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Effects of temperature and food availability on liver fatty acid composition and plasma cortisol concentration in age-0 lake sturgeon: Support for homeoviscous adaptation

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ABSTRACT

Overwintering survival in north temperate fishes involves a series of adaptive responses to multiple environmental stressors. Homeoviscous adaptation includes changes in membrane lipid composition in response to reduced environmental temperature, which may be driven by changes in hormones involved in the endocrine stress response. We examined how reduced temperature and food availability may act in concert to influence hepatic fatty acid composition of phospholipids and triglycerides, in addition to plasma concentration of cortisol in age-0 lake sturgeon (*A. fulvescens*). At 153 days post hatch (dph), temperature was decreased from 16 °C to 1 °C at a rate of 0.5 °C per day, and at 200 dph, fish were either fed every other day or deprived of food for 45 days to simulate an overwintering event. Liver fatty acid composition of phospholipids and triglycerides were assessed before temperature manipulation (16 °C; 153 dph), when fish had been at 1 °C for 16 days (199 dph), 25 days of overwintering (225 dph) and 45 days of overwintering (245 dph). Plasma cortisol concentration was assessed at 153, 225 and 245 dph. When temperature was decreased, both mono- and polyunsaturated fatty acids significantly increased in phospholipids and triglycerides. Total omega-6 fatty acids significantly increased in phospholipids while total omega-3 fatty acids did not. During the simulated overwintering, there was no obvious difference in fatty acids of phospholipids and triglycerides between diet treatments and no difference in circulating cortisol concentration between baseline and post-stressed fish in the fasted group. Our results provide support for homeoviscous adaptation to cold temperatures in lake sturgeon.

1. Introduction

Physiological adaptation to changing environments is particularly important in young of the year fish as it strongly dictates the subsequent cohort and population dynamics (Johnson et al., 2014; Sogard, 1997). The significance of this concept is well illustrated in temperate and northern aquatic species as they approach the first winter of life (Fernandes and McMeans, 2019; Michaletz, 2010; Sogard and Olla, 2000). While physiological responses to cold environments have been reasonably well described (Donaldson et al., 2008), our knowledge is still lacking in terms of how extended periods of food deprivation, typical of a northern winter, can influence these responses. For example, scarcity in food during winter months may lead to β-oxidation of endogenous fatty acids to sustain vital cellular processes (Bar, 2014; Mommsen et al., 1999), however, this requirement often needs to be balanced with similar biochemical processes required for the synthesis of mono- and polyunsaturated fatty acids that play a key role in maintenance of membrane fluidity in response to cold temperatures (Homeoviscous adaptation; HVA) (Monroig et al., 2018).

In fishes, HVA has been well reported where exposure to colder environments results in an increase in monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) with concomitant decreases in saturated fatty acids (SATs) in both liver and muscle tissues (Hazel, 1995; Hsieh et al., 2003; Snyder et al., 2012; Wijekoon et al., 2021). Eicosapentaenoic acid (EPA, C20:5n3), arachidonic acid (AA, C20:4n6) and docosahexaenoic acid (DHA, C22:6n3) are long chain polyunsaturated fatty acids (LC-PUFAs) that are commonly found in phospholipids and thought to play a key role in HVA (Hazel, 1997;

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Parrish, 2009; Pernet et al., 2007; Sargent et al., 1993; Tocher and Sargent, 1990; Wallaert and Babin, 1994). During HVA, these LC-PUFAs could be supplied from dietary intake as fatty acids of triglycerides that are transferrable to phospholipids *via* acylation processes catalyzed by phospholipases, acyltransferases, and transacylases (Yamashita et al., 2014) or LC-PUFAs could be converted *in vivo* from either alpha-linolenic acid (C18:3n3) or linoleic acid (C18:2n6) through a series of desaturation, elongation and peroxisomal β-oxidation (Li et al., 2010; Monroig et al., 2018; Park et al., 2009; Sprecher, 2000; Tocher, 2010). Specifically, it has been shown that enzymatic activity levels of Δ 6 desaturase, a rate limiting enzyme for PUFA synthesis, increase in low temperature acclimated teleosts providing a mechanistic link between phospholipid restructuring and cold adaptation - HVA (Hagar and Hazel, 1985; Schünke and Wodtke, 1983; Tocher et al., 2004).

It is hypothesized that fish cannot synthesize C18:3n3 nor C18:2n6 due to a lack of Δ 12 and 15 desaturases (Monroig et al., 2018). Since *in vivo* production of LC-PUFAs is possible through substrate input of C18:3n3 and C18:2n6 from the diet (see Fig. 1), reduced access to food sources during winter months may limit the capacity for appropriate cold adaptation. Food deprived fish catabolize fatty acids from triglyceride stores to support vital processes (Bar, 2014), but it has also been suggested that prolonged food deprivation may deplete such stores, leading to a breakdown of membrane lipids (Brückner and Heethoff, 2020). Fatty acids released from triglycerides could be metabolized to synthesize various molecular species of phospholipids such as phosphatidylcholine, phosphatidylethanolamine and phosphatidylserine, all of which are known to play a key role in maintaining membrane fluidity in fish (Coleman and Lee, 2004; Tocher et al., 2008; Tocher and Sargent, 1990). As the capacity for HVA may depend on the availability of pre-existing substrates or PUFAs (Alhazzaa et al., 2013; Craig et al., 1995; Kelly and Kohler, 1999; Wijekoon et al., 2021), it is hypothesized that under cold, food deprived conditions, synthesis of LC-PUFAs in phospholipids may be facilitated by catabolism of fatty acids from triglycerides and the lipolysis of triglycerides may be mediated by cortisol, a glucocorticoid hormone involved in regulation of metabolites in response to a cold stress (Dave et al., 1979; de Gomez Dumm et al., 1979; Donaldson et al., 2008; Mommsen et al., 1999). Indeed, recent work in age-0 lake sturgeon (*A. fulvescens*) showed that 28 days of fasting at 1 °C resulted in a gradual decrease in plasma triglyceride concentrations followed by a significant increase in triglycerides post-fasting concomitant with a significant increase in whole body cortisol concentration (Deslauriers et al., 2018).

