

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. Aside factors such as water chemistry (Bromage et al., 1992), photoperiod (Carrillo et al., 1989), or overripening of eggs (Bromage et al., 1994) the main driver of egg quality seems to be the mothers nutrition (Brooks et al., 1997). The most discussed biochemical group in relation to mothers' nutrition and egg quality are lipids (Izquierdo et al., 2000), a vast group of molecules fulfilling many roles in living organisms. Amongst the lipids, polyunsaturated fatty acids are the prime candidates explaining egg (Carrillo et al., 1994) and larval quality (Ferron and Leggett, 1994). Several studies showed that adjusting lipid levels or -types in broodstock diets revealed higher egg quality (reviewed by Fernández-Palacios et al., 2011),

In the current study we used ballan wrasse eggs (*Labrus bergylta*). The ballan wrasse is a species in the limelight in aquaculture due to its use as cleaner fish in salmon aquaculture. According to most recent data available, only about 30% of the 2.5–3.5 million ballan wrasse used per year are farmed fish, the rest being wild caught (Fiskeridirektoratet, 2021). Due to the nature of the sticky eggs, none of the above-mentioned measures but size at hatch can easily be assessed in ballan wrasse at present, hence the nature of the eggs in this species makes a wider search for suitable proxies for egg quality necessary.

To lay a knowledge foundation, we studied different egg characteristics with the potential to predict egg (and offspring) quality. We tested most of the abovementioned traits but female size/age, as the egg batches we used were from group spawning events. Due to the overwhelming importance of lipids in egg quality we used a lipidomics approach to investigate if any of the investigated traits correlate with lipid species in the eggs. Migaud et al. (2013) states in a review in gamete quality that, " … the molecular picture of the good quality oocyte remains fuzzy." This paper aims at contributing to decrease this fuzziness by providing insights into the interplay between lipidomics, fatty acids and various oocyte traits of ballan wrasse eggs.

2. Material and methods

Eggs used in the current study were purchased from two commercial hatcheries in Norway between September and November 2019. We studied 6 different egg batches, spawned, and fertilized naturally on spawning mats placed in the spawning tanks. This procedure unfortunately prevented us from analysing fertilization success of the eggs, commonly used as a egg quality descriptor. Ballan wrasse spawn at night in the dark. Right before shipment, the eggs spawned during the last 12 h were scraped of the spawning mats and placed in double plastic bags (13l) with oxygenized water (saturation upon on arrival >90% O_2) and transported in a Styrofoam box the same day to SINTEF Sealab, Trondheim where the experiments took place. Batch 1 was spawned by an established broodstock, while batches 2–6 were spawned by wild fish which has been in captivity between 1 and 3 years. The different broodstocks were kept at 11–11.5 °C at a 12:12 light: dark light regime.

Upon arrival eggs were acclimatized to the new water chemistry till the following morning. We divided each batch into 12 sub-batches (approximately 200 eggs) were transferred into incubators consisting of 50 ml plastic tubes with a 120 μm plankton mesh (SEFAR NITEX) fixed at the top and bottom of the tubes. The incubators were supplied with fresh sea water in a flow-through system until 16 days post fertilization (dpf). It was impossible to distinguish between fertilized, unfertilized, and dead eggs upon introduction to the incubators because the eggs were clumped together, and debris and fibres from the spawning mats was still attached to the eggs, hence we could not establish hatching rates either. The eggs were sampled 14 times until 16 days post-fertilization. We analysed several biometric measurements using microscopy and computer aided image analysis, respiration rates, egg dry weight, elemental analysis (carbon & nitrogen), bone/cartilage development and lipidomics.

The markers analysed in this study can broadly be grouped into markers related to (a) size, (b) energy content (c) metabolic activity and (d) larval deformities (a) length at hatch, body area at hatch, eye diameter, chorion diameter, total egg diameter, dry weight, and gum layer thickness, (b) egg yolk volume, yolk area, yolk fraction, carbon and nitrogen content, and C:N of the egg, and (c) rate of change in C:N, dry weight, respiration rates, carbon and nitrogen content, and egg respiration at fertilization (Table 1).

2.1. Biometry

Standardised images of larvae were used for biometric analyses of egg size, chorion size, gum layer thickness, standard length, eye diameter and body area. The biometric analysis were automated using deep learning with Mask R-CNN neural net architecture (He et al., 2017; Kvæstad et al., 2022) trained on manually annotated images of ballan wrasse eggs and larvae at different developmental stages.

