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Pikeperch *Sander lucioperca* egg quality cannot be predicted by total antioxidant capacity and mtDNA fragmentation

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ABSTRACT

In farmed pikeperch, there is a high variability in egg quality restraining the propagation of this species in aquaculture. The identification of reliable biomarkers for predicting successful embryo development already at an early stage (unfertilized oocyte) could help improve production efficiency. Total antioxidant capacity (TAC) and the quantification of mitochondrial DNA (mtDNA) fragmentation have been established as biomarkers for oxidative stress and damage of macromolecules, potentially influencing embryo development. Therefore, we evaluated these biomarkers in eggs of commercially farmed pikeperch (44 females). We measured egg TAC, as well as lesion rates per 10 kb of 12S and cytochrome b (cytb) as target regions within the mitochondrial genome by qPCR. It was tested whether these markers correlate with embryo development (fertilization rate, embryo survival, hatching rate). There was no significant relation of mtDNA lesion rates or TAC with these egg quality parameters. We detected average lesion rates (±SD) of 1.50 (±1.57) and 1.89 (±2.14) in 12S and cytb mtDNA respectively. Lesion rates in 12S and cytb were highly correlated within samples ($P < 0.0001$) and were independent of the observed TAC. The results suggest that TAC does not prevent mtDNA fragmentation and that embryos rather seem to be able to cope with the observed fragmentation of mtDNA. However, in post-ovulatory aged eggs of three females with little to no fertilization success, lesion rates of cytb were significantly elevated, whereas TAC was significantly lower compared to other females, suggesting a possible role of oxidative stress during post-ovulatory ageing.

1. Introduction

Aquaculture is one of the fastest growing sectors of food production worldwide (FAO, 2014). To increase species diversification in this sector, new candidate fish species are introduced to farming to meet market demands. However, to overcome the candidate status of new species towards domestication, the reliable production of high quality gametes is a major prerequisite (Migaud et al., 2013). Only the constant availability of stocking material allows for an up-scaling of production. In intensive aquaculture, reproduction of fish can be achieved e.g., through photothermal induction of maturation with or without the use of stimulating hormones (cf., Donaldson, 1996; Mylonas et al., 2010 for review). As in wild fish, such controlled induction of reproduction results in strong

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inter-individual variability of gamete quality (Brooks et al., 1997; Bobe and Labbe, 2010). As a consequence, production costs remain on a high level, because broodstock and hatchery capacities need to be large enough to cope with losses caused by low biological quality. Accordingly, the identification of female and/or batch-specific biomarkers, which can reliably predict future developmental success of offspring remains a major task (Bobe and Labbe, 2010; Migaud et al., 2013). Understanding the underlying biochemical and molecular drivers of gamete quality can potentially improve reproductive performance and hence optimize the reproductive management of species in aquaculture (e.g., via food additives or gamete handling procedures).

Oxidative stress, the imbalance of reactive oxygen species (ROS) and inherent defense mechanisms, has been identified as potential cause affecting cell functioning and early embryo development (Aitken and Baker, 2004; Dennery, 2007; Metcalfe and Alonso-Alvarez, 2010) and analyses dealing with this fragile balance could be used as biomarkers of gamete quality. Oxidative stress can cause damage of essential genetic information, such as DNA strand breaks and base modifications especially in mitochondria, which exhibit high chronic ROS exposure (Yakes and VanHouten, 1997; Rothfuss et al., 2010). Therefore, an egg with impaired antioxidant status is likely to exhibit abnormal development. There are two hierarchical levels affecting oxidative stress where the application of biomarkers seems most useful: the inherent defense mechanisms against oxidative stress and the damage caused as consequence of oxidative stress.