Every year northern fish populations experience periods of near 0 °C and limited resources that can last for in excess of 5 months (Hrenchuk et al., 2017). Therefore, it is reasonable to expect that an appropriate HVA response would be particularly important for age-0 fish as they approach their first winter of life (Donaldson et al., 2008; Hazel, 1997; Hurst, 2007). Lake sturgeon is a species endangered or at risk across its natural range (Bruch et al., 2016) and conservation aquaculture has been in operation to support populations across its range (Osborne et al., 2020). In Manitoba, Canada, lake sturgeon fall fingerlings or spring yearlings are released annually to enhance existing populations, but estimated survival is substantially lower in fall fingerlings compared to spring yearlings (McDougall et al., 2020; McDougall et al., 2014). One factor that likely reduces survival of fall fingerlings is the first winter of life. It has been shown that freshwater alewife (*Alosa pseudoharengus*) exhibited increased mortality during winter months which may be the result of an inappropriate HVA response (Snyder et al., 2012; Snyder and Hennessey, 2003). Therefore, understanding how overwintering conditions could influence fatty acid metabolism and plasma cortisol concentration of age-0 lake sturgeon would inform current rearing practices and may ultimately result in improved post-release survival rates for fall fingerlings.

In this study, we examined the effects of reduced environmental temperature on hepatic fatty acid composition and tested the prediction that a reduced temperature would increase the proportion of MUFAs and PUFAs in phospholipids. We chose to measure fatty acid in liver tissue as liver is the central organ of fatty acid metabolism for phospholipids and triglycerides (Enjoji et al., 2018). We also examined the added effects of food deprivation and tested the prediction that fasted fish would have decreased hepatic MUFAs and PUFAs in triglycerides compared to fed fish during reduced environmental temperature, which may in part be driven by an increase in plasma cortisol concentration.

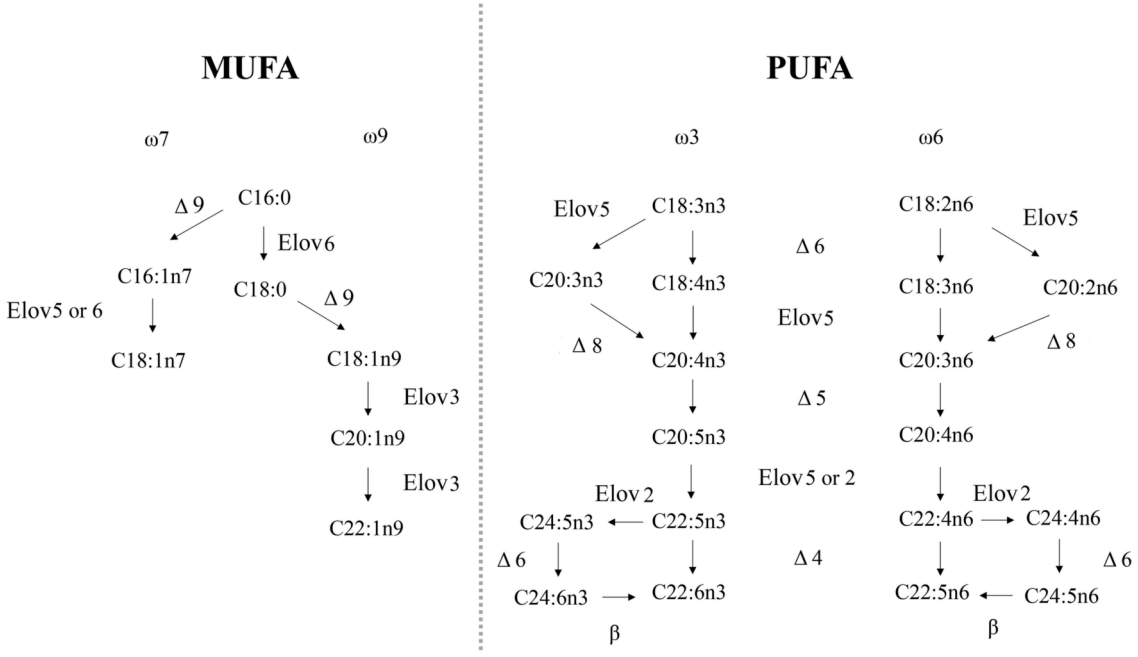

Fig. 1. *In vivo* mono (MUFA) and polyunsaturated fatty acids (PUFA) pathway in vertebrates. Elov 2, 3, 5 and 6 denote fatty acid elongase 2, 3, 5 and 6. Δ 4, 5, 6, 9 represent Δ 4, 5, 6, 9 desaturase, respectively. β is for peroxisomal oxidation for shortening fatty acid chain.

