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Modulations of lipid metabolism and development of the Atlantic salmon (*Salmo salar*) fry in response to egg-to-fry rearing conditions

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Abstract In stocking program, the use of artificial incubation conditions in hatcheries from the fertilisation of eggs to the release of unfed fry could reduce their ability to adapt to the natural environment. This study evaluates the effects of three factors on the fitness and physiology of salmon fry at their emergence, the origin of water (river vs drilling), the type of support in the incubator (support matrix vs plastic sheets) and the type of incubators (Californian vs vertical trays), and compares them to a semi-natural incubation method in river. Key biological functions including nutritional and immune status were compared among experimental conditions using biometric parameters, lipid composition and gene expression analyses. Our findings demonstrated that fry incubated in vertical trays supplied with river water had no significant difference in growth and lipid composition compared to those in semi-natural incubators. Besides, fry incubated on a substrate matrix in Californian trays exhibited phenotypic characteristics closest to those incubated in river. This support matrix improved fish growth, lipid consumption and distribution compared to fry on plastic sheets. Moreover, the large amounts of several PUFAs in these fry could allow a better membrane fluidity ensuring a better adaptation to temperature variation under cold conditions. In addition, drilling water improved the survival rate compared to river water due to lower numbers of fine particles, known to be responsible for the clogging of eggs. To conclude, using a substrate combined with drilling water in artificial incubators could increase fry fitness and its adaption to wild life.

Keywords Egg incubation · *Salmo salar* · Rearing conditions in hatchery

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Introduction

Decline in anadromous Atlantic salmon *Salmo salar* populations is observed as a consequence of a combination of factors including overfishing (Parrish et al. 1998), dam construction and habitat degradation (Renardy et al. 2020), river pollution (Coghlan and Ringler 2005), genetic introgression and acid rain (Glover et al. 2017). To maintain or restore wild salmon populations, artificial reproduction from wild salmon breeders is a common method to restock rivers with fry or parr stages (Bams 1985; Jonsson and Jonsson 2009). As previously described, brood stock origin, breeding strategy, individual adaptation potential, method of release, density of individuals in the

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river, selected habitat, predation and competition are essential factors that can be manipulated to improve the efficiency of restocking (Farooqi and Aprahamian 1995; Jonsson and Jonsson 2011). For example, the use of large-scale rearing methods derived from intensive fish farming production could reduce the survival of salmon that could be the consequences of its adaptability to a natural environment after stocking in the river (Saloniemi et al. 2004). In nature, salmon embryo development depends on natural conditions such as the presence of fine particles, concentration of water oxygen, daily variation of water temperatures, pathogens, pollutants and benthic prey which contribute to salmon adaptation after emergence (Murray and McPhail 1988; Iida et al. 2017). Particularly, temperature is a major factor affecting embryo and larval development and the time from fertilisation to hatching fry was described to last about 500°, and 300 to 350° from hatching to emergence (this thermal sum decreases above 8 degree-days) (Kane 1988; Jonsson and Jonsson 2011).

The average survival rate from egg deposition to emergent fry varies from 0 to 90% among fry nests in the same river, the large majority of records stating a survival rate of 2 to 35% (Dumas and Marty 2006). Several studies reported that a major cause of high mortality rates observed in rivers are due to floods, which are more frequent during the season of the embryonic phase (Greig et al. 2005; Dumas et al. 2007; Sear et al. 2016). Indeed, floods carry a large quantity of fine particles (< 1 mm) that clog the interstices of the substrate and deposit on the surface of the eggs, thus reducing the oxygen input with consequences for the development of the embryo (Greig et al. 2005; Sear et al. 2016). Moreover, during violent floods, the substrate is washed away and the eggs resuspended, and through the action of erosion may become non-viable. A high demand for oxygen is essential during the egg stage which further increases during hatching (Dumas et al. 2007). Furthermore, as described by Beacham and Murray (1990), incubation temperature plays a major role in fry development as it affects their growth performances.

Conversely, the use of controlled conditions in fish culture such as low turbidity (reduction of fine particles) and controlled water flow, daily removal of dead eggs/larvae, allows a high survival rate of fry at the emergent stage. But, raising animals in non-selective and controlled environments could promote traits leading to fish adapted to captivity but not for life in nature (Heath 2003). Particularly, in chinook salmon, Heath (2003) highlighted that a low selection in hatchery rearing limited favouring of large eggs, allowing fecundity selection to drive exceptionally rapid evolution of small eggs. In addition, this low selection pressure could affect the adaptive capacities of those fry and consequently lead to low survival in natural streams after stocking. In stocking programs, embryos and yolk sac fry are kept in hatcheries to maximise the number of eggs that can reach the emergent fry stage. Nevertheless, the survival of fry resulting from these artificial incubation modes remains very low once they are released into rivers compared to their congeners hatched under natural river conditions (Kallio-Nyberg et al. 2015). Henderson and Letcher (2003) observed mortalities of up to 60% 2 days after stocking salmon fry into the wild due to predation (Henderson and Letcher 2003). This could indicate that the conventional incubations used in hatcheries fail to adequately prepare fry for wildlife.

In nature, salmonids lay their eggs in redds in the stream bed and cover them with gravel. The gravel selected by the female protects the offspring against predators but also serves to guard against shifts in gravel caused by other females, flooding, desiccation during periods of low water and freezing (Fleming 1996; Bardonnet and Baglinière 2000). Then, after hatching, yolk sac salmon fry live for several weeks (corresponding to about 300–350° day) within this gravel (Bardonnet and Baglinière 2000). Surprisingly, in hatcheries, it is still common practice to rear eggs on a simple plastic or metal tray without any support matrix. This forces the fry to invest a lot of energy to maintain an upright position limiting the conversion of yolk into body tissue (Hansen and Torrissen 1985). In order to imitate incubation conditions similar to the natural environment, several authors proposed to use a semi-natural system (Bams 1985; Bamberger 2009a). Bams (1985) firstly compared the performances of three instream incubation techniques to rear coho salmon (*Oncorhynchus kisutch*) eggs: a shallow matrix made by in fish- and light-tight boxes, a deep matrix formed by cleaned gravel enclosed in areas of existing riffles and baskets buried in cleaned areas of the riffles. The shallow matrix proved to be the least effective technique, while the basket gave the best mean survival and growth performance. Later,

Bamberger (2009a, b) demonstrated that rearing fry using a substrate matrix could increase growth performances and improved their survival rates when stocked into a river (Bamberger 2009b).

In the present study, fry fitness at emergence was assessed by analysing growth performance and lipid metabolism that give an insight on energy allocations of the yolk sac, and the expression of genes involved in key physiological pathways. Among those genes, two involved in the innate immunity of fish were evaluated: the lysozyme, a catalytic innate immune enzyme that hydrolyses peptidoglycans in the wall of Gram + bacteria (Saurabh and Sahoo 2008) and C3a component of the complement, known to encode a protein involved in pathogen elimination mechanisms by destroying the target via the formation of a transmembrane channel, thus allowing the entry of water molecules into the cell (Holland and Lambris 2002). In addition, genes associated to energy metabolism and swimming activity were evaluated: *Glut1* a gene coding for a glucose transporter responsible for the low level of basal glucose uptake required to maintain respiration in all cells (Jensen et al. 2006), and the *myogenin*, encoding a protein involved in the coordination of skeletal muscle development, myogenesis and muscle repair (Campos et al. 2013). Three genes involved in development were also selected: the *neurotrophin* gene is encoding a protein involved in survival and neuron development (Lukiw and Bazan 2008), *Sox11* gene is involved in the regulation of embryonic development and cellular determination and neurogenesis (Jiang et al. 2015), *CryB1*, a gene encoding crystallin B1, a protein involved in the maintenance of transparency and the refractive index of the lens of the eye (Zou et al. 2015). The influence of different rearing conditions on those parameters has been investigated along egg-to-fry development to evaluate the fitness status of fry. Therefore, we evaluated the benefits of supplying the incubator with water containing few fine particles (vertical tray supplied with drilling water vs Aisne river water) to avoid egg clogging, and of depositing eggs on a substrate matrix (Californian tray with or without pebble stones) to allow fry to spare some energy. In addition, a semi-natural incubation was carried out under river conditions (the Aisne, a tributary of the Ourthe, in Belgium) in order to compare the physiological parameters recorded in fry from hatcheries with those of wild fry. The objective of the study was to optimise hatchery incubation methods to guarantee a high survival rate from egg to fry without compromising growth and health.