2.2. Respiration & elemental analysis

Respiration rates were measured using a MicroRespiration System (Unisense, Denmark) which consisted of two oxygen sensors (OX-MR) connected to a MicroSensor Multimeter, and a MicroRespiration Rack (MR2-Rack) equipped with a MicroRespiration Stirrer controller (MR2-Co). The chambers placed in a water bath to ensure constant temperature during the analysis. All analyses were performed at a water temperature of 10 °C. Respiration was measured several times during egg development and during the first days after hatch, before mouth opening. 5–6 live eggs or larvae were randomly selected and were gently pipetted into micro respiration chambers (1 ml volume) filled with pre-chilled filtered sea water. The magnet stirrer was set to 600 rotations per minute to avoid the development of oxygen gradients in the chamber. Respiration measurements were started after an approximately 5 min long adaptation period and the measurement lasted for at least 10 min. Respiration was measured and respiration rates were calculated using SensorTrace Rate software (application version 2.8.200.21688). After the measurement, eggs were pipetted from the respiration chamber into distilled water to rinse salt water from the eggs. Rinsed eggs were then placed into pre-weighed tin capsules for later weighing and elemental analysis. Larvae were flushed into a pH buffered, overdosed MS222 (tricaine methanesulfonate) and sea water solution (500 mg l^{-1}). Dead larvae where then rinsed in distilled water and put into pre-weighed tin capsules. Eggs and larvae were dried at 60 °C. Once dried, larvae were weighed and shipped to NC Technologies, Milano, Italy to be analysed for carbon and nitrogen content.

2.3. Deformities

Cartilage staining tests to detect malformations were performed for larvae on 16 days post fertilization. Cartilage staining was performed using Alcian Blue with an adapted protocol from Kjørsvik et al. (2009 h). Photographs were taken with a dissecting microscope (Leica M205, Leica Microsystems, Germany) equipped with a camera (AxioCam ERc 5 s, Zeiss Inc., Germany). These images were analysed for deformation, such as jaw and head deformations.

2.4. Lipidomics

Lipidomic analysis were performed on pools of 50 to 100 eggs. Lipids were extracted with the Folch method (Folch et al., 1957). Roughly 100 mg 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). Samples were spiked with 10 μl of a heavy isotope-labelled internal standard mixture (SPLASH® II LIPIDOMIX® Mass Spec Standard (from Avanti Polar Lipids). Samples were reconstituted in 1 ml chloroform and stored at −80 °C. For lipidomics

Table 1
Data used as indicators of egg quality parameters in six different groups of ballan wrasse. For a better understanding, parameters were grouped into categories such as eggs size-, metabolic activity-, and energy-related parameters. Data given as mean ± SD (N). BG1, Stord1, Stord2, NOV-A, NOV-B and NOV-C refers to the tank names at MOWI Stord, Norway in which the eggs were laid.