2. Material and methods

2.1. Animal husbandry and environment manipulation

Gamete collection and animal rearing were performed as previously described (Earhart et al., 2020) and 5 month-old fish were used for the present study. There were three flow-through fish tanks (11 L; 27 × 18 × 40 cm) per treatment. At 153 days post hatch (dph), the protocol for a simulated overwintering event was performed by decreasing water temperature from 16 °C to 1 °C by 0.5 °C per day. All experiments were conducted in a controlled environment chamber (CMP 6050, Conviron, Manitoba, Canada) which allowed for cooling air temperature to near 0 °C. Cold water inflow was at approximately 2 °C; thus, a combination of reduced air temperature allowed us to maintain aquarium temperature at approximately 1 °C for overwintering. When temperature reached 1 °C, fish were either fed every other day to satiation with bloodworm (Hikari, California, USA) or fasted for 45 days. Fish were sampled for the measured variables on four separate occasions during the course of the study (1) at 153 dph, 16 °C prior to any temperature manipulation or food deprivation; (2) at 199 dph when fish had been at 1 °C for 16 days (3) at 225 dph, 25 days after the initiation of feeding/fasting protocol at 1 °C; (4) at 245 dph, 45 days after the initiation of the feeding/fasting protocol at 1 °C.

2.2. Sampling

At each sampling point, 8 fish were haphazardly captured from tanks by dipnet and sacrificed with an overdose of anesthetic (250 mg·L^{-1}, MS-222, Syndel Laboratory, British Columbia, Canada) buffered with an equal amount of sodium bicarbonate. Then, total length and body mass were measured to 1 mm and 0.001 g, respectively. Following euthanization, whole blood was taken from fish by severing the tail and collecting blood with hematocrit tubes. Blood was transferred to 1.5 mL Eppendorf tubes and centrifuged at 10,000*g* for 5 min to separate plasma from red blood cells. The plasma was removed from the sample and frozen at −80 °C until subsequent cortisol analysis. The liver was then removed from the abdominal cavity and again stored at −80 °C for subsequent fatty acid analysis.

2.3. Fatty acid analysis

We analyzed total fatty acid composition of the diet (bloodworm) and fatty acid composition of phospholipids and triglycerides in the liver tissue. In brief, dietary fatty acid profile of bloodworm was assessed through the direct saponification and methylation method with slight modifications (Kang and Wang, 2005). In terms of fatty acid profile in phospholipids and triglycerides of the liver tissue, tissue was homogenized with 0.025% $CaCl_2$ and total lipids were extracted (n = 8 per treatment) by chloroform:methanol (2:1, *v*/v) with butylated hydroxytoluene as an antioxidant (Folch et al., 1957). Analysis of fatty acids from phospholipid and triglyceride fractions were performed as previously described (Feltham et al., 2019). The extracted lipids were separated on silica gel G plates in a solvent system consisting of ether: diethylether:acetic acid in a ratio of 80:20:1 (by vol) to separate phospholipid and triglyceride. The corresponding lipid classes were visualized with 0.1% 8-anilino-1-naphthalene-sulfonic acid under UV light and scraped for saponification and methylation. Fatty acid methyl esters were prepared using 14% BF_3 (Suh et al., 2009). Fatty acid methyl esters were analyzed *via* gas chromatography (Vista 6010 GLC and Vista 402 data system; Varian Instruments, Ontario, Canada) with a micro-capillary column (15 m × 0.1 mm inner diameter; BPX70; SGE analytical science, North Carolina, USA). Hydrogen was used as a carrier gas at a flow rate of 0.5 mL.min^{-1}. The initial oven temperature held at 130 °C and increased to 175 °C at 20 °C.min^{-1}, held for 1 min then increased to 200 °C at 6 °C.min^{-1} with no hold, and finally increased to 280 °C at 30 °C.min^{-1}. Peaks were identified using the fatty acid methyl ester standard mixture 461 (Nucheck Prep Inc., Minnesota, USA). It is important to note that for triglyceride fatty acid identification and quantification, we chose to report only major fatty acids from C14:0 to C18:3n3 because the small sample size combined with low concentrations of PUFAs did not allow us to reliably quantify LC-PUFAs. Fatty acid concentration was expressed in mol %.

2.4. Plasma cortisol concentration

We assessed baseline and peak plasma cortisol concentrations as previously described (Earhart et al., 2020; Zubair et al., 2012). In brief, baseline cortisol level was assessed by immediate collection (< 3 min) as described above. Peak cortisol concentration was induced by stressing fish to elicit a cortisol response where fish were gently prodded in the tail with a plastic pipette for 5 min and left undisturbed for 30 min to recover. Following this period, fish were sacrificed by immersion in an overdose of MS-222 (250 mg·L^{-1}) buffered with equal volumes of sodium bicarbonate and blood was collected as described above. Plasma cortisol concentration was analyzed through radio immunoassay as previously established (Hare et al., 2015). All unknown cortisol values were determined by interpolating against a standard curve generated in each assay. Intra-assay variation was 11%, inter-assay variation was 9%, and extraction efficiency was 99.5 ± 1.3%.

