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Lipid content and fatty acid profile during lake whitefish embryonic development at different incubation temperatures

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ABSTRACT

Lipids serve as energy sources, structural components, and signaling molecules during fish embryonic development, and utilization of lipids may vary with temperature. Embryonic energy utilization under different temperatures is an important area of research in light of the changing global climate. Therefore, we examined percent lipid content and fatty acid profiles of lake whitefish (*Coregonus clupeaformis*) throughout embryonic development at three incubation temperatures. We sampled fertilized eggs and embryos at gastrulation, eyed and fin flutter stages following chronic incubation at temperatures of 1.8, 4.9 and 8.0 °C. Hatchlings were also sampled following incubation at temperatures of 3.3, 4.9 and 8.0 °C. Fertilized eggs had an initial high percentage of dry mass composed of lipid (percent lipid content; ~29%) consisting of ~20% saturated fatty acids (SFA), ~32% monounsaturated fatty acids (MUFA), ~44% polyunsaturated fatty acids (PUFA), and 4% unidentified. The most abundant fatty acids were 16:0, 16:1, 18:1(n-9c), 20:4(n-6), 20:5(n-3) and 22:6(n-3). This lipid profile matches that of other cold-water fish species. Percent lipid content increased during embryonic development, suggesting protein or other yolk components were preferentially used for energy. Total percentage of MUFA decreased during development, which indicated MUFA were the primary lipid catabolized for energy during embryonic development. Total percentage of PUFA increased during development, driven largely by an increase in 22:6(n-3). Temperature did not influence percent lipid content or percent MUFA at any development stage, and had inconsistent effects on percent SFA and percent PUFA during development. Thus, lake whitefish embryos appear to be highly adapted to low temperatures, and do not alter lipids in response to temperature within their natural incubation conditions.

1. Introduction

Fertilized fish eggs contain nearly all the required nutrients for embryonic development, with respiratory gases and metabolic heat being exchanged with the environment (Blaxter, 1969; Kamler 2008). Within the egg, lipids, proteins and carbohydrates are sources of energy for the developing embryo (Cetta and Capuzzo, 1982; Vetter et al., 1983). In addition to serving as energy storage and substrates for ATP production, lipids also act as signaling molecules and important components of membranes (Zechner et al., 2009; Patterson and Green, 2014). The initial amount of lipid contained in the egg varies between species, and species with lengthier incubation times tend to have higher initial percent lipid content (Kaitaranta and Ackman, 1981). Eggs that contain oil globules also have high percent lipid content (Eldridge et al., 1983; Jobling et al., 1995; Tocher, 2003), and while oil globules may aid buoyancy in some species, the high lipid content indicates an important role for nutrition.

The utilization of lipids during fish embryonic development, including the use of different lipid classes and fatty acids, varies between species, but some overall trends do exist. The phospholipid and neutral lipid rich yolk is high in polyunsaturated fatty acids (PUFA), while oil globule neutral lipids are high in monounsaturated fatty acids (MUFA) (Wiegand, 1996). The high level of PUFA following fertilization is often maintained or increased during development, while MUFA are preferentially utilized for energy (Tocher et al., 1985; Wiegand et al., 1991). Likewise, individual types of fatty acids may be preferentially

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used as energy sources while others are retained or incorporated into tissue. For example, 22:6(n-3), which is often the most abundant fatty acid, is retained during embryonic and larval development of Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), Atlantic herring (*Clupea harengus*), gilthead seabream (*Sparus aurata*) and goldfish (*Carassius auratus*) (Cowey et al., 1985; Tocher et al., 1985; Fraser et al., 1988; Mourente and Odriozola, 1990; Wiegand et al., 1991).

Investigating how lipid stores are used during the embryonic period is important for understanding and predicting how environmental conditions may alter developmental processes (Dabrowski and Luczynski, 1984). In addition, changing local or global environmental patterns may permanently or transiently alter energy use, storage or availability for fishes. Fish can selectively catabolize certain fatty acids, while retaining others (Hazel et al., 1987; Tocher, 2003). Temperature can differentially regulate these processes, with desaturation and elongation of fatty acids occurring during cold acclimation, resulting in an increase in PUFA and a decrease in SFA in adult fish (Miller et al., 1976; Hazel and Prosser, 1979; Bell et al., 1986; Brodte et al., 2008). Temperature can also alter survival, development rate and oxygen consumption rate of fish embryos (Mueller et al., 2011; Eme et al., 2015). Previous studies have shown that temperature affected yolk conversion efficiency and energetic cost of development in embryonic fishes (Hamor and Garside, 1977; Heming, 1982; Rombough, 1994; Mueller et al., 2011; Mueller et al., 2015). At 20 °C, sturgeon (*Acipenser transmontanus*) embryos have shown a lower lipid content compared to embryos at 11, 14 and 17 °C, and this suggests that yolk depletion occurs faster with increasing incubation temperature in *A. transmontanus*, embryos (Wang et al., 1987). Incorporation of PUFA into larval bodies is altered by rearing temperature in goldfish (Wiegand et al., 1991). Such studies suggest that temperature influences how endogenous energy stores, particularly the lipids contained in yolk and oil globules, are catabolized.