Materials and methods

Experimental design

On November 28, 2016, a batch of 160,000 Atlantic salmon eyed-eggs were obtained from a single source of pooled eggs resulting from the artificial reproduction of 5 wild males F0 and 124 captive females F1 (3 years old), provided by the National Conservatory of Wild Salmon (Chanteuges, France). Briefly, after striping the males and females to collect the sperm and ova, batches of 5000 eggs were fertilised with a ratio of 1 to 4 million spermatozoa per egg to reach a total of 186,000 fertilised eggs. A survival rate of 86% was observed from fertilisation to eyed stage. Before their transfer to the Conservatory of Meuse salmon (Erezée, Belgium), eggs were incubated in 12 vertical incubator trays supplied by L'Allier river water until 43.9% development, calculated according to the Kane formula (Kane 1988) (that relates the number of days from fertilisation to initial feeding (Y) to the mean temperature (X) by the predictive equation $\log e^{Y} = 6.003e^{-0.0307X}$). The experiment was composed of 5 experimental eyed-eggs-to-fry rearing conditions:

(1) semi-natural incubators filled with pebble stones and disposed in a salmonid river (the Aisne river, AR). A week prior to the incubation of eyed-eggs, incubators were built with perforated extruded plastic cylinders and plastic connections. Each incubator was filled with pebble stones collected in Aisne river, washed and dried before use (with a length of 34.51 ± 16.1 mm, with an interstitial space of ~ 30% calculated three times by measuring the volume of water that fills the space between the stones). One hundred fifty eyed-eggs were added per incubator. In the Aisne river, three redd sites were selected, and 4 incubators were disposed in each site summing up to 1800 incubated eyed-eggs in total. The details geographic coordinates, height of water column

and flow rate at the position of the different incubators are presented in the Table 1.

(2) vertical trays purchased at Aqualor (France) containing 8 trays each (dimension of each tray: 0.83 m he. × 0.6 m wid. × 0.64 m depth). An artificial substrate consisting of a plastic mat containing 500 pimples of 2 cm he. × 0.5 cm wid. separated by 2 cm from each other was deposited on the bottom of the tray, and supplied with Aisne river water (RT).

(3) Identical to incubator 2 and supplied with drilling water (DT). The drilling water was taken from underground water showing no contamination with sediment, with values of turbidity that were always beyond the level of detection. All the trays of both vertical trays were filled with eggs during the experiment, but only three trays of the eight available per incubator were used for the present experiment. Both vertical trays (RT and DT) were filled with an optimal density of 10 000 eyed-eggs (obtained from previous experiments for each type of incubator, unpublished data).

(4) California trays (dimension: 2.33 × 0.50 × 0.17 m) purchased at Aqualor (France) were filled with pebble stones collected in Aisne river, washed and dried before use (with a length of 34.51 ± 16.1 mm, with an interstitial space of ~ 30% calculated three times by measuring the volume of water that fills the space between the stones) and supplied with Aisne river water (SC).

(5) California trays (dimension: 2.33 × 0.50 × 0.17 m) without substrate and supplied with Aisne river water (EC). California trays (EC and SC) were filled with an optimal density of 8000 eyed-eggs respectively (obtained from previous experiments for each type of incubator, unpublished data).

Each incubation method (from 2 to 5) was conducted in triplicate.

Water parameters including pH, conductivity, dissolved oxygen concentration and turbidity were measured twice a week, whereas temperature in each system was recorded continuously with a water temperature recorder (for experiments in the river, the temperature of each redd was monitored individually). The flow rate was adapted to reach the optimal condition, specific for each incubation mode (California trays with pebble stones need a higher flow rate than empty California trays) in order to get an optimal oxygenation of the eggs.

In addition, the incubators were checked daily to remove dead/white eggs. For each replicate, the number of dead egg/fry was divided by the initial number of eggs deposited in the tray or incubator to calculate the survival rate.

Table 1 Geographical coordinates, height of water column, river flow rates and survival rates in each incubator placed in Aisne river

	Incubators (with geographic coordinates)	Flow speed ($m\ s^{-1}$)		Height of water column (cm)		Highest height (cm)	Lowest height (cm)	Survival rate (%)
		Mean	SD	Mean	SD			
Redd A (up-stream)	A1 (50° 15′ 25.9″ N 5° 35′ 43.4″ E)	0.52	0.17	29.62	12.26	53	13	8.7
	A2 (50° 15′ 25.9″ N 5° 35′ 42.8″ E)	0.44	0.25	27.69	12.36	52	11	0
	A3 (50° 15′ 25.7″ N 5° 35′ 41.8″ E)	0.63	0.28	20.54	13.08	49	4	6
	A4 (50° 15′ 25.5″ N 5° 35′ 41.3″ E)	0.71	0.20	24.62	13.36	55	8	50.7
Redd B (middle-stream)	B1 (50° 15′ 25.0″ N 5° 35′ 37.9″ E)	0.44	0.35	31.00	13.32	57	13	44.7
	B2 (50° 15′ 25.1″ N 5° 35′ 37.2″ E)	0.68	0.37	32.62	14.55	64	15	40
	B3 (50° 15′ 25.2″ N 5° 35′ 36.5″ E)	0.80	0.37	34.15	14.03	64	17	20
	B4 (50° 15′ 25.3″ N 5° 35′ 35.5″ E)	0.62	0.23	29.46	14.09	58	11	4
Redd C (down-stream)	C1 (50° 15′ 27.0″ N 5° 35′ 30.4″ E)	0.50	0.31	31.77	12.57	55	16	44.7
	C2 (50° 15′ 27.0″ N 5° 35′ 29.7″ E)	0.46	0.29	30.31	13.49	58	13	42.7
	C3 (50° 15′ 27.1″ N 5° 35′ 29.0″ E)	0.58	0.27	34.54	12.45	57	18	31.3
	C4 (50° 15′ 27.1″ N 5° 35′ 27.5″ E)	0.60	0.20	29.08	14.01	58	12	2.7

Sampling procedure

The average daily water temperature value was used to calculate the daily percent of development based on the Kane formula as described in the "Experimental design" section (Kane 1988). In each experimental rearing system, 60 fry (20 per incubator replicate in the hatchery and 5 per incubator in the Aisne river (AR)) were sampled between 95 and 97% of development, measured from the front of the head to the fork, and weighed. In addition, for each incubator replicate ($n=3$ sites for AR, and $n=3$ trays for hatchery), 6×5 fish were pooled for lipid analysis and 9 fish were sampled for gene expression analysis, snap-frozen and stocked at − 80 °C. The condition factor was calculated using the Fulton's formula: $K=100\times W/L^3$, with W: weight of the fish in mg, and L: length of the fish in mm.

Parameters of the water during rearing from eyed-egg to emerging fry

The physicochemical parameters of the Aisne river (in situ and in the hatchery) and the drilling water were monitored throughout the experiment (Figs. 1 and 2). Overall, the oxygen conditions were similar between all incubation modes with values around 12 mg of dissolved O_2 l^{-1} (Fig. 1a). pH measurements showed that the Aisne river water was more acidic than the drilling water, with values ranging from 7.26 units at the beginning of incubation to 8.01 units at fry emergence, while pH values of the drilling water ranged at