2.5. Statistical analysis

Due to the non-factorial design and bimodal distribution of our data, we used a Student *t*-test to examine the temperature effect in total length, body mass and fatty acids from each lipid class between 153 and 199 dph. Also, we used two-way ANOVA to examine the effects of food deprivation and its duration on fatty acids from each lipid class between 225 and 245 dph. When ANOVA indicated a significance ($p < 0.05$), a Tukey's HSD was performed to compare difference between individual treatments. With respect to plasma cortisol two-way ANOVA was performed within baseline or peak concentrations as described above, and a t-test was used to compare difference between baseline and peak cortisol concentrations at each sampling time. Data were transformed through square root, log, cube root or Tukey power where the assumption of normality or homogeneity of variance was not met (Mangiafico, 2021). Assumptions of normality and homoskedasticity were visually assessed as previously described (Zuur et al., 2010). Full results of data analysis are provided as Supplementary tables (Table A1–A5). All data analysis was conducted in R (R Core Team, 2021).

All experiments were approved by the University of Manitoba Animal Care Committee under the requirements established by the Canadian Council for Animal Care under protocol number F015–007.

3. Results

3.1. Dietary fatty acid composition (bloodworm)

Overall, the most abundant fatty acid group in the diet was PUFA (39.41 ± 0.24%; mean ± S·D) followed by SAT (34.02 ± 0.14%) and MUFA (26.57 ± 0.13%) (Table 1). Specifically, the most abundant SAT was C16:0 (15.53 ± 0.11%.) followed by C18:0 (10.29 ± 0.15%). Among MUFA, the most abundant fatty acid was C18:1 (11.15 ± 0.13%) followed by C16:1t (7.97 ± 0.16%). Also, C18:2n6 (23.81 ± 0.23%) was higher than C18:3n3 (8.54 ± 0.09%), contributing to an omega-6 PUFA dominated diet. Lastly, we found that DHA (C22:6n3) was very low in the diet (0.02 ± 0.02%).

3.2. Fatty acid composition in liver phospholipids and triglycerides

Overall, we saw that fatty acid compositions in phospholipids and triglycerides appear to be similar (Tables 2, 3). For example, in phospholipids, the most abundant SAT was C16:0, MUFA was C18:1, and

Table 1
Summary of fatty acid profile in diet (bloodworms; n = 6) used in the present study. Data are expressed in mean ± S.D.

Fatty acid	mol %
C14:0	2.2 ± 0.19
C14:1	1.82 ± 0.1
C15:0	1.54 ± 0.04
C16:0	15.53 ± 0.11
C16:1	1.12 ± 0.11
C16:1t	7.97 ± 0.16
C17:0	2.58 ± 0.03
C18:0	10.29 ± 0.15
C18:1	11.15 ± 0.13
C18:1n7c	4.17 ± 0.05
C18:2n6	23.81 ± 0.23
C18:3n6	0.84 ± 0.06
C18:3n3	8.54 ± 0.09
C20:0	1.43 ± 0.05
C20:1	0.13 ± 0.03
C20:2n6	0.12 ± 0.03
C20:3n6	0.14 ± 0.01
C20:3n3	0.15 ± 0.02
C20:4n6	3.5 ± 0.06
C20:5n3	2.26 ± 0.07
C22:0	0.32 ± 0.02
C22:1	0.22 ± 0.03
C22:4n6	0.03 ± 0.02
C22:6n3	0.02 ± 0.02
C24:0	0.13 ± 0.02
Σ SAT	34.02 ± 0.14
Σ MUFA	26.57 ± 0.13
Σ PUFA	39.41 ± 0.24
Σ n3	10.97 ± 0.21
Σ n6	28.44 ± 0.25
U/S ratio	1.94 ± 0.01

PUFA was DHA. Similarly, in triglycerides, C16:0 and C18:1 were the most abundant SAT and MUFA respectively.

When temperature decreased from 16 to 1 °C between 153 and 199 dph, we observed that in phospholipid, total SATs significantly declined with a significant increase in total MUFAs ($p < 0.001$; Table 2, Fig. 2a,b) and total PUFAs also increased although this was statistically insignificant (Fig. 2c). Specifically, C16:0 and C18:0 significantly decreased whereas C16:1, C18:1, C18:1n7c and C20:1 significantly increased ($p < 0.05$; Table 2). Meanwhile, C20:4n6 significantly increased ($p < 0.05$) whereas C20:5n3and C22:6n3 did not change (Table 2). The ratio of unsaturated fatty acids to saturated fatty acids (U/S Ratio) significantly increased with decreasing temperatures ($p < 0.001$; Table 2, Fig. 2d). Total omega-3 PUFAs did not change with temperature whereas total omega-6 PUFAs significantly increased as temperature decreased ($p < 0.001$; Table 2, Fig. 3 a,b). Finally, the unsaturation index (UI) did not change throughout the experiment (Fig. 3c). We saw a similar trend in triglycerides where C14:0 significantly decreased when temperature decreased from 153 to 199 dph ($p < 0.01$; Table 3) and C18:1, C18:1n7c, C18:3n6 and C18:3n3 significantly increased between 153 and 199 dph ($p < 0.01$; Table 3).

During the overwintering event of food deprivation at 1 °C for 45 days, we did not observe notable trends of changes in fatty acids in both phospholipids and triglycerides (Tables 2, 3).

