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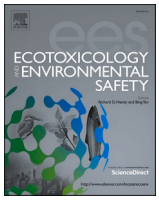

Monitoring ocean water quality by deployment of lumpfish (*Cyclopterus lumpus*) eggs: *In situ* bioaccumulation and toxicity in embryos

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ABSTRACT

Fish embryos can bioaccumulate and are particularly sensitive to a wide range of contaminants, which makes them suitable sentinels for environmental biomonitoring. However, fish embryos are very rarely utilized in environmental monitoring surveys, possibly due to their fragility and seasonality. In the present work, we assessed the applicability of caged lumpfish (*Cyclopterus lumpus*) eggs for *in situ* biomonitoring of exposure and effects of organic contaminants focusing on polyaromatic hydrocarbons and phenolic compounds. Fertilized eggs (1 dpf) were transplanted for 17–19 days at different locations that differed in terms of contaminant load, depths and weather conditions, namely at three stations close to the city of Trondheim (two harbour areas and a one in the Fjord) and three stations at a coastal aquaculture facility. High survival upon retrieval after deployment showed that lumpfish eggs are relatively robust and survive encaging in different environments. Bioaccumulation of organic contaminants (PAHs and phenolic compounds) was measured and potential effects on hatching, development, survival and larvae morphometry were determined. Chemical analyses showed that especially PAHs were effectively accumulated in eggs in contaminated sites, with concentrations of ΣPAHs being 15 – 25 times higher in harbour areas compared to those at the aquaculture facility. A higher incidence of embryonic deformations was observed in the most polluted deployment location, but larvae morphometry revealed no evidence of toxicity related to pollutant body burden. In conclusion, the *in-situ* exposure method was proven to work well, making it attractive for implementations in environmental monitoring programs.

1. Introduction

The marine environment acts as a sink for anthropogenic contaminants from numerous sources. Organic contaminants of concern include petrogenic compounds, like polycyclic aromatic hydrocarbons (PAHs) and phenolic compounds, which are ubiquitous in the environment (Hammam et al., 2015; Lawal, 2017). PAH concentrations are hight near industrial or urban locations, such as harbour areas, and near oil sand mines, and near offshore oil and gas installations discharging produced water (Lawal, 2017). The major sources for phenolic compounds include municipal wastewater treatment plants and a range of different industries and refineries (pulp, paper, cooling, wood, steel, metal, petrochemical, paint, textile) (Breton et al., 2003; Hammam et al., 2015). Alkylated phenols of most concern are bisphenol A (BPA), nonylphenol (NP) and octylphenol (OP) due to their endocrine disruption properties. NP and OP are degradation products of alkylphenolpolyethoxylates, which are commercially important nonionic surfactants with different industrial, agricultural, and domestic applications (Talmage, 2020). BPA is an important raw material in epoxy and polycarbonate plastics, used largely in food packaging coating, thermal papers, household appliances, and adhesives (Noonan et al., 2011).

To assess contaminant release and negative environmental impacts, biomonitoring programs can be implemented, and the use of biota to monitor contaminants in the environment is essential under the European Union's Water Framework Directive (WFD) (Besse et al., 2012; Commission, 2000). In Norway, a long-term monitoring program has surveilled the environment around offshore oil production installations in the North Sea for decades (Hylland et al., 2008).

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Biomonitoring can be performed by measuring i) the contaminant load in an organism, ii) and/or a biological signal (biomarkers) tightly coupled to the response of an organism towards specific contaminants, or iii) by an effect assessment using biotests, biosensors, and reporter organisms for monitoring (Seifert et al., 2003). For biomonitoring, either native organisms (bioindicators) can be collected in the field, or organisms can be transferred to and caged in the environment under investigation with sampling, chemical and/or toxicological assessments performed after a defined period. Caging of organisms allows for better control of confounding factors like species, biological parameters (weight, size, sex, age, genetics, reproductive status) and previous exposure history (location and duration) that may affect interpretations of data gathered from wild bioindicator animals (Besse et al., 2012).

Caged and wild-caught fish are used for biomonitoring in both freshwater and marine environments, as they are sensitive to a wide range of organic and inorganic contaminants (Van der Oost et al., 2003). However, caging of fish can pose certain challenges, such as obtaining suitable species and individuals from clean reference sites or hatcheries, and the underlying effects of starving and stress of caged animals that can bias observations (Oikari, 2006). To date, caging of fish for biomonitoring purposes has mostly been limited to juvenile or adult fish. Encaging fish embryos can offer several advantages. The embryonic and yolk-sac larvae stages of fish can accumulate contaminants and are highly sensitive to exposure (Hansen et al., 2021; Perrichon et al., 2021; Sørhus et al., 2015). They further display a wide range of morphological abnormalities as response to contaminant exposure that can be useful for toxicological assessment following *in situ* exposure. In controlled laboratory experiments, exposure to petrogenic compounds causes acute toxicity as well as sub-lethal effects, like retarded development, deformations, pericardial edema and yolk sac edema in early live stages (ELS) of marine fish. For example, Atlantic cod (*Gadus morhua*) and Atlantic haddock (*Melanogrammus aeglefinus*) embryos exposed to oil dispersions showed craniofacial deformations at concentrations as low as 0.76 (haddock) and 2.8 (cod) μg total PAH/L (Sørensen et al., 2017). Similarly, Hansen et al. (2019b) reported cardiotoxicity and severe craniofacial deformations in cod larvae after embryonic exposure to 50–500-fold dilutions of produced water. In one of the few studies where embryos were used for *in situ* exposure, Incardona et al. (2012) subjected Pacific herring (*Clupea pallasii*) embryos to oiled shoreline habitats three months after the Cusco Busan accident, resulting in tissue necrosis, lethality and cardiotoxicity in exposed individuals. Two years later, exposure to oiled sediments still caused cardiotoxic impacts. West et al. (2019) also used Pacific herring embryos for *in situ* monitoring of effect of removing creosote-treated pilings in a nearshore marine habitat of Puget Sound.

Advantages of using fish embryos for *in situ* biomonitoring are the use of genetically comparable treatment groups (single males and females to provide oocytes and milt for fertilization), accessibility of spawning products from commercial hatcheries or facilities holding brood stocks, standardized procedures for cryopreservation and *in vitro* fertilization for many species (Draper and Moens, 2009; Peuß et al., 2019; Żarski et al., 2015). Further, using ELS of fish for chemical testing is regarded as a better alternative compared to juvenile and/or adults considering reduction of pain and distress (Embry et al., 2010). Housing of fish embryos will also be considerably smaller than for larger fish, enabling a wider range of habitats to be surveyed. Challenges, however, using fish eggs, are that they may be sensitive to handling and physical stress, and thus, may be less tolerant to wave action unless sufficiently protected. This may be a particular concern for pelagic and positively buoyant fish eggs, like Atlantic cod and haddock, but may be less for demersal eggs like lumpfish (*Cyclopterus lumpus*). Lumpfish is a common fish species of the North Atlantic. While adult lumpfish can be found in open water, they migrate to the coast for spawning where females can lay up to 400,000 eggs (Brown et al., 1992; Davenport, 1985; Moen and Svensen, 2004). The eggs are demersal, 1.8–2.6 mm in diameter and stick to each other before hardening in contact with saltwater (Davenport, 1985; Lönning et al., 1984). They are also relatively robust to variations in temperature (Imsland et al., 2019) and oxygen tension (Davenport, 1983). Due to their use as cleaner fish in marine aquaculture (Powell et al., 2018), reproducing adults (and eggs) are available from hatcheries several times per year. This increases the time window to assess environmental impacts of acute spills of contaminants, *e.g.*, acute oil spills, and allows for temporal trends in environmental contamination. Lumpfish eggs develop relatively slowly, taking more than one month to hatch (Lønning et al., 1988), which increases the length of time they can be exposed in the environment.

In this work we describe a method to prepare, transfer and cage lumpfish embryos in the environment for *in situ* exposure. We tested the method through a field survey in which we caged/exposed lumpfish embryos at different locations in the Trondheimsfjord and in an aquaculture facility (ACE) located on the Norwegian coast. The locations featured varying environmental conditions such as contamination levels/type, depth and weather exposure. Bioaccumulation of PAHs and phenolic compounds were studied, as well as a suite of toxicological endpoints investigated in the exposed embryos and larvae, to evaluate the use of lumpfish embryos for biomonitoring purposes.