Parameter	Category	Time point	BG-1	NOV-A	NOV-B	NOV-C	Stord1	Stord2	Average	difference between min & max as % of average
Dry weight (μg)	Size	1 dpf	83.46 ± 21.6 (4)	91.46 (1)	95.52 (1)	87.94 (1)	90.64 (1)	98.57 (1)	91.27	17
Total egg diameter (μm)	Size	1 dpf	1098 ± 47 (6)	1070 ± 48 (6)	1084 ± 29 (6)	1050 ± 27 (6)	1110 ± 30 (6)	1100 ± 27 (6)	1085	6
Chorion diameter (μm)	Size	1 dpf	899 ± 40 (6)	843 ± 87 (6)	881 ± 21 (6)	855 ± 9 (6)	902 ± 36 (6)	859 ± 21 (6)	870	7
Gumlayer diameter (μm)	Size	1 dpf	99.5 ± 13.7 (6)	113 ± 30 (6)	101 ± 11 (6)	97.6 ± 11.3 (6)	104 ± 5 (6)	121 ± 21 (6)	0.11	22
Eye diameter (mm)	Size	8 dpf (hatch)	0.21 ± 0.01 (35)	0.21 ± 0.02 (8)	0.20 ± 0.01 (6)	0.18 ± 0.01 (6)	0.18 ± 0.02 (6)	0.19 ± 0.01 (10)	0.20	18
Body area (mm^2)	Size	8 dpf (hatch)	1.15 ± 0.05 (35)	1.10 ± 0.08 (8)	1.04 ± 0.05 (6)	1.08 ± 0.09 (6)	1.05 ± 0.05 n(6)	1.04 ± 0.06 (10)	1.08	10
Standard length (mm)	Size	8 dpf (hatch)	3.7 ± 0.1 (35)	3.5 ± 0.1 (10)	3.4 ± 0.1 (6)	3.4 ± 0.1 (6)	3.4 ± 0.1 (6)	3.5 ± 0.1 (10)	3.52	11
Egg respiration ($nmolO_2$/h/egg)	Metabolic activity	1 dpf	1.39 ± 0.42 (4)	1.74 (1)	3.00 (1)	1.99 (1)	1.74 (1)	1.33 (1)	1.87	90
Δrespiration rate	Metabolic activity	Change between 1 and 16 dpf	0.435 (1)	0.498 (1)	0.416 (1)	0.8211 (1)	0.2734 (1)	0.368 (1)	0.47	117
ΔDW/ΔT (μg/16 days)[a]	Metabolic activity	Change between 1 and 16 dpf	−3.023 (1)	−3.0584 (1)	−3.5871 (1)	−2.9593 (1)	−2.6539 (1)	−4.0167 (1)	−3.22	42
ΔC/ΔT (μg/16 days)[b]	Metabolic activity	Change between 1 and 16 dpf	−2.354 (1)	−1.694 (1)	−1.8554 (1)	−1.6825 (1)	−1.6633 (1)	−1.8098 (1)	−1.84	37
ΔN/ΔT (μg/16 days)[c]	Metabolic activity	Change between 1 and 16 dpf	−0.6222 (1)	−0.3525 (1)	−0.3846 (1)	−0.3459 (1)	−0.3447 (1)	−0.4022 (1)	−0.41	68
ΔC:N/ΔT[d]	Metabolic activity	Change between 1 and 16 dpf	0.0639 (1)	−0.0149 (1)	−0.0171 (1)	−0.0118 (1)	−0.0132 (1)	0.0021 (1)	0.00	5400
C egg (μg)	Energy	1 dpf	43.48 ± 0.34 (4)	45.43 (1)	45.89 (1)	42.04 (1)	44.87 (1)	44.78 (1)	44.42	9
N egg (μg)	Energy	1 dpf	9.85 ± 0.06 (4)	9.88 (1)	9.93 (1)	8.94 (1)	9.67 (1)	9.89 (1)	9.69	10
C:N ratio egg	Energy	1 dpf	4.43 ± 0.05 (4)	4.60 (1)	4.62 (1)	4.70 (1)	4.64 (1)	4.53 (1)	4.59	6
Egg yolk volume (mm^3)	Energy	1 dpf	0.246 ± 0.034 (6)	0.094 ± 0.043 (6)	0.124 ± 0.038 (6)	0.145 ± 0.012 (6)	0.087 ± 0.020 (6)	0.098 ± 0.023 (6)	0.13	120
Yolk area (mm^2)	Energy	8 dpf (hatch)	0.2787 ± 0.034 (35)	0.2854 ± 0.055 (8)	0.2025 ± 0.041 (6)	0.3068 ± 0.046 (6)	0.3028 ± 0.041 (6)	0.2802 ± 0.037 (10)	0.28	38
Yolk fraction (proportion yolk of total egg)	Energy	8 dpf (hatch)	0.24 ± 0.03 (35)	0.26 ± 0.04 (8)	0.19 ± 0.04 (6)	0.28 ± 0.03 (6)	0.29 ± 0.03 (6)	0.27 ± 0.04 (10)	0.26	36
% deformities[e]		16 dpf	30.8 (25)	10.7 (27)	7.1 (23)	0 (11)	7.4 (22)	13.3 (10)	11.55	266

[a] Change in dry weight from 1 to 16 dpf divided by number of days (15).
[b] Change in carbon content from 1 to 16 dpf divided by number of days (15).
[c] Change in nitrogen content from 1 to 16 dpf divided by number of days (15).
[d] Change in C:N from 1 to 16 dpf divided by number of days (15).
[e] Percentage of the total number of stained fish (N) displaying any type of deformation.

analysis, samples were dried under nitrogen and re-dissolved 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 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 60-min gradient of (A) acetonitrile-water (40:60) and (B) isopropanol-acetonitrile (90:10), both containing 10 mM ammonium formate and 0.1% formic acid. Each sample was injected three times with each injection analysing a different set of lipids (phospholipids, glycerides and free fatty acids, and sphingolipids). We performed widely targeted lipidomics using multiple reaction monitoring mode (MRM). That is, we generated an expansive list of mass transitions from previously-observed transitions in the literature and scanned for all of them on each run (Jouhet et al., 2017; Takeda et al., 2018; Tsugawa et al., 2015; Woodfield et al., 2018; Xuan et al., 2018). To further validate the identity of the lipids, all MRMs were scanned on a sample of lipid extract from different pooled sources to obtain retention times using the observation that acyl chain length increases and desaturation decreases with increased retention time on a reverse phase column (Bromke et al., 2015; Giavalisco et al., 2011). Data from LC-MS/MS analyses were analysed using the Agilent MassHunter Quantitative Analysis software package. All peaks were controlled for retention time drift, peak symmetry, and minimum intensity. An exogenous standard comprised of a small aliquot from each sample was also run with different injection volumes to run as a surrogate standard curve, which was used to determine the response for each mass transition. A regression curve was generated for each transition and a R^2 cut-off of 0.7 was used, under which samples were excluded. Next, the peak intensities

were normalized to heavy isotope-labelled standards of the same lipid class in each sample, which controls for both instrument response drift and extraction efficiency. Finally, signals were normalized to total sample weight.