3.3. Plasma cortisol concentration

At 153 dph, there was no difference between baseline and peak cortisol concentration in the fed group ($p > 0.1$; Table A1, Fig. 4). Between 225 and 245 dph, baseline plasma cortisol concentration was significantly influenced by both food availability and its duration ($p < 0.001$; Table A2, Fig. 4). Specifically, within the fed group, baseline cortisol concentration was significantly higher at 245 dph compared to 225 dph ($p < 0.001$; Fig. 4). At 225 dph baseline plasma cortisol concentration did not differ between fed and fasted fish, but at 245 dph,

Table 2
Summary of fatty acid profile in phospholipid of age-0 Lake sturgeon (*Acipenser fulvescens*) during a simulated overwintering event. * represents significant difference in values between 153 and 199 days post hatch (dph) whereas different letters indicate significant difference in values between 225 and 245 dph. Fatty acids equal or less than 0.1% were not listed (C22:0, C22:1, C24:0 and C24:1). Data are expressed in mol % (mean ± S.D.).

Fatty acid (mol %)	153 dph (16°C)	199 dph (1°C)	225 dph (1°C)		245 dph (1°C)	
	Fed		Fed	Fasted	Fed	Fasted
C14:0	1.48 ± 0.66	0.53 ± 0.06*	0.5 ± 0.07	0.55 ± 0.27	0.51 ± 0.1	0.59 ± 0.08
C14:1	0.25 ± 0.19	0.32 ± 0.04	0.27 ± 0.04	0.32 ± 0.24	0.26 ± 0.09	0.33 ± 0.08
C15:0	0.76 ± 0.12	0.71 ± 0.05	0.72 ± 0.11	0.74 ± 0.09	0.73 ± 0.06	0.76 ± 0.08
C16:0	34.99 ± 6.1	27.07 ± 2.66*	29.85 ± 2.15ab	30.82 ± 2.79a	31.38 ± 3.34ab	31.92 ± 4.12b
C16:1	0.38 ± 0.11	0.54 ± 0.08*	0.69 ± 0.16	0.67 ± 0.08	0.65 ± 0.11	0.7 ± 0.05
C16:1t	1.59 ± 0.48	2.13 ± 0.38*	2.47 ± 0.42	2.07 ± 0.81	2.68 ± 0.57	2.73 ± 0.27
C17:0	1.08 ± 0.28	1.06 ± 0.44	0.72 ± 0.13a	1.06 ± 0.26b	0.62 ± 0.28a	0.76 ± 0.16ab
C18:0	15.33 ± 2.86	8.33 ± 1.52*	7.22 ± 1.36	10.03 ± 2.17	7.99 ± 2.47	7.09 ± 2.58
C18:1	9.89 ± 0.48	12.09 ± 0.74*	10.85 ± 1.11	10.98 ± 1.53	11.14 ± 2.26	10.72 ± 0.54
C18:1n7c	4.42 ± 0.46	9.43 ± 0.7*	9.44 ± 0.67a	8.98 ± 0.36ab	8.27 ± 0.92b	9.00 ± 0.45ab
C18:2n6	3.13 ± 0.28	3.71 ± 0.35*	3.3 ± 0.35	3.23 ± 0.55	3.67 ± 0.66	3.35 ± 0.27
C18:3n6	0.25 ± 0.05	0.94 ± 0.06*	0.95 ± 0.08	0.96 ± 0.11	0.85 ± 0.1	0.95 ± 0.11
C18:3n3	0.46 ± 0.31	1.02 ± 0.2*	0.74 ± 0.14ab	0.52 ± 0.38a	0.84 ± 0.19ab	0.93 ± 0.27b
C20:0	0.35 ± 0.22	0.42 ± 0.11	0.44 ± 0.1ab	0.54 ± 0.13a	0.39 ± 0.11b	0.44 ± 0.09ab
C20:1	1.25 ± 0.23	2.02 ± 0.33*	1.97 ± 0.36	2.03 ± 0.36	1.63 ± 0.2	1.9 ± 0.28
C20:2n6	0.89 ± 0.26	1.20 ± 0.16*	1.10 ± 0.16	0.98 ± 0.16	1.52 ± 1.6	0.94 ± 0.15
C20:3n6	0.61 ± 0.18	1.84 ± 0.66*	1.37 ± 0.39ab	1.15 ± 0.17a	1.52 ± 0.29ab	1.64 ± 0.39b
C20:3n3	0.37 ± 0.09	0.69 ± 0.09*	0.56 ± 0.09	0.57 ± 0.14	1.04 ± 1.39	0.52 ± 0.07
C20:4n6	4.16 ± 1.81	6.73 ± 0.77*	7.41 ± 0.68	6.84 ± 0.78	6.67 ± 1.16	7.00 ± 1.19
C20:5n3	3.79 ± 1.61	2.51 ± 0.46	1.93 ± 0.19	1.67 ± 0.48	2.21 ± 0.49	2.23 ± 0.66
C22:4n6	0.09 ± 0.03	0.24 ± 0.07*	0.25 ± 0.04	0.29 ± 0.06	0.25 ± 0.02	0.24 ± 0.06
C22:5n3	1.08 ± 0.36	1.67 ± 0.39*	1.50 ± 0.22	1.40 ± 0.28	1.57 ± 0.13	1.77 ± 0.59
C22:6n3	12.99 ± 7.09	14.51 ± 3.00	15.44 ± 2.64	13.18 ± 2.99	13.27 ± 4.73	13.14 ± 2.97
Σ SAT	54.29 ± 9.85	38.31 ± 4.65*	39.63 ± 3.58	44.03 ± 5.07	41.86 ± 5.63	41.8 ± 5.93
Σ MUFA	17.89 ± 1.18	26.64 ± 1.11*	25.82 ± 1.1	25.17 ± 2.49	24.73 ± 1.91	25.49 ± 0.99
Σ PUFA	27.81 ± 10.3	35.05 ± 4.53	34.55 ± 3.66	30.8 ± 4.44	33.4 ± 6.76	32.71 ± 5.69
Σ n3	18.69 ± 8.75	20.39 ± 3.47	20.17 ± 3.04	17.34 ± 3.54	18.92 ± 6.25	18.58 ± 3.92
Σ n6	9.13 ± 2.13	14.66 ± 1.37*	14.38 ± 0.77	13.46 ± 0.99	14.48 ± 1.57	14.13 ± 1.82
U/S ratio	0.89 ± 0.31	1.64 ± 0.28*	1.54 ± 0.24	1.3 ± 0.25	1.43 ± 0.37	1.43 ± 0.34
UI	150.26 ± 55.37	185.73 ± 23.72	185.93±19.86	166.2 ± 23.93	174.04 ± 35.62	173.98 ± 28.26