2. Materials and methods

2.1. Lumpfish egg and milt acquisition and egg fertilisation

Unfertilized eggs were obtained from wild-caught females from two different sources (Namdalen Rensefisk AS and Skjerneset AS). Eggs from Namdalen Rensefisk AS were used for the Trondheimsfjorden deployment, and the eggs from Skjerneset AS were used for the deployment at the ACE aquaculture facility. After strip-spawning, eggs were transferred to ziplock bags and transported on ice to SINTEF Sealab within 4 h. Cryopreserved milt from one single male was obtained from Cryogenetics (Hamar, Norway), and eggs were fertilized *in vitro* using their protocol for the use of cryopreserved milt. Briefly, 1 mL cryopreserved milt was added to 50 mL eggs and stirred gently before the addition of 50 mL filtered (1 μm) seawater. After three minutes, circular egg monolayers (CEML) were prepared in circular molds (diameter 2 cm) containing approximately 80 eggs each. Eggs were kept submerged until they hardened and placed in holding frames custom-built from polycarbonate (each holding frame containing 16 CEML).

2.2. Holding frames and field deployment

Within the holding frame, CEML were contained in holes drilled on a 2 mm polycarbonate sheet and restricted by two layers of 250 μm plankton mesh that were supported by matching polycarbonate plates secured by nylon screws (Fig. 1A and B). These holding frames were mounted into aluminum holders, which were, in turn, mounted into rigs (Fig. 1C). The rigs were custom built from welded aluminum parts (Fig. 1C) with slots for placing four egg holders per rig. During transfer of egg holders to the frame, eggs were sheltered from direct sun light and kept under water during assembly. The holding frame was anchored on rope and floating buoy and/or attached to a pole in the water. The eggs were kept under water throughout the whole *in situ* exposure period.

Altogether 7 holding frames were deployed at six different locations, three locations within Trondheimsfjorden (4 frames; 17 days deployment) (Fig. 2, Table 1) and three off the coast in Trøndelag (3 frames; 19 days deployment) (Fig. 2B, Table 1).

Two of the three stations located in Trondheimsfjorden were in presumably contaminated harbour areas, namely Brattørkaia (BK) and Nyhavna (NH). BK is the one of the main harbours for small maritime traffic in Trondheim. NH has been an important industry area since the late 1800 s, and has included *e.g.* a hardwood company, mechanical workshops and a shipyard. During the second World War, two submarine bunkers (Dora I and Dora II) were built; now serving as storage facilities. Today, the NH harbour area consists of a shipyard and a local

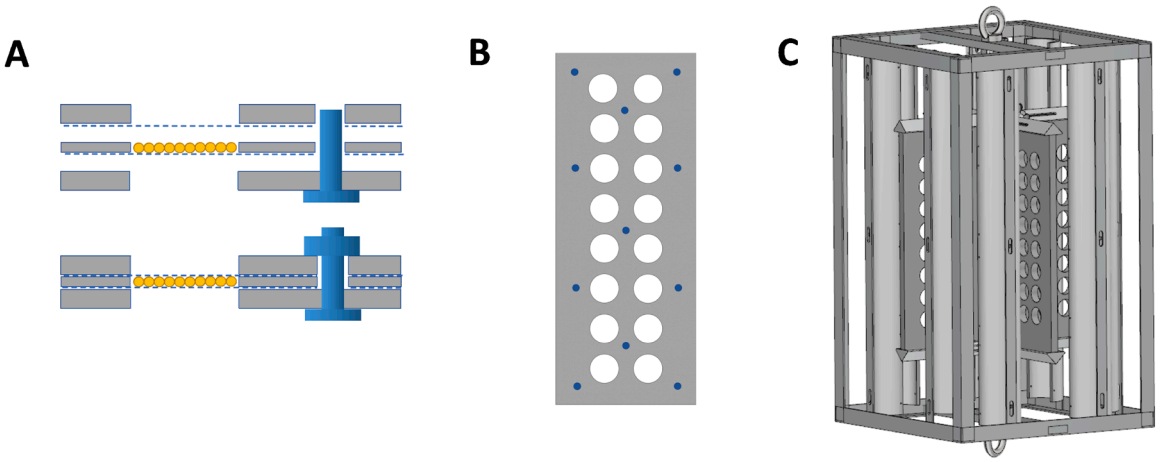

Fig. 1. Design of the system for field application. A: The circular egg monolayers (CEML, yellow circles) were contained in holes in a 2 mm polycarbonate sheet and restricted by two layers of 250 μm plankton mesh that were supported by matching polycarbonate plates secured by nylon screws. B: Polycarbonate sheet containing 16 holes for insertion of CEML. C: The aluminum frame holding up to four plates.

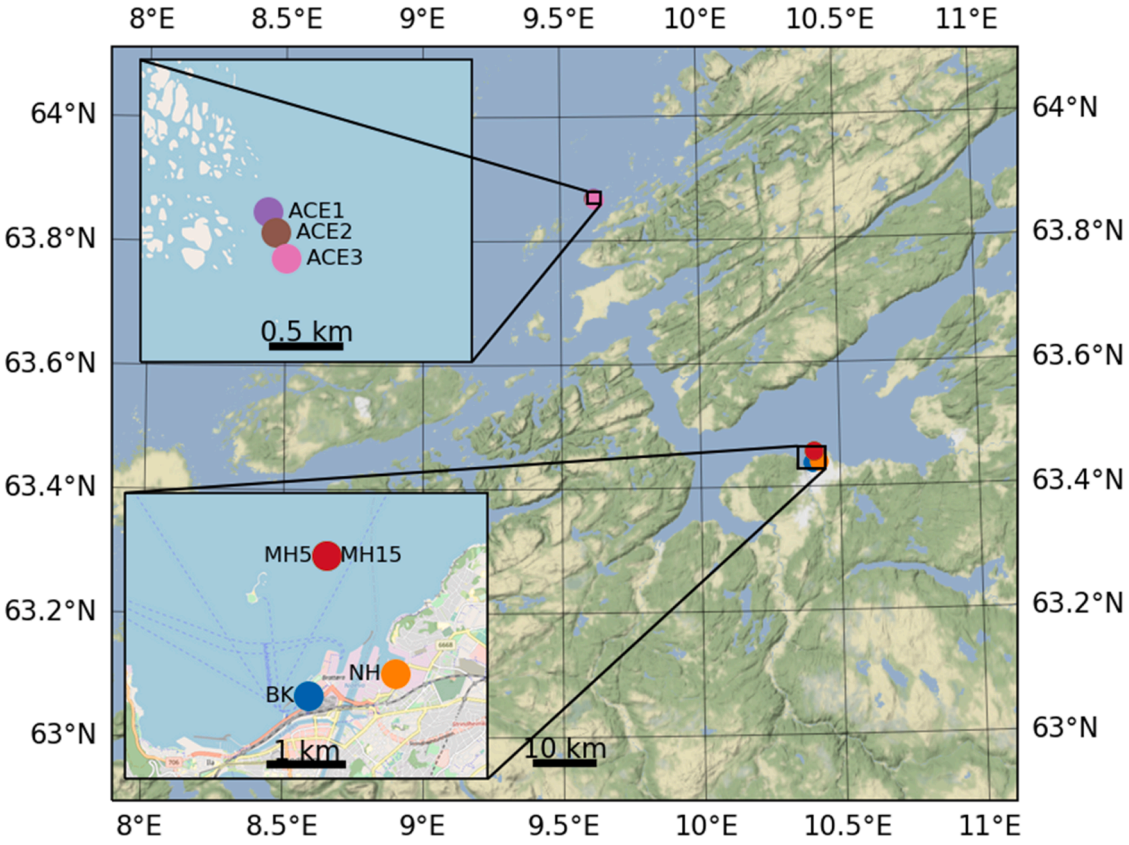

Fig. 2. : Map of the locations of the seven stations in Trøndelag, Norway, where lumpfish eggs were deployed for environmental monitoring.

Table 1
Locations of the seven stations in Trøndelag, Norway, where lumpfish eggs were deployed for environmental monitoring, including type of exposure, depth of deployment, number of days of field deployment and sea water temperature logged during deployment (mean ± standard deviation).

Station name	Position (N,E)	Exposure type	Depth (m)	No. days deployed	Temp (°C)
Brattørkaia (BK)	63.4387032, 10.3980235	Harbour	5	17	9.9 ± 0.5 (8.6–11.2)
Nyhavna (NH)	63.4411812, 10.4227135	Harbour	5	17	9.9 ± 0.5 (8.9–10.8)
Munkholmen 5 (MH5)	63.456252, 10.404144	Fjord (city reference)	5	17	9.6 ± 0.5 (8.4–10.7)
Munkholmen 15 (MH15)	63.456252, 10.404144	Fjord (city reference)	15	17	10.1 ± 0.6 (8.9–11.3)
Ace 1 (ACE1)	63.8699734, 9.6192513	Coast, Aquaculture (downstream)	5	19	7.7 ± 0.7 (6.9–9.5)
Ace 2 (ACE 2)	63.8685794, 9.6204002	Coast, Aquaculture (mid)	5	19	7.7 ± 0.6 (6.9–9.5)
Ace 3 (ACE3)	63.8668579, 9.6217961	Coast, Aquaculture (upstream, reference)	5	19	7.7 ± 0.6 (6.9–9.4)

industrial hub. As reference site to the Trondheim survey, one rig was placed at 5 m depth and one at 15 m depth (MH5 and MH15) in the fjord north of the city of Trondheim.

