

Spawning manipulation, broodfish diet feeding and egg production in farmed Atlantic salmon

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ABSTRACT

Atlantic salmon aquaculture relies on continuous supply of high quality eggs. Broodfish nutrition and manipulation of ovulation time (photoperiod and temperature) are key factors. The optimum feeding period with broodfish diet has not been investigated before. The present study examined how feeding period with broodfish diet (9 vs. 17 months) interacted with manipulation of ovulation time (early (Nov), normal (Dec), late (Feb)) on broodstock egg production capacity and egg quality in two-sea-winter female Atlantic salmon (~12 kg). All groups were fed until June 2021 when they were transferred to tanks and starved until ovulation.

There were no measurable main or interaction effects of feeding period. Manipulation of ovulation gave several significant effects compared to normal ovulating females:

1. Early ovulating females had higher viscerosomatic index, smaller eggs with higher swelling rate (early 1.36, normal 1.27, late 1.23), and lower egg quality shown as reduced survival from fertilization to the eyed egg stage (mortality: early 11%, normal 5%, late 14%).
2. Late ovulating females: (i) were emaciated as shown by low condition factor calculated on gutted weight, and hepatosomatic and viscera indexes; (ii) had larger gonads caused by larger eggs; (iii) had reduced egg quality measured as elevated mortality from fertilization to first-feeding (eyed egg – first feeding: early 11%, normal 8%, late 23%).
3. All groups spawned a similar number of eggs per female.

The current results show that the feeding period with broodstock diet may be set to 9 months. This would make Atlantic salmon egg production more economical and environmentally sustainable. The results also show that manipulation of ovulation time reduce egg quality but not fecundity. Further research should investigate feeding periods with broodstock diet shorter than 9 months and determine the threshold for jeopardized fish welfare with respect to starvation time for manipulated late ovulating females.

1. Introduction

Time of gamete release can be manipulated by artificial environments in salmonids. In its natural habitat in the North Atlantic Ocean, anadromous Atlantic salmon (*Salmo salar*) experience marked seasonal

changes in daylength and temperature, which times and regulates the developmental speed of smoltification (Hoar, 1988; Solbakken et al., 1994; Handeland et al., 2004) and sexual maturation (Hansen et al., 1992; Taranger et al., 1998). While precocious sexual maturation in freshwater is common in juvenile males, adult fish usually enters

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puberty in seawater and migrates back to freshwater where they complete sexual maturation and spawn (Klementsen et al., 2003). Wild Atlantic salmon may stay in seawater from one to five years (Klementsen et al., 2003), while farmed broodstock are mainly based on fish that mature as two-sea-winter fish (Taranger et al., 1998). Following a normal maturation cycle, spawning takes place in November and December (Taranger et al., 1998). Inspired by pioneer work on rainbow trout (Whitehead et al., 1978; Duston and Bromage, 1986), Taranger et al. (1991) showed that an abrupt change from long to short day length in the summer advances, while continuous light from July delays ovulation. Next, Taranger and Hansen (1993) showed that elevated temperature can delay ovulation. Combined, this knowledge allowed the development of a flexible salmon production where gametes are produced from September to February and smolts are put into seawater throughout the season. This has been one of the important success factors behind the steady increase in production of farmed Atlantic salmon, which had reached over 2.6 million tons world-wide in 2020 (FAO, 2022).

To optimize the nutritional composition of the eggs, salmon broodstock is fed specialized broodstock diets which contain a higher level of marine ingredients (fish oil and -meal) and enhanced supplementation of several micronutrients (Rennie et al., 2005, own data). In contrast, marine ingredients are successfully replaced by vegetable ingredients in grow-out diets (Sargent and Tacon, 1999; Torstensen et al., 2000; Bell et al., 2003), which lower costs and make them more sustainable. Replacement of fish meal and oil with vegetable meals and oils can decrease reproductive performance in fish (Hernandez De-Dios et al., 2022). Partial replacement with vegetable ingredients altered the egg nutritional composition without affecting fertilization rate, mortality, or first-feeding fry weight (Rennie et al., 2005). Feeding periods with broodfish diet have been reported to be between 9 and 14 months (Skjærven et al., 2020, 2022). There is, however, no knowledge on whether the length of the feeding period with broodfish diet affects egg production capacity or quality. Egg number or fecundity along with egg size are good parameters to assess egg producing capacity, while egg quality is commonly assessed by fertilization rate and survival rates at eying, hatch and first-feeding (Bromage, 1995). Furthermore, Żarski et al. (2012) recorded that cortisol reaction intensity during the swelling process was an objective and reliable egg quality indicator in pikeperch (*Sander lucioperca*).

Maturing farmed adult Atlantic salmon have decreased appetite from early June (Kadri et al., 1997), and salmon broodstocks are consequently not fed following transfer from sea-cages to freshwater tanks, but depend on reallocation of nutrients from somatic tissues to the developing ovary. It has been shown that deposition of nutrients in the eggs was affected when Atlantic salmon females were manipulated to ovulate either early or late compared to the normal ovulation period in December (Hamre, 2015). Later, Skjærven et al. (2022) reported that advanced ovulation impaired optimal deposition of nutrients into the eggs, while delayed ovulation enhanced the muscular catabolic activity of the broodfish and elevated levels of catabolized amino groups in the eggs.

The main aim of this study was to examine how feeding period with broodfish diet in combination with manipulation of ovulation time affected egg production capacity and quality in Atlantic salmon.

2. Material and methods

In this study, female two-sea-winter Atlantic salmon broodstock (previously fed a commercial grow-out diet) were fed a commercial broodfish diet for either 17 or 9 months prior to transfer to on-land tanks in early June, and then manipulated with photoperiod and temperature to ovulate early (November) or late (February) or kept at simulated ambient photoperiod and temperature for normal ovulation time (December). The fish were not fed after transfer to tanks in June, and all eggs were fertilized with cryopreserved sperm from the same male.

Response parameters were broodfish growth, fecundity and egg size and swelling rate, and off-spring fertilization rate, survival from fertilization to first-feeding, and size at first-feeding.

2.1. Ethical statement

All experiments were performed at the Institute of Marine Research (IMR), Matre (60° N, 5° E, Western Norway), which is authorized for animal experimentation (Norwegian Food Safety Authority, facility 110) in accordance with International guidelines. This study was certified using Norwegian research permit number 2021.

2.2. Fish stock

On 16 May 2019, the female broodfish (in the current study) arrived at the Institute of Marine Research, Matre (Western Norway) as yearling smolt ($n = 2000$, ~ 180 g). They had been produced at Mowi Terningen (Mid-Norway), where they had been pit-tagged and sexed by genotyping (Yano et al., 2013). At arrival, the fish were randomly allocated between two 5x5x7 meter sea cages and fed a common grow-out diet under natural light.

2.3. Experimental design

The overall experimental design is shown in Fig. 1. On 15 January 2020, the fish were randomly re-allocated between four 5x5x7 m sea cages where fish in two cages were fed a grow-out diet, and the other two cages were fed a broodstock diet under natural light (Fig. 1). The fish in all four cages were fed the same broodstock diet between 15 September 2020 and 01 June 2021. All mature one-sea-winter fish were euthanized (0.5 g L^{-1} Finquel) on 17 November 2020. On 01 June 2021, the remaining fish were sedated (0.1 g L^{-1} Finquel), had their PIT tag number recorded, length and weight measured, and maturity status evaluated by ultrasound examination (Næve et al., 2018). Then 9 maturing females from each of the 4 sea cages were transferred to 9 different circular tanks (3 m diameter) on land (totally 81 fish from each cage), ending up with 36 fish in each tank with 9 fish from each cage in common garden. After completing the fish transfer, groups of 3 tanks were subjected to three different environmental regimes combining photoperiod and temperature to produce three different ovulation times (Fig. 2):

2.3.1. Normal

Simulated natural photoperiod (day length including civil twilight of 61° N, Western Norway) and a temperature regime simulating the temperature of Matre River (using the average of historical temperature data but limiting the highest and lowest temperature to 16 °C and 4 °C respectively). Matre River is a local salmon and sea trout river and was selected as reference. This temperature regime increased from 10 °C at transfer to reach 15 °C on 26 June 2021, was stable between 15 °C and 16 °C until 1 September when it was reduced according to the Matre River data.

2.3.2. Early

A photoperiod regime with LL from 02 June 2021 (transfer to tanks), reduced to 8 L:16D on 13 July, combined with temperature which was increased to reach 16 °C on 6 June and reduced from 26 August to reach 6 °C on 1 September 2021. The abrupt change from long to short day length advance ovulation (Taranger et al., 1991), which is further advanced by a drop in temperature (Taranger et al., 2003; King and Pankhurst, 2007; Vikingstad et al., 2008). We used the approach of King and Pankhurst (2007) and introduced the drop in temperature 1200°-days after the drop in day length to ensure that the developing ovary responded sufficiently.