2.5. Statistics

Data analysis and multivariate statistics were performed using the mixOmics R package (Lê Cao et al., 2009; Rohart et al., 2017). We compared two datasets: first, the lipidomics dataset consisted of a table with sample names as rows and relative lipid abundances (obtained from MassHunter and processed as described above) as columns. Second, the quality parameter dataset included egg quality marker data instead of lipidomics. We analysed the lipidomics dataset for zero-variance columns and removed them. We then aimed to integrate the two data sets measured on the same samples by extracting correlated information using sparse partial least square analysis keeping the 50 lipids with highest covariance. The data was visualized with a clustered image map (CIM), which hierarchically clustered the matrix of lipids and egg quality parameters simultaneously. The comp parameter, which controls which latent components are used to determine the similarity between features was set to "1:6." The distance measure for row and column clustering, which determines which clusters are combined as the dendograms are being generated weas set to "euclidean." The clust. method parameter, which controls the agglomeration method, was set to "complete."

3. Results & discussion

The potential egg quality descriptors we analysed all showed

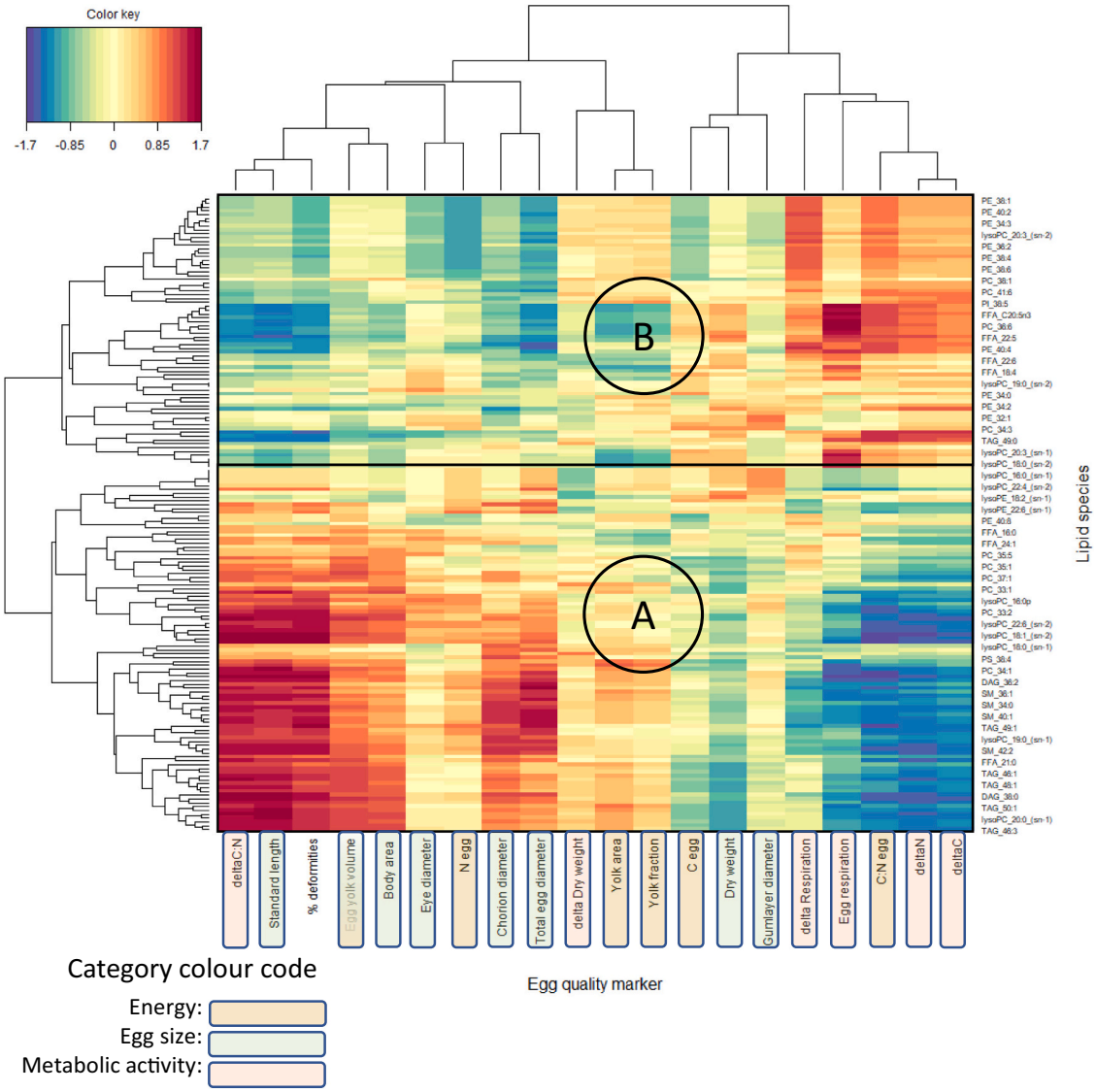


Fig. 1. Comparison of lipidomics of ballan wrasse eggs with egg quality markers. Clustered Image Map of the top lipid species that most contribute to the variance. (A) and (B) denotes the two main clusters or the dendrogram on the left side of the figure grouping lipids.
Cluster A: PE 38:0, PE 40:2, PE 34:3, lysoPC 20:3 (sn-2), PE 36:2, PE 38:4, PE 36:6, PC 38:1, PC 41:6, PI 38:5, FFA 20:5n3, PC 36:6, FFA 22:5, PE 40:4, FFA 22:6, FFA 18:4, lysoPC 19:0 (sn-2), PE 34:0, PE 34:2, PE 32:1, PC 34:3, TAG 49:0, lysoPC 20:3 (sn-1), lysoPC 18:0 (sn-1).
Cluster B: lysoPC 16:0 (sn-1), lysoPC 22:4 (sn-1), lysoPE 18:2 (sn-1), lysoPE 22:6 (sn-1), PE 40:8, FFA 16:0, FFA 24:1, PC 35:5, PC 35:1, PC 37:1, PC 33:1, lysoPC 16:0, PC 33:2, lysoPC 22:6 (sn-2), lysoPC 18:1 (sn-2), lysoPC 18:0 (sn-1), PS 38:4, PC 34:1, DAG 36:2, SM 36:1, SM 34:0, SM 40:1, TAG 49:1, lysoPC 19:0 (sn-1), SM 42:2, FFA 21:0, TAG 46:1, TAG 48:1, DAG 38:0, TAG 50:1, lysoPC 20:0 (sn-1), TAG 46:3.
PC: Phosphatidylcholines, PE: Phosphatidylethanolamines, lysoPC: Lysophosphatidylcholine, lysoPE: Lysophosphatidylethanolamines, PI: Phosphoinositides, TAG: Triacylglicerids, DAG: Diacylglycerols, SM: Sphingomyelins, PS: Phosphoserine, FFA: Free fatty acids, sn-1 or sn-2 indicate different isomers.