A second survey focused on testing the system at a rougher coastal site, and at the same time monitor the environment around an aquaculture facility (ACE) serving as a full-scale aquaculture research laboratory on the coast of Trøndelag. The facility holds full-scale salmon production run by SalMar Farming. Three rigs were placed along a transect following the main current through the aquaculture facility (ACE1–3).

During rig deployment and recovery, CTD (conductivity, temperature and depth) measurements were taken (depth profiles, Castaway®-CTD, SonTek). Temperature (°C) was monitored (HOBO Pendant® Temperature 64 K Data Logger, Onset Computer Corporation) in 5 min intervals throughout the deployment period. After the deployment period, rigs were recovered and transported back to the laboratory facility in transport boxes containing ambient seawater.

2.3. Sampling and laboratory incubation of eggs

After 17–19 days exposure in the field, the rigs were retrieved, and the eggs transported to the laboratory. Immediately after arrival in the laboratory, 4 CEML replicates from each location were rinsed with filtered (1 μm) seawater and immediately frozen (−20 °C) until extraction and chemical analyses as described below. The remaining CEML were distributed into separate incubator chambers, each provided with a steady flow-through of natural seawater (filtered 1 μm) from Trondheimsfjord (80 m depth below thermocline; salinity 34‰; pH 7.6).

All CEML were imaged in the laboratory using a handheld camera and through a macroscope (model Z6APO, Leica Microsystems, Germany) connected to a CMOS camera (MC170HD, Leica Microsystems, Germany). The BK, NH and MH groups were imaged five days after retrieval (23 dpf and 220–229 d°), and the ACE groups were imaged directly upon arrival in the laboratory (20 dpf and 156–157 d°). The images were used to determine the number of unfertilized eggs as well as eggs displaying normal and deformed embryos or developmentally delayed embryos (see Fig. 4 for examples).

The eggs were kept under controlled conditions at 9.5 °C (water and ambient temperature) and a constant light cycle of 12 h light/12 h darkness. The eggs were incubated to determine hatching success, and the larvae monitored up until 2 days post hatch (dph) to determine mortality and deformations, as described below. Hatching and mortality of larvae were assessed daily, and dead individuals were counted and removed.

2.4. Extraction and contaminant target screening

Extractions of eggs were performed as described in Sørensen et al. (2016b). Briefly, samples were accurately weighed (sample mass range 0.35–0.66 g) and transferred to glass vials. After addition of n-hexane/DCM (1:1 v/v, 4 mL) and surrogate standards (25.08 ng naphthalene-*d*8, 5.00 ng phenanthrene-*d*10, 4.86 ng chrysene-*d*12, 5.08 ng perylene-*d*12, 2533.4 ng phenol-*d*6, 104.2 ng p-cresol-*d*8, 137.4 ng 4-n-propylphenol-*d*12), the samples were homogenized using a disperser (IKA 10 basic ULTRA-TURRAX®; IKA-Werke, Staufen, Germany), sodium sulfate was added followed by a brief vortex and centrifuged (2000 rpm (720 g), 2 min). The supernatant was collected, and the extraction repeated two additional times. Extracts were subjected to clean-up by gel permeation chromatography (GPC). Samples (500 μL) were injected with DCM as a mobile phase (5 mL/min) and separated using an Envirogel column (19 × 300 mm, 15 μm; Waters Milford. MA, USA). Chromatograms were monitored at 210, 254 and 280 nm UV. After initial optimization, analyte fractions were collected from 10.1 to 14.5 min with pre-added *n*-hexane in the collection vials as a keeper. The sample volume was adjusted to 0.4 mL by solvent evaporation (40 °C under a gentle flow of N_2) and a recovery internal standard (100 ng fluorene-*d*10) was added prior to analysis.

Extracts were analyzed by an Agilent 7890 gas chromatograph coupled with an Agilent 7010B triple quadrupole mass spectrometer fitted with an EI source and collision cell was used (Agilent Technologies, Santa Clara, CA, USA). Two Agilent J&W HP-5MS UI GC-columns (30 m × 0.25 mm x 0.25 μm) were coupled in series through a purged ultimate union (PUU). The carrier gas was high purity helium at constant flow (1.2 mL/min). Samples (1 μL) were injected at 310 °C split less. For PAH and alkyl PAH analysis, the oven temperature was kept at 40 °C for 1 min, then ramped to 110 °C by 40 °C/min, to 220 °C by 6 °C/min and finally to 325 °C by 4 °C/min.

For AP analysis, the oven temperature was kept at 40 °C for 1.5 min, then ramped to 110 °C by 40 °C/min and finally to 325 °C by 20 °C/min. For the other target compounds, samples were injected at 250 °C, the oven temperature was kept at 40 °C for 1.5 min, then ramped to 110 °C by 40 °C/min, to 310 °C by 5 °C/min and held for 20 min

For all methods, the temperature was finally held at 330 °C for 5 min, while the first column was backflushed. The transfer line temperature was 300 °C, the ion source temperature was 230 °C and the quadrupole temperatures were 150 °C. The EI source was operated at 70 eV. N2 was used as collision gas at a flow of 1.5 mL/min and helium was used as a quench gas at a flow of 2.25 mL/min. Target analytes were identified by two unique MRM transitions and quantified by the most intense peak as previously described (Oppegård et al., 2020; Sørensen et al., 2016a, 2016b)x. Bisphenol A was monitored by transitions 213–91 (35 eV) and 228–213 (5 eV) and quantified using the response of the former. Standards were run for each 12 sample injections to monitor system performance and a variation of no more than 25% was accepted. Method limits of detection (LOD) based on laboratory blanks, as well as recovery and precision of the method determined from previous validation is provided in Table SI.1.

2.5. Larvae morphometry and deformations

The larvae (2 dph) were embedded in a methylcellulose gel, and imaged with a macroscope (model Z6APO, Leica Microsystems, Germany) using a CMOS camera (MC170HD, Leica Microsystems, Germany). To assess potential impacts on larvae growth and development, biometric traits (standard length, myotome height, body area, yolk sac area, lipid area and eye diameter) were automatically determined using AutoMOMI (Automated Morphometrics On Microscope Images (Kvæstad et al., 2022). AutoMOMI utilizes the MASK-R CNN neural net architecture (He et al., 2017) trained on 364 manually annotated images of lumpfish larvae. The neural net architecture classifies the body-, eye-, lipid- and yolk outline, where measurements such as area and length were calculated using automated image processing techniques such as Topological Structural Analysis (TSA) (Suzuki, 1985) and skeletonize (Zhang and Suen, 1984). See Fig. 3 for outlines of annotated parameters. Detected outliers were manually checked for accuracy of the automated process.

2.6. Lipid peroxidation

LPO was assessed in lumpfish eggs and larvae by determining the concentrations of MDA and 4-HNE, following the method described by Erdelmeier et al. (1998). Briefly, two pooled egg samples (20 eggs each, wet weight 0.1224–0.1765 g) were homogenized in 0.02 M Tris-HCl containing 0.5 M butylated hydroxytoluene BHT (pH 7.4) with an UltraTurrax homogenizer at 24000 rpm for 1 min at 4 °C. One pooled larvae sample (15 larvae, wet weight 0.0645–0.0843 g) was homogenized using a Precellys 24 Lysis and Homogenization (Bertin Technologies, Montigny-le-Bretonneux, France) in 20 nM Tris-HCl (pH 7.4) containing 0.5 M BHT at 6000 rpm for 1 min at 4 °C. Homogenates were centrifuged at 15,000 g for 20 min at 4 °C and the resulting supernatants used for LPO and total protein analysis. LPO analysis is based on the reaction of two moles of N-methyl-2-phenylindole, a chromogenic

