Fate or independency: Is batch-specific larval performance determined by egg traits? A case study in farmed pikeperch (*Sander lucioperca*)

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Fate or independency: Is batch-specific larval performance determined by egg traits? A case study in farmed pikeperch (*Sander lucioperca*)

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Abstract

Fish embryos and larvae undoubtedly depend on maternal provisioning of essential egg components, such as fatty acids (FA), during early ontogeny. But which aspects and stages of batch-specific larval development are modulated by these inherent oocyte traits? This question is of major importance from an ecological, as well as from an aquacultural perspective. We examined the effects of batch-specific non nuclear-genetic egg traits (fatty acid (FA) profiles, egg size, mtDNA fragmentation, cortisol content) already in unfertilized oocytes of pikeperch (*Sander lucioperca*) and studied their influence on survival (15 dph) and larval performance (length at hatch, stress resistance, first feeding, swimbladder inflation rate, yolk sac and lipid droplet size, growth in weight and length) after hatching under stable laboratory conditions (n = 12 batches). While larval survival and performance were independent of mtDNA fragmentation and cortisol levels, FA profiles affected specific larval traits. Especially FA of the neutral fraction were positively correlated with larval size and growth (16:0, 18:0, 22:1n-9, total saturated FA), swimbladder inflation (arachidonic acid 20:4n-6, total FA) and early first feeding (16:0, 18:0, total saturated FA). Important FA of the polar fraction included 15:0 and 16:1n-7, which showed alternate effects on larval survival, first feeding and growth (length). Furthermore, larval length at hatch was positively correlated with egg diameter. Other larval parameters (yolk sac and lipid droplet size and stress response) were not or only marginally affected by egg traits. Consequently, high oocyte FA deposition fuels fast rates of growth and development during early ontogeny. On the other hand, larvae develop independently of assumed disadvantageous properties (cortisol content, mtDNA damage). Knowledge of this relation allows for the improvement of aquaculture practice, as well as predicting recruitment success in the wild.

Keywords: aquaculture; egg quality; embryogenesis; fatty acids; hatching; larval development; reproduction

List of abbreviations:

ARA	arachidonic acid
BW	body weight
DHA	docosahexaenoic acid
dph	day post hatch
EPA	eicosapentenoic acid
FA	fatty acid
FAME	fatty acid methyl esters
HUFA	highly unsaturated fatty acid
MUFA	monounsaturated fatty acid
SBI	swim bladder inflation
SD	standard deviation
SFA	saturated fatty acid
SL	standard length

1. Introduction

The postulation *omne vivum ex ovo* – every living thing comes from an egg – is a fundamental principle of life science. However, not every egg develops into a living organism. This is in particular the case in animals, which produce large amounts of eggs, such as most fish species, to cope with high mortalities especially during early ontogeny. In pikeperch (*Sander lucioperca*) for example, a pikeperch female can produce more than 400,000 eggs per kg body weight (Lappalainen et al. 2003). Furthermore, larval performance, e.g., size and growth, as well as resistance towards stress and food scarcity, are important modulating factors beyond sheer survival being of major interest for fish ecology and fisheries management, as well as from an aquacultural perspective.

Factors modulating early ontogenetic survival and development are both, external and internal. In addition to predation and fertilization failure caused by low sperm quality or quantity, fish eggs and larvae are exposed to a variety of threats, such as insufficient nutritional reserves and starvation, disease, e.g., fungal infections, predation, varying environmental conditions, e.g., suboptimal temperature or oxygen supply, as well as mechanical injuries or drift (e.g., Feiner and Höök 2015). Furthermore, developing embryos and larvae may suffer from suboptimal inherent (internal) conditions, such as stress or insufficient nutrition, which are transferred from the parents to the offspring (e.g., Brooks et al. 1997; Izquierdo et al. 2001; Bobe and Labbé 2010). Such perturbation may lead to critical levels of adverse detergents or damage within the egg further impeding or even disabling successful development.