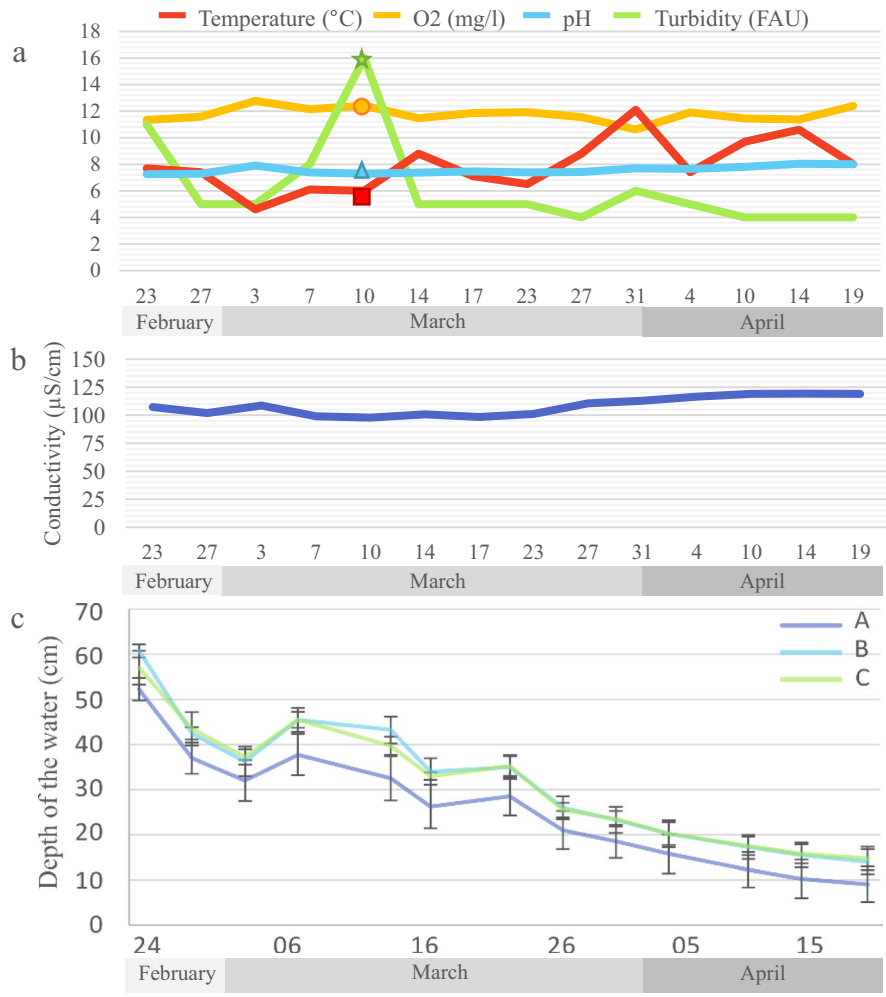


Fig. 1 Physicochemical parameters of the Aisne river during incubation of Atlantic salmon eggs. **a** Monitoring of dissolved O_2 (mg l^{-1}, yellow circle), turbidity (FAU, green star), temperature (°C, red square) and pH (blue triangle). **b** Conductivity monitoring (µS cm^{-1}). **c** Mean depth of the water (4 sites per reeds) of the three redds selected and during the experiment

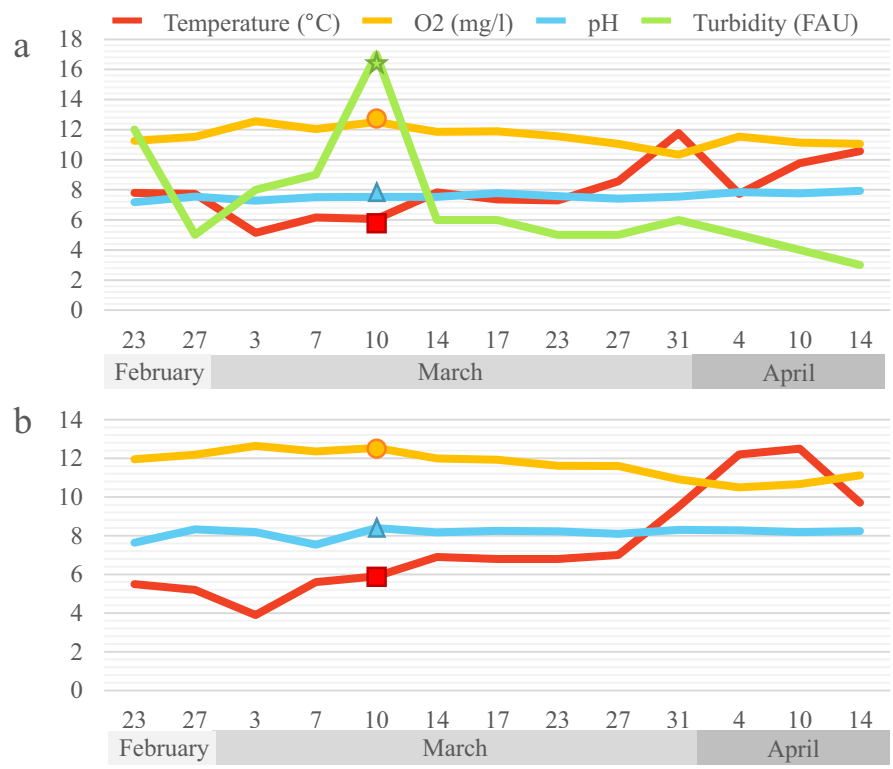


Fig. 2 Physicochemical parameters of river (**a**) or drilling (**b**) water in incubators during incubation of salmon eggs at CoSMOS station. Monitoring of dissolved O_2 (mg l^{-1}, yellow circle), turbidity (FAU, green star), temperature (°C, red square) and pH (blue triangle). In (**b**), the turbidity was not represented as its value was always under the detection level

8.20 throughout the incubation period. Turbidity was measured as the decrease of transmitted light through a water sample at an angle of 180° with a spectrophotometer. This parameter highlighted the occurrence of several floods in the Aisne river during the experiment (Fig. 1b). At the beginning of the experiment, the eyed-eggs incubated in Aisne river and in the incubators of the CoSMOS station supplied by Aisne water were exposed to a first flood (respective turbidity of 11 to 12 Formazin Attenuation Units (FAU)) and then to a second major flood observed on March 10 (respective turbidity of 16 and 17 FAU). Temperature measurements were crucial in order to predict the moment of fry emergence as accurate as possible. As the winter was relatively mild, water temperatures in the river and in the hatchery were between 4 and 6 °C at the beginning of this experiment and reached between 10 and 12 °C at the end of the incubation period. Finally, the water depth of the Aisne river decreased in every site of the three selected redds in the same way over the experimental period (Fig. 1c).

Determination of fatty acid profiles of fry

Total lipids were extracted with methanol:chloroform:water (2:2:1.8, v:v:v) as described by Bligh and Dyer (Bligh and Dyer 1959). A solution composed of 1,2-dipentadecanoyl-sn-glycero-3-phosphatydylcholine (Larodan, Sweden), triheptadecanoin (Larodan, Sweden) and tridecanoic acid (Larodan, Sweden) was used as internal standard. Total lipid extracts were separated by solid-phase extraction into three lipid fractions, i.e. neutral lipids, free fatty acids and phospholipids, as described by Schneider and collaborators (Schneider et al. 2012). Neutral lipid, free fatty acid and phospholipid extracts were methylated as described by Schneider et al. with minor modifications to ensure sufficient concentration of samples. Eluted fractions were briefly evaporated to dryness under a stream of N2 at 30 °C and methylated at 70 °C through the addition of 1 ml methanol containing KOH (0.1 M) and 1 h incubation followed by the addition of 0.4 ml of acidified methanol (1.2 M HCl) and 15-min incubation. The resulting fatty acid methyl esters (FAMEs) were then extracted using 2 ml hexane. FAMEs were separated by gas chromatography as described by Schneider et al. (Schneider et al.

2012), with minor adaptations as in Ferain et al. (Ferain et al. 2018). The chromatograph (GC Trace-1310, Thermo Scientific) was equipped with an RT2560 capillary column (100 m×0.25 mm internal diameter, 0.2 µm film thickness: Restek, USA), a TriPlus TP100 autosampler (Thermo Fisher Scientific, Italy) and a flame ionisation detector (FID: Thermo Quest, Italy). The carrier gas used was H2 at constant pressure (200 kPa) and flow rate (2 ml min^{-1}). The FID was continuously flowed by H2 (35 ml min^{-1}), air (350 ml min^{-1}) and N2 (40 ml min^{-1}) and kept at a constant temperature of 255 °C. The temperature program was as follows: an initial temperature of 80 °C, which increased at 25 °C min^{-1} up to 175 °C, a holding temperature of 175 °C during 25 min, a new increase at 10 °C min^{-1} up to 200 °C, a holding temperature of 200 °C during 20 min, a new increase at 10 °C min^{-1} up to 220 °C, a holding temperature of 220 °C during 5 min, a last increase at 10 °C min^{-1} up to 235 °C and a final holding temperature of 235 °C during 15 min. FAMEs were finally identified and quantified by comparing the obtained chromatograms (retention times and peak areas) with that of a standard containing 44 FAMEs (Larodan, Sweden) at known concentrations. The chromatogram management software used was ChromQuest (version 5.0, Thermo Finnigan, Italy). Data from the technical duplicates were averaged. The resulting spectra were processed with the ChromQuest software (version 4.2, Thermo Finnigan, Italy). Methyl-undecanoate (Larodan, Sweden) was used as injection standard. Peak identification and quantification were enabled by the use of an external standard, composed of 42 FAMEs (Larodan, Sweden).