A Atlantic salmon brood fish feeding in seawater



B Manipulation of ovulation time in freshwater and incubation of eggs until 3 weeks of first-feeding

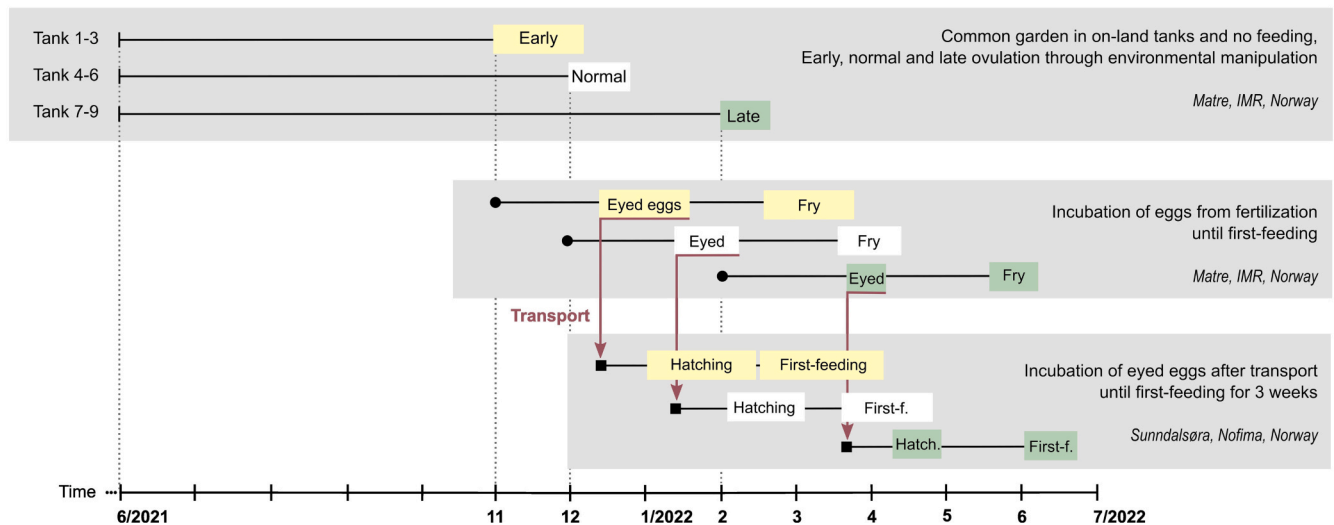


Fig. 1. (A) Two-sea-winter Atlantic salmon broodstock was reared in seawater from January 2020 to June 2021 and fed either a commercial broodfish diet for 17 months (long-term) or a grow-out diet for 8 months followed by the broodfish diet for 9 months (short-term) in duplicate sea cages. (B) Nine fish (PIT tagged) from each sea cage were transferred to 9 tanks on land with freshwater, to be reared in common garden. The fish were not fed in this period. Fish in triplicate tanks were manipulated to ovulate early (Nov), normal (Dec) or late (Feb, see Fig. 2). Eggs from each female of each ovulation group were fertilized with sperm from one single male and then incubated until the eyed stage. Eyed stage embryos were then either transported to Sunndalsøra (Norway) or remained at Matre (Norway) until first-feeding (fry). Incubation at Sunndalsøra included a three weeks first-feeding period. Time of ovulation from the first to the last ovulating female, and accordingly eyed stage, hatching and first-feeding periods are indicated by rectangles representing each period.

2.3.3. Late

A simulated natural photoperiod reaching LL on 21 June 2021 and kept on LL until 3 January 2022 when it was reduced to 8 L:16D. The temperature regime was identical to the NORMAL regime until 1 September. From then the LATE group were kept on 15 °C until 21 September when it was reduced in 1 °C steps to reach 12 °C on 10 November. From 12 °C the temperature was dropped in steps from 03 January 2022 to reach 6 °C on 05 January. Continuous light (Taranger et al., 1991) and elevated temperature (Taranger and Hansen, 1993) delay ovulation. Taranger (1993) suggested that water temperature above 11 °C inhibit ovulation in Atlantic salmon. Hence, we reared the fish at LL and ~12 °C and then reduced temperature and day length to stimulate ovulation.

The salinity in all groups was kept at approx. 25 ppt from transfer, between 15 and 20 ppt from 20 July 2021 and changed to freshwater (0.8 ppt) on 25 September (early), 15 October (normal) and 23 December (late). The flow rate was 0.4 l per minute per kg biomass, and the inlet water was supplied with oxygen so that the oxygen saturation of the outlet water was kept above 80%.

The fish were checked for ovulation in the period 5 October 2021 to 21 February 2022. The fish were sedated (AQI-S vet. MSD Animal Health, 5 mg L⁻¹) and examined within their rearing tanks. The same operator checked for ovulation throughout the ovulation period. Per ovulation group (early, normal, late), 21 in early, otherwise 24 ovulated fish (8 per tank, 2 per earlier sea-cage) were sampled for different tissues and had their eggs (i) measured for evaluation of broodstock egg production capacity and (ii) fertilized for evaluation of egg quality and offspring performance.

All eggs were fertilized with cryopreserved sperm from the same male and incubated at ~7 °C until 328–388-days post fertilization (supplementary Table S1). At the eyed stage, 250 ml, i.e. approximately 2000 eggs per female were packed on ice and transported to NOFIMA Sunndalsøra (Mid-Norway) for completion of incubation, hatching, and finally evaluation of first-feeding growth performance for three weeks. The remaining eggs per female were kept at Matre for evaluation of hatching rates, juvenile offspring size, deformity types and rate at first-feeding.

At the arrival of eyed eggs sent to Nofima, dead eggs were removed and counted. Approximately 50 ml were used for samples, 100 ml were incubated in each of two small incubators (16.0 cm × 13.5 cm). One of the duplicate incubators was used for sampling of alevins and fry ready for first-feeding. The second incubator was undisturbed until first feeding, except for removal of dead eggs and alevins. Mean water temperature in incubators was 7.1 °C and dissolved oxygen 96–98%. When ready for first feeding, fry from the undisturbed incubators were transferred to 60 × 60 cm flat bottomed tanks, 30 cm water depth, 24 h light, temperature 12 °C and dissolved oxygen approximately 95%. Feed was supplied continuously (every 15 min) using electrically driven belt feeders (Imenco Smart Solutions, Aksdal, Norway). The feeders released feed for 45 s followed by a 14 min and 15 s pause.

2.4. Diets

The diets used in the present study were produced by Mowi Feed, Valsneset (Mid-Norway). At start, broodfish in the long-term feeding (LF) group were fed on a commercial 9 mm extruded broodfish diet (BRS

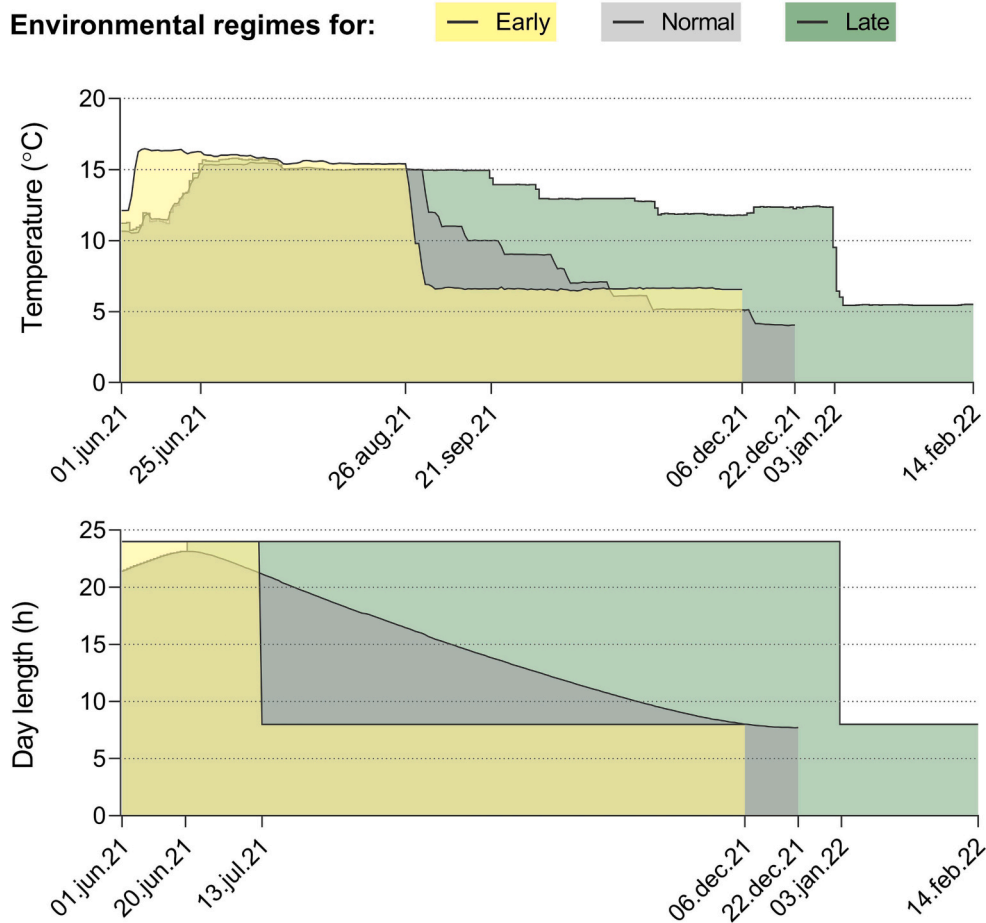


Fig. 2. Temperature and day length regimes during the freshwater period in on-land tanks.