Albeit the occurrence of parental care, for example in form of mouth-breeding, nest building or – as in the case of pikeperch – nest guarding, the offspring appear to be most often left alone after spawning or latest after hatching (Feiner and Höök 2015). However, the offspring is not entirely unprepared to face these challenges. Besides nuclear-genetic information, the parents – especially the females – invest substantial resources ideally providing optimum ‘start-up’ conditions for each embryo while producing large number of eggs to account for inevitable mortalities (Lubzens et al.2010). These resources fuel successful embryogenesis and hatching, as well as enable the larvae to cope with suboptimal conditions, e.g., sufficient nutrients stored in the yolk-sac to resist starvation until sufficient prey becomes available.

In an aquaculture setting, farmers may benefit from this reproductive strategy. By providing optimal conditions in the absence of inter-species and inter-cohort predation or cannibalism during egg incubation and larval rearing, one could expect high numbers of offspring from a limited number of well-conditioned spawners. Still, mortalities occur or larval performance may be poor, which cannot be solely explained by disease, suboptimal rearing or intra-cohort cannibalism (Feiner and Höök 2015). Indeed, the reliable production of high quality larvae is to date a major bottleneck and mortalities during early life-stages remain high, especially in candidate species such as pikeperch (Overton et al. 2015; Schaerlinger and Žarski 2015).

To date, only a limited number of studies observed the influence of batch-specific egg traits on larval performance parameters under stable and presumably optimal rearing conditions. We addressed this topic in eggs and larvae of pikeperch, a freshwater predator with a large distribution in Europe (Lappalainen et al. 2003). The species does not only sustain substantial inland fisheries, both commercial and recreational, but is also considered as a prime candidate for European inland aquaculture diversification (Mylonas and Robles 2014). Here, batch-specific larval performance was observed in rearing experiments and related to egg characteristics, which have been determined in sub-samples of unfertilized oocytes. The performance parameters consisted of the fundamental life-history traits size and growth, as well as survival. In addition, yolk and lipid droplet size, as quantitative measures of nutritional reserves, swimbladder inflation rate, day of first feeding, and stress resistance (salinity change) were determined. Egg parameters considered were fatty acid (FA) profiles and egg size, as well as mtDNA lesion rates and cortisol levels as potentially harmful traits.

Especially the FA are acknowledged as integrative parameters modulating several aspects of early life-history (Dabrowski et al. 2015; Kestemont and Henrotte 2015; Schaerlinger and Žarski 2015). The FA, especially the highly unsaturated FA (HUFA) are important structural components, serve as nutrient reserves and are precursors of eicosanoids (Bell et al. 1986; Tocher 2003). In developing pikeperch embryos, specific FA are related to fertilization success and survival (Schaefer et al. 2018a). Egg size on the other hand is often related to a larger size at hatch, which in turn is supposed to be beneficial for

115 larval survival in a natural environment (Brooks et al. 1997). During embryogenesis in incubated eggs
116 however, size proved to have adverse effects on development in pikeperch, whereas embryos gained
117 increasing independence from the inherent egg composition until hatching (Schaefer 2016; Schaefer et
118 al. 2018a). Still, it appears likely that these traits come into effect after hatching modulating larval
119 performance. The neutral FA fraction for example, which is predominantly stored within the oil globule
120 and not mobilized during embryogenesis, is likely to affect larval performance parameters after hatch
121 prior to first feeding (Wiegand 1996; Žarski et al. 2012). It was shown in free swimming percid larvae
122 that life-traits, e.g., susceptibility towards stress, are modulated by specific HUFA (Henrotte et al.
123 2010). However, these studies observed effects of alternating nutritional FA levels and knowledge of the
124 influence of inherent FA profiles is scarce.
125 Consequently, specific aspects of the inherent egg characteristics may well be stage- and/or trait-
126 specific. Knowledge of egg components, which – positively or negatively – modulate larval
127 performance, as well as ontogenetic effect strength, can support optimization of farming protocols. In
128 parallel, information on the level of predetermination of batch- and stage-specific development may
129 contribute to fisheries management in terms of prediction of recruitment success. Here, we present the
130 combined analysis of data obtained by a two-part project, which aimed at understanding broodstock
131 effects on gamete quality (Schaefer et al. 2018a,b) and on larval performance (Tielmann, 2017) .
132