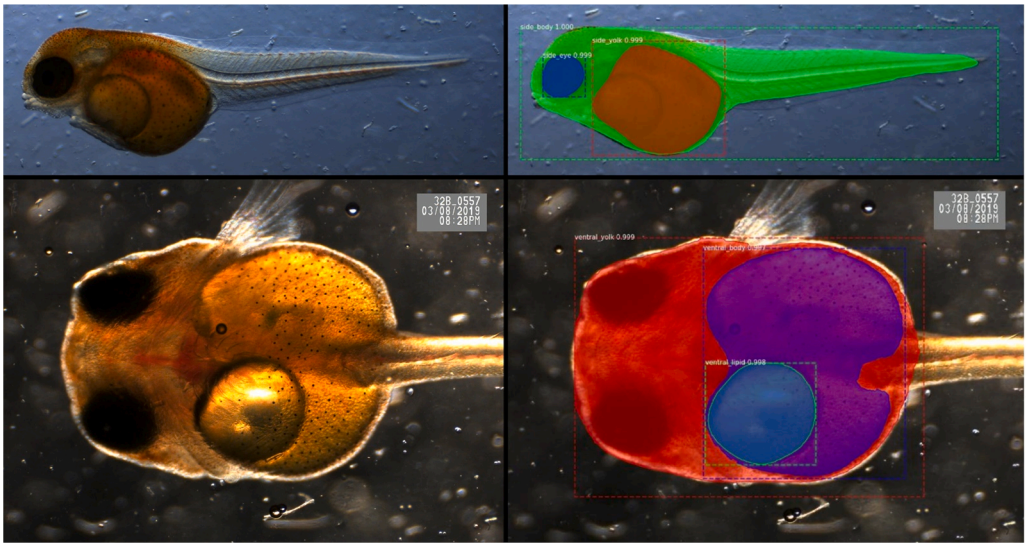

Fig. 3. Annotations of the biometric parameters that were measured on lumpfish larvae using automated analyses of standardized images taken from the side and ventrally at 1 day post hatch. Top left: Raw side image. Top right: Output file from automated analyses displaying detected regions of interest (ROI): green = body area, brown = yolk sac area, blue = eye area. Bottom left: Raw ventral image. Bottom right: Output file from automated analyses displaying detected regions of interest (ROI): red = body area, purple = yolk sac area, blue = lipid area.

reagent, with one mole of either MDA or 4-HNE under acidic conditions at 45 °C for 60 min. This reaction yields a stable chromophore that has maximum absorbance at 586 nm using malondialdehyde bis-(1,1,3,3-tetrametoxypropane) as a standard. Total protein was determined following the Lowry method (Lowry, 1951) using Bovine Immunoglobulin G (IgG) as a reference standard. LPO concentrations were expressed as nmol MDA + 4-HNE per gram of total protein.

2.7. Statistical analyses

Data treatment and statistical analyses were conducted with GraphPad Prism V9.00 (GraphPad Software, Inc., CA, USA). Comparisons between treatments were performed with one-way ANOVA, followed by Tukey's multiple comparisons test or Kruskal-Wallis test, followed by Dunn's multiple comparison test for non-normal distributed data sets according to D'Agostino & Pearson omnibus normality test. Significance level was set to $p < 0.05$ unless otherwise stated. A nonlinear curve fit (third-order polynomial) was applied in figures assessing hatching as a function of time (days post fertilization).

3. Results

3.1. Egg integrity and embryo development

When collected, the integrity of the whole rig was assessed visually, and no damages were observed to any of the components. The presumably weakest component, the mesh holding the eggs inside the egg holder which keep a constant interaction between eggs and the

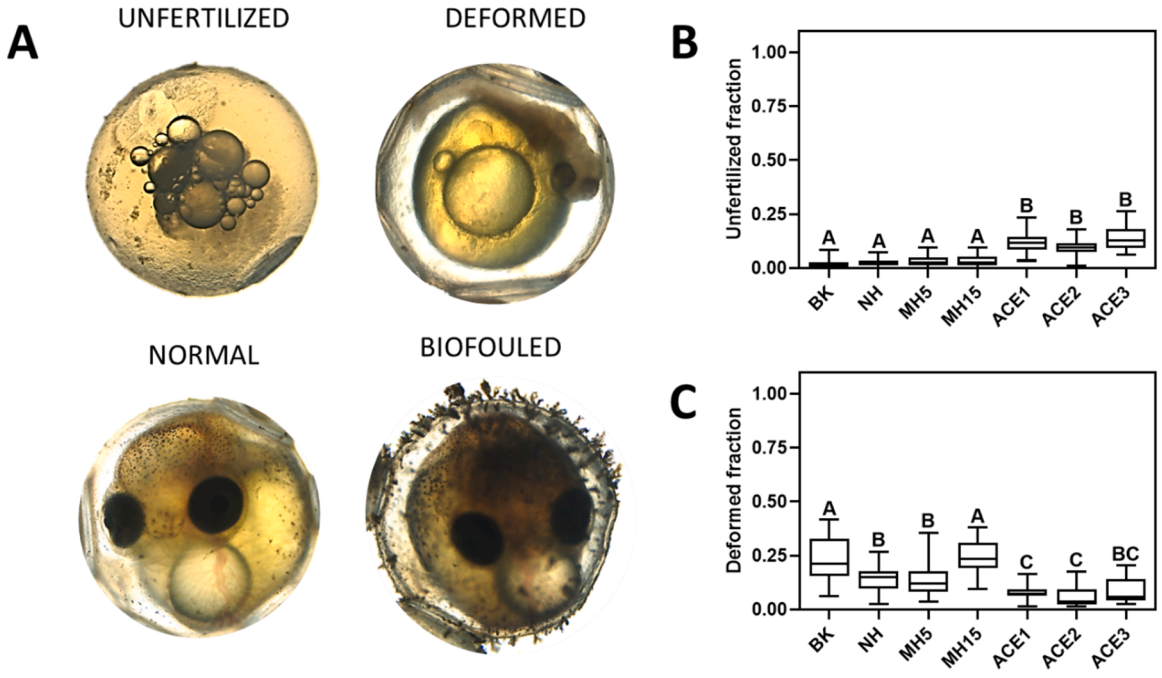

Fig. 4. A: Example images of lumpfish eggs retrieved from *in situ* exposure (20 dpf) displaying an unfertilized egg (top left), egg with a deformed embryo (top right), an egg displaying a normal embryo (bottom left) and an egg with biofouled chorion but normal embryo (bottom right). B: Fraction of unfertilized eggs from the different locations. C: Normal fraction of eggs retrieved from the different locations. D: Fraction of fertilized eggs displaying a normal embryo. Data are given as whiskers (median, 5 and 95 % confidence intervals and min / max values). Groups displaying significant differences are denoted with different letters (N = 16-32).

surrounding water, was completely intact after field deployment. Temperature differed slightly between the caging locations, with locations in Trondheimsfjorden (BK, NH, MH5 and MH15) approximately 2 °C warmer compared to the ones off the coast (ACE1–3). For the Trondheimfjorden survey, eggs were also deployed for shorter time (17 days) than for the ACE survey (19 days) (Fig. 2). Temperature and deployment time affected the period (in degree-days) the eggs were deployed in their respective locations: The Trondheimsfjorden eggs were exposed for 168–172 d° (1–18 dpf), whereas the ACE eggs were exposed for 146–147 d° (1–20 dpf). Images taken after retrieval were used to determine the fraction of unfertilized eggs and eggs with normal or deformed embryos (representative images shown in Fig. 4A). The fertilization success for the eggs was generally high, but numbers varied slightly between the two egg batches. The batch used for the Trondheimsfjorden survey (BK, NH and MH) had 1.6–3.1% unfertilized eggs (mean values for each station, N = 32), whereas the batch used for ACE had 9.6–13.8% unfertilized eggs (Fig. 4B). No significant differences were found in fertilization success between stations from the same egg batch. Eggs from BK and MH15 displayed significantly ($p < 0.05$) more deformed embryos (BK: 24 ± 9%. MH15: 24 ± 7%) compared to eggs from the other locations (Fig. 4C). The MH5, considered as a reference for the Trondheimsfjorden survey displayed 13 ± 8% deformed embryos, and the ACE3, considered as the reference for the ACE survey displayed 8 ± 6% deformed embryos. In the MH15 location most of the eggs further displayed biofouling of chorions (see Fig. 4A, bottom right), which presumably was sporophyte growth. This was not observed in any of the other locations. There was no significant difference in deformed embryos between the three ACE stations. Furthermore, eggs from the ACE locations had a significantly ($p < 0.05$) lower fraction of deformed embryos compared to the eggs incubated in Trondheimsfjorden (Fig. 4C).

3.2. Accumulation of polycyclic aromatic hydrocarbons (PAHs) and phenolic compounds

Of the 44 analyzed PAHs and alkyl PAH homologue groups (see Table SI 1 for exhaustive list), 9 were detected in eggs from all sites with all replicates above LOD (naphthalene, C1-naphthalenes, C3-naphthalenes, biphenyl, dibenzofuran, C1-C2-fluorenes, C1-phenanthrenes and fluoranthene), and an additional 10 were found in all stations, but not in all replicates within each station (C2-naphthalenes, fluorene, C3-fluorenes, C2-phenanthrenes, C3-phenanthrenes, C4-phenanthrenes and C1-C4-dibenzothiophenes). For the PAHs with highest log K_{OW} and thus lowest water solubility, levels above LOD were only observed in some of the replicates from BK and NH, and four (indeno[1,2,3-*cd*] pyrene, dibenz[*ah*]anthracene, benzo[*ghi*]perylene and benzo[*e*]pyrene) were not detected in any replicates from any location. The BK station was, by far, the location where highest PAH accumulation was observed in the fish eggs, followed by the NH location (Fig. 5A). This is reflected on a total PAH basis, and within PAH groups separated by ring-numbers (Fig. 5B-D). See Supporting Information (Table SI 1) for full details.