Table 3
Analysis of fatty acid profile in triglyceride of age-0 Lake sturgeon (*Acipenser fulvescens*) during a simulated overwintering event. * represents significant difference in values between 153 and 199 days post hatch (dph) whereas different letters indicate significant difference in values between 225 and 245 dph. Data are expressed in mol % (mean ± S.D.).

Fatty acid (mol %)	153 dph (16°C)	199 dph (1°C)	225 dph (1°C)		245 dph (1°C)	
	Fed		Fed	Fasted	Fed	Fasted
C14:0	8.24 ± 1.36	2.79 ± 0.39*	2.93 ± 0.51	3.5 ± 1.06	2.94 ± 0.21	2.69 ± 0.26
C14:1	1.76 ± 1.3	2.77 ± 0.27*	2.08 ± 0.64ab	1.47 ± 0.6a	2.25 ± 0.57ab	2.50 ± 0.38b
C15:0	1.39 ± 0.46	1.40 ± 0.14	1.28 ± 0.34	1.43 ± 0.58	1.32 ± 0.21	1.13 ± 0.1
C16:0	31.17 ± 4.66	27.45 ± 2.98	32.15 ± 9.25ab	38.3 ± 5.28a	32.67 ± 6.93ab	25.85 ± 4.47b
C16:1	1.12 ± 0.3	1.06 ± 0.18	1.2 ± 0.54ab	0.79 ± 0.71a	0.99 ± 0.35ab	1.24 ± 0.28b
C16:1t	10.05 ± 2.23	8.77 ± 1.45	6.57 ± 3.22	3.90 ± 3.64	7.59 ± 3.71	10.32 ± 2.45
C17:0	1.67 ± 1.29	1.16 ± 0.25	2.04 ± 2.21	3.75 ± 4.11	2.38 ± 1.94	1.06 ± 0.58
C18:0	7.43 ± 5.97	6.38 ± 2.34	13.93 ± 9.81	28.06 ± 8.26	14.15 ± 10.66	5.22 ± 6.34
C18:1	21.02 ± 4.87	25.46 ± 2.28*	20.37 ± 9.09ab	9.13 ± 7.54a	18.96 ± 8.35ab	26.21 ± 4.42b
C18:1n7c	5.70 ± 1.39	11.36 ± 1.29*	9.08 ± 5.17ab	4.19 ± 3.47a	7.91 ± 4.35ab	12.74 ± 2.01b
C18:2n6	8.15 ± 1.72	7.40 ± 1.49	4.91 ± 2.24a	1.95 ± 1.27b	5.19 ± 1.83a	6.90 ± 1.67a
C18:3n6	0.59 ± 0.12	1.00 ± 0.17*	0.77 ± 0.33a	0.42 ± 0.47a	0.87 ± 0.28ab	1.28 ± 0.17b
C18:3n3	1.3 ± 0.7	2.37 ± 0.88*	1.53 ± 0.81a	0.16 ± 0.25b	1.44 ± 0.91a	2.46 ± 1.02a

baseline plasma cortisol concentration was significantly higher in the fed group compared to the fasted group ($p < 0.001$; Fig. 4). Our two-way ANOVA indicated a significant effect of food availability on peak cortisol concentration ($p < 0.01$; Table A2), but the *post hoc* analysis of individual group comparison did not support this conclusion ($p > 0.1$; Fig. 4, Table A2). At 225 dph, there was significant differences between baseline and presumptive peak cortisol concentration in the fed group ($p < 0.001$; Fig. 4, Table A1), but not in the fasted group. At 245 dph, there was no difference between baseline and peak cortisol in either the fed or fasted group ($p > 0.05$, Fig. 4, Table A1).

3.4. Body mass

Both student *t*-test and two-way ANOVA indicated that body mass did not significantly change throughout the study ($p > 0.05$; Table A2, Fig. 5).