133 **2. Material and Methods**

134 2.1 Fish origin and egg sampling

135 All egg batches (n = 12) were sampled at a commercial aquaculture facility (AquaPri, Egtved,
136 Denmark) during routine reproduction procedures in accordance with EU and National legislation for
137 animal welfare in fish production. AquaPri maintained four separated, seasonally shifted, but otherwise
138 identically reared broodstocks of the same genetic origin to achieve four spawning seasons per year
139 (intervals of three months). Spawning was induced once per year in each broodstock using an identical
140 protocol. After four months of wintering below 14 °C the temperature was raised to ~16 °C for the

induction of natural ovulation (no hormone treatment). The samples of the present study were obtained during two consecutive years including six different spawning seasons from a total of twelve individual females. Average female size (measured to the nearest cm) was 69.5 cm ranging from 63.0 to 78.0 cm. After introduction to the broodstock, all females were allowed to acclimatize for a full annual cycle before being stripped for the first time. Prior to stripping, spawners were anesthetized (Kalmagin 20%; Centrovet, Santiago de Chile, Chile). Sub-samples of unfertilized eggs were taken, transported to the laboratory in liquid nitrogen and stored at -80 °C until analyses. The remaining majority of eggs were fertilized according to the hatchery protocol using one to three different running males (*in vitro* fertilization, depending on the availability of running males). Fertilization rates observed ~2 h post-fertilization ranged from 85.0 to 98.0% with an average of 92.1% fertilized eggs confirming functionality of the protocol. Fertilized eggs were transferred to Zuger-jars and incubated at 15 to 16 °C. Hatching rates (all eggs and not only fertilized eggs taken into account) were 86.9% on average with a minimum of 71.2% hatched larvae per batch. Large portions of the fertilized eggs were transported to the experimental rearing facilities after 10 to 40 degree days (d°C) (cf. 2.3).

2.2 Egg parameter analyses

Egg FA analysis was performed in ten out of twelve batches according to the protocol by Boëchat et al. (2014) with changes described by Schaefer et al. (2018a). In brief, FA were extracted from homogenized eggs after dry-freezing with chloroform-methanol (2:1 V:V). Neutral and polar lipids were separated by solid phase extraction using Strata NH2 (55 µm; 70 Å) 1000 mg/6 mL columns (Phenomenex, Torrance, CA, USA). After methylation of each fraction, the re-suspended FA methyl esters (FAME) were analyzed in an Agilent 6890N gas chromatograph (Agilent, Santa Clara, CA, USA) equipped with an Agilent 5973N mass selective detector (Agilent) and a fused silica capillary column (J&W CP-Sil 88 for FAME; Agilent). Detection limit was 0.1 µg mg^{-1} and determination threshold was 0.4 µg mg^{-1}. Specific FA below quantification limit were excluded from further analyses. Egg size was determined for 10 eggs per batch using ImageJ software (version 1.44; National Institute of Health,

USA). Egg cortisol content of 30 µl manually crushed eggs were analyzed in duplicate using a cortisol-specific enzyme-linked immunosorbent assays (ELISA; IBL, Hamburg, Germany) according to Hermelink et al. (2011). A dilution series was used to calculate cortisol concentrations. Determined recoveries (spiking experiment) exceeded 91%. Mitochondrial DNA (mtDNA) fragmentation for two mtDNA regions (12S, cytochrome B) was assessed by qPCR according to Schaefer et al. (2016). Specifications of the qPCR assays are displayed in table 1. Lesion rates were calculated as described by Rothfuss et al. (2010).