2500, Batch B1 and B2) whereas broodfish in the short-term feeding (SF) group continued on a 9 mm extruded commercial grow-out diet (Jupiter 2500, Batch G1 and G2) from January to August 2020. Both groups were fed the same 11 mm extruded broodfish diet from September 2020 onwards (BRS 5000, Batch B3). The BRS diets have a higher sum of EPA & DHA and vitamin and mineral levels compared to the Jupiter grow-out feed. All feed ingredients used in the diets are based on selected well known raw materials (Table 1). All vitamins, minerals and amino acids were added according to NRC requirements and levels are in proprietary of Mowi Feed AS (1). For further description of Mowi sustainability raw material sourcing please see the www.mowi.com/policies. Five batches of feed were analysed for proximate composition, fatty acid, cholesterol, amino acids, macro- and microminerals, and fat- and water-soluble vitamins. Analysed feed composition is listed in Table 2a and supplementary Table S2. The nutrient composition of the diets and the dry matter of eggs were measured by ISO certified routine methods at IMR, Bergen. Table 2b presents an overview of the methods with analysis principles and references.

The commercial feed used for 3 weeks of first-feeding at Nofima research station was EWOS MICRO START 015 (Cargill, Norway).

2.5. Sampling procedures

2.5.1. Broodfish

Prior to sampling, fish was starved for one day and euthanized (0.5 g L⁻¹ Finquel) followed by an accurate and forceful blow to the skull performed by trained personnel (percussive stunning). Broodfish were sampled five times during the sea cage period in January 2020, May 2020, September 2020, January 2021, and June 2021 before transfer to

on-land tanks, and once in the middle (3 months before spawning) of the tank period and at the end of each ovulation period (early, normal, late). Samples of female broodfish at sea and on land, were taken in the same manner. Fork length, body weight, liver, viscera (excluding liver and gonads), and gonad weight were recorded. Gutted weight was recorded after removing the viscera and gonads. Whole fish and samples from homogenized liver and muscle tissue (NQC), visceral fat, scales, vertebra, plasma, gonads, unfertilized and fertilized eggs were taken for further nutrient analyses (not content of this publication).

2.5.2. Unfertilized eggs

At time of ovulation, total egg weight from each female was registered, and average individual egg weight calculated by weighing three egg batches with a known number of eggs. Dry matter (DM) content was analysed in both unfertilized and fertilized eggs 24 h post fertilization to estimate egg swelling.

2.5.3. Fertilization until transport of eyed eggs

Fertilized eggs were identified the day after fertilization. An egg sample were cleared in 10% acetic acid and eggs with cell cleavage (2 or 4 cell stage) were, counted and divided by total of sampled eggs per batch to determine fertilization %. Approximately 800 mL of eggs from each female were incubated. Dead eggs were removed and counted regularly, and each batch of eyed eggs was divided into 3 (at 328–388 dC). Approximately 250 ml was sent to Sunndalsøra for a juvenile growth study, 200 mL was frozen for nutrient analyses, and the rest were kept at Matre and was incubated until first-feeding. The procedure for sending eggs was the same as used commercially, with eggs from each female packed in chambers in a styrofoam box with wet ice on the top

Table 1

Diet formulation of grow-out diets and brood fish diets used during the experiment (all values are g/kg).

	G1	G2	B1	B2	B3
Diet type	Grow-out diet	Grow-out diet	Brood fish diet	Brood fish diet	Brood fish diet
Feeding period	01/2020–5/2020	05/2020–9/2020	01/2020–5/2020	05/2020–9/2020	9/2020–6/2021
Formulations					
Fishmeal (Scandinavia)	98,9	78,2	252,7	250,9	271,2
Fishoil (Anchovetas)	25,2	18,8	–	–	8,0
Fishoil (Menhaden)	41,5	–	73,9	148,1	214,9
Fishoil (Scandinavia)	33,5	99,6	71,4	–	–
Rapeseed oil	217,2	220,1	145,3	148,1	74,3
Linseed oil	16,7	–	–	–	–
Soy protein concentrate	106,3	168,1	201,1	144,2	50,0
Corn gluten meal	52,0	51,5	–	50,5	–
Wheat gluten meal	149,9	160,1	34,7	72,3	160,0
Pea protein concentrate	38,4	–	46,6	–	–
Guar protein	52,0	25,8	–	–	–
Wheat whole grain	36,4	25,8	145,0	75,1	133,6
De-hulled horse beans	80,5	98,4	–	49,8	25,0
Amino acids, vitamins and minerals ¹	23,8	24,0	14,8	17,3	20,5
Pigment (10% astaxanthin)	0,5	0,5	0,5	0,6	0,6
Feed phosphorus (MAP)	16,0	17,8	9,3	10,0	10,4
Water balance	9,6	10,3	4,2	32,9	31,5

and melting water trickling down on the eggs. The transport by plane and on the road lasted 1–2 days. The weight of each egg batch at the eyed stage was measured and used to calculate the number of remaining eggs per female. When adding mortality data, the number of eggs incubated from ovulation could be calculated, together with fertilization %, hatching %, mortality % before and after the eyed stage.

2.5.4. Fry at first-feeding

Twenty euthanized (0.5 g L⁻¹ Finquel) fry from each ovulation batch were patted dry on tissue paper prior to weighing. Standard length and myotome height at the anus of the fry were quantified by image analysis using Inkscape. At the same time, ~200 fry were euthanized from each ovulation batch and screened for externally visible deformities and deviating phenotypes. These were classified as curled (curled body axis), curved (curved body axis), twin (conjoined twins), yolk sac (abnormal size and shape of yolk sac), tail fin (deviating tail fin), and albino (pale blue/white coloration).

2.5.5. After transport of eyed eggs until after first-feeding

Samples for analyses were collected at arrival (eyed eggs), at hatching (alevins), at first feeding, and after approximately 3 weeks of feeding. Alevins and fry were euthanized (0.5 g L⁻¹ Finquel) and samples were frozen in liquid nitrogen before storage at –80 °C for later analyses.

2.6. Calculations

Broodfish condition factor was expressed as Fulton's condition factor and calculated based on total body weight and gutted weight. Condition factors, organ indexes, egg weight, egg swelling, fertilization rate, hatching rate and mortality rate were calculated using the following formulas (BW, body weight; DM, dry matter; GW, gutted weight; L, fork length; t, time; W, weight):

$$\text{Condition factor on BW} = \frac{BW}{L^3} \times 100$$

$$\text{Condition factor on GW} = \frac{GW}{L^3} \times 100$$

$$\text{Organ indexes} = \frac{W_{\text{organ}}}{BW} \times 100$$

$$W_{\text{fertilized eggs}} (\text{g}) = \frac{W_{\text{unfertilized eggs}} (\text{g}) \times DM_{\text{unfertilized eggs}} (\%) }{DM_{\text{fertilized eggs}} (\%)}$$

$$\text{Egg swelling} = \frac{W_{\text{fertilized eggs}} (\text{g})}{W_{\text{unfertilized eggs}} (\text{g})}$$

$$\text{Fertilization\%} = \frac{\text{Egg count}_{\text{fertilized}}}{\text{Egg count}_{\text{total}}} \times 100$$

$$\text{Hatching\%} = \frac{\text{Egg count}_{\text{hatched}}}{\text{Egg count}_{\text{total}}} \times 100$$

$$\text{Mortality\%} = \frac{\text{Dead egg count } t_1 - t_2}{\text{Total egg count } t_1} \times 100$$

2.7. Statistics

The software Statistica (TIBCO Software Inc., version 13, <http://tibco.com>) was used for the statistical treatment. All variables with percent data were arcsine transformed. Data were subjected to Levene's test to detect nonhomogeneous distributions. The data were first subjected to a factorial design with feeding regime and ovulation time as independent variables. If feeding regime did not have a significant effect, the data were analysed by one-way ANOVA with ovulation time as the independent variable. When the variances were not homogenous, the data were analysed with nonparametric methods (Kruskal-Wallis ANOVA and median test). Differences and effects were considered significant at $P < 0.05$.

3. Results

3.1. Broodstock growth and organ indexes

There were no differences in body weight, condition factor, hepatosomatic index, gonadosomatic index between the LF and SF feeding groups throughout the trial period (Fig. 3). Mean body weights were 2.7 kg in January 2020 (start broodstock diet LF), 6.5 kg in September 2020 (start broodstock diet SF), and 12.0 kg in June 2021 (transfer to tanks).