Gene expression analysis

For each incubation condition, 9 fish were used (3 fish per replicate). Total RNA was extracted from the entire body of the fry (excluding the tail that was removed prior to extraction) using Tri Reagent solution (Ambion, Thermo Fisher Scientific) according to the manufacturer's instructions. The pellet, containing RNA, was dried and resuspended in 100 µl of RNase-free water. Total RNA concentration was determined with NanoDrop 2000 spectrophotometer (Thermo Scientific) and the integrity was checked with the bioanalyzer 2100 (Agilent). Genomic DNA was digested for 15 min at 37 °C with 1U of rDNAse I (Thermo Fischer Scientific) and quantified again with NanoDrop-2000 spectrophotometer. Then, 1 µg of total RNA was reverse-transcribed using RevertAid RT kit (Thermo Fischer Scientific) according to the manufacturer's instructions. Resulting cDNA were used to measure the expression in real-time qPCR of several molecular markers involved in different functions including cognition, larval development, immunity and swimming capacity. The geometric mean of two housekeeping genes (β-actin and elongation factor 1α (EF1α)) were used as reference. The list of specific primers is given in Table 2. Real-time qPCR was carried out with iTaq universal SYBR green supermix (Bio-Rad Laboratories) using a 1:100 dilution of the cDNA for target and reference genes. Primers for target and reference genes were used at 500 nM. The thermal conditions were 2 min at 95 °C, followed by 40 cycles at 95 °C for 10 s and 60 °C for 20 s, and melting curves were analysed to verify the absence of multiple amplicons. All reactions were performed using StepONE device (Applied Biosystems) and the relative gene expression was calculated according to the standard curve method. Values for each sample were expressed as normalised relative expression (NRE) of the target gene on the mean of the reference genes.

Statistical analyses

Statistical analyses were performed using RStudio software (version 3.2.1). Normality and homogeneity of variances of data were assessed with Shapiro–Wilk and Bartlett tests, respectively. When homoscedasticity and normality were respected, the effect of incubation mode was analysed using one-way ANOVA. Post hoc comparisons at a 5% significant level were performed with Tukey test. Data with no normality and/or homoscedasticity were tested with Kruskal–Wallis tests and post hoc pairwise Wilcoxon comparison tests.

Results

Emerging dynamics and survival rate

Using Kane's formula and daily collected river temperature data, a time window was defined during which daily surveys and/or samples were taken. In the Aisne river, fry was sampled when they reached 95.6% of emergence according to Kane's formula. Mortality in incubators varied greatly depending on the site in the redds (Table 1). Indeed, although many incubators showed survival rates around 30%, we also

Table 2 Primer sequences used for analyses of selected gene expression in *Salmo salar*

Gene	Accession number	Sequences (5′-3′)
Neurotrophin 3	XM_014185212.1	Fw-TGTCGGACTGCCAAACCT
		Rv-ACAACGCACAGACACAGGAA
Crystallin, beta B1 (*CryB1*)	XM_014128318.1	Fw-GGACAGAGTGCGCAGTATCA
		Rv-GGCAGTCGCTACGGTAAGA
Myogenin	DQ294029.2	Fw-CAGGAGAACGACCAGGGAA
		Rv-GGAGGTCCTCGTTGCTGTA
Glucose transporter member 1-like (*glut1*)	XM_014177444.1	Fw-TTGGCTTGGAGTCTCTGCT
		Rv-CCTGCTCCTCCTTGTTCTG
Lysozyme g (*lysog*)	NM_001146413.2	Fw-TGACCTTGCTGCCATGAACA
		Rv-GGCTGGGGTAGTGTCAATC
Complement C3 (*c3*)	XM_014186867.1	Fw-GTGACAGGTGGAGAGCAGA
		Rv-CCAGGCCAATATCCTCCCA
SRY-box 11 (*sox11*)	NM_001173797.1	Fw-CGAACGCAGCTCCTAACAGA
		Rv-GCGTCTTCACTGGAACTGGA
Elongation Factor 1α (*ef1α*)	AF321836	Fw-AGAACCATTGAGAAGTTCGAGAAG
		Rv-GCACCCAGGCATACTTGAAAG
Actin beta	BG933897	Fw-ACTGGGACGACATGGAGAAG
		Rv-GGGGTGTTGAAGGTCTCAAA

noticed the failure of some incubators with mortality ranging from 91 to 100%. In general, survival varied greatly between redds and sites.

Regarding the emergence of fry incubated in the hatchery, the indicative value of development rate according to Kane's formula on the sampling day was 96% for incubators with empty California trays (EC) or with substrate (SC), as well as for vertical trays supplied with river water (RT) and 94% for trays with drilling water (DT). The survival rate in the hatchery was very high for all incubation modes (Fig. 3a). Although survival rates were all greater than 97%, significant differences were observed between the incubation modes ($p < 0.0001$). SC had a higher survival rate compared to EC and RT.

Biometric parameters

The analyses of biometric parameters of fry at emergence highlighted differences in size and weight depending on the incubation modes or between hatchery modalities and river conditions. Mean weight of fry incubated in EC and RT was significantly lower than of fry from other incubations (Fig. 3b). As for the length, fry from RT were the smallest, followed by EC, DT and SC. The longest fish were observed in AR incubators (Fig. 3c). Regarding the condition factor (K), it highlighted the impact of the short length of fry from RT (Fig. 3d) ($p < 0.001$). K was indeed significantly higher in the fry of RT compared to all other conditions. On the contrary, the condition factor of fry from EC incubator was the lowest suggesting those fry are skinnier.

Lipid composition

The total concentration of lipid expressed as the percentage of the dry weight in SC-incubated fry was significantly lower compared to EC, DT and RT (Fig. 4a). Regarding the concentration of total fatty acid (in mole/fish) per lipid fraction (i.e. free fatty acids (FFA), neutral lipids (NL) and phospholipids (PL)), it appeared that the lipid repartition was different among incubation modes (Fig. 4b-d). Indeed, total fatty acid concentration (in mole/fish) was significantly lower in fry from EC compared to all other incubation modes for free fatty acids and compared to AR and DT for phospholipids ($p < 0.05$). However, for neutral lipids, fry from DT exhibited a significantly higher concentration of lipid than all other groups ($p < 0.05$). Finally, eyed-eggs were rich in lipids (> 8% of wet weight) and mainly contained neutral lipids (with mainly

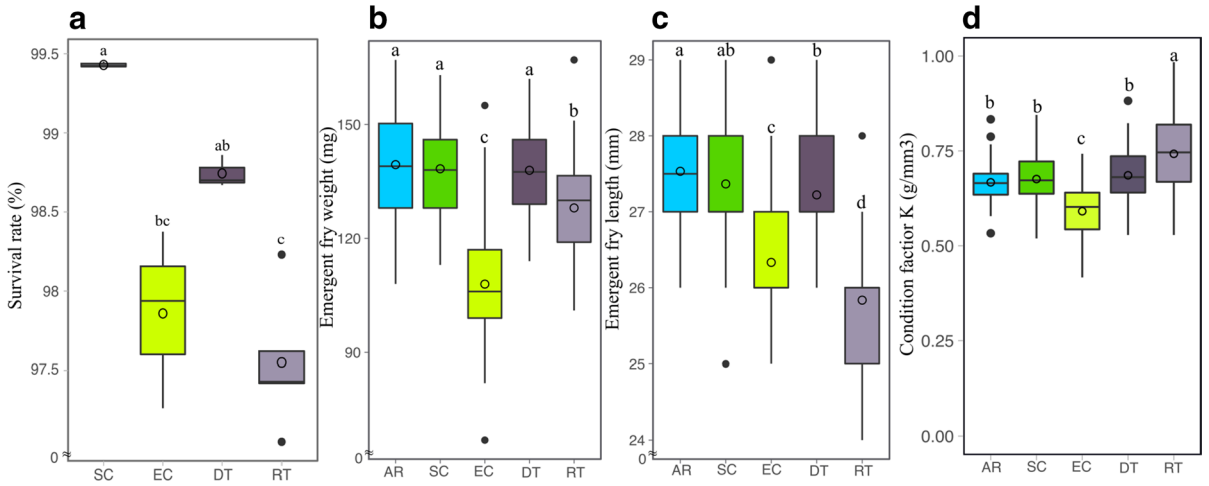


Fig. 3 Box plot representing the quartile distribution of the survival rates from eyed-eggs to emerging fry in hatchery in the 4 different modes of incubations (**a**), the weight (**b**), size (**c**) and condition factors (**d**) of emerging fry with the different modes of incubation. AR, Aisne river (blue); SC: California tray with substrate (dark green); EC, California tray empty (light green); RT, vertical tray with Aisne river water (dark purple); DT, vertical tray with drilling water (light purple). Empty circle represents the mean of all replicates of each incubation mode. Statistically significant differences between incubation are denoted with a, b and c letters ($n = 60$/condition, in hatchery: 20 fish × 3 replicates; in wild: 5 fish × 4 incubators × 3 sites)

saturated (C16:0), C18:1(n-9)c, EPA, DHA and LA) and phospholipids (with mainly saturated (C16:0 and C18:0), C18:1(n-9)c, EPA and DHA). In NL and PL fractions, the concentrations (in moles/fry) of the different FA decreased by about 50% between eyed-egg to emerging fry in all groups.