The T-PAH concentrations in individual samples ranged within 10–752 ng/g, and significantly higher T-PAH concentrations were found in BK (523 ± 165 ng/g) and NH (291 ± 79.2 ng/g) samples compared to the other stations (Fig. 5A). Embryos exposed in BK was also significantly higher in T-PAH compared to NH. In MH5, considered the reference station in the Trondheimsfjorden survey, T-PAH concentrations were almost an order of magnitude lower (84.1 ± 75.7 ng/g). The lowest was ACE3 (13.6 ± 3.3 ng/g). No significant differences were found between MH5, MH15 and the three ACE locations. By grouping PAHs based on number of aromatic rings (but regardless of alkylation), identical trends were observed for sum naphthalenes (Fig. 5B), which

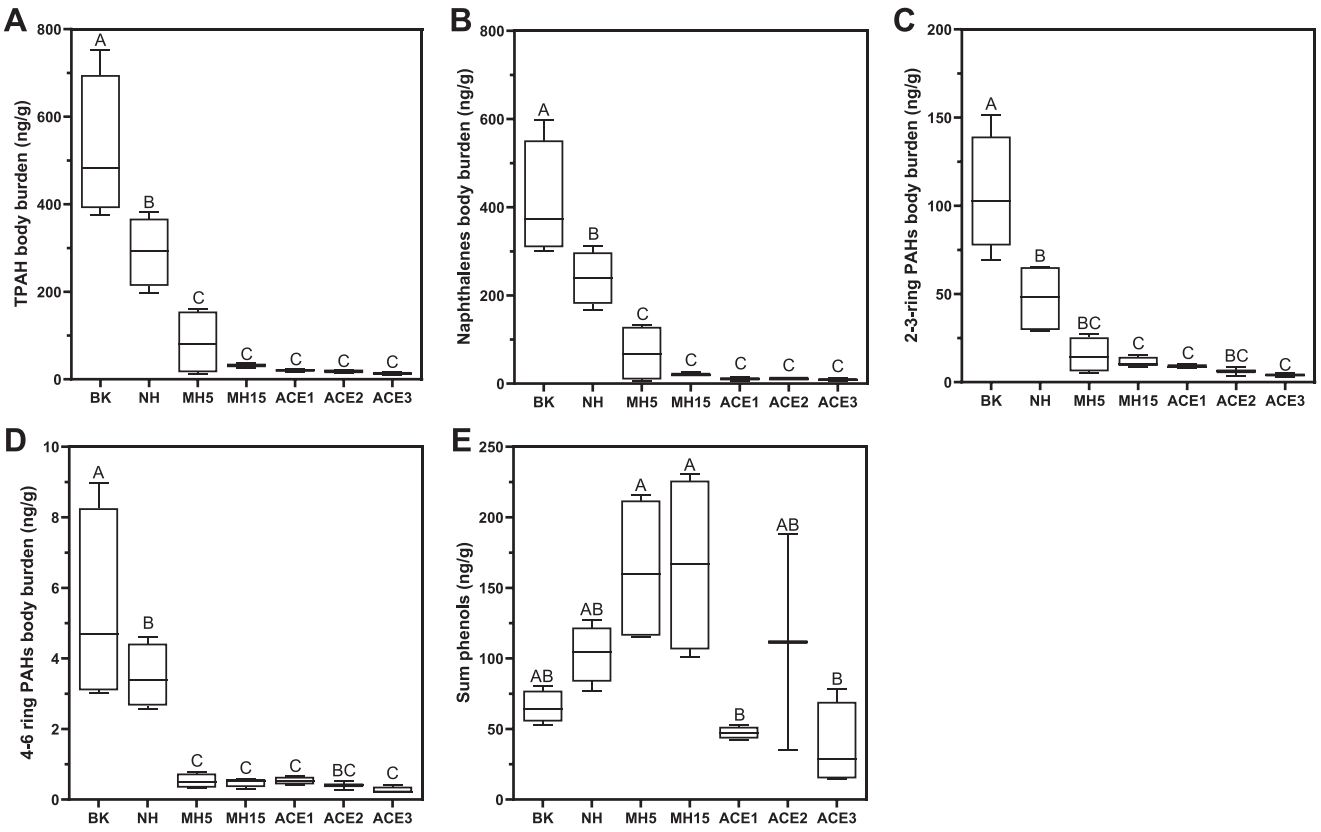

Fig. 5. Concentrations of PAHs and phenolic compounds in lumpfish eggs after exposure *in situ* at seven different stations. A: T-PAH as the sum of all 44 measured PAHs and alkylated homologues. B: Sum naphthalenes and C1-C4 homologues. C: Sum 2–3 ring PAHs and their alkylated homologues. D: Sum 4–6-ring PAHs and alkylated homologues. E: Sum phenolic compounds. Body burden data are presented as whiskers (median, 5% and 95% confidence intervals and min / max values). Groups displaying significant differences are denoted with different letters (N = 4), except ACE2 (N = 2). Please note different scaling on the axes.

was the dominating group. For 2–3-rings and for 4–6-rings, similar trends were observed, being significantly higher ($p < 0.05$) for BK and NH samples compared to the other stations.

The sum of phenolic compounds analyzed displayed a different pattern than observed for the PAHs (Fig. 5E), where the highest concentrations were found in the Trondheimsfjord MH5 (161.3 ± 52.3 ng/g) and MH15 (165.3 ± 64.0 ng/g) stations. The lowest concentrations were observed in ACE1 (47.2 ± 4.4 ng/g), ACE3 (37.4 ± 29.8 ng/g) and BK (65.5 ± 11.6 ng/g). Of the 30 phenolic compounds analyzed, the most abundant was phenol which was detected at highest concentrations in MH5 (72.6 ± 25.8 ng/g) and lowest in BK (20.6 ± 3.7 ng/g). Three of the targeted phenolic compounds analyzed have known endocrine disrupting capacity: Bisphenol A (BPA), 4-*n*-octylphenol and 4-*n*-nonylphenol.

BPA was detected above LOD in all samples in BK station (min-max: 6.2–15.0 ng/g), three samples from NH (min-max: 8.2–11.3 ng/g) and in one sample from MH15 (9.1 ng/g). 4-*n*-Nonylphenol was only detected in eggs from two replicates in station NH (11.1 – 14.5 ng/g), and 4-*n*-octylphenol was detected in one sample from BK (24.7 ng/g) and two samples from NH (19.9–21.8 ng/g). 13 phenolic compounds (4-*n*-butylphenol, 4-isopropyl-3-methylphenol, 2-*tert*-butyl-4-methylphenol, 4-*n*-pentylphenol, 4-*tert*-butyl-2-methylphenol, 4-*n*-hexylphenol, 4-*n*-heptylphenol, 4-(1-ethyl-1-methylpropyl)− 2-methylphenol, 2,4-di-*sec*-butylphenol, 2,6-di-*tert*-butylphenol, 4-*n*-nonylphenol and 2-methyl-4-*tert*-octylphenol) were not detected above LOD in any samples. See Supporting Information (Table SI 1) for full details.

3.3. Lumpfish hatching and survival

Eggs incubated in Trondheimsfjorden hatched earlier compared to those incubated at ACE (Fig. 6). The eggs from BK, NH and MH5 hatched 31 dpf, whereas MH15 hatched one day before (30 dpf). All groups exposed at the ACE stations hatched 34 dpf. This was likely correlated to lower ambient seawater temperatures at the ACE locations. Measured in degree-days (d° = temperature (°C) x days) the eggs incubated at ACE hatched after 285 day-degrees, whereas hatch age for the eggs incubated in Trondheimsfjorden ranged 292–301 day-degrees (Fig. 6C). Hatching success was high (> 85%) for all groups (Fig. 6B), with BK displaying significantly higher hatching success ($p < 0.05$) than ACE1 and ACE3. No significant differences ($p > 0.05$) in hatching success were shown for any of the remaining groups.

3.4. Lipid peroxidation

Lipid peroxidation (LPO) concentrations were measured in eggs and larvae sampled after incubation in the seven different field locations. LPO levels were generally higher in eggs than in larvae, and some significant differences were observed between lumpfish incubated at different locations (Fig. 7). For eggs, highest levels were observed in the ACE2 location. For the remaining locations, no differences were observed, suggesting no impact on eggs being incubated in the polluted harbours. Interestingly, in larvae, the lowest concentration was observed in MH5 and highest observed in MH15. No clear pollution-induced responses in LPO concentrations in eggs and larvae were observed.

3.5. Larvae morphometry

Morphometric measurement data of larvae (1 day post hatch) incubated at the different stations is shown in Fig. 8. No differences were observed between groups in terms of standard length and ventral body area. MH15 group displayed significantly ($p < 0.05$) larger body area (5.4 ± 0.2 mm^2) compared to all other groups, except ACE1 (5.2 ± 0.2 mm^2). Further, side and ventral yolk areas and ventral lipid areas were smaller in MH15 larvae (Side yolk area: 0.9 ± 0.05 mm^2. Ventral yolk area: 1.7 ± 0.1 mm^2) compared to several of the other groups ($p < 0.05$).