2.3 Egg incubation and larval rearing

Egg incubation and larval rearing protocols are described in detail by Tielmann (2017). In brief, fertilized eggs of 12 females were transported to the rearing facilities after 10 to 40 d°C at 16-17 °C over the course of two years. Eggs were incubated at the experimental facility of the Gesellschaft für Marine Aquakultur (GMA; Büsum, Germany) in McDonald-type hatching jars (Pentair Hatching Jar, Apopka, United States) at 18 °C and 50 lx ambient light. Stopping the water inflow after 72 d°C induced hatching. Larvae were stocked into 40 L tanks in triplicates at a density of 100 larvae L^{-1} and a temperature of 18±0.5 °C (n = 12,000 larvae per batch). Water turbidity was maintained at 2-5 Nephelometric Turbidity Units (NTU) using algae concentrate (BlueBioTech *Nannochloropsis* sp., Büsum, Germany). Light (24 h) was provided by light tubes (Lampen XXL CMI T 36W IP 65, Kronau, Germany) with an intensity of 500-700 lx. From 4 days post hatch (dph) onwards, larvae were fed *Artemia* spec. nauplii (INVE AF Specialty Cysts, Dendermond, Belgium) at a density of 10 nauplii ml^{-1} every 6 h until the end of experiment at 21 dph.

2.4 Larval performance parameters

The sampling for the assessment of larval performance parameters started immediately at 0 dph every 3 days until 21 dph. Sampling took place between 2 and 4 pm on each sampling day (after second feeding). Per sampling, 30 larvae (10 larvae from each rearing tank) were transferred into a solution of

193 pH-stabilized tricaine methansulfonate (MS222) for sedation. Images of sedated larvae were taken
194 (Olympus SZ61, Tokio, Japan). Afterwards, the larvae were rinsed under deionized water, individually
195 stored in 1.5 ml Eppendorf tubes and snap-frozen at -80 °C.

196 Standard length (SL; tip of the upper jaw to the posterior end of the last vertebra), yolk-sac size and
197 lipid-droplet size (both at 0 dph) were determined from the images using ImageJ software. For the
198 determination of body weight (BW), frozen larvae were freeze-dried for 24 h (Christ Alpha LD 1-2,
199 Osterode, Germany) and individually weighed (Sartorious Cubis Balance MSA2.7S, Göttingen,
200 Germany). Average sizes (SL and BW) of larvae were combined per sampling day for the calculation of
201 respective growth parameters (slope and intercept of the growth curve). Growth in SL and BW followed
202 a natural exponential curve with $size_{time} = intercept * e^{(slope*time)}$. The proportion of swim bladder inflation
203 (SBI) was recorded after 15 dph for the 30 larvae sampled per batch.

204 Day of first feeding (50% of larvae with visible food ingestion) was determined by observing the
205 gut fullness of 30 randomly selected larvae from 3 dph onwards. On day 15 post hatch total number of
206 larvae in each tank were counted to calculate the survival rate taking sampled larvae into account (n =
207 4,000 larvae $tank^{-1}$ at 0 dph). On the same day, a stress test was conducted by exposing larvae to an
208 acute salinity challenge. A total of 360 larvae (120 from each tank) were transferred to a 1 L glass
209 beaker containing saltwater (19 PSU; 18 °C). Cumulative mortality (larvae lying motionless on the
210 bottom) was determined over a period of 2 h in intervals of 5 min as described by Dagar et al. (2010).
211

212 2.5 Data analyses

213 Data are presented as mean ± standard deviation (SD). Correlation analysis was performed with
214 Spearman's correlation using SPSS (version 22; IBM, Armonk, NY, USA). Due to a lack of data
215 (missing sampling points) not all larval performance parameters could be assessed for all 12 batches.
216 For specific parameters (growth curves, rates of survival, SBI, stress test survival) only 9 datasets were
217 available for correlation analysis.
218

3. Results

3.1 Egg and larval parameters

Egg FA profiles for the ten observed egg batches of both, neutral and polar fractions, are presented in table 2. The mtDNA lesion rates for the two tested genes were 2.0 ± 2.2 for 12S ranging from 0 to 5.9 and 0.6 ± 0.1 for cytochrome B with a minimum of 0.5 and a maximum of 0.8 lesions per 10 kbp. Egg cortisol level was 78.2 ± 31.0 ng mL^{-1} on average with 37.0 as lowest and 134.7 ng mL^{-1} as highest observed concentration. Average egg diameter was 1.2 ± 0.1 mm ranging from 1.0 to 1.3 mm. Larval parameters are listed in table 3.