The mitochondrial DNA (mtDNA) is an ideal target for detecting ROS-induced damage in eggs. First, mtDNA is presumably more prone to damage compared to nuclear DNA (Carton-Garcia et al., 2013; Sawyer et al., 2003; Yakes and VanHouten, 1997). Second, in most cases the entirety of mitochondria is inherited maternally, so paternal influences can – to a large extent – be excluded (Schwartz and Vissing, 2002; Wolff and Gemmell, 2008) resulting in maternal (egg) specific markers. In addition, the mitochondrial genome is present in a high copy number in each fish oocyte, which allows for a representative assessment (Artuso et al., 2012). However, the majority of methods for detecting DNA fragmentation (e.g., comet assays) are relatively unspecific, only partially quantitative and do not allow a separated analysis of mtDNA and nDNA damage (Tice et al., 2000). A new sensitive method to quantify DNA lesion rates has been recently developed (Rothfuss et al., 2010). The method utilizes the quantification of a short and a long fragment of a specific target region by qPCR for the quantification of DNA lesions (Rothfuss et al., 2010). This method has successfully been applied to detect DNA lesions caused by cryopreservation of zebrafish *Danyo rerio* primordial germ cells (Riesco and Robles, 2012) and gilt-head bream *Sparus aurata* sperm (Carton-Garcia et al., 2013). However, the method has not yet been applied for the use as a biomarker for the assessment of egg quality in fish and knowledge about the physiological consequences of mtDNA lesions remains vague.

The determination of total antioxidant capacity (TAC) has previously been used as proxy for sperm quality (Mahfouz et al., 2009; Gurler et al., 2015) and is generally regarded as potent marker of oxidative stress (Ghiselli et al., 2000; Kusano and Ferrari, 2008), but to our best knowledge, it has not yet been used as biomarker for egg quality in fish. Measurement of TAC can potentially be a valuable determinant of egg quality, since several studies have shown that certain antioxidants, such as α-tocopherol and vitamin C, are beneficial, if not crucial for embryonic development in fish (cf., review by Izquierdo et al., 2001).

The main aim of the present study was the evaluation of the applicability of mtDNA damage and TAC as biomarkers for the prediction of egg quality in pikeperch *Sander lucioperca* under hatchery conditions. Over the course of two consecutive years, a total of 44 unfertilized egg batches from pikeperch broodstock were sampled on a commercial farm and fertilization, survival of embryos (after 24, 42 and 72 h) and hatching rate were assessed as estimates of egg quality. In addition to TAC, lesion rates in two target regions of the mitochondrial genome encoding for 12S and cytochrome b (cytb) were determined by qPCR. We hypothesized that elevated TAC prevents mtDNA lesions. Further, we expected high numbers of mtDNA lesions to negatively influence embryo development, whereas elevated TAC levels are expected to be beneficial. This study contributes to understanding the consequential effects of oxidative stress and inherent protection through antioxidants during embryogenesis. By identifying inter-individual differences in these parameters, broodstock composition as well as reproductive management may be optimized in commercial aquaculture of pikeperch.

2. Materials and methods

2.1. Sampling

Ovulated eggs were collected from a total of 44 female pikeperch over two consecutive years at a commercial aquaculture facility (AquaPri, Denmark). Broodstock maturation was induced by wintering below 14 °C for three months and subsequent warming to ~16 °C to trigger ovulation. No hormone treatment was used. Broodstock fish of both sexes were reared together.

At time of ovulation, female fish were anesthetized (Kalmagin 20%, Centrovet, Santiago, Chile) and eggs were stripped. A subset of eggs from each of the females was immediately frozen at −20 °C and transported to the IGB Berlin in liquid nitrogen for further storage at −80 °C. For the majority of females (n = 33), the remaining eggs were fertilized with freshly stripped sperm and transferred into Zuger-jars until hatching. A minimum of 50 eggs were considered at the designated time points in triplicates to determine fertilization rate (%), survival after 24, 48, 72 h (%) and hatching rate (%) on day four post fertilization by use of a microscope (Stereozoom IT-TR, Gundlach, Marburg, Germany). In some cases, not all five quality parameters could be assessed, due to rearing limitations or hatchery related operations.

2.2. DNA extraction and fragmentation standards

For each sample ~30 mg (wet weight) of eggs were extracted using a commercial kit (peqGOLD Tissue DNA