The duration of the period with broodstock diet (LF vs SF) did not affect weight, condition factor, hepatosomatic index, gonadosomatic index, or relative viscera or gutted weights in any ovulation groups (Early, Normal, Late) either three months before or at ovulation (Fig. 4). There was a significant effect of ovulation time on weight, condition factors, hepatosomatic index, viscera (%) (one-way ANOVAs, $P < 0.00001$), and gonadosomatic index (Kruskal-Wallis ANOVA, $P < 0.00001$) 3 months before ovulation (all groups were measured 3 months before their ovulation time). At this time point: (i) condition factor calculated both on body weight and gutted weight, viscera (%) and gonadosomatic index were significantly different between all ovulation times and decreasing with later ovulation time for the three

Table 2a

Analysed nutrient composition of five batches of broodfish diet given to Atlantic salmon during the seawater period. The table provides a selection of nutrients, and full list is given in the supplementary Table S2.

		G1	G2	B1	B2	B3
	Diet type	Grow-out diet	Grow-out diet	Broodfish diet	Broodfish diet	Broodfish diet
	Feeding period	1/2020–5/2020	5/2020–9/2020	1/2020–5/2020	5/2020–9/2020	9/2020–6/2021
Proximate composition						
Crude protein	g/100 g ww	39	38	39	39	38
Fat	g/100 g ww	35	33	32	32	33
Carbohydrates ¹	g/100 g ww	15,8	16,8	16,6	15,1	14,8
Dry matter	g/100 g ww	94	92	93	92	91
Ash	g/100 g ww	4,2	4,2	5,4	5,9	5,2
Lipids						
Cholesterol	mg/kg ww	716	1225	852	1862	1572
Amino acids						
Hydroxy-Proline	mg/g ww	<0.6	<0.6	1,7	1,5	1,5
Histidine	mg/g ww	8,3	7,5	8,5	7,6	7,7
Taurine	mg/g ww	0,75	0,6	1,52	1,25	1,93
Serine	mg/g ww	17,8	17,1	17,6	17,8	16,8
Arginine	mg/g ww	21,7	18,5	22,6	20,2	18,2
Glycine	mg/g ww	15,4	13,8	18,8	17,7	17,3
Aspartic acid	mg/g ww	29,1	27,8	36	33	28,1
Glutamic acid	mg/g ww	83	81	67	72	86
Threonine	mg/g ww	15,2	14,4	17	15,6	16,5
Alanine	mg/g ww	15,9	15,1	18,3	19,3	16,5
Proline	mg/g ww	26,7	26,2	21	22,4	26,4
Lysine	mg/g ww	22,6	22,2	25,3	26,3	25,3
Tyrosine	mg/g ww	13,3	11,4	12,2	12	11,7
Methionine	mg/g ww	8,8	7,8	8,9	8,6	8,9
Valine	mg/g ww	15,8	15,1	17,5	16,4	16,5
Isoleucine	mg/g ww	14,5	13,9	15,7	14,9	15,5
Leucine	mg/g ww	29,3	27,9	28,3	29,9	26,7
Phenylalanine	mg/g ww	18,7	16,7	17,6	16,8	18,7
Fatty acids						
20:4n-6 (ARA)	mg/g ww	0,62	1,1	0,99	1,96	2,41
20:5n-3 (EPA)	mg/g ww	10,83	10,07	14,23	16,49	31,35
22:5n-3 (DPA)	mg/g ww	1,34	0,96	1,9	3,32	5,23
22:6n-3 (DHA)	mg/g ww	7,41	12,47	12,15	13,57	19,66
Sum fatty acids	mg/g ww	288	322	271	281	282
Sum saturated	mg/g ww	47,9	51,8	53	62,1	80,1
Sum monounsaturated	mg/g ww	140	169	130	118	91,1
Sum EPA + DHA	mg/g ww	18,2	22,5	26,4	30,1	51
Sum n-3	mg/g ww	49,8	49,7	49,5	55,3	74
Sum n-6	mg/g ww	45,6	46,1	32,4	35,5	24,2
Sum polyunsaturated	mg/g ww	95,8	97,1	83	93,2	102
n-6/n-3	–	0,9	0,9	0,7	0,6	0,3
Vitamins						
Biotin (B7)	mg/kg ww	0,78	0,76	1	1	1,3
Cobalamin (B12)	mg/kg ww	0,3	0,24	0,25	0,41	0,34
Folate (B9)	mg/kg ww	6	9,1	7,4	15	16
Niacin (B3)	mg/kg ww	140	130	190	190	160
Pantothenic acid (B5)	mg/kg ww	54	58	85	89	89
Pyridoxine (B6)	mg/kg ww	15	14	23	22	25
Riboflavin (B2)	mg/kg ww	17	20	25	30	25
Thiamine -HCl (B1) ²	mg/kg ww	25	24	37	43	31
Vitamin C equivalent (AAE)	mg/kg ww	522,58	123,77	419,44	398,81	378,18
Vitamin D3	mg/kg ww	0,12	0,13	0,13	0,12	0,09
Vitamin E (alpha-tocopherol)	mg/kg ww	340	250	580	450	430
Vitamin K (sum) ³	mg/kg ww	544,8	463,5	414,7	373,5	266,9
Vitamin K3 (menadione)	µg/kg ww	1,58	0,832	6,42	1,84	3,4
Minerals						
Ca	mg/kg ww	3600	3800	7700	11,000	9100
Na	mg/kg ww	1600	1400	4100	3800	4200
K	mg/kg ww	6000	7200	7500	8200	5400
Mg	mg/kg ww	1300	1500	1700	1700	1400
P	mg/kg ww	8400	9200	9300	11,000	9100
Trace elements						
Iodine	mg/kg ww	2,4	2	1,3	2,9	3,3
Mn	mg/kg ww	54	39	39	37	34
Fe	mg/kg ww	230	200	180	190	140
Cu	mg/kg ww	9,8	7,7	6,8	7,1	6,2
Zn	mg/kg ww	190	130	150	140	130
Se	mg/kg ww	0,59	0,62	0,92	0,93	0,97

¹ Levels represent the subtracted values of protein, fat and ash from dry matter content.

² Hydrochloride (HCl) makes up 10,7% in thiamine -HCl.

³ Sum of vitamin K1 and K2 forms measured. See supplementary Table S2.

Table 2b

Analytical methods.

Analyte	Principle	Reference
Dry matter	Gravimetric after drying at 104 °C	
Protein	N x 6.25, Leco N Analyzer	Hamre and Mangor-Jensen (2006)
Total lipids	Gravimetric after acid hydrolyses	(EU directive 84/41983)
Cholesterol	Saponification extraction and GC/FID	Araujo et al. (2006)
Fatty acids	Transmethylation extraction and GC/FID	Lie and Lambertsen (1991)
Ash	Gravimetric after combustion at 550 °C	
Thiamine	HPLC	CEN (2003b))
Riboflavine	Microbiological	Maeland et al. (2000))
Niacine	Microbiological	Maeland et al. (2000)
Folat	Microbiological	Maeland et al. (2000)
Vitamin B6	HPLC	CEN (2005)
Vitamin C	HPLC	Maeland and Waagbø (1998)
Vitamin A	HPLC	Moren et al. (2002)
Vitamin D	HPLC	CEN (1999)
Vitamin E	HPLC	Hamre et al. (2010)
Sum vitamin K3	HPLC	CEN (2003a))
Trace elements	ICP-MS	Julshamn et al. (2004)
Minerals	ICP-MS	Liaset et al., 2003

first parameters while increasing with ovulation time for the latter; (ii) hepatosomatic index was significantly lower in the Early than the Normal and Late females (Fig. 4).

At the time of ovulation, there was a significant effect of ovulation time on hepatosomatic index, gonadosomatic index, condition factor on gutted weight (one-way ANOVAs, $P < 0.00001$), and viscera (%) (Kruskal-Wallis ANOVA, $P < 0.00001$). At this time point: (i) Late females had significantly lower hepatosomatic index, and significantly higher gonadosomatic index than Early and Normal females; (ii) viscera (%) was significantly different between all ovulation times and decreased with ovulation time; (iii) Late females had a significantly lower condition factor calculated on gutted weight than Early females (Fig. 4 and supplementary Table S3).

3.2. Ovulation time and egg production

Broodstock diet feeding period in seawater did not affect time of ovulation, total egg weight, or egg counts and individual weights. Ovulated Early females were detected in the period 01 November to 06 December 2021, Normal in the period 29 November to 22 December 2021, and Late in the period 31 January to 14 February 2022 (Fig. 5A). Late females had significantly higher total and individual egg weights compared to Early and Normal females at the time of ovulation (Fig. 5B, supplementary Table S3 and Table S4).

3.3. Egg quality

Broodstock diet feeding period in seawater did not affect any of the measured egg quality parameters (Table 3). Ovulation time had a significant effect on egg weight after fertilization (one-way ANOVA), fertilization rate, hatching rate, egg weight before fertilization, swelling, and mortality rates in periods between fertilization and first-feeding (Kruskal-Wallis ANOVA). Early and Late had significantly higher mortality rates from fertilization to the eyed egg stage compared to Normal females (Table 3). Late had significantly lower fertilization and hatching rates, and significantly higher egg weight before fertilization, and mortality rates (Matre and Sunndalsøra) between the eyed egg stage and first-feeding compared to the Early and Normal females (Table 3). Swelling rate was significantly higher in Early than in Late females, while Normal had intermediate values (Table 3). Egg weight after

fertilization was significantly different between all ovulation time points and increasing with ovulation time for SF fish, while for LF fish, Early and Late females were significantly different and Normal had intermediate values (Table 3).