FA composition in each lipid fraction

FA composition in FFA

Since the concentration of FA in mole/fish depends on the total amount of lipids, fry incubated in EC

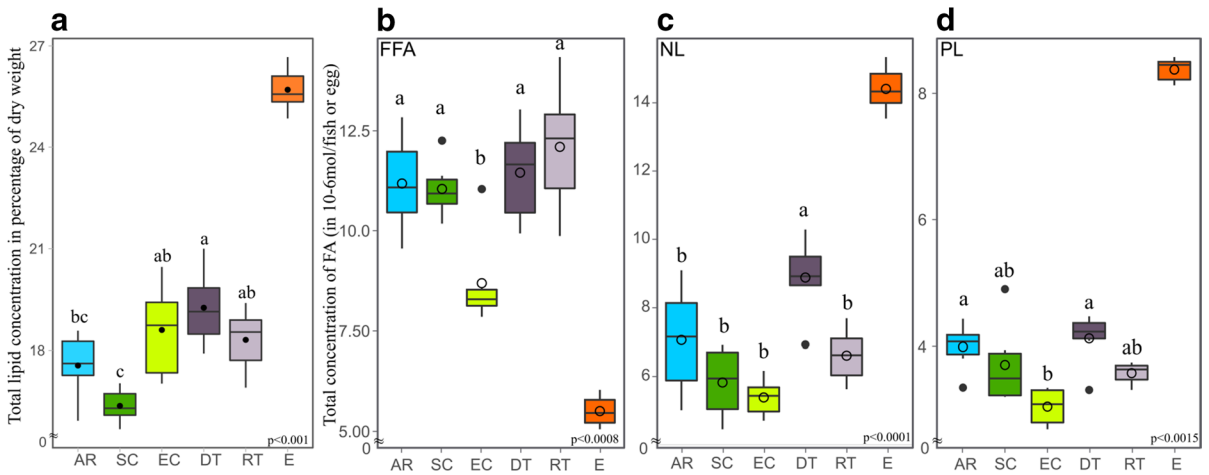
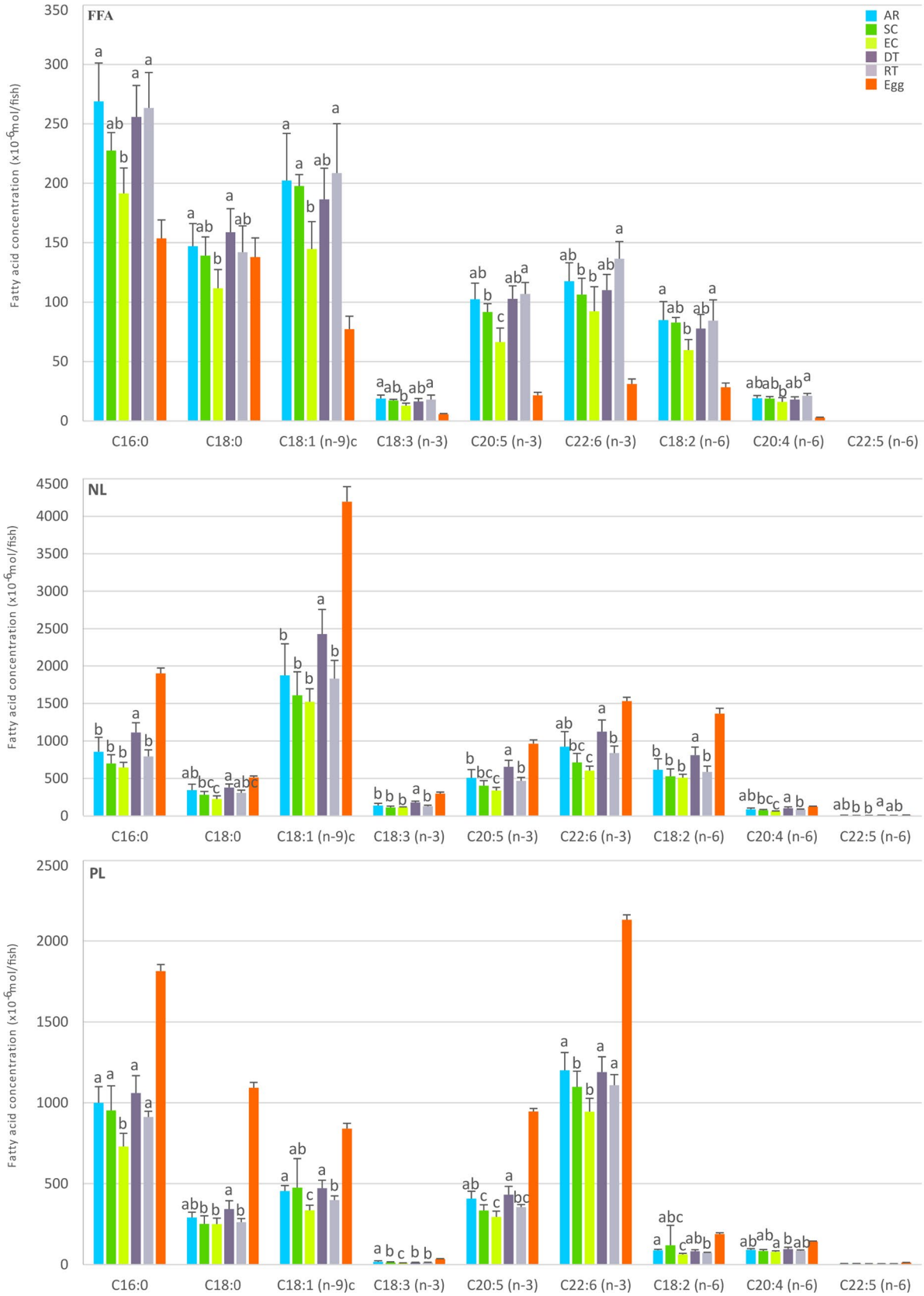


Fig. 4 Concentration of fatty acids (in 10^{-6} mol/fish or egg) in the different lipid fractions: Free fatty acids (FFA), neutral lipids (NL) and phospholipids (PL) in the 5 incubation modes. **a** Fatty acid composition expressed in 10^{-6} mol/fish or egg in the 3 lipid fractions FFA (**b**), NL (**c**) and PL (**d**), in fry from the following incubation: AR, Aisne river (blue); SC, California tray with substrate (dark green); EC, California tray empty (light green); RT, vertical tray with Aisne river water (dark purple); DT, vertical tray with drilling water (light purple); E, eyed-egg (orange). Statistically significant differences between incubation are denoted with a, b and c letters ($n = 6$, with 2 pools of 5 fry/incubation mode × 3 replicates)


FFA
NL
PL
AR
SC
EC
DT
RT
Egg
Fatty acid concentration (x10-6mol/fish)
C16:0
C18:0
C18:1 (n-9)c
C18:3 (n-3)
C20:5 (n-3)
C22:6 (n-3)
C18:2 (n-6)
C20:4 (n-6)
C22:5 (n-6)

◂**Fig. 5** Fatty acid composition expressed in 10^{-6} mol/fish or egg from the following incubation (represented from the left to the right for each FA): AR, Aisne river (blue); SC, California tray with substrate (dark green); EC, California tray empty (light green); DT, vertical tray with drilling water (light purple); RT, vertical tray with Aisne river water (dark purple); E, eyed-egg (orange). Values are expressed as mean ± sd. Statistically significant differences between incubation are denoted with a, b and c letters ($n = 6$, with 2 pools of 5 fry/incubation mode × 3 replicates)