4. Discussion

We observed significant changes in hepatic fatty acids during cold adaptation, supporting the process of HVA in age-0 lake sturgeon entering their first winter of life. Specifically, we found significant increases in MUFAs of C16:1 and C18:1 in phospholipids from the liver in cold adapted fish. These increases may have been the result of selective retention of diet derived C16:1 and C18:1 during adaptation to colder temperatures or could be endogenous synthesis. Coupled with significant decreases in hepatic phospholipids of C16:0 and C18:0, our data suggests an increase in Δ9 desaturase activity. Δ9 desaturase (also known as stearoyl-CoA desaturase) is an enzyme involved in the synthesis of MUFAs, converting C16:0 and C18:0 to C16:1 and C18:1, and is the rate limiting step inserting a double bond (*cis* form) into the 9th carbon from the carboxyl end in the acyl chain (Miyazaki and Ntambi, 2003) prior to further conversion to C18:1n7c and C22:1 *via* fatty acid

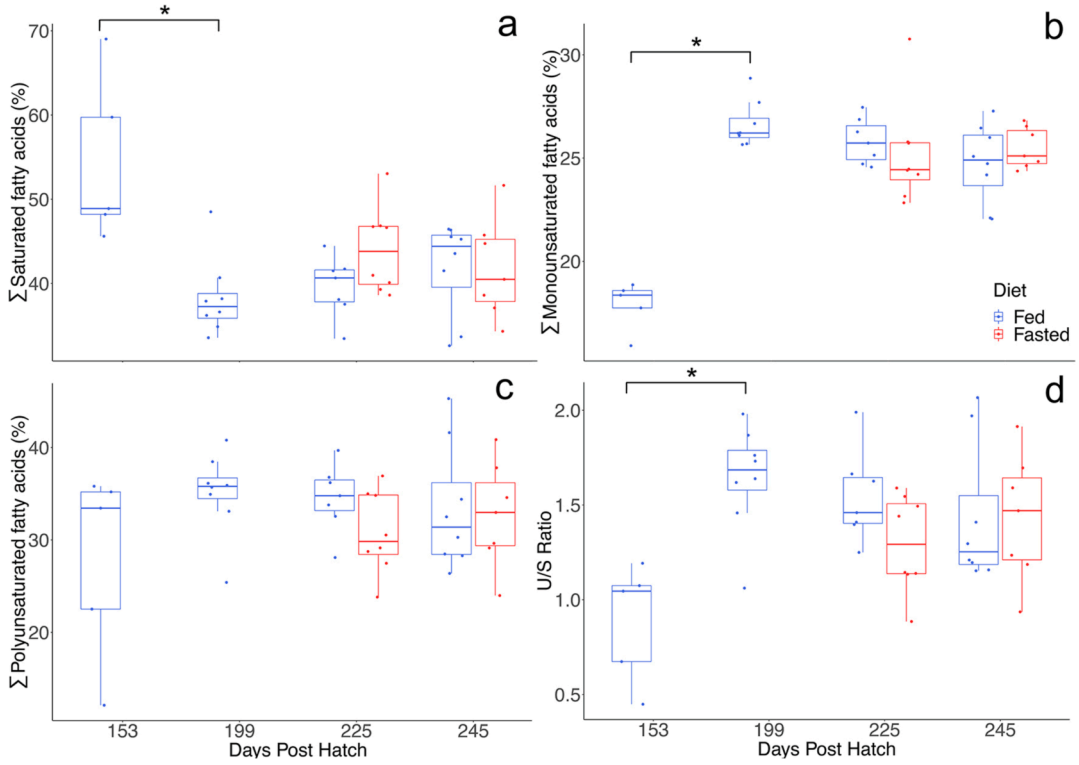

Fig. 2. Changes in saturated (a), monounsaturated (b), polyunsaturated fatty acid (c) and unsaturated to saturated fatty acids ratio; U/S ratio (d) in the phospholipid of liver tissue of age-0 lake sturgeon (*Acipenser fulvescens)*. From 200 days post hatch (dph), fish were either fed every other two days (blue) or fasted for 45 days (red). * denotes significant difference between 153 and 199 dph (t.test; $p < 0.001$). Dots represent individual data points. Data are not transformed for the graphical purpose. $n = 5$–8. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