substantial variability between batches. The more conservative quality descriptors, such as larval length, egg diameter and egg weight, showed less variability than the more dynamic ones, such as the decrease in dry weight, or decrease in carbon content per egg between one- and 16-days post-fertilization. A notable exception in terms of variability on the data was the change of C:N ratio between 1- and 16-days post-fertilization, which was 5400% of the average. This reflects the nature of ratios, that tiny changes result in large differences. We focussed on these potential egg quality descriptors, as the two probably most prominent descriptors of egg quality, fertilization - and hatching rates cannot easily be used in ballan wrasse. Both rates could not be analysed, as the eggs stemmed from group spawning at night at a commercial hatchery. Ballan wrasse spawn very sticky eggs, the resulting clusters of eggs does not allow for a proper discrimination between fertilized and unfertilized eggs.

We quantified 245 different lipid species in the ballan wrasse eggs, of which 56 correlated to the 20 potential egg quality markers we used in this study (Fig. 1). These markers can broadly be grouped into markers related to (a) size, (b) energy content, and (c) metabolic activity (Table 1). Those lipids which were related to the egg quality markers grouped into two main clusters: Cluster A (the lower cluster in the clustered image map Fig. 1) showed a positive covariance with size related measures such as larval length at hatch, or yolk volume at 2 days post hatch, and negatively with measures related to metabolic activity, such as changes in respiration rate between fertilization and hatch or the decrease of nitrogen and carbon in the egg. This group consisted of phosphatidylcholines (PC), lysophosphatidylcholine (lysoPC), and triacylglycerides (TAG) and three different sphingomyelins (SM). This first cluster was associated with various measures related to larval size, so it can be depicted that larger eggs result in larger larvae with more energy reserves. This would resemble a fish species producing eggs without oil globules. Eggs from such species usually utilize PC and TAG as the major lipid energy source during embryonic development (Sargent et al., 1999). The FAs in the PC usually decrease unselectively at the same rate, as they primarily serve as an energy source. In TAG, however, monounsaturated fatty acids decline faster. There could also be a transfer from PC PUFAs to TAG PUFAs, (Fraser et al., 1988). Ballan wrasse eggs have droplets (D'Arcy et al., 2012), and the correlations with TAGs, and especially PCs is potentially related to the energy expenditure pattern of using the high energy dense oil globule as an energy reserve after hatch, but not during egg development (Finn et al., 1995).