3.2 Links between egg and larval parameters

Correlation analysis showed links between larval performance after hatch and egg parameters (table 2). Especially the FA profile affected the larvae. Interestingly, only 15:0 and 16:1n-7 of the polar FA fraction showed linkage with larval performance whereas a variety of FA of the neutral fraction, including ARA, were involved in modulating larval traits. If the polar FA affected standard length growth parameters, the neutral ones were correlated with larval weight gain. Within the polar fraction, 15:0 and 16:1n-7 seem to have alternating effects. Where 15:0 was positively correlated with larval parameters, 16:1n-7 had a negative relation and the other way around. The only larval trait which was not significantly correlated with any egg parameters was the lipid-droplet size. There were no correlations found in regard to egg cortisol levels and mtDNA damage. Egg diameter however, was correlated not only with larval standard length at hatch (Fig. 1) and growth, but was also associated with swimbladder inflation rate.

4. Discussion

The influence of broodstock properties, such as individual size, age, nutritional status and stress, and their effect on egg composition has been studied in numerous fish species including percids (Schreck et al. 2001; Kestemont and Henrotte 2015). On the other hand, there is only very little information

245 available on how variability in inherent batch-specific egg traits is related to early larval performance
246 after hatch under similar conditions. In previous efforts we could – to a certain extent – identify a
247 critical modulating role of FA profiles, mainly of FA within the polar fraction, during the first 48 h post
248 fertilization in pikeperch (Schaefer et al. 2018a). This influence however, was of lesser significance
249 during late embryo development and hatching. It was hypothesized that specific egg traits, mainly FA of
250 the neutral fraction, may exert a strong influence on larval performance after hatch due to the
251 conservation of these components during early embryogenesis (Moodie et al. 1989; Wiegand 1996).
252 Presence of such modulating effects of FA, especially HUFA, during early ontogeny is supported by
253 observations made in other percids (Henrotte et al. 2010; Lund and Steenfeldt 2011). Therefore, the
254 analysis of the effects of inherent egg traits was extended beyond the critical hatching stage.
255 Indeed, we could detect correlations between FA of the neutral fraction (16:0, 18:0, SFA) and
256 important early life history traits, especially in regard to growth and size characteristics. High deposition
257 of specific FA also induced advanced first feeding. Levels of ARA and total FA positively affected SBI.
258 Consequently, specific larval capabilities seem to be pre-determined by the inherent egg composition,
259 which could be detected prior to fertilization. The importance of FA during reproduction and early
260 ontogeny was studied and described for a variety of fish species including percids (e. g., Czesny and
261 Dabrowksi 1998; Dabrowski et al. 2015; Kestemont and Henrotte 2015). In Eurasian perch (*Perca*
262 *fluviatilis*), modulating effects of dietary FA on the broodstock levels could be detected, which were
263 reflected in the FA composition of the eggs (Henrotte et al. 2010). Especially the levels and composition
264 of HUFA are of major importance during early development. Here, ARA was positively correlated with
265 SBI. At 15 dph it is most likely that the critical phase of SBI had ended (Steenfeldt 2015). Arachidonic
266 acid is the main precursor of eicosanoids, which are in turn essential for embryonic development. (Bell
267 et al. 1986; Sargent et al. 2002; Tocher 2003). Interestingly, we did not detect critical influence of other
268 HUFA. It is likely, that onset of external feeding from 3 dph onwards may have masked these effects. It
269 was shown for pikeperch larvae that varying HUFA composition in larval diets from 3 to 21 dph affect
270 larval performance (Lund and Steenfeldt 2011).

On 15 dph the larvae were exposed to an acute salinity challenge to test for the capabilities to perform osmoregulation and to counteract stress (Kestemont et al. 2007; Lund and Steenfeldt 2011; Lund et al. 2012). While batch-specific survival rates were highly variable, the capabilities were only marginally correlated with egg FA profiles and were thus relatively independent of the observed traits. Interestingly, Tielmann (2017) observed a strong and significant link between lipid-droplet size and stress test survival in pikeperch larvae and hypothesized that this might be due to the availability of MUFA, which are primarily stored in the oil droplet in larvae of teleost fish (Wiegand 1996; Schaerlinger and Žarski 2015). In Eurasian perch Henrotte and colleagues (2010) observed differences in osmotic stress tolerance in larvae depending on the HUFA levels in the broodstock diet, which was reflected in the egg composition. Such patterns could not be confirmed by the findings of the present study.