Pictures of random unfertilized, fertilized, and eyed eggs from the different ovulation groups are shown in Fig. 6, note the observable larger eggs in the Late females.

3.4. Offspring size

Fry weight at first-feeding was significantly higher in offspring from Late than from Early and Normal females among offspring kept at Matre (IMR) (Fig. 7 and supplementary Table S4). Morphometric analysis of offspring kept at Matre showed significantly higher body length and myotome height in Late than in Early and Normal offspring (Fig. 7). Assessment of malformations in first-feeding fry showed no effect of either period on broodfish diet or ovulation time (Table 4).

4. Discussion

4.1. 105 Days from first to last ovulation

Ovulated females were sampled in the period 01 November 2021 to 14 February 2022. Earlier studies have shown that gamete release can be advanced by five to six months by manipulation of photoperiod and temperature in both male and female Atlantic salmon (Johnston et al., 1992; Fjelldal et al., 2011; Skjærven et al., 2020). Such advancement is, however, not possible to achieve when maturing two-sea-winter fish are reared in sea-cages until transfer to tanks during the summer, as in the current study. Using this approach allows gamete release during the autumn and winter (Taranger et al., 1998; Skjærven et al., 2022). The fish needs to be reared at elevated temperature during the winter to induce gamete release during the spring (Johnston et al., 1992; Fjelldal et al., 2011) or summer (Hansen et al., 2015; Skjærven et al., 2020). For this purpose, salmon farmers rear the fish in recirculated aquaculture systems (RAS) (Skjærven et al., 2020).

4.2. Excessive broodfish nutrition?

A reduction of the period on broodstock diet from 17 to 9 weeks had no measurable negative effects, independent of ovulation time. Even though we should look at nutrient dynamics before solid conclusions can be drawn, it seems that broodstock production of Atlantic salmon can become more economically and environmentally sustainable by restricting the broodstock diet feeding period to 9 months. Our selected feeding time periods (9 vs 17 months) are within and outside those used in commercial salmon farming, which have been reported to vary between 9 and 14 months depending on the environmental manipulation (Skjærven et al., 2020, 2022). How feeding broodstock diets for periods shorter than 9 months affects egg production capacity and quality, and possible interactions with manipulation of ovulation remains to be elucidated.

4.3. Early ovulating females produced small eggs with high swelling rate

Early ovulating females had the smallest eggs, which were significantly smaller compared to late ovulation. Similarly, early ovulation caused by environmental manipulation reduced egg size in rainbow trout (*Oncorhynchus mykiss*) (Bromage et al., 1984) and Atlantic cod (*Gadus morhua*) (Hansen et al., 2001; Penney et al., 2006). In contrast, equal egg size has been observed in normal ovulating and manipulated early ovulating sea bass (*Dicentrarchus labrax*) (Carrillo et al., 1989) and turbot (*Shophthalmus maximus*) (Polat et al., 2021). Early females had higher relative viscera weights than normal, which most probably reflects a larger abdominal lipid store. Skjærven et al. (2022) compared commercially produced spawns originating from September (early),

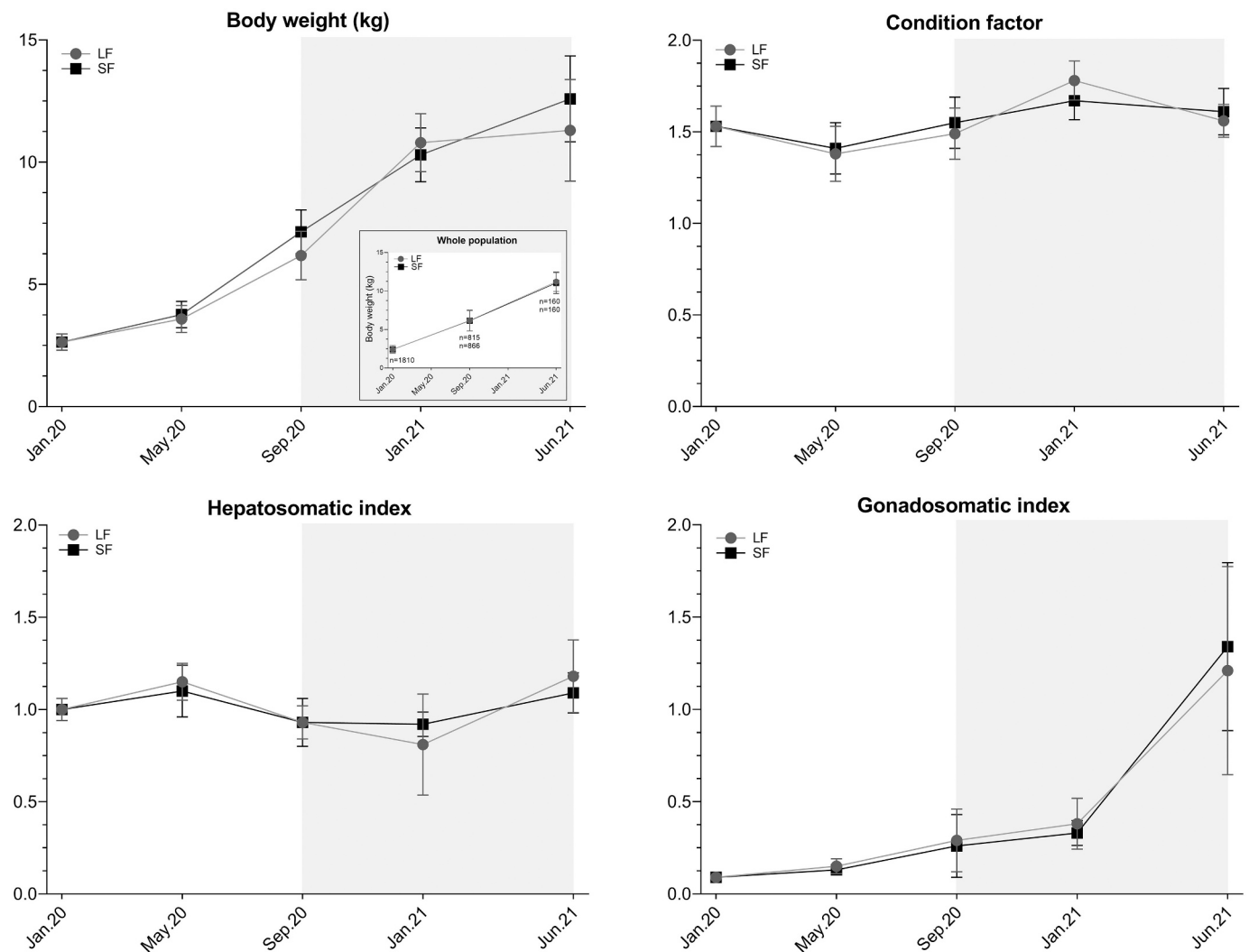


Fig. 3. Growth performance, condition factor, hepatosomatic and gonadosomatic indexes of female Atlantic salmon broodfish at five data collection timepoints during the seawater feeding period from January 2020 to June 2021. Broodfish from both the long-term (LF) and short-term (SF) feeding group were fed the same broodfish diet (Batch B3) from September 2020 onwards indicated by grey background colour. Single data points represent the mean $\pm$ SD of sampled fish ($n = 7-10$) from two sea cages per feeding group. Body weight for the whole population ($n > 800$) is given as an insert in the figure.

November (normal) and January (Late) and found smaller livers, eggs, ovaries, and first-feeding offspring size in early compared to normal ovulating Atlantic salmon broodstock. All these differences were not observed in the current study. The reason behind this difference is most probably that Skjærven et al. (2022) compared early females that were transferred to tanks (environmental manipulation and starvation) in mid-April with females that were transferred in August, while the current study compared early and normal females that were both transferred to tanks in early June. Together with the smaller egg size observed in early than in normal females, their higher viscera weights could suggest a lower maternal investment. Indeed, their lower hepatosomatic index three months prior to ovulation indicates that less energy was allocated through the liver to the developing ovaries. Liver size increases during ovarian development due to its role in vitellogenesis (van Bohemen et al., 1981), and then decreases sharply some weeks before ovulation (Tveranger, 1985). Lower hepatosomatic index in early females three months before ovulation may have reduced their vitellogenin producing capacity. In turn, since fecundity was unaffected, this reduced capacity may have resulted in their reduced egg size.