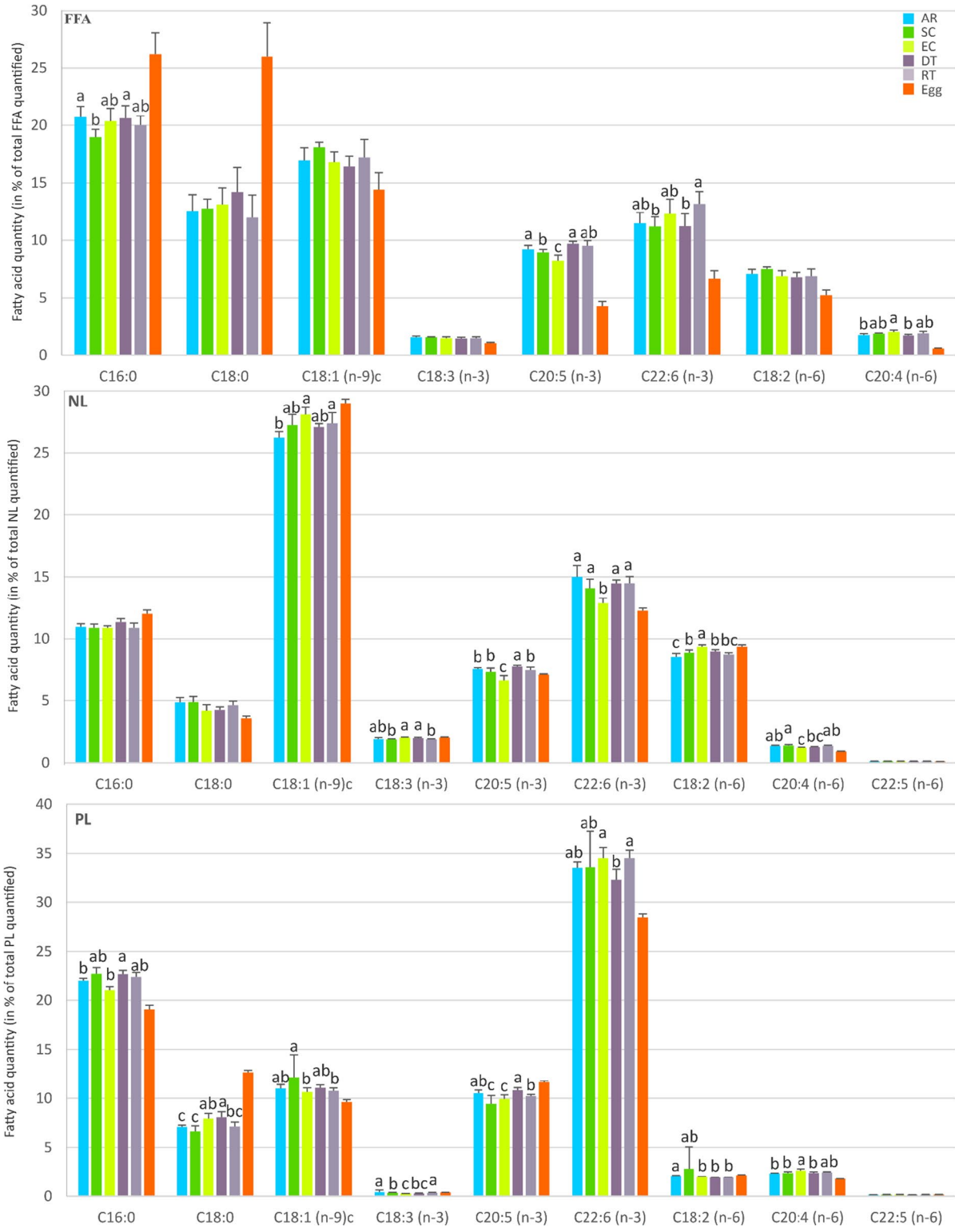
often showed the lowest FA concentrations. Fry incubated in AR, DT and RT had a higher saturated and mono-unsaturated fatty acid content than fry from EC (Fig. 5). For omega-3 fatty acids, while alpha-linolenic acid (ALA) content was significantly lower in EC compared to AR and RT, the levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were significantly higher in fry from RT in comparison with SC and EC. As for the free fatty acid content of the omega-6 family, the levels of linoleic acid (LA) were significantly higher in fry from DT in comparison with other incubation conditions. And while no significant differences were observed in docosapentaenoic acid (DPA) ($p > 0.05$), the concentration of arachidonic acid (ARA) was lower in fry from EC than DT ($p < 0.05$). However, when the fatty acid composition was expressed in percentage of total FFA identified, and thus, independent of the concentration of lipid in the fry, different FA distributions were observed (Fig. 6). In saturated and mono-unsaturated fatty acids, only two FA groups were significantly different. Indeed, C16:0 was higher in fry from AR and DT in comparison with SC and the amount of C20:1n-9 was lower in DT compared to AR, SC and EC. Regarding n-3 fatty acids, only EPA and DHA differed in that the percentage of EPA was significantly the lowest in EC followed by SC in comparison with AR and DT, whereas for DHA, its percentage appeared higher in RT compared with DT and SC. Regarding the n-6 family, AA relative content was lower in AR and DT than in EC.

FA composition in NL

As in free fatty fraction, the concentration in mole/fish being influenced by the total amount of lipid, fry incubated in EC often showed the lowest FA concentrations. Nevertheless, differences between other groups were also observed (Fig. 5). For several saturated, mono-unsaturated, n-3 (ALA, EPA and DHA) and omega-6 FA (LA), fry from DT exhibited the highest concentration in comparison with all other incubation modes. When the FA composition is expressed as the percentage of total NL identified in the NL fraction, we observed that the mono-unsaturated FA C18:1n-9c was significantly higher in EC and RT than in AR (Fig. 6). Moreover, concerning the omega-3 family, the percentage of ALA in NL fraction was higher in EC and DT compared to SC and RT, while for EPA, the highest percentage was observed in fry from DT followed by AR, SC and RT, and then EC, and the percentage of DHA was the lowest in EC in comparison with all other incubation modes. Finally, regarding the n-6 FA family, LA was significantly the most represented in EC followed by DT, SC and RT, and then AR ($p < 0.05$). The percentage of AA was significantly higher in fry from SC compared to those from EC and DT.

FA composition in PL

Similarly, in the PL fraction, it appeared that the concentration in mole/fish of fry from EC was lower than of fry from other incubation modes for several FA including the saturated, mono-unsaturated, n-3 and n-6 (Fig. 5). In addition, for the mono-unsaturated FA C18:1(n-9)c, fry from AR and DT exhibited the highest concentration followed by SC, RT and finally EC. Concerning the n-3 family, AR showed the highest concentration of ALA and EC the lowest. AR and DT had the highest amount of EPA and DHA compared to fry incubated in California trays. In terms of omega-6 FA, unlike for DPA, the concentration of the precursor of this family (LA) was significantly more abundant in AR groups than in RT and EC ones, while the concentration of AA was also higher in DT than in EC. When considering the FA quantity (expressed in % of total PL identified), significant differences are observed in saturated and mono-unsaturated FA. Indeed, C18:1(n-9)c were present in larger quantities in fry from SC compared to those from EC and RT (Fig. 6). In addition, fry from California trays exhibited the lowest percentage of ALA and EPA when compared to AR and RT, while DT had the lowest percentage of DHA in comparison with EC and RT. Finally, concerning the omega-6 family, the percentage of LA was the highest in fry from AR compared to EC and RT. The percentage of AA was significantly higher in fry from DT compared to EC.


FFA
AR
SC
EC
DT
RT
Egg
Fatty acid quantity (in % of total FFA quantified)
C16:0
C18:0
C18:1 (n-9)c
C18:3 (n-3)
C20:5 (n-3)
C22:6 (n-3)
C18:2 (n-6)
C20:4 (n-6)
NL
Fatty acid quantity (in % of total NL quantified)
C16:0
C18:0
C18:1 (n-9)c
C18:3 (n-3)
C20:5 (n-3)
C22:6 (n-3)
C18:2 (n-6)
C20:4 (n-6)
C22:5 (n-6)
PL
Fatty acid quantity (in % of total PL quantified)
C16:0
C18:0
C18:1 (n-9)c
C18:3 (n-3)
C20:5 (n-3)
C22:6 (n-3)
C18:2 (n-6)
C20:4 (n-6)
C22:5 (n-6)

◂**Fig. 6** Fatty acids composition expressed in percentage of total FA identified in the studied fraction (FFA, NL or PL) in fry from the following incubation (represented from the left to the right for each FA): AR, Aisne river (blue); SC, California tray with substrate (dark green); EC, California tray empty (light green); DT, vertical tray with drilling water (light purple); RT, vertical tray with Aisne river water (dark purple); E, eyed-egg (orange). Values are expressed as mean ± sd. Statistically significant differences between incubation are denoted with a, b and c letters ($n = 6$, with 2 pools of 5 fry/incubation mode × 3 replicates)

Gene expression

The analyses of the immune genes *lysozyme* and the *C3a complement* showed a high variability between individuals but no differences between groups were detected regardless of the modes of incubation (data not shown).

The results showed that this gene was differently expressed among the different modes of incubation (Fig. 7). Fry incubated in vertical trays (RT and DT) and in the Aisne river had a lower *glut1* expression (up to two times less for RT) than those incubated in EC and SC. In addition, fish in EC had a significantly higher expression than those in SC. Regarding *myogenin* gene, it was also differently regulated among the 5 incubation modes, with a higher level in AR compared to the other 4 modes, and lower expression level in fry incubated in RT compared to incubations in the river and in California trays (Fig. 7).