Not all egg traits, which showed significant correlations with larval performance, proofed to be advantageous for the developing larvae. Especially the composition of FA within the polar fraction had inconsistent impacts. For example, the growth intercept of the SL was positively correlated with 15:0 and negatively correlated with 16:1n7. It is documented that an excess of specific FA can exert negative effects during early ontogeny (Fernández-Palacios et al. 1995; Broach et al. 2017; Schaefer et al. 2018a). Yet, it is not clear which underlying patterns may explain for these observed counteracting effects. The indicative or predictive value of FA regarding egg and larval quality are somewhat limited due to high variability and might not be generalized (Schaerlinger and Žarski 2015).

In addition to FA profiles, morphometric traits are among the first parameters to be studied in relation to egg and larval characteristics. Several studies explored the relations involved in egg size and developmental success, but there seems to be no generally valid pattern (Brooks et al. 1997; Kamler 2005). Positive effects of large egg sizes have often been associated with a larger larval size at hatch and an optimized nutritional state of the embryo. In turn, sizes at hatch were correlated with growth and survival of larvae and juveniles in the field (Meekan and Fortier 1996; Benôit and Pepin 1999) and in laboratory studies (Trippel et al. 2005). Indeed, we detected a positive correlation of larval SL at hatch

297 and egg diameter, which was well in range of previous reports on pikeperch oocytes prior to fertilization
298 (Demska-Zakes et al. 2005; Žarski et al. 2012). In addition, elevated egg size was associated with
299 increased SBI at 15 dph further supporting the critical role of size at hatch.
300 The influence and fate of cortisol during fish reproduction is still being controversially discussed
301 (Milla et al. 2009). There are indications that the initial egg cortisol levels are diluted during the uptake
302 of water (Hwang et al. 1992; Brooks et al. 1997; Stratholt et al. 1997). Here, egg cortisol had no adverse
303 effect, similar to the observations made during embryogenesis (Stratholt et al. 1997; Schaefer et al.
304 2017). While larval performance was independent of egg cortisol content, it was shown that the FA
305 profiles are linked to this primary stress marker in pikeperch oocytes, which in turn could potentially
306 affect early ontogeny (Schaefer et al. 2018a). Similar to cortisol, the detected mtDNA fragmentation had
307 no visible effect on larval traits. The mtDNA is a suitable target for the detection of damage induced by
308 reactive oxygen species since it is more prone to fragmentation compared to the nuclear DNA (Sawyer
309 et al. 2003). It seems that either potent repair mechanisms (Rothfuss et al. 2010; Alexeyev et al. 2013)
310 or partial degradation of damaged DNA (Alexeyev et al. 2013) prevented adverse effects as discussed
311 by Schaefer et al. (2016) for pikeperch embryos.
312 As observed in a previous study, the variability in egg traits was relatively high (Schaefer et al.
313 2018a). This pattern could be followed into the larval stage here. Such differences and variability in egg
314 composition may arise from a variety of extrinsic and intrinsic factors including stress, nutrition, nuclear
315 and non-nuclear genetic information, female size, age, spawning experience and condition or individual
316 metabolism, as well as environmental conditions (e.g., Schreck et al. 2001; Kestemont and Henrotte
317 2015; Schaefer et al. 2018b). In addition, fertilization success and offspring performance is also
318 influenced by sperm traits (Bobe and Labbé 2010). However, the offspring seem to be able to cope with
319 a variety of inherent characteristics including potentially harmful ingredients or damage. In the context
320 of the high variability observed here, the detected correlations of specific traits may highlight crucial
321 characteristics.
322

Conclusions

In this case study, we have discovered a relatively high level of independency for the observed batch-specific larval performances in response to the inherent egg traits under similar and seemingly ideal conditions. However, specific aspects –in regard to FA profiles and egg size – were associated with early larval characteristics. Consequently, high oocyte FA deposition (neutral fraction) fuels fast rates of growth and development during early ontogeny after hatch. On the other hand, larvae developed independently of assumed disadvantageous properties (cortisol content, mtDNA damage). Knowledge of this relation allows for the improvement of aquaculture practice, e. g. in terms of early larval and broodstock feed formulation, as well as predicting recruitment success in the wild.