Group B clustered related to the same measures, but in the opposite way with the exception that size related measures showed a weaker association. This group of lipid species contained several phosphatidylethanolamines (PE) and free fatty acids. Especially the increase in respiration rates was related to free fatty acids. Free fatty acids are present in any catabolizing organisms, as they are the intermediary between *e.g.* TAG and acetyl-CoA (Gyamfi et al., 2019; Sargent et al., 2003), demonstrating the relationship between respiration and lipids which are representative for metabolic activity.

Several quality parameters exhibited a stronger covariance with lipids than others. For example, egg weight, egg carbon content at fertilization, the thickness of the gum layer or the eye diameter at hatch (as a representative of size) did not covary well with any of the 56 lipid species. Contrarily, the difference in C:N ratio, larval length at hatch, the percentage of larval deformities and the body area, as well as C:N at fertilization, and the decrease in carbon and in nitrogen content of the eggs covaried strongly with certain lipid species. Interestingly, the degree of deformities covaried with the same lipids as delta C:N, larval length and the yolk volume at fertilization did. For instance, LysoPE 22:6 sn-2 (a DHA derivate in the form of lysophosphatidylcholine) covaried positively with length, and delta C:N but also with the amount of deformities. The relation to the change in C:N ratio is also reflected in covariances with the nitrogen content and the C:N ratio of the egg. LysoDHA is an important lipid, as it has been demonstrated being a DHA carrier into the brain of mice (Sugasini et al., 2017), hence can be expected to be a rather beneficial, meaning the possibility of a relation to deformed larvae should be further investigated.

Another example is the thickness of the gum layer, which has been put forward being an easily measurable quality parameter. We expected to find the gum layer thickness to be related to several lipids in the egg, as the gum layer thickness is reported to decreases with ongoing spawning season, similar to egg size, indicating energy deficiencies of the repeated spawning mothers (Grant et al., 2016). This measure was only positively related to few lysoPCs.

Even though several studies investigating the effect of female lipid nutrition showed that adjusting lipid levels or types in broodstock diets revealed higher egg quality (reviewed by Fernández-Palacios et al., 2011) there are contrasting opinions, which would strengthen the approach of using lipidomics in egg quality assessment. Tocher and Sargent (1984) very thoroughly investigated lipids in the eggs of seven temperate marine fish species, and they reported that the fatty acids profiles in the neutral fraction were very variable between species, while the fatty acid composition in the polar fraction, and especially in the phosphatidylcholine was very similar between species. This would suggest a general and not species-specific pattern and point towards a tight regulation and the importance of PCs. Taking this thought further, in pike yolk sac fry phosphatidylcholine was selectively incorporated into larval bodies while the levels of other lipid classes remained unchanged in the yolk (Desvilettes et al., 1997), again pointing towards PC-fatty acids being very important building blocks of rapidly developing fish embryos.

In conclusion, the pattern of several lipid species being positively related to size and negatively to metabolic activity while another group is showing exactly the opposite deserves further investigation. Based on Tocher & Sargents's work (1984) recurrent patterns can be expected, and by linking lipid groups to function we would ultimately gain mechanistic understanding. To our knowledge this is the first study applying lipidomics approaches to assess its potential as an egg quality measure. This study only scratches the surface of this complex topic but can serve as a basis for further identifications of important groups of lipids. The future task would be to identify recurring patterns within species and to test to which extend these are species specific or general, and ultimately try to achieve a mechanistic understanding of the importance and the interplay of these groups.

Author declaration

Arne M. Malzahn: wrote the article, contributed to analysis, performed experiments.

Antonio Sarno: ran all lipid analysis and lipidomics bioinformatics, contributed to writing.

Andreas Hagemann: contributed to experimental planning, execution of experiments and writing.

Julia Farkas: contributed to execution of experiments and writing.

Luciana Aves Musialak: analysed deformities, contributed to writing.

Elin Kjørsvik: contributed to planning of experiments, supervision of students and contributed to writing.

Bjørn Henrik Hansen: Planned the experiments, analysed data, contributed to writing.

Declaration of Competing Interest

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

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

Acknowledgements

We thank FHF (Norwegian Seafood Research Fund, Norway) for funding of the work (FHF #901561). The project war carried out in the

framework of the national research infrastructure “Norwegian Center for Plankton Technology” (Norwegian Research Council #245937/F50).

References

Barneche, D.R., Robertson, D.R., White, C.R., Marshall, D.J., 2018. Fish reproductive-energy output increases disproportionately with body size. Science 360, 642–645.

Bromage, N., Jones, J., Randall, C., Thrush, M., Davies, B., Springate, J., Barker, G., 1992. Broodstock management, fecundity, egg quality and the timing of egg production in the rainbow trout (*Oncorhynchus mykiss*). Aquaculture 100, 141–166.

Bromage, N., Bruce, M., Basavaraja, N., Rana, K., Shields, R., Young, C., Gamble, J., 1994. Egg quality determinants in finfish: the role of overripening with special reference to the timing of stripping in the Atlantic halibut *Hippoglossus hippoglossus*. J. World Aquacult. Soc. 25, 13–21.