Finally, 3 genes involved in development were studied. Among them, the *neurotrophin* gene was not modulated by the incubation modes (data not shown). However, the analysis of *Sox11* gene expression showed significant differences within the incubation modes. Indeed, fry incubated in RT had an expression that was 2 times lower than incubations in AR, EC and SC. In addition, fry in AR had a significantly higher *Sox11* gene expression level than those in SC (Fig. 7). It should be noted, however, that the expression of this gene appeared to be highly variable between individuals. Finally, the analysis of *CryB1*, a gene encoding crystallin B1, a protein involved in the maintenance of transparency and the refractive index of the lens of the eye, revealed that the fry incubated in RT had a lower gene expression than in AR and SC (Fig. 7).

Discussion

In this study, the different effects observed on growth performance, lipid metabolism and gene expression will be discussed in three steps in order to evaluate (1) the physiological differences between fry raised in semi-natural incubators vs in hatchery, (2) the role of a matrix substrate in the development of the fry and (3) the importance of water quality on larval development.

Comparison of artificial incubations in the hatchery with a semi-natural incubation in the river

Firstly, differences in survival rates from eyed-eggs to emerging fry were observed between incubation modalities in the hatchery and under incubations conditions in the river. As expected, the mean survival rate in the river was about 30% with a high variability among redds ranging from 0 to 45%. Other authors using the same incubation material described an average survival rate in Nivelle rivers between 2 and 35% from egg deposition to fry emergence (Dumas and Marty 2006). Several studies reported that floods, which are more frequent during the embryonic phase of salmonids, can be a major cause of high mortality rates in the river (Mackenzie and Moring 1988; Greig et al. 2005; Sear et al. 2016). As stated above, high quantities of fine sediments can clog the interstices of the substrate and deposit on the surface of the eggs, thus reducing the oxygen availability required for the development of embryos (Sear et al. 2016). A high supply of oxygen is essential during egg stage and the demand further increases during hatching (Dumas and Marty 2006). In addition, the clogging of space around eggs accumulates toxic metabolic waste that cannot be removed (ammonium, ammonia and nitrites) (Greig et al. 2005). In our experiment, the recorded turbidity revealed the several occurrences of floods that could partly explain embryo mortality, particularly at the moment of the installation of eyed-eggs in the incubators. Thus, dissolved organic matter could have entered into the incubators and deposited on the eggs limiting oxygen uptake of embryos. In addition, it is possible the position of the incubator placed in the substrate limits gas exchange and water renewal inside the incubator. In addition, the high difference we observed between

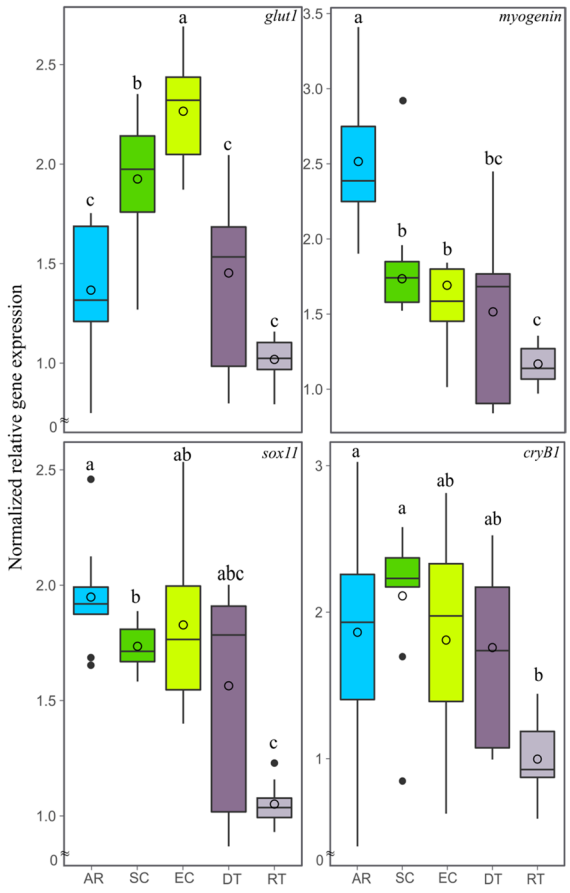


Fig. 7 Box plot representing the quartile distribution of the relative expression of the *glucose transporter 1* (*glut1*) and the myogenin, *sox11* and *crystallin B1* (*CryB*), normalised to the mean expression of reference genes: *elongation factor 1α* and *β-actin*. AR, Aisne river (blue); SC, California tray with substrate (dark green); EC, California tray empty (light green); RT, vertical tray with Aisne river water (dark purple); DT, vertical tray with drilling water (light purple). Empty circle represents the mean of all replicates of each incubation mode. Statistically significant differences between incubation are denoted with a, b and c letters ($n = 9$, 3 fry × 3 replicates)

redds is probably due to their position (water depth combined with light exposure). Over the duration of the experiment, water flow was influenced by variable river water levels, and potentially by deposition of organic matter and oxygen supply. Besides, some incubators were found close to the surface, and some redds were sunnier than others, with consequences for fry development. Finally, because of the design of this semi-natural incubator, the mortality survey and dead egg removal were not possible, thus microorganism such as fungi or bacteria developing on dead eggs could spread to healthy eggs/larvae and contaminate them. Further studies should apply the use of a new incubator design, the Bamberger-Box, that mimics the conditions in natural redds, which, under normal climatic conditions, should improve the survival rates of eyed-eggs-to-emerging fry to > 93% as the authors reported (Bamberger 2009a, b). As seen previously, the survival rates in the hatchery were higher than those observed in the river and are comparable to those observed for trout (Dumas and Marty 2006). Regarding fish health, no diseases or pathogen infections were detected in emerging fry, and the selected innate immune-related genes encoding lysozyme or *c3a* complement component proteins were not significantly influenced by the incubation modes. However, *c3a* expression was highly variable among individuals. But such heterogeneity within the groups is not surprising since immune parameters are known for their strong inter-individual heterogeneity (Cornet et al. 2018), especially in the absence of stimuli of pathogens, which tends to reduce such differences.

In this study, the analysis of qualitative and quantitative compositions of fry lipids combined with the condition factor K also highlighted differences among the incubation modes. The lipid content and fatty acid profiles of unfed emerging fry are provided by the yolk sac. In Atlantic salmon, triacylglycerols are known to be particularly catabolised throughout the development, and neutral lipids are particularly catabolised after hatching (Tocher 2003). Catabolism of lipids results in the release of FFA which can be mobilised either for energy or for the formation of rapidly developing tissues. Here, the results of total lipid concentration (in percentage of dry weight) showed that fry from AR and SC had the lowest content particularly compared to fry from DT, highlighting a higher lipid consumption strategy all along the experiment. Moreover, a similar condition factor K of fry in AR and DT with a lowest content of total lipid could suggest that fry in river had consumed more energy to reach a similar size. Regarding FFA concentration, fry from incubation in the hatchery had the same content than those from the semi-natural incubation in the river.

In order to evaluate some developmental processes, we investigated the expression of several genes involved in fry development and particularly in neural development, namely *glut1*, *Sox11* and *CryB1* genes. We found that fry incubated in Californian

trays showed a higher expression level of *glut1* than fry from the other incubation modes. In zebrafish, it has been shown that this glucose transport gene plays a crucial role during embryonic development and more particularly in the brain as the lack of this protein causes numerous cerebral malformations (Jensen et al. 2006). Since this glucose transporter is involved in brain development, the cognitive capacities of fry incubated in SC could be slightly more developed compared to those incubated in AR, DT and RT. However, a behavioural analysis would be needed to evaluate these differences. In addition, we also showed that the expression levels of *Sox11* and *Crystalline B1* were reduced in fry incubated in RT compared to those in the Aisne river and in California trays. In teleost fish, *Sox11* is highly expressed during embryogenesis, larval development and neurogenesis. In *Larimichthys crocea*, Jiang et al. showed that *Sox11a* is not only found throughout embryogenesis with a maximum expression in stage 7 (closure of the blastopore), but is also expressed in gonads, brain and eyes in adult fish (Jiang et al. 2015). Similarly, Zou et al. have shown the importance of a crystalline A in the development of the retina (Zou et al. 2015). The lower expression of those two developmental genes in fry incubated in RT could suggest a delay in the developmental processes of those individuals compared to those in AR and California trays.