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Conflict of Interest: The authors declare that they have no conflict of interest.

Ethical approval: Fish rearing and reproduction were performed in accordance with EU and National legislation for animal welfare in fish production.

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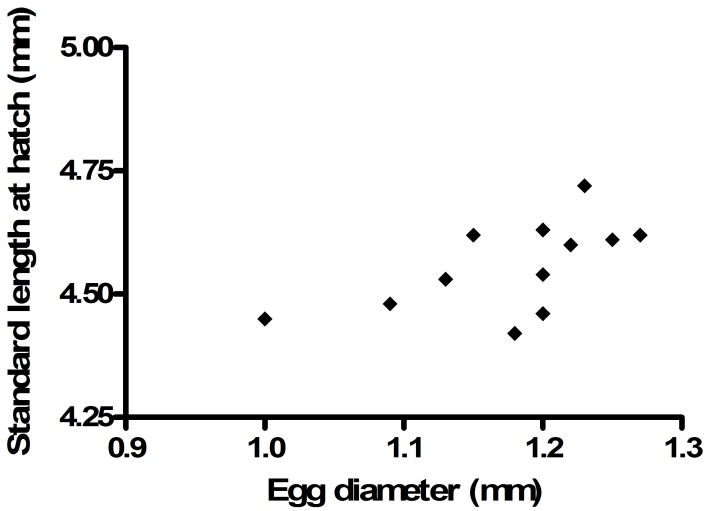
Figure captions

Fig. 1. Egg diameter versus larvae standard length at hatch (n = 12).



Tables

Table 1. Specifications of qPCR assays targeting short (s) and long (l) segments of the 12S and cytochrome B (cytb) genes for the determination of lesion rates per 10 kb. The table includes primer sequences, amplicon length in base pairs (bp) and qPCR efficiency (Eff). Annealing temperature was 60 °C for all targets.

Target gene (fragment)	Primer	5'-3' sequence	Size [bp]	Eff [%]
l12S	f	GAACTCAGCAGTGATAGACA	739	86.0
	r	GTACACTTACCATGTTACGA		
s12S	f	GAACTCAGCAGTGATAGACA	249	95.0
	r	CGTAGCTTTCGTGGGTTCAG		
lcytb	f	ACAACGCACTAGTTGACCTA	1066	96.5
	r	GAGAGCCTTGTTTTCAACCCAT		
scytb	f	ATGTTCCATTCTTACCTGA	141	96.4
	r	GAGAGCCTTGTTTTCAACCCAT		

461

Table 2. Absolute FA content (µg mg^{-1} dry weight) and standard deviation (SD) major fatty acids (FA)[1] and selected ratios in polar and neutral FA for ten observed egg batches (prior to fertilization).

	Polar fraction		Neutral fraction	
Fatty acid (FA)	Mean	± SD	Mean	± SD
14:0	1.32	± 0.25	2.07	± 0.45
15:0	0.50	± 0.25	0.38	± 0.24
16:0	9.94	± 2.92	12.93	± 2.16
16:1(n-7)	0.40	± 0.25	10.87	± 4.42
18:0	4.06	± 1.82	8.37	± 1.55
18:1(n-9)	0.80	± 0.28	14.19	± 5.32
18:2(n-6)	N/A		9.93	± 4.90
18:3(n-3)	N/A		1.51	± 0.73
20:4(n-6), ARA[2]	N/A		1.01	± 0.42
20:5(n-3), EPA[3]	0.94	± 0.34	6.72	± 3.31
22:6(n-3), DHA[4]	3.35	± 1.49	27.27	± 8.71
Sum SFA[5]	16.21	± 4.92	23.78	± 3.82
Sum MUFA[6]	1.99	± 1.16	27.12	± 10.15
Sum n-3 HUFA[7]	4.29	± 1.83	35.51	± 12.47
DHA/EPA	3.47	± 0.39	4.36	± 0.91
EPA/ARA	N/A		6.55	± 1.03
Sum FA	23.60	± 5.88	97.35	± 27.67

[1] Other FA routinely found (below quantification limit of 0.4 µg mg^{-1} dry weight): 12:0, 17:0, 20:0, 20:1(n-9), 20:2 (unknown isomer), 22:0, 22:1(n-9), 24:0, 24:1 (unknown isomer).