Bromke, M.A., Hochmuth, A., Tohge, T., Fernie, A.R., Giavalisco, P., Burgos, A., Brotman, Y., 2015. Liquid chromatography high-resolution mass spectrometry for fatty acid profiling. Plant J. 81, 529–536.

Brooks, S., Tyler, C.R., Sumpter, J.P., 1997. Egg quality in fish: what makes a good egg? Rev. Fish Biol. Fish. 7, 387–416.

Carrillo, M., Bromage, N., Zanuy, S., Serrano, R., Prat, F., 1989. The effect of modifications in photoperiod on spawning time, ovarian development and egg quality in the sea bass (*Dicentrarchus labrax* L.). Aquaculture. 81, 351–365.

Carrillo, M., Zanuy, S., Prat, F., Mañanós, E., Cerdá, J., Bromage, N., Kah, O., 1994. Nutritional and photoperiodic effects on hormonal cycles and quality of spawning in sea bass (*Diceatrarchus Labrax* L.). Neth. J. Zool. 45, 204–209.

D'Arcy, J., Dunaevskaya, E., Treasurer, J.W., Ottesen, O., Maguire, J., Zhuravleva, N., FitzGerald, R.D., 2012. Embryonic development in ballan wrasse *Labrus bergylta*. J. Fish Biol. 81, 1101–1110.

Desvilettes, C., Bourdier, G., Breton, J.C., 1997. Changes in lipid class and fatty acid composition during development in pike (*Esox lucius* L) eggs and larvae. Fish Physiol. Biochem. 16, 381–393.

Fernández-Palacios, H., Norberg, B., Izquierdo, M., Hamre, K., 2011. Effects of broodstock diet on eggs and larvae. In: Holt, G.J. (Ed.), Larval Fish Nutrition. Wiley, pp. 151–181.

Ferron, A., Leggett, W.C., 1994. An appraisal of condition measures for marine fish larvae. Adv. Mar. Biol. 30, 217–303.

Finn, R.N., Henderson, J.R., Fyhn, H.J., 1995. Physiological energetics of developing embryos and yolk-sac larvae of Atlantic cod (*Gadus morhua*). 2. Lipid metabolism and enthalpy balance. Mar. Biol. 124, 371–379.

Fiskeridirektoratet, 2021. Use of Cleaner Fish in the Production of Atlantic Salmon and Rainbow Trout.

Folch, J., Lees, M., Stanley, G.H.S., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Fraser, A.J., Gamble, J.C., Sargent, J.R., 1988. Changes in lipid content, lipid class composition and fatty acid composition of developing eggs and unfed larvae of cod (*Gadus morhua*). Mar. Biol. 99, 307–313.

Giavalisco, P., Li, Y., Matthes, A., Eckhardt, A., Hubberten, H.M., Hesse, H., Willmitzer, L., 2011. Elemental formula annotation of polar and lipophilic metabolites using ^{13}C, ^{15}N and ^{34}S isotope labelling, in combination with high-resolution mass spectrometry. Plant J. 68, 364–376.

Grant, B., Davie, A., Taggart, J.B., Selly, S.L.C., Picchi, N., Bradley, C., Migaud, H., 2016. Seasonal changes in broodstock spawning performance and egg quality in ballan wrasse (*Labrus bergylta*). Aquaculture. 464, 505–514.

Gyamfi, D., Ofori Awuah, E., Owusu, S., 2019. Chapter 2 - lipid metabolism: an overview. In: Patel, V.B. (Ed.), The Molecular Nutrition of Fats. Academic Press, pp. 17–32.

He, K., Gkioxari, G., Dollár, P., Girshick, R., 2017. Mask R-CNN. In: 2017 IEEE International Conference on Computer Vision (ICCV).

Hixon, M.A., Johnson, D.W., Sogard, S.M., 2013. BOFFFFs: on the importance of conserving old-growth age structure in fishery populations. ICES J. Mar. Sci. 71, 2171–2185.

Izquierdo, M.S., Socorro, J., Arantzamendi, L., Hernandez-Cruz, C.M., 2000. Recent advances in lipid nutrition in fish larvae. Fish Physiol. Biochem. 22, 97–107.

Johnston, T.A., Leggett, W.C., 2002. Maternal and environmental gradients in the egg size of an iteroparous fish. Ecology 83, 1777–1791.

Jouhet, J., Lupette, J., Clerc, O., Magneschi, L., Bedhomme, M., Collin, S., Rebeille, F., 2017. LC-MS/MS versus TLC plus GC methods: consistency of glycerolipid and fatty acid profiles in microalgae and higher plant cells and effect of a nitrogen starvation. PLoS One 12, e0182423.