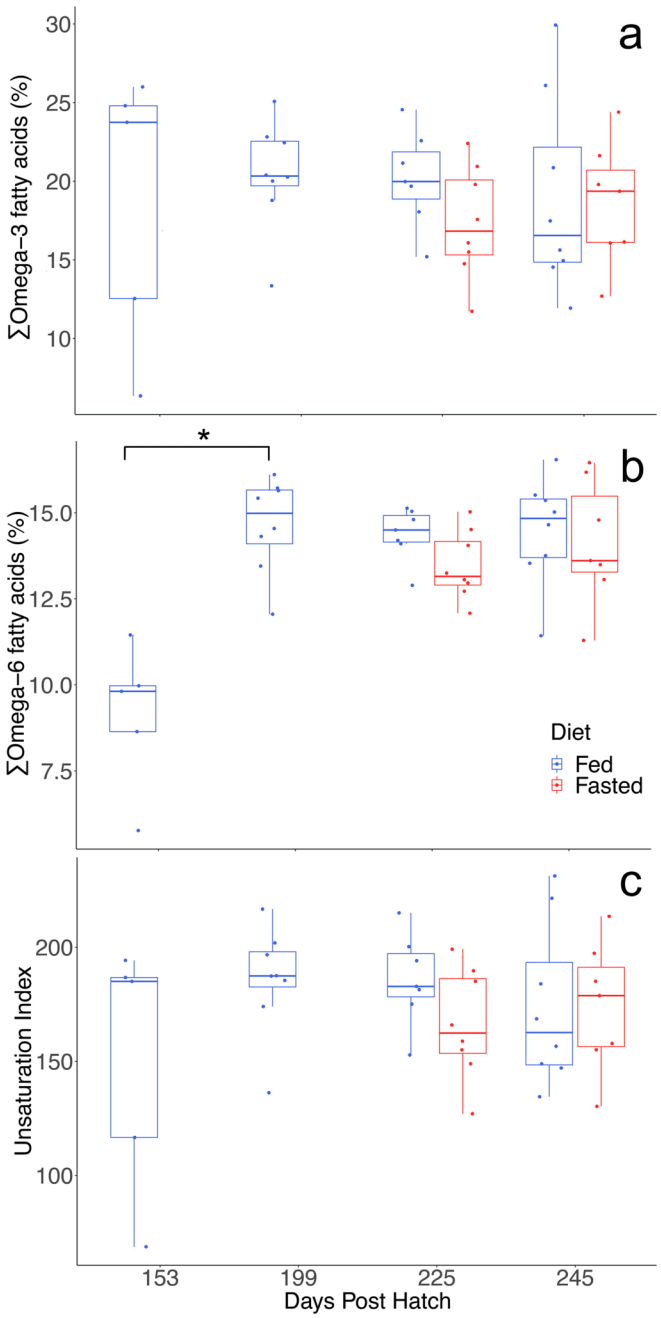

Fig. 3. Changes in omega 3 (a), 6 fatty acids (b) and unsaturation index (c) in the phospholipid of liver tissue of age-0 lake sturgeon (*Acipenser fulvescens)*. From 200 days post hatch (dph), fish were either fed every other two days (blue) or fasted for 45 days (red). * denotes significant difference between 153 and 199 dph (t.test; $p < 0.001$). Dots represent individual data points. Data are not transformed for the graphical purpose. n = 5–8. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

elongases (Burdge, 2018; Ferreri et al., 2020). In teleosts such as carp (*Cyprinus carpio*) and milkfish (*Chanos chanos*), cold exposure increased gene expression of Δ 9 desaturase (Hsieh et al., 2003; Trueman et al., 2000). Similarly, a recent study in our lab showed that age-0 lake sturgeon significantly increased hepatic gene expression of Δ 9 desaturase when environmental temperature was decreased to 3.5 °C from 16 °C (Bugg et al. *unpublished*).

Interestingly, we saw that when temperature decreased, total omega-6 PUFAs in phospholipids significantly increased whereas total omega-3

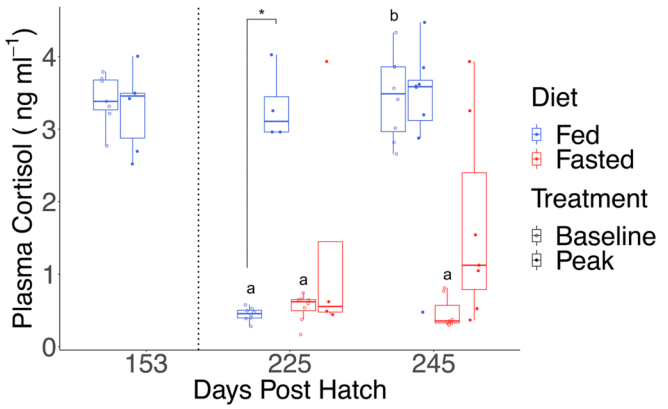

Fig. 4. Changes in plasma cortisol concentration in age-0 lake sturgeon (*Acipenser fulvescens)*. Baseline (open dots) was assessed by immediate collection of blood from the caudal vein whereas peak (filled dots) was induced by chasing fish 5 min and 30 min of recovery. From 200 days post hatch (dph), fish were either fed every other two days (blue) or fasted for 45 days (red). * denotes significant difference between baseline and peak concentrations (t.test; $p < 0.001$). Different letters indicate significant difference within baseline group between 225 and 245 dph (two-way ANOVA, $p < 0.001$). Dots represent individual data points. Data are not transformed for the graphical purpose. n = 4–8. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

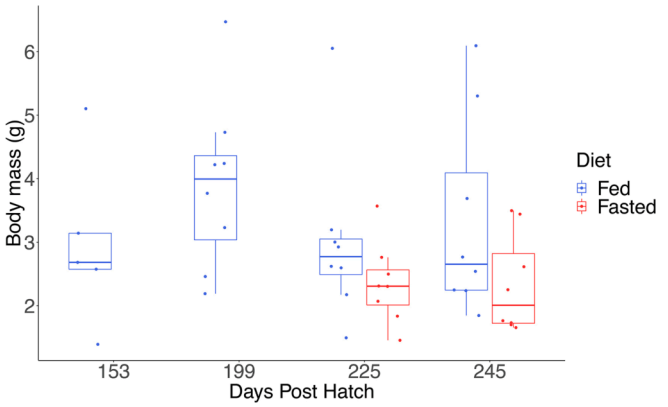

Fig. 5. Changes in body mass of lake sturgeon (*Acipenser fulvescens*) in the present study. From 200 days post hatch (dph), fish were either fed every other two days (blue) or fasted for 45 days (red). Dots represent individual data points. Data are not transformed for the graphical