Although early females produced smaller un-fertilized eggs, their eggs showed the highest swelling rate post fertilization. Swelling reflects the formation of the perivitelline space (PVS), which forms between the

chorion and the perivitelline membrane. During this process, an exocytic expulsion of colloid material from the cortical alveoli causes a water influx from the ambient medium, which results in PVS formation (Lønning and Davenport, 1980; Eddy and Talbot, 1983; Li et al., 1989). The pressure Atlantic salmon eggs can resist decreases following fertilization (Zotin, 1958), which reflects an increase in the elasticity in the chorion. The turgor pressure caused by colloid material in the PVS fluid and the elasticity of the chorion, together, determines the final volume of the fluid in the PVS and the swelling capacity of the egg. Later, the toughness of the chorion increases dramatically so that Atlantic salmon eggs can resist a pressure as high as 2500–3500 g (Zotin, 1958). The hardening process in the chorion is controlled by maternal factors such as Ca originating from the ovarian fluid (Kusa, 1949a, 1949b). The degree of hardening may further impact on the swelling capacity of the egg (Kusa, 1949a, 1949b).

The higher swelling rate among eggs from early females in our study most probably reflects a relatively larger PVS in this group. Whether the higher swelling rate observed among early females was related to altered chorion elasticity, PVS turgor pressure or ovarian fluid composition, is at the current state unknown. Smaller eggs with higher egg dry weight have been reported in manipulated early ovulating Atlantic cod (Penney et al., 2006, 2009). If this is transferable to Atlantic salmon, it

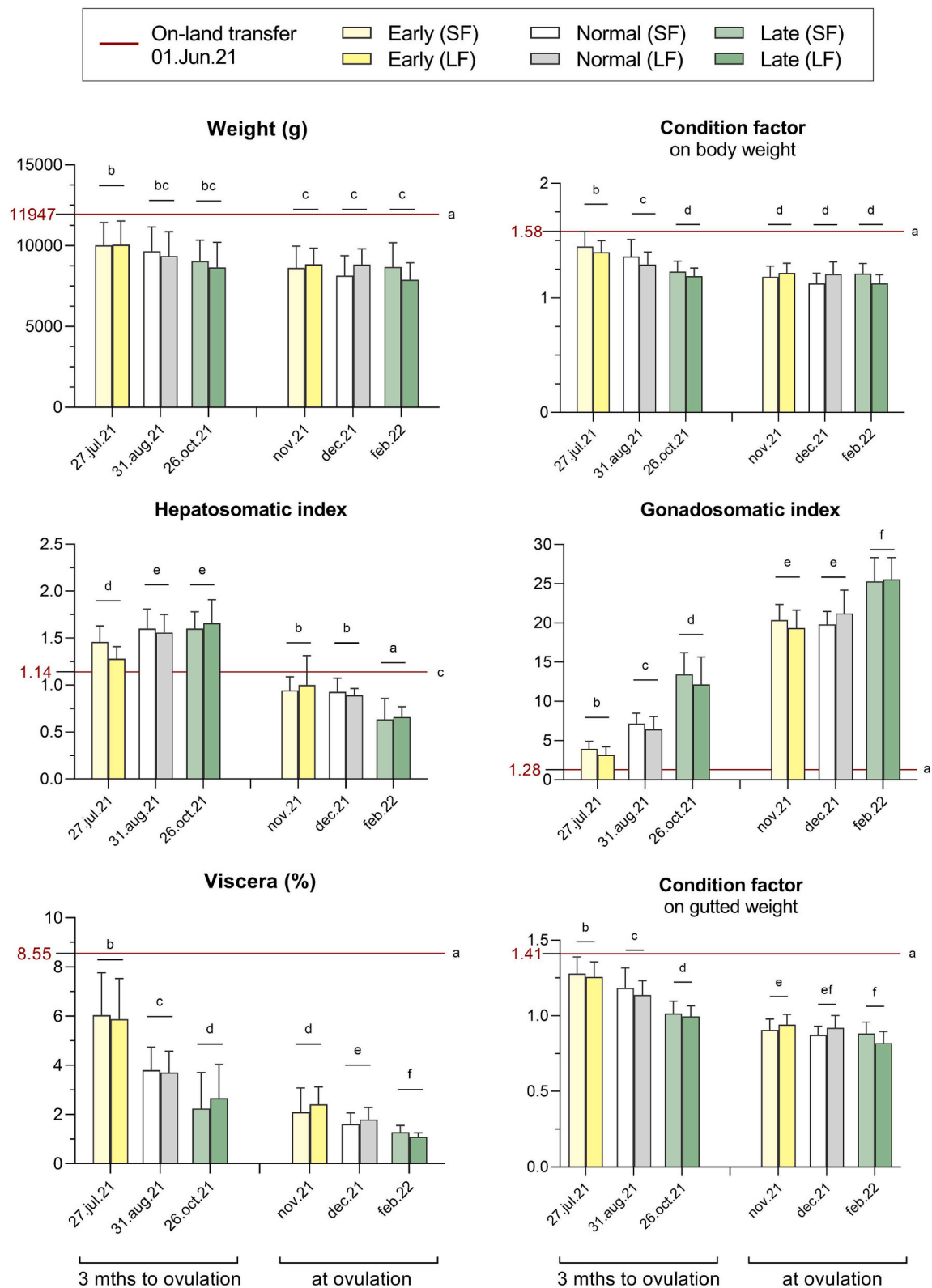
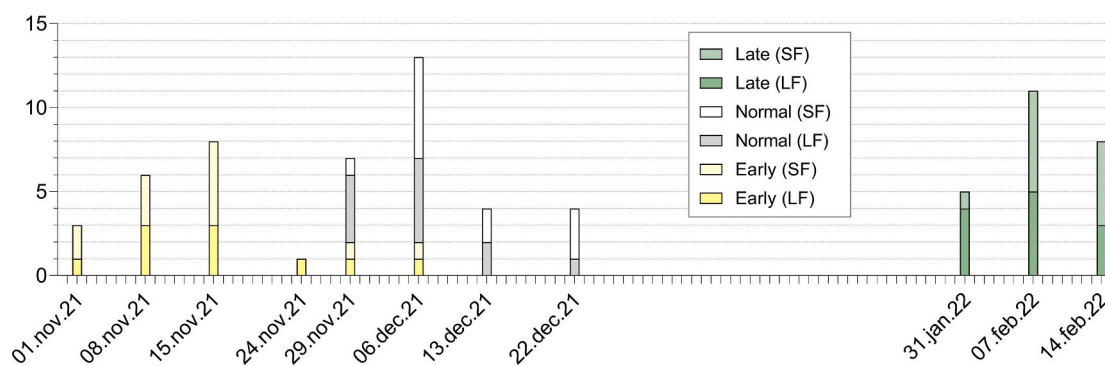


Fig. 4. Changes in body weight, condition factor, hepatosomatic index, gonadosomatic index, viscera (%) and condition factor calculated on gutted weight among female Atlantic salmon broodfish kept in freshwater on-land tanks to ovulate early, normal or late. Broodfish from both the long-term (LF) and short-term (SF) feeding group were kept in common garden from June 2021 until ovulation at different times. Red horizontal lines represent average values of all groups when transferred on-land. Bars represent mean $\pm$ SD ($n = 12$) and letters represent significant differences between ovulation groups ($p < 0.05$). Gonadosomatic index at ovulation is presented as percent roe weight (Table S3). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

A Number of mature females per stripping time point



B Egg production capacity

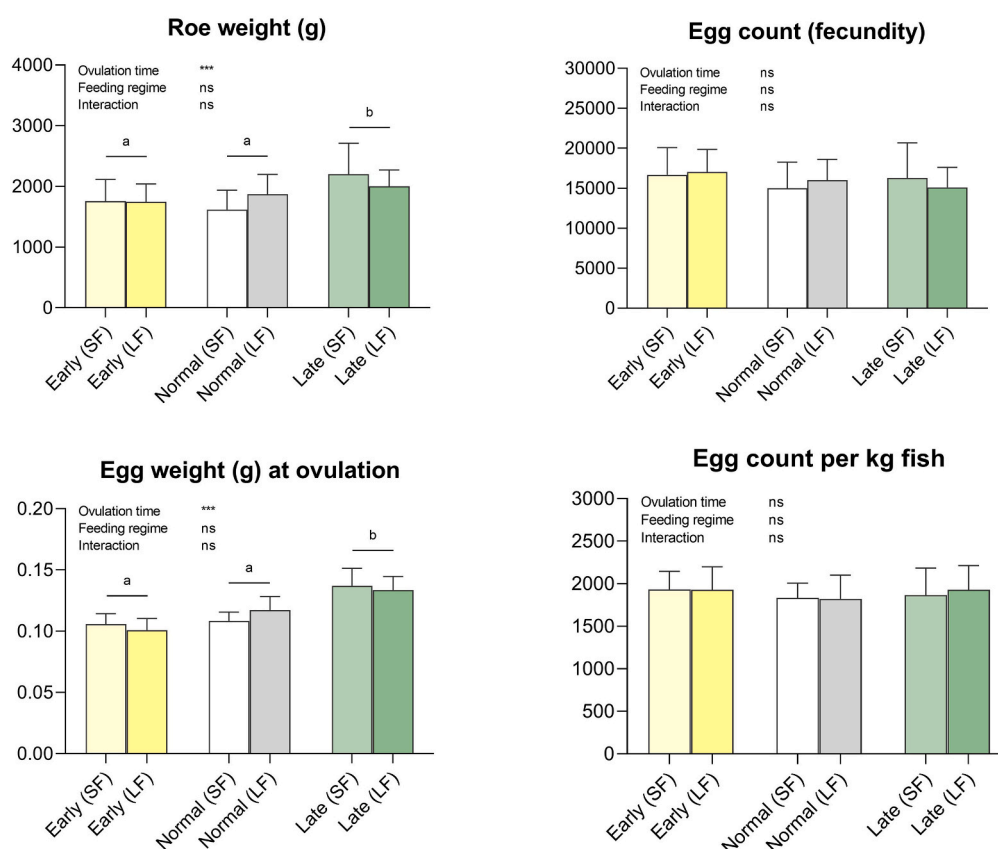


Fig. 5. Number of mature females per stripping time point (A) and egg production capacity (B) separated by ovulation time and seawater feeding group. At time of stripping, total roe weight and average individual egg weight were recorded to calculate fecundity and egg count per kg fish. Egg production capacity data is shown as mean $\pm$ SD ($n = 10\text{--}12$) and different letters indicate significant differences between ovulation groups ($p < 0.05$). An overview of each individual and egg production capacity statistics are provided in the supplementary data (Table S1 and Table S4).