Kjesbu, O.S., Solemdal, P., Bratland, P., Fonn, M., 1996. Variation in annual egg production in individual captive Atlantic cod (*Gadus morhua*). Can. J. Fish. Aquat. Sci. 53, 610–620.

Kjørsvik, E., Mangor-Jensen, A., Holmefjord, I., 1990. Egg quality in fishes. Adv. Mar. Biol. 26, 71–113.

Kjørsvik, E., Hoehne-Reitan, K., Reitan, K., 2003. Egg and larval quality criteria as predictive measures for juvenile production in turbot (*Scophthalmus maximus* L.). Aquaculture 227, 9–20.

Kvæstad, B., Hansen, B.H., Davies, E.J., 2022. Automated morphometrics on microscopy images of ichthyio- and zooplankton using mask R-CNN and classical machine vision techniques. MethodsX 9, 101598.

Lasne, E., Leblanc, C.A.-L., Gillet, C., 2018. Egg sqize versus number of offspring trade-off: female age rather than size matters in a domesticated arctic charr population. Evol. Biol. 45, 105–112.

Lê Cao, K.-A., González, I., Déjean, S., 2009. integrOmics: an R package to unravel relationships between two omics datasets. Bioinformatics. 25, 2855–2856.

Lim, J.N., Senior, A.M., Nakagawa, S., 2014. Heterogeneity in individual quality and reproductive trade-offs within species. Evolution 68, 2306–2318.

Malzahn, A.M., Boersma, M., 2007. Year-to-year variation in larval fish assemblages of the southern North Sea. Helgol. Mar. Res. 61, 117–126.

Migaud, H., Bell, G., Cabrita, E., McAndrew, B., Davie, A., Bobe, J., Carrillo, M., 2013. Gamete quality and broodstock management in temperate fish. Rev. Aquac. 5, S194–S223.

Morrongiello, J.R., Bond, N.R., Crook, D.A., Wong, B.B.M., 2012. Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish. J. Anim. Ecol. 81, 806–817.

Pavlov, D., Kjorsvik, E., Refsti, T., Andersen, O., 2004. Brood stock and egg production. In: Moksness, E., Kjørsvik, E., Olsen, Y. (Eds.), Culture of Cold-Water Marine Fish. Blackwell Publishing, London, pp. 129–203.

Rohart, F., Gautier, B., Singh, A., Le Cao, K.-A., 2017. mixOmics: an R package for 9omics feature selection and multiple data integration. bioRxiv 108597.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999. Lipid nutrition of marine fish during early development: current status and future directions. Aquaculture. 179, 217–229.

Sargent, J.R., Tocher, D.R., Bell, J.G., 2003. The lipids. In: Halver, J.E., Hardy, R.W. (Eds.), Fish Nutrition. Academic Press, pp. 181–257.

Smith, C.C., Fretwell, S.D., 1974. The optimal balance between size and number of offspring. Am. Nat. 108, 499–506.

Srivastava, R., Brown, J., 2011. The biochemical characteristics and hatching performance of cultured and wild Atlantic salmon (*Salmo salar*) eggs. Can. J. Zool. 69, 2436–2441.

Sugasini, D., Thomas, R., Yalagala, P.C.R., Tai, L.M., Subbaiah, P.V., 2017. Dietary docosahexaenoic acid (DHA) as lysophosphatidylcholine, but not as free acid, enriches brain DHA and improves memory in adult mice. Sci. Rep. 7, 11263.

Takeda, H., Izumi, Y., Takahashi, M., Paxton, T., Tamura, S., Koike, T., Shiomi, M., 2018. Widely-targeted quantitative lipidomics method by supercritical fluid chromatography triple quadrupole mass spectrometry. J. Lipid Res. 59, 1283–1293.

Tocher, D.R., Sargent, J.R., 1984. Analyses of lipids and fatty acids in ripe roes of some northwest European marine fish. Lipids. 19, 492–499.

Tsugawa, H., Ohta, E., Izumi, Y., Ogiwara, A., Yukihira, D., Bamba, T., Arita, M., 2015. MRM-DIFF: data processing strategy for differential analysis in large scale MRM-based lipidomics studies. Front. Genet. 5, 471.

Woodfield, H.K., Cazenave-Gassiot, A., Haslam, R.P., Guschina, I.A., Wenk, M.R., Harwood, J.L., 2018. Using lipidomics to reveal details of lipid accumulation in developing seeds from oilseed rape (*Brassica napus* L.). Biochim. Biophys. Acta 1863, 339–348.

Xuan, Q., Hu, C., Yu, D., Wang, L., Zhou, Y., Zhao, X., Xu, G., 2018. Development of a high coverage pseudotargeted lipidomics method based on ultra-high performance liquid chromatography–mass spectrometry. Anal. Chem. 90, 7608–7616.