[2] Arachidonic acid.

[3] Eicosapentaenoic acid.

[4] Docosahexaenoic acid.

[5] Saturated fatty acid.

[6] Monounsaturated fatty acid.

[7] Highly unsaturated fatty acid.

492 Table 3. Mean ± standard deviation (SD), minimum (min) and maximum (max) values for larval
493 parameters. Growth characteristics are presented as intercept and slope of the growth curve ($size_{time}$ =
494 intercept*$e^{(slope*time)}$).

Parameter	Mean	± SD	Min	Max	*n*
Survival 15 dph (%)	68.20	± 31.14	16.33	99.33	9
Stress test survival 15 dph (%)	54.13	± 32.17	4.67	98.67	9
Swimbladder inflation 15 dph (%)	39.59	± 26.83	0	73.33	9
Standard length at hatch (mm)	4.56	± 0.09	4.42	4.72	12
Yolk-sac size (mm^2)	0.48	± 0.38	0.04	1.32	12
Lipid-droplet size (mm^2)	0.17	± 0.03	0.12	0.21	12
Day at 50% first feeding	6.83	± 1.34	5	10	12
Growth (BW^1) intercept	0.09	± 0.03	0.05	0.12	9
Growth (BW^1) slope	0.13	± 0.02	0.09	0.16	9
Growth (SL^2) intercept	4.66	± 0.16	4.33	4.90	9
Growth (SL^2) slope	0.05	± 0.02	0.04	0.09	9

495 [1] Body weight.
496 [2] Standard length.
497

498 Table 4. Spearman correlation of egg and larval parameters (only for parameters with one or more
499 significant correlations). Growth characteristics are presented as intercept and slope of the growth curve
500 ($size_{time}$ = intercept*$e^{(slope*time)}$). Significant correlations are indicated by dark grey highlights. Level of
501 significance is indicated by asterisks (*$p < 0.05$; **$p < 0.01$).

		Polar FA[1]		Neutral FA[1]						
	Egg diameter (mm)	15:0	16:1n-7	15:0	16:0	18:0	22:1n-9	20:4n-6[2]	SFA[3]	Total FA[1]
n	9	9	9	8	8	8	8	8	8	8
Survival (15 dph)	.53	.73*	-.67*	-.34	.62	.50	.38	.62	.62	.48
Stress test survival (15 dph)	.47	-.06	-.06	-.76*	.19	.02	.41	.12	.19	-.05
Swimbladder inflation rate (15 dph)	.67*	.69*	-.43	-.30	.50	.34	.25	.76*	.50	.65*
Standard length (0 dph)	.58*	-.09	.22	.28	-.35	-.58*	-.27	-.08	-.39	-.06
Yolk-sac size	-.33	-.63*	.40	-.19	-.52	-.41	.04	.08	-.44	.09
Day of first feeding (50%)	.09	-.65*	.69**	.02	-.76 **	-.82 **	-.36	-.10	-.76 **	-.02
Growth (SL^4) intercept	.67*	.88**	-.75*	-.05	.31	.21	.28	.62	.31	.52
Growth (SL^4) slope	.18	.61*	-.59*	.30	.17	.20	-.02	-.22	.17	-.14
Growth (BW^5) intercept	.05	.55	-.51	-.44	.76*	.71*	.73*	.50	.76*	.33
Growth (BW^5) slope	-.37	.02	-.03	.78*	-.31	-.17	-.47	-.33	-.31	-.14

502 [1] Fatty acids.
503 [2] Arachidonic acid.
504 [3] Saturated fatty acids.
505 [4] Standard length.
506 [5] Body weight.