4. Discussion

4.1. Lumpfish eggs for field application

This work represents one of the first field applications of fish eggs for environmental monitoring and the first study using lumpfish. Considering the high survival and hatching success, our method, which included *in vitro* fertilization, molding of CEML and transferring and keeping lumpfish eggs in a field location for over two weeks, was successful. The eggs were kept alive within a very confined space with good exchange with surrounding water, and they developed under environmental conditions. Very little biofouling was observed on the eggs during field application, except from the MH15 group, where most eggs were overgrown by algae sporophytes. This may have impacted hatching success and even some morphological traits in the larvae, which was

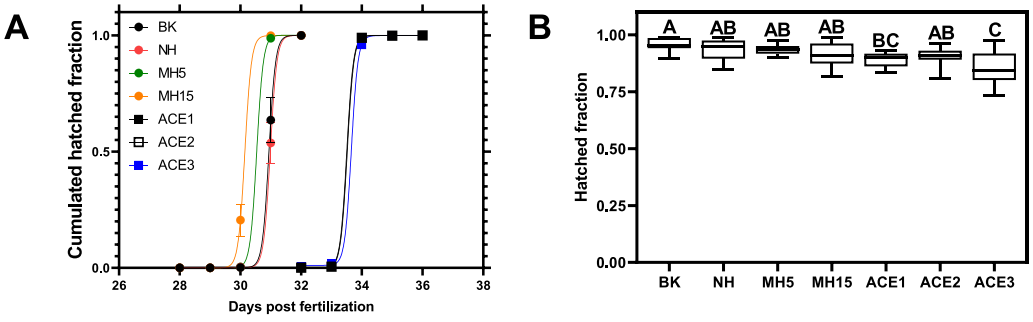

Fig. 6. A: Cumulative hatching of lumpfish eggs over time (days post fertilization, dpf). B: Hatching success, displayed as hatched fraction of lumpfish eggs incubated at seven different stations. Data are given as whiskers (median with 5/95% quartiles and min/max values) and groups displaying significant differences are denoted with different letters (N = 16–36). C: Mean temperatures and number of days lumpfish eggs spent in the laboratory and field location with estimated 50% hatch timing for the different groups. Hatch days are given as days post fertilization (dpf) as well as day-degrees (d°).

C

	Mean temperature (°C)		Number of days			50% hatch age	
Location	Field	Lab	Pre-field	Field	Post field till hatch	DPF	d°
BK	9.89	9.5	1	17	13	31	301
NH	9.9	9.5	1	17	13	31	301
MH5	9.6	9.5	1	17	13	31	292
MH15	10.1	9.5	1	17	12	30	297
ACE1	7.74	9.5	1	19	14	34	285
ACE2	7.74	9.5	1	19	14	34	285
ACE3	7.69	9.5	1	19	14	34	285

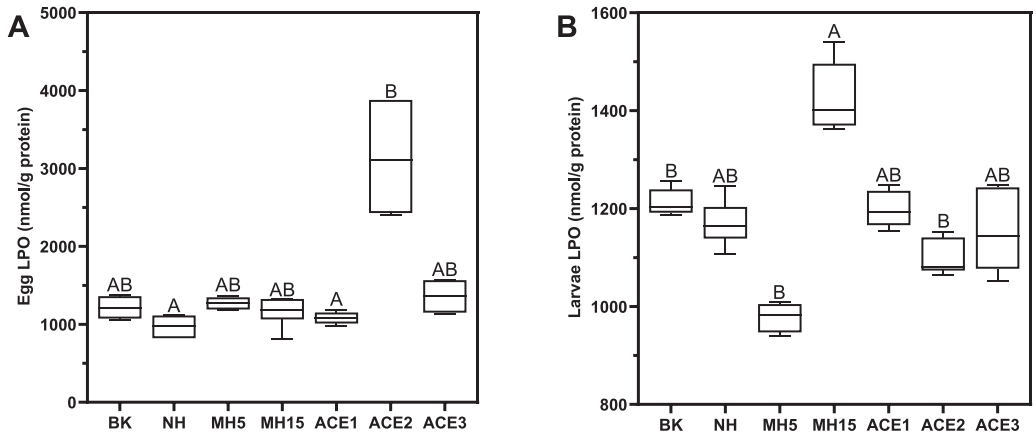

Fig. 7. LPO concentrations in lumpfish eggs (A) and larvae (B) incubated at seven different locations. Data are given as whiskers (median with 5/96% quartiles and min/max values) and groups displaying significant differences (Kruskal-Wallis test, $p < 0.05$) are denoted with different letters (N = 6 throughout).

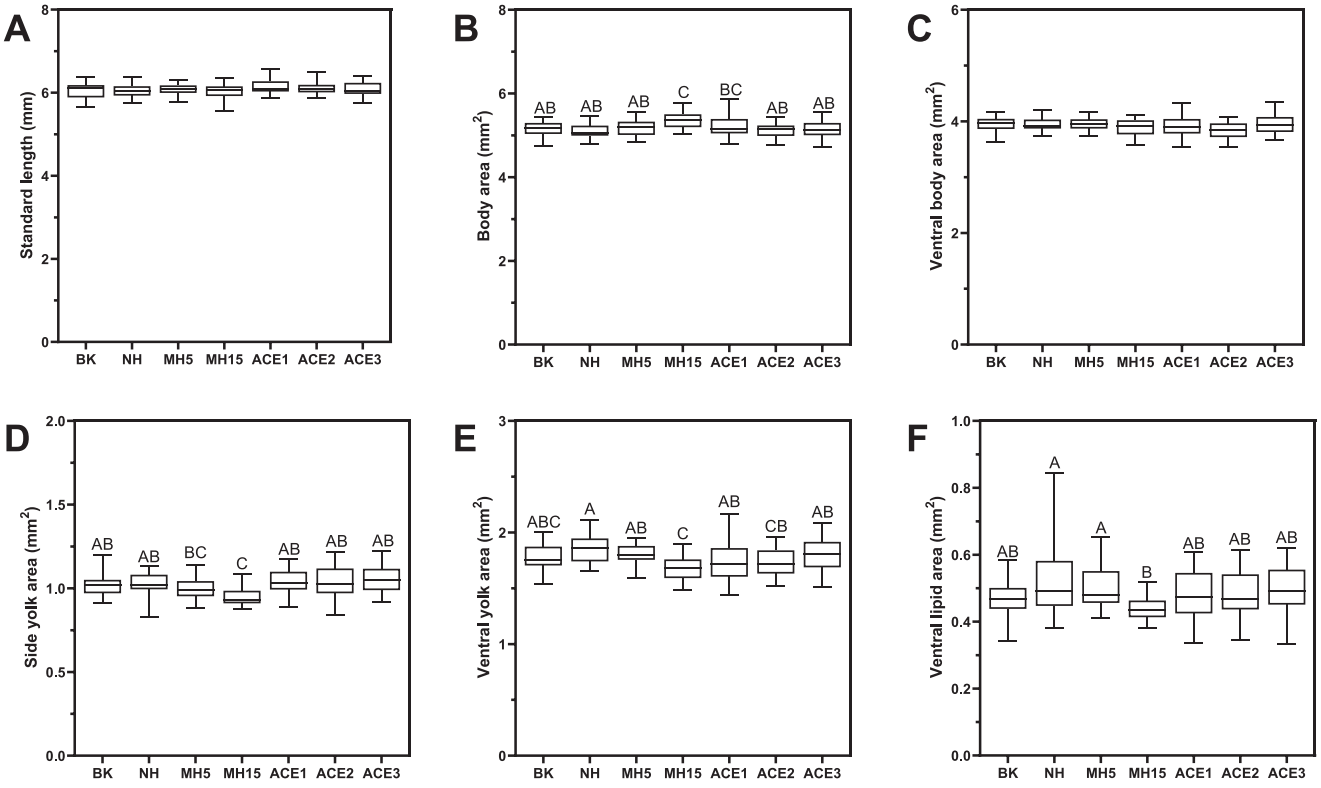

Fig. 8. Morphometric data on lumpfish larvae (2 days post fertilization) incubated for 17 (BK, NH, MH5 and MH15) or 19 (ACE1, ACE2 and ACE3) days in the field followed by incubation in the laboratory until hatch. Data are given as whiskers (median with 5/95% quartiles and min/max values) and groups displaying significant differences are denoted with different letters (N = 24).

found to differ at this station compared to the other locations (see below).