would suggest a higher turgor pressure as the root cause. Although early and normal females were equal across most of the measured parameters, early females had lower egg quality shown by elevated mortality rate from fertilization to the eyed egg stage. Likewise, Taranger et al. (1998) found reduced survival to the eyed egg stage in manipulated early ovulating Atlantic salmon females. If a high turgor pressure is the root cause for the increased swelling in early eggs, this may have decreased embryo survival rate (Zarski et al., 2012).

4.4. Heavy maternal investment among late females

Late females had the lowest condition factor based on gutted weight showing that their carcasses were emaciated. They also had the highest gonadosomatic index caused by the combined effects of larger eggs, lower viscera and liver size, and carcass emaciation. Manipulated late ovulating females have been reported to produce smaller eggs in Atlantic cod (Hansen et al., 2001), similar sized eggs in sea bass (Carrillo et al., 1989), and larger eggs in rainbow trout (Bromage et al., 1984).

Table 3

Fertilization and hatching rates, egg swelling (weight fertilized egg / weight unfertilized egg), survivals before and after eyed stage, with and without transport. Kruskal-Wallis ANOVA and median test, except for egg DM after fertilization, which was analysed by factorial ANOVA. Different letters indicate significant differences. DM, dry matter.

Ovulation time	Early		Normal		Late		P
Feeding regime	SF	LF	SF	LF	SF	LF	Ovulation time
N	12	9	12	12	12	12	
Fertilization %	99 ± 2 ^a	99 ± 1 ^a	100 ± 0 ^a	100 ± 0 ^a	74 ± 22 ^b	79 ± 23 ^b	<10 ⁻⁴
Hatching %	94 ± 4 ^a	92 ± 15 ^a	95 ± 5 ^a	94 ± 7 ^a	88 ± 7 ^b	81 ± 28 ^b	0.04
Egg DM % before fertilization	36 ± 1 ^a	36 ± 3 ^a	37 ± 1 ^a	37 ± 2 ^a	39 ± 1 ^b	39 ± 1 ^b	<10 ⁻⁴
Egg DM % after fertilization	27 ± 3 ^a	27 ± 3 ^a	30 ± 1 ^b	29 ± 2 ^{ab}	33 ± 2 ^c	31 ± 2 ^{bc}	<10 ⁻⁶
Swelling	1.35 ± 0.15 ^a	1.37 ± 0.22 ^a	1.25 ± 0.04 ^{ab}	1.28 ± 0.08 ^{ab}	1.19 ± 0.05 ^b	1.26 ± 0.07 ^b	0.001
Mortality % fertilization – eyed stage	10 ± 7.5 ^b	12 ± 9.2 ^b	3 ± 2.5 ^a	7 ± 5.6 ^a	14 ± 13 ^b	14 ± 12 ^b	0.004
Mortality % eyed stage – First-feeding Matre	10.8 ± 10.1 ^a	14.7 ± 27.0 ^a	7.5 ± 6.2 ^a	10.5 ± 10.4 ^a	21 ± 12 ^b	25 ± 28 ^b	<10 ⁻³
Mortality % eyed stage – First-feeding Sunndalsøra	7.6 ± 5.1 ^a	10.0 ± 13.8 ^a	7.5 ± 6.8 ^a	7.0 ± 10.9 ^a	26 ± 13 ^b	18 ± 15 ^b	<10 ⁻⁴

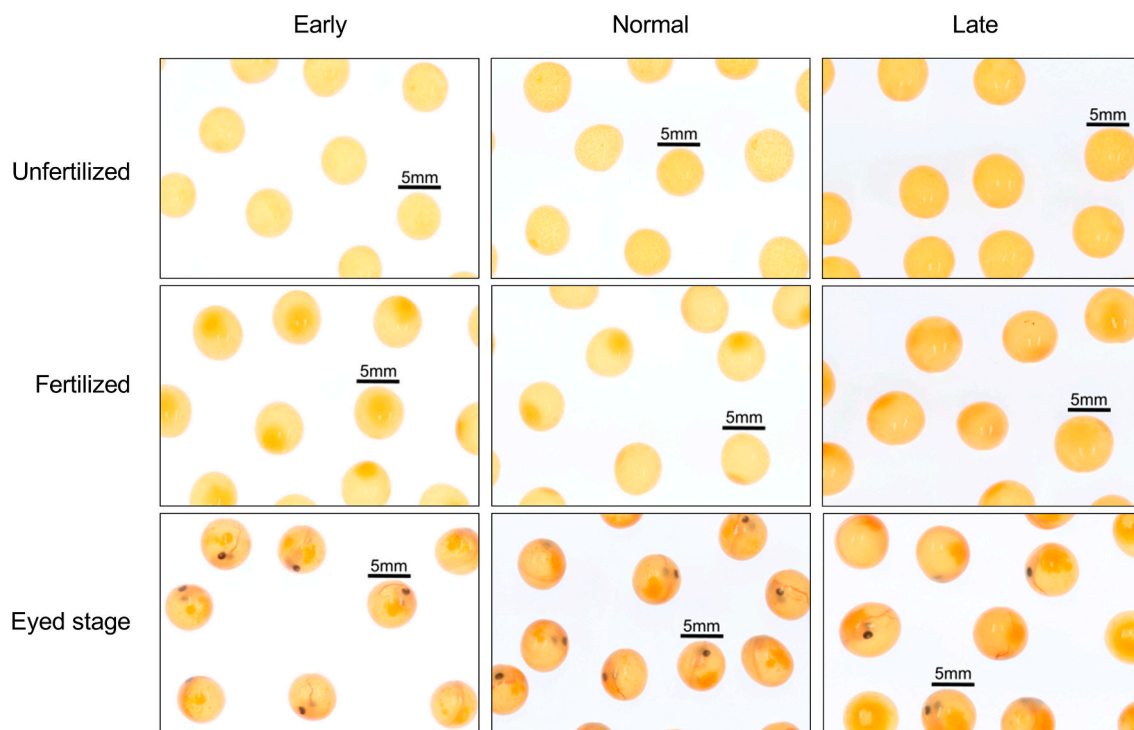


Fig. 6. Illustration of unfertilized and fertilized eggs, and eyed stage embryos from early, normal and late ovulating Atlantic salmon. Pictures are taken for illustration purpose of egg size of a randomly selected egg batch in an ovulation group regardless of LF or SF treatment in seawater. Differences in colour tones are due to varying lighting conditions when the pictures were taken.

compared to normal ovulating females. Lower hepatosomatic and viscera indexes among late females show that they were more energy depleted than early and normal females at ovulation. When normal and manipulated late ovulating females were transferred from sea-cages to tanks in August (compared to early June in our study), late females also had lower hepatosomatic index and higher ovary weight and larger eggs at ovulation compared to normal females (Skjærven et al., 2022). Our results show that the increase in gonadosomatic index and ovary weight among late females is not related to increased fecundity, but an increase in egg size.

Larger eggs in late females confirms their enhanced maternal investment as indicated by emaciation and low viscera and hepatosomatic indexes. When normal and late ovulating females were starved for 120 and 166 days, their hepatosomatic indexes were 1.25 and 0.92% (Skjærven et al., 2022). In the current study when maximum starvation time (latest ovulation date for each group) were 204 and 258 days for normal and late females, their hepatosomatic indexes were 1.0 and 0.75%. This indicates that early transfer from sea cages to tanks and

prolonged starvation period downshifts hepatosomatic indexes in normal and late ovulating females. In the current study, normal females had good egg quality even if they were starved for up to 204 days. Skjærven et al. (2022) analysed samples collected at a commercial farm and found lower lipid levels and catabolism of muscle proteins which influenced the nutrient composition of the newly fertilized eggs among late spawners (starvation: 166 days). Together with the low egg quality among late females in our study these results indicate that it is not the long starvation period per se that challenges egg quality in manipulated late ovulating females, but the environmental manipulation. One possible reason is the prolonged inhibition of ovulation by elevated temperature (Vikingsstad et al., 2015).