The purpose of this study was to examine how incubation temperature altered percent lipid content and fatty acid profile of lake whitefish (*Coregonus clupeaformis*) embryos throughout embryonic development. The North American lake whitefish is a commercial, cold-adapted species, producing eggs that develop over 60–150 days in winter months at temperatures of 0.5–8.0 °C (Brooke, 1975; Mitz et al., 2014; Mueller et al., 2015). The eggs of this species, like other coregonid fishes, contain lipid in both yolk and oil globule compartments (Dabrowski et al., 1984; Sutela and Huusko, 1997). The initial percent lipid content of lake whitefish eggs, how lipids may be utilized during embryonic development, and how lipid catabolism is altered by temperature are unknown. Recent studies have shown that incubation at different temperatures influences survival, whole embryo physiology, and energy use of lake whitefish (Eme et al., 2015; Lee et al., 2015; Mueller et al., 2015). In particular, an increase in temperature from 2 to 8 °C decreased yolk conversion efficiency and increased cost of development in lake whitefish embryos (Mueller et al., 2015). We hypothesized that weight percentage lipid content of lake whitefish eggs would decrease during embryonic development, indicative of lipid catabolism. We also hypothesized that at lower incubation temperatures, PUFA would be selectively retained and that this may be related to the maintenance of membrane integrity, i.e. homeoviscous adaptation, at near freezing temperatures.

2. Materials and methods

2.1. Embryo acquisition and rearing

Male ($N = 30$; 1.39 ± 0.04 kg, 49.6 ± 0.4 cm fork length) and female ($N = 18$; 1.51 ± 0.07 kg, 50.9 ± 0.7 cm fork length; ± SEM) lake whitefish were caught by gill net near the Fishing Islands of lake Huron adjacent to South Bruce Peninsula, Ontario, Canada on November 21, 2013 (44°42′37.74″N 81°18′38.94″W; lake water surface temperature = 7.0 °C; Ontario Ministry of Natural Resources Permit UGLMU2013–08.). Eggs (N ~ 120,000; ~3 mm diameter fertilized eggs) and milt were stripped from all individual fish caught ($N = 48$), pooled, and fertilized without water for 4 min. Fertilized eggs were promptly put into fresh lake water at a 50:50 ratio by volume of eggs:fresh lake water, exposed to 0.5% iodine for 30 min, then thoroughly rinsed three times with lake water (iodine exposure helped to reduce any possible fungal or bacterial 'contamination'). Eggs were transported to the laboratory in containers with 50:50 ratio of eggs:fresh lake water, on ice. Eggs were distributed into 3–6 McDonald Bell hatching jars at 1.8 ± 0.3 °C, 4.9 ± 0.1 °C and 8.0 ± 0.1 °C at a density of 4500–5500 per liter of clean dechlorinated tap water, and water was recirculated, filtered and UV sterilized within custom incubators (Mitz et al., 2014). A 50% water change was performed weekly and dead embryos were removed from the jars as necessary. Water quality (pH, ammonia, nitrates, and nitrites) were measured frequently, and levels were always within adequate levels.

Ten 15-ml tubes each containing ~100 fertilized eggs at 0 days post fertilization (dpf) were snap frozen in liquid nitrogen and stored at −80 °C. Embryos were monitored during development for the following stages (with dpf at which sampling occurred at each temperature indicated in parentheses): end of gastrulation (~15% development, 2 °C = 23dpf, 5 °C = 14dpf, 8 °C = 8dpf), eyed stage (~30% development, 2 °C = 39dpf, 5 °C = 25dpf, 8 °C = 18dpf), constant fin flutter (~60% development, 2 °C = 88dpf, 5 °C = 56dpf, 8 °C = 37dpf) and hatching (prior to exogenous feeding, 3.3 °C = 130dpf, 5 °C = 95dpf, 8 °C = 82dpf). These stages were chosen as they align with our previous studies examining physiological function of the embryos (Eme et al., 2015; Mueller et al., 2015). A small subset of eggs were removed from a bell jar, placed on ice and staged according to Price (1934a, 1934b, 1935) and Sreetharan et al. (2015) under a Zeiss AXIO Zoom V16 microscope (Carl Zeiss AG; Oberkochen, Germany). When embryos had reached the target stages, approximately 1000 eggs or 500 hatchlings were selected from four bell jars and transferred to ten 15 ml tubes (each tube contained embryos from a single bell jar) and snap frozen in liquid nitrogen. The frozen samples were then stored at −80 °C until lipid extraction. Due to a malfunctioning incubator, when 2 °C embryos reached constant fin flutter, incubation temperature was changed to 3.9 ± 0.3 °C until hatching. This resulted in an average incubation temperature throughout development of 3.3 ± 0.2 °C, and thus we refer to hatchlings from this group as being incubated at 3.3 °C throughout the manuscript.

2.2. Total lipid extraction

A sample of frozen eggs or hatchlings was freeze-dried (FreeZone™ 4.5 L Freeze Dry System, Labconco Corporation, Kansas City, MO, USA) overnight and lipids extracted by a modification of the method described by Folch et al. (1957). Each extraction sample consisted of ~50–100 eggs or ~25–50 hatchlings, depending on developmental stage. Freeze-dried samples (~50–90 mg) were crushed until all eggs/hatchlings were broken, and then the mass was determined in a pre-weighed eppendorf tube on an electronic balance (±0.01 mg, model XS205DU, Mettler Toldeo, Greifensee, Switzerland). Chloroform:methanol (2:1 *v*/v) solvent was added to the samples, which were placed in an ultrasonic cleaner (model FS30, Fisher Scientific, Pittsburgh, PA, USA) for 30 min and then spun in a microcentrifuge (model 5418, Eppendorf AG, Hambrug, Germany) at 7000 rpm for 5 min to separate the solvent containing lipids from other egg components. The solvent was removed and placed into a pre-weighed eppendorf tube. This extraction process was repeated three times per sample and the removed solvent containing lipids was combined. The solvent was evaporated in a Savant™ DNA120OP SpeedVac Concentrator (Thermo Electron Corporation, Milford, MA, USA) before total lipid mass was gravimetrically measured (±0.01 mg, balance XS205DU, Mettler Toldeo, Greifensee, Switzerland).