Male lumpfish, who usually guard eggs in the environment, use their tail to fan eggs right after fertilization and just prior to hatching. This activity removes ammonia and increases oxygen tension (Davenport, 1985), but for the most part of embryonic development, lumpfish eggs are relatively tolerant to low oxygen tension (Davenport, 1983). Although oxygen uptake was not measured in field-deployed eggs in this study, high survival suggests that oxygen tension was sufficient for the monolayers of eggs. Fertilized lumpfish eggs develop over a period of up to 300 dd (Imsland et al., 2019), and can thus be incubated in the environment for monitoring purposes for several weeks, taking ambient temperatures into consideration. In this study we utilized a field period of 17–19 days with ambient temperatures of 7.7–10.1 °C, before they were transferred back to the laboratory for an additional 12–14 days (at 9.5 °C) incubation until hatch (30–34 dpf, 285–301 dd). This is comparable with findings by Imsland et al. (2019), where eggs incubated at 10 °C (in the laboratory) hatched after 28 dpf (279 dd). In the same study, eggs were also incubated at 4–6 °C, which lead to an onset of hatching at 63 dpf (285 dd). Thus, the eggs could have been kept in the field for a longer time period in our study. However, it would not be advisable to extend the field incubation period to hatch, as the housing utilized and described in the present work is small (designed to fit approximately 80 eggs) and will thus have limited space for (active) larvae. Further, the availability of food for the larvae will inevitably be inadequate behind the mesh used to hold the fish eggs.

The long incubation time is typical for demersal eggs, like lumpfish eggs, in comparison to pelagic eggs, like cod and haddock. Demersal fish eggs typically have a thicker and more complex chorion which makes them more resistant to mechanical damage (Lønning et al., 1988). This is an advantage for field-deployment applications but may also reduce uptake of contaminants in embryos. On the other hand, lumpfish eggs are sticky for a short period of time after fertilization, and adhesion of particles (*e.g.*, oil droplets) can therefore be expected. Differences in chorion properties of eggs between marine fish species have previously shown to cause marked differences in adhesion of crude oil droplets facilitating uptake of PAHs in embryos (Hansen et al., 2018; Sørensen et al., 2019). This is discussed further below.

High lipid content facilitates bioaccumulation of organic hydrophobic contaminants (Spacie and Hamelink, 1982; Van der Oost et al., 2003). Particularly, elimination rates are low when lipid content in exposed organisms is high (Øverjordet et al., 2018). Demersal eggs contain heterogenous yolk, that partly consist of oil droplets, and therefore have a high lipid content (Lønning et al., 1988). Lipid content in fresh lumpfish roe (oocytes) has been reported to be 4.3–6.1% (wet weight) (Basby et al., 1998; Vasconi et al., 2020). Lipid content measured in unfertilized egg (oocytes) batches used in the current experiments was 3.5% (N = 1, eggs used for BK, NH, MH5 and MH15) and 3.7% (N = 1, ACE locations). As lipid content was not measured in the same samples as contaminants, lipid-adjusted body burden concentrations cannot be reported accurately.

4.2. Contaminant accumulation

Fish eggs incubated in the two harbour areas (BK and NH) accumulated higher concentrations of PAHs (Fig. 5A). This was not unexpected as these two harbour areas have been industry-heavy areas and are still important for maritime transportation as well as land-based industries. Both areas have been remediated (ended in 2016) by dredging and covered with crushed limestone. Our results show that there is still PAH contamination in the harbours, and it may be suggested that this is primarily from ferry/boat traffic or from creosote-treated piling (finalized in 2012), and not from sediment leakage. The highest T-PAH concentration observed was in one of the BK replicates, where T-PAH levels were 752 ng/g. In a multi-year study by West et al. (2019) where herring eggs were exposed in the Seattle harbour near creosote-treated piles, T-PAH concentration in herring eggs ranged between 15 and 2300 ng/g, and levels were 90 times greater than a reference area. Our reported levels are also in good agreement with those reported in a recent review by Wallace et al. (2020), in which the authors presented PAH content of 1–5171 ng/g in biota in the Canadian environment. The highest levels (TPAH) were detected in adult fish copper redhorse (*Moxostoma hubbsi*) in Richelieu River in Canada (4000 ng/g) (de Lafontaine et al., 2002) and populations of multixenobiotic-resistant killifish (*Fundulus heteroclitus*) from the Sydney Tar Ponds, Nova Scotia, Canada (457–5171 ng/g). However, a direct comparison to values reported in literature is often challenging as the number of PAHs included in T-PAH analyses varies between studies, with most studies reporting less than in this work (44 components). Fish residing in sites along the Athabasca River close to oil sands areas in Canada ranged between 57 and 510 ng/g, and the composition was dominated by C0-C3-naphthalenes and C4-phenanthrenes (Evans et al., 2019), in line with results in our study on lumpfish embryos (Fig. 5 and Table SI.1). Fish T-PAH levels reported by AMAP (2010) were 72 ng/g for parent PAHs and 425 ng/g for alkylated T-PAHs, which is also comparable to levels observed in the lumpfish in our experiments. Compared to juvenile lumpfish exposed to crude oil dispersions (nominal exposure range: 30–710 mg crude oil/L), reported by Frantzen et al. (2015), T-PAH body burden in the field-exposed lumpfish eggs were very low. Exposure of juvenile lumpfish to crude oil resulted in T-PAH body burdens ranging 2284–82656 ng/g.

Several laboratory studies have investigated toxicity of crude oil to Atlantic haddock, and most of these studies were utilizing dispersed oil exposure (Lie et al., 2019; Sørensen et al., 2017; Sørhus et al., 2021, 2015, 2017, 2016). As haddock appears to have a sticky chorion surface for oil droplets facilitating adhesion (Hansen et al., 2018; Sørensen et al., 2017; Sørhus et al., 2015), the body burden levels include both PAHs associated with oil droplets on the chorion surface, as well as PAHs actually penetrating the chorion (Sørensen et al., 2019). Exposure to filtered oil dispersions caused lower body burdens than exposure to unfiltered dispersions suggesting oil droplet adhesion to be a significant contributor to body burden levels (Hansen et al., 2019a; Sørhus et al., 2021). This was further evidenced for haddock as dispersion exposures of eggs at the surface (containing oil sheen) caused higher PAH body burdens (up to 7800 ng/g T-PAH) than exposure sub-surface (droplet exposure only) (Sørhus et al., 2021). Sub-surface incubation of lumpfish eggs in the BK harbour area might have caused exposure to fuel oil droplets as this area is the ferry center for the city of Trondheim, and oil sheen is from time to time observed on the surface of the water. Lumpfish eggs are very sticky for a few hours after fertilization, but their adhesion capacity for oil droplets is unknown. Thus, it is not known to what extent oil droplets may have facilitated higher TPAH body burdens for this group. Nevertheless, the body burden levels observed in lumpfish eggs in the BK station were in the low range of what has been found in the haddock studies where toxicity was observed.

Importantly, TPAH concentrations reported from field-sampled fish are usually from juvenile and/or adult fish, not embryos. This is of significance, as embryos and juveniles/adults differ from embryos in their ability to metabolize PAHs. When and if lumpfish embryos can metabolize PAHs through induction of *cyp1a* is currently not known. Another issue with measuring PAH levels in wild-caught fish is that the long-term exposure history is not known, whereas for the lumpfish eggs used in the current study, exposure time and environmental conditions are well defined.

Compared to PAHs, body burden data on phenolic compounds in fish exposed in the natural environment are scarce, and primarily focusing on phenols that are considered as endocrine disruptors (EDCs; 4-NP, 4-*t*-OP and BPA). For most of our samples, concentrations of these components in lumpfish eggs were below LOD. BPA was, however detected in all samples in BK and ¾ of the samples in NH. The concentrations of 4-NP, 4-*t*-OP and BPA in lumpfish eggs were low compared to those reported in juvenile and adult wild fish (8 different freshwater species) from the Pearl River system in China (Lv et al., 2019). Lv and co-authors report that BPA, 4-*t*-OP and 4-NP concentrations vary between tissues, with highest levels observed in bile, ranging from 0 to 13070, 35.9–2625 and 3216–27420 ng/g wet weight for BPA, 4-*t*-OP and NP, respectively. Lowest concentrations were found in muscle tissues with BPA, 4-*t*-OP and NP concentrations ranging 0.70–2053, 0–6.98 and 9.54–329 ng/g wet weight, respectively (Lv et al., 2019). In muscle tissue of fish from Dianchi Lake, China, 4-*t*-OP, 4-cumylphenol, NP and BPA were detected with maximal concentrations of 4.6, 4.4, 18.9 and 83.5 ng/g dry weight (dw), respectively (Liu et al., 2011). In a controlled laboratory experiment, phenol exposure to fish (adult Oreochromis niloticus) through a phenol-containing diet (0.7–2.8 μg/g; 16 weeks) caused phenol accumulation in muscles, gills and liver, with highest concentration observed in liver (18 000 ± 920 ng/g) (Gad and Saad, 2008). These concentrations are around three orders of magnitude higher compared to concentrations found in the caged eggs in this study. Differences in uptake and accumulation can be attributed to different exposure concentrations or uptake-modes. Interestingly, levels of phenolic compounds in eggs did not seem to be related to PAH levels suggesting different sources. Two municipal wastewater treatment plants are located around Trondheim and could be a potential source of phenolic compounds in the MH stations which were higher (although not significant) than the two harbour stations.