Influence of the substrate matrix in hatchery incubation modes

In our hatchery, high survival rates were observed in all incubation modes, with the highest survival in incubator with a substrate matrix. Moreover, the lack of a substrate matrix dramatically reduced the weight and length of emerging fry in empty Californian trays (EC). These results are in accordance with other studies showing that the use of semi-natural incubation systems using a support for fry resulted in significantly larger fry compared to conventional incubation systems (Bamberger 2009a; Hansen and Torrissen 1985). In their new semi-natural model "B-box", Bamberger suggested that a substrate matrix could help producing significantly larger fry and could also reduce deformities (Bamberger 2009a). Hansen et al. suggested that this support provided by a substrate matrix could satisfy the innate righting response of eleuthero embryos and allow the fish to rest (Hansen and Torrissen 1985). The artificial incubation mode SC used in our study aimed at imitating at best the natural environment where calibrated stones were deposited in the rack with an interstitial space of ~ 30% allowing constant water flow and, thus, improving larval development. We observed that the fry incubated without substrate in EC had the lowest condition factor (meaning those are longer than larger compared to fry from other conditions) combined with the smallest amount of lipid when expressed in percentage of fry's dry weight compared with fry incubated on a substrate matrix. This could reflect that a larger part of the energy contained in the yolk sac was not used for the growth of the larvae. Other studies already described that total lipid content could vary in embryos and larvae during development depending on environmental conditions such as temperature, oxygen supply, and on energy requirement (Wirth and Steffens 1996; Dantagnan et al. 2007). Here, the support provided by the substrate spared energy that could be allocated for growth. On the contrary, in the empty Californian tray "EC", larvae had to invest a lot of their lipid stock to maintain an upright position, and thus converted less yolk into body tissue which will probably affect fish physiology. Particularly, in the PL fraction, which reflects the lipid composition of the cell membrane, we observed that the percentages of several fatty acids (such as C18:1 (n-9)c, EPA and DHA) involved in membrane fluidity (Wiegand 1996) were higher in fry from SC compared to those in EC. It has been reported that the insertion of this mono-unsaturated fatty acid at the sn-1 position of phosphatidylethanolamine, which is essential as energetic resource, allowed an adequate adaptation of membrane fluidity under cold conditions (Dey et al. 1993). Thus, economising such PUFA could be a real advantage for these fry that are continuously subjected to daily temperature fluctuations.

Generally, the presence of environmental bacteria on the substrate, as is the case in SC incubators, could be an advantage for the development of fry intestines since the digestive tract could rapidly be colonised by commensal bacteria that are essential for the digestion of many feed items (Reinoso Webb et al. 2016; Kelly and Salinas 2017). Moreover, the substrate could be a source of prey allowing the fry to start feeding while they still have some yolk reserves leading to larger

emerging fry as hypothesised in Bamberger (2009b). Further investigations of fry stomach would be necessary to confirm the hypothesis of a first feeding in the substrate matrix before emergence.

Influence of water quality on embryo/larval development

Here, the results showed that the use of water river exerted a slight negative influence on fry survival in comparison with drilling water, probably due to floods that increased water turbidity inducing the deterioration of water quality. In our hatchery, the water was derived from the Aisne river in order to supply fry incubators and tanks hosting emergent fry and adult salmons. Despite a preceding decantation process, some small particles (< 0.1 mm) remained in the water. In addition, flooding events increased the turbidity in the river resulting in large amounts of organic particles that can clog the eggs' chorion or fish gills while drilling water is nearly free of organic particles. Floods have already been pointed out as a major cause of high mortality rates in several studies (Mackenzie and Moring 1988; Greig et al. 2005; Sear et al. 2016). They carry large quantities of fine particles (0.063–0.125 mm) that clog the interstices of the substrate and deposit on the surface of eggs, thus reducing the oxygen availability required for the development of embryos (Sear et al. 2016). Therefore, the use of drilling water appears to be a good alternative to improve survival rates as it limits the quantity of those fine particles.

Furthermore, fry incubated in trays supplied with drilling water (DT) were significantly bigger and taller than those incubated in river water (RT) with the condition factor K that was significantly higher in RT reflecting that fry from RT had larger shape than in DT. These differences could be partially explained by the slight differences of water temperature observed along the experiment. Temperature has been shown to affect numerous aspects of embryos and larval development (Kane 1988; Ojanguren et al. 1999). In particular, Murray et al. showed that high incubation temperatures significantly decreased fry size in 5 different species of salmonids (Murray and McPhail 1988). Although we adjusted drilling water temperature daily to mimic the temperature of the Aisne river, the average daily temperature of the river water was about 1 °C higher than drilling water during most of time of embryo and fry development. In addition, the temperature in the Aisne river could fluctuate profoundly within a day depending on the weather while drilling water temperature remained stable.

Differences were further observed in fry lipid content and FA composition. Neutral lipid content was higher in fry from DT compared to RT. As the yolk sac is mostly composed of NL lipid, it is possible that the higher content in DT reflects the remaining NL of the egg. Combined with the results on growth performances and the condition factor, it suggests that fry from DT had a better but slower consumption of their energy contained in the yolk sac. In addition, FA composition was impacted by water quality as in free fatty acid fraction, the percentage of DHA was higher in RT than in DT, while in neutral lipid fraction, DT had the highest percentage of EPA. In fry from DT, the use of saturated FA for catabolism rather than fatty acids like EPA and DHA that are spared for other biological functions could attest a certain quality of the eggs (Tocher 2003). Indeed, EPA and DHA are essential FA involved in several biological functions including inflammatory processes, immunity and oxidative stress (Geay et al. 2015; Ferain et al. 2016, 2018; Cornet et al. 2018). The relative stable temperature of drilling water (DT) compared to the Aisne river could affect phospholipid DHA composition. LC-PUFA (n-3) are known to be essential components in fish neuronal activity and are involved in schooling behaviour (Mourente 2003). In Atlantic Bluefin tuna (*Thunnus thynnus*), DHA was shown to enhance vision and prey acquisition of larvae (Koven et al. 2018). The importance of DHA for the proper development of neural tissues has also been demonstrated in larval Atlantic herring (*Clupea harengus*) (Mourente 2003). However, although essential to membrane function, PUFAs and particularly DHA are also more sensitive to oxygen or organic attacks by radicals and a high quantity of DHA in cell membranes increases the risk of oxidative stress (Tocher 2003). Indeed, in a recent study performed on rainbow trout juveniles, fish fed with a DHA-enriched diet and exposed to a low dose of Cd produced higher amounts of ROS, which potentially lead to a higher inflammation status and cell damage (Cornet et al. 2018). But such a relationship could not be established in this study since no oxidative stress biomarkers were tested. The differences in the levels of PUFAS we observed had limited physiological

impacts on the target immune genes tested in this study as their expression seemed comparable between fry whatever the incubation mode. More immune parameters would merit investigation to confirm such a hypothesis on immunity. Likewise, selected genes associated with larval development and swimming capacity did not seem to be influenced by water quality.

Conclusions

To conclude, we showed that the incubation mode of California trays with a substrate matrix possessed several advantages since an incubation on calibrated stones mimics best the incubation in a natural environment. Fry had a lower percentage of total lipids reflecting a different strategy of energy distribution of the yolk sac potentially allowing a better development of muscle tissue which possibly renders such fry more "adapted" for swimming. In addition, the presence of large amounts of oleic, EPA and DHA fatty acids allowing a good membrane fluidity under cold conditions could be of advantage to adapt to an environment with highly variable temperatures. In comparison, fry from empty Californian trays displayed a lower size and weight associated to lower levels of lipids and fatty acids, reflecting an over consumption of energy to resist against water flow.

The use of drilling water proved to be advantageous as it increased the survival rates compared to river water probably due to a limited number of particles present which may lead to egg clogging. Besides, drilling water also improved growth and lipid metabolism as fry were larger than those from RT and had higher levels of neutral lipids and, to a lesser extent, phospholipids. fry incubated in river water did not show any significant difference in growth and lipid composition compared to those from incubation in the Aisne river. However, fry from Aisne river had a lower expression of certain genes involved in swimming, larval, cerebral development than fry from other incubation modes. This could indicate that the development of these fry was slightly retarded compared to other fry. To conclude, this study suggested that a substrate matrix in Californian trays and drilling water could improve fry survival, growth performance and lipid metabolism essential for a successful first feeding in hatcheries. The combination of these two factors in hatcheries could help to reach optimal rearing conditions for fry destined to restocking.

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Author contribution Ideas: FG, PK, YL, XR and SNMM; in vivo experiment: FG; data generation: VC and EF; data analysis: VC; manuscript preparation: VC, FG, SNMM, PK, YL and XR.

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Data availability The authors confirm that the data supporting the findings of this study are available within the article and upon request to the corresponding author.

Code availability Not applicable.

Declarations

Ethics approval The care and use of experimental animals complied with local Ethic Committee for Animal Research of the University of Namur (Belgium) animal welfare laws, guidelines and policies. However, this experiment was using embryos and non-autonomous fry and thus was not subjected to Ethic Committee approval.

Consent to participate The authors declare that they consent to participate to this paper.

Consent for publication The authors declare that they consent to publish the findings exposed in this paper.

Competing interests The authors declare no competing interests.

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