The enhanced emaciation among late females was most probably a result of long-lasting starvation. Eight weeks of starvation had no effect on welfare in post-smolt Atlantic salmon (Hvas et al., 2022). In our study, however, eight weeks covers only the increase in starvation time between normal and late females. Maturing wild Atlantic salmon do not consume much energy after entering freshwater (Johansen, 2001). Thus,

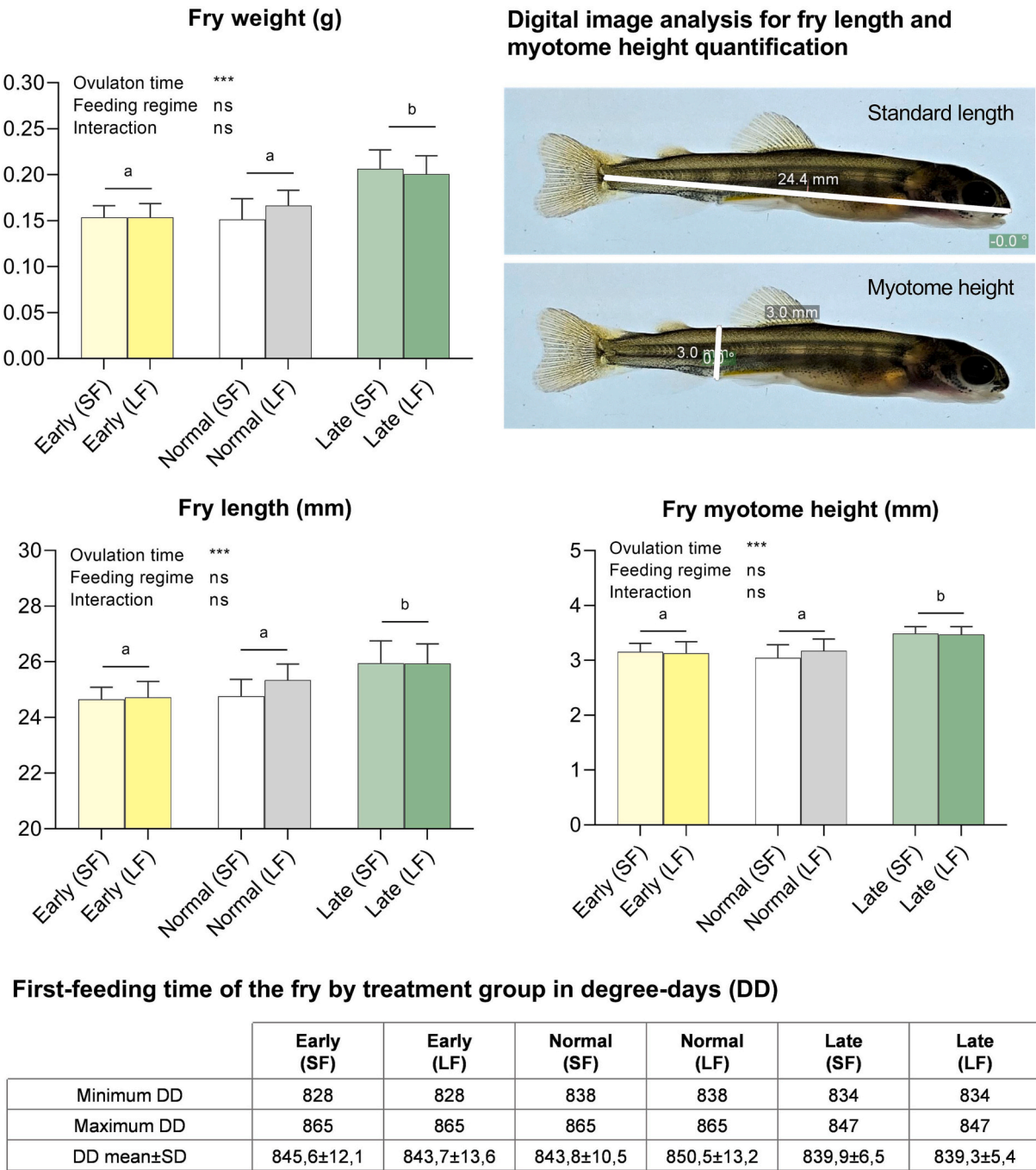


Fig. 7. Fry weight, length, and myotome height at first-feeding. Individual length and myotome height (at anus) were quantified by image analysis using Inkscape. Growth data is shown as mean ± SD of 10–12 fry batches per treatment group (20 individuals per batch). fry size statistics are provided in the supplementary Table S4. Letters indicate significant differences between ovulation groups ($p < 0.05$).

in the current study, normal females experienced close to natural conditions, while late females did not, which may have jeopardized fish welfare. There is a need to further elaborate starvation time in manipulated late ovulating females to determine a threshold for sustained welfare.

Late females produced the largest offspring as evaluated by size at first feeding. In agreement, alevin length prior to onset of exogenous feeding is positively related to egg size in Atlantic salmon (Solberg et al., 2014). How this would affect further growth, and if this is a beneficial trait in late spawners is at present unclear. Laboratory studies on offspring of wild Atlantic salmon have shown diverging results. Pri-vol'nev et al. (1964) and Glebe et al. (1979) reported that egg size and

juvenile size were still correlated 81 days and 8 months after hatching, respectively, while Hayes and Armstrong (1942) and Thorpe et al. (1984) reported that larger eggs gave rise to larger alevins compared to smaller eggs, but that the size difference had disappeared 35 days post-hatch and through the first year of growth, respectively. There are also diverging results from studies on other salmonids. In brown trout (*Salmo salar*), Ojanguren et al. (1996) reported a strong positive association between egg size and fry length 52 and 92 after hatch, which was also positively associated with maternal size. Springate and Bromage (1985) studied offspring from 2 and 3 year old broodfish of rainbow trout (*Oncorhynchus mykiss*), and found a positive association between egg and first feeding fry size that were lost four weeks after first feeding. In

Table 4
Deformities in first-feeding fry (both feeding regimes). Kruskal-Wallis ANOVA and median test.

Ovulation time	Early		Normal		Late		P
	SF	LF	SF	LF	SF	LF	
N	12	10	12	12	12	12	
Tail fin %	1.21 ± 1.11	2.58 ± 3.25	0.89 ± 1.12	1.05 ± 1.53	1.29 ± 3.26	0.16 ± 0.47	ns
Curved %	1.37 ± 3.23	1.11 ± 1.56	2.21 ± 2.13	0.95 ± 0.72	1.80 ± 2.75	1.80 ± 2.51	ns
Yolk sac %	0.09 ± 0.22	0.15 ± 0.22	0.07 ± 0.12	0.13 ± 0.25	0.45 ± 0.64	0.29 ± 0.55	ns
Twin %	0.03 ± 0.09	0.03 ± 0.10	0.02 ± 0.07	0.02 ± 0.08	0.03 ± 0.11	0.00	ns
Albino %	0.16 ± 0.39	0.03 ± 0.10	0.05 ± 0.11	0.10 ± 0.20	0.13 ± 0.26	0.30 ± 0.50	ns
Curled %	0.05 ± 0.12	0.00	0.00	0.10 ± 0.15	0.61 ± 1.37	0.33 ± 0.49	ns
Total %	2.91 ± 4.23	3.91 ± 4.40	3.30 ± 3.07	2.37 ± 2.62	4.38 ± 6.07	3.02 ± 3.60	ns

chinook salmon (*Oncorhynchus tshawytscha*), fry originating from big eggs retained larger size after several weeks of feeding compared to those from smaller eggs (Fowler, 1972). There are no studies on the effect of egg size on long term growth performance in farmed Atlantic salmon. In case the relative difference between early and late spawners is constant throughout life it would mean that total production time from first feeding to harvest could be several weeks shorter in offspring of late compared to early spawners.

5. Conclusions

Based on the biological results reported here, the feeding period with broodstock diet may be set to 9 months, making Atlantic salmon egg production less costly and perhaps more sustainable due to lowered consumption of marine resources. Manipulation of ovulation time reduced egg quality, but not fecundity. In order to have access to smolts all year round, it is also possible to design other regimes for manipulation of ovulation that are less stressful to the fish than the methods used in this study.

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CRedit authorship contribution statement

Per Gunnar Fjelldal: Methodology, Validation, Investigation, Writing – original draft. Anne-Catrin Adam: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization. Gerd M. Berge: Conceptualization, Methodology, Investigation, Writing – review & editing. Ingrid Lein: Conceptualization, Methodology, Investigation, Writing – review & editing. Ernst M. Hevrøy: Methodology, Resources, Writing – review & editing. Ingun Næve: Methodology, Investigation, Writing – review & editing. Rudi R. Seim: Methodology, Resources, Writing – review & editing. Maren Mommens: Methodology, Resources, Writing – review & editing. Kaja H. Skjærven: Conceptualization, Methodology, Writing – review & editing. Tom Hansen: Methodology, Investigation, Writing – review & editing. Kristin Hamre: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – review & editing, Project

administration, Funding acquisition.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Kristin Hamre reports financial support was provided by Norwegian Seafood Research Fund.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aquaculture.2023.740227>.

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