4.3. Ecotoxicity

Analyses of images taken of egg monolayers upon return from field to the laboratory revealed that the fertilization rate was lower for the eggs deployed at ACE than the eggs deployed in Trondheimsfjorden. This difference is likely not related to the exposure, but rather to the use of two different egg batches for the surveys. No significant differences in fertilization success were observed between groups in Trondheimsfjorden or between groups from the ACE location (Fig. 4B). The use of cryopreserved milt, using milt from the same male to fertilize all eggs from both egg batches, removes any implications of paternal effect on fertilization and hatching success. However, the fertilization success of on average 97.4% and 88% for the two batches used in our study is higher than reported for lumpfish eggs fertilized with cryopreserved milt in the literature (Imsland et al., 2021).

The fraction of the embryos that were normally developed were significantly lower in two locations, BK and MH15. Deformed embryos were characterized as smaller and less developed than the other eggs (example given in Fig. 4A). The BK station also displayed the highest bioaccumulation of PAHs, indicating that there may be a relationship between exposure and embryonic deformations. For the MH15 group, chorion biofouling (and/or lower light intensity) may also have contributed to an increase in embryonic deformations, as the eggs incubated 10 m above (MH5) had a significantly lower fraction of deformed embryos. Even with deviating incidences of embryo deformations between the different stations, hatching success was high throughout (>85% for all locations), with the ACE3 site (85 ± 7%) being the only group displaying significantly lower hatching success than the other groups. Differences in hatch timing can be attributed to differences in temperature between locations, and maybe somewhat dependent on the egg batch, since the calculated developmental time in day-degrees differ slightly between the two batches (ACE and Trondheimsfjorden). Furthermore, only minor morphological differences were observed in larvae deployed as embryos at the different locations (Fig. 7). In one of the few exposure studies reported on lumpfish, Frantzen et al. (2015) observed high mortality in juvenile lumpfish exposed to the highest concentration of oil (710 mg/L, nominal). In contrast, they found no signs of stress or toxicity in lower exposures (30 mg/L, nominal), where PAH body burden (muscle tissue) levels were lower (approximately 20,000 ng/g) (Frantzen et al., 2015). In contrast, the BK group in our study, which had accumulated 522.9 ± 164.6 ng/g TPAH and 56.0 ± 11.6 ng/g phenolic compounds, displayed significantly increased incidence of deformed embryos compared to most other groups (except MH15).

Increased production of reactive oxygen species (ROS) due to contaminant exposure can overwhelm the antioxidant capacity of cells and consequently cause oxidative damage in DNA, proteins and lipids. One of the most common examples of biochemical and physiological damages associated with oxidative stress is lipid peroxidation (LPO). The formation of lipid peroxides is characterized by the presence of the by-products malondialdehyde (MDA) and 4-hydroxyalkenals (4-HNE) and is considered one of the essential endpoints when studying oxidative stress (Livingstone, 2001).

In developing fish, lipid reserves are generally characterized by the presence of polyunsaturated fatty acids that are highly susceptible to oxidative stress and consequently LPO. LPO concentrations were measured in eggs and larvae sampled after incubation in the seven different field locations. LPO levels were generally higher in eggs than in larvae, consistent with a higher lipid content, and some significant differences were observed between lumpfish incubated at different locations (Fig. 7). For eggs, highest concentrations were observed in the ACE2 location. For the remaining locations, no differences were observed, suggesting no impact on eggs being incubated in the polluted harbours. Interestingly, lowest concentration in larvae was observed in MH5 and highest observed in MH15, with the latter possibly explained by the increased chorion biofouling, as seen for hatching success and embryonic development. Oxidative stress in fish is known to originate not only oxidative damage in DNA, proteins and lipids but also lead to decreased growth rate and survival, as well as increased deformities and muscular lesions (Betancor et al., 2012). No clear pollution-induced responses in LPO concentrations were observed, even though a different pattern was detected for eggs and larvae. This can possibly be explained by a differential triggering of the antioxidant defense system of eggs and larvae to protect against oxidative stress. Very few studies have evaluated the protective role of antioxidant defenses (enzymatic and non-enzymatic) against ROS formation between embryonic and larval stages. For example, in *Salmo trutta* and *S. salar*, an increase in antioxidant enzymatic activities was detected from fertilized eggs to hatched larvae, whereas the levels of antioxidant molecules decreased (Arslan et al., 2016; Cowey et al., 1985). Nonetheless, the antioxidant capacity of early developmental stages of lumpfish is not well known and additional studies are needed to evaluate the shifts and efficiency of antioxidant defense system of eggs and larvae, and its correlation to oxidative stress, hatching success and embryonic development.

Compared to pelagic fish larvae, demersal fish like lumpfish, are more developed at hatch (Lönning et al., 1984; Lønning et al., 1988) and their larvae are more pigmented and thus less transparent. This makes them less suitable for assessing cardiotoxic (pericardial edema, poor looping) and other developmental deformations (jaw and craniofacial deformations), known to be caused by embryonic PAH exposure (Incardona et al., 2015, 2004). Several studies with Atlantic haddock indicate that T-PAH body burden correlates well to ecotoxicological parameters (Cresci et al., 2020; Lie et al., 2019; Sørensen et al., 2017; Sørhus et al., 2021, 2015, 2017, 2016). Haddock exposed to oil dispersions (80 μg oil/L) as eggs, reaching TPAH body burden of 330 ± 124 ng/g, displayed 75-fold increase in *cyp1a* expression and significantly lower swimming speed in larvae (Cresci et al., 2020). A 7-day embryonic exposure of haddock to dispersed oil (600 μg oil/L) resulted in TPAH body burdens of 2961 ± 1258 ng/g associated with severe cases of craniofacial deformations and cardiac abnormalities. A pulsed exposure caused lower uptake (218 ± 64 ng/g) and moderate deformation phenotypes (Sørhus et al., 2016). Pelagic cold-water species, like cod and haddock, are considered a highly sensitive to organic contaminants (Hansen et al., 2021; Sørhus et al., 2015). A comparative study using 2-methylnaphthalene exposures on fish eggs on a range of different species ranked lumpfish as the least sensitive species, followed by capelin (*Mallosus villosus*), plaice (*Pleuronectes platessa*), flounder (*Platichthys flesus*), long rough dab (*Hippoglossoides platessoides*), and cod (Stene and Lønning, 1984).

5. Concluding remarks and recommendations

Our study, which included different types of locations in terms of contaminant load, depths and weather conditions, showed that lumpfish eggs are relatively robust and survive encaging for several weeks in different target environments. The eggs further accumulated organic contaminants, like PAHs and phenolic compounds, and can thus be suitable for biomonitoring purposes. The ecotoxicological parameters studied here included embryonic deformations, hatching success and larvae morphology, and, except for higher indices of embryonic deformations in two of the stations, little evidence of toxicity was observed. This could be related to relatively low contaminant concentrations in the chosen locations. More sensitive and in-depth toxicological parameters should be included to evaluate potential effects, *e.g.*, *cyp1a* transcription and more detailed investigations on the antioxidant defense system of eggs and larvae and developmental impacts. Importantly also, long-term impacts of the environmental exposure were not addressed in the current work (terminated 2 dph) and should therefore be considered in future studies. Nevertheless, the *in situ* exposure method has been proven to work well, making it attractive for implementations in monitoring programs, *e.g.* the offshore oil and gas water column monitoring program in Norway. Bioaccumulation data on PAHs

and phenolic compounds from produced water discharges could contribute to understand the potential for fish ELS toxicity related to these discharges as well as validating of risk assessment modelling tools, like DREAM (Nepstad et al., 2021).

CRediT authorship contribution statement

Bjørn Henrik Hansen: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Writing - original draft; Writing - review & editing, **Trond Nordtug**: Conceptualization; Data curation; Validation; Writing - original draft; Writing - review & editing, **Ida Beathe Øverjordet**: Conceptualization; Methodology, **Lisbet Sørensen**: Data curation; Validation; Writing - original draft; Writing - review & editing, **Bjarne Kvæstad**: Methodology; Software; Data curation; Writing - review & editing, **Emlyn John Davies**: Methodology; Writing - review & editing, **Sonnich Meier**: Supervision; Writing - review & editing, **Tania Cristina Gomes:** Data curation; Formal analysis; Writing - review & editing, **Steven Brooks:** Data curation; Formal analysis; Writing - review & editing, **Julia Farkas**: Conceptualization; Data curation; Formal analysis; Supervision; Validation; Writing - original draft; Writing - review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Bjorn Henrik Hansen reports financial support was provided by SINTEF Ocean.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2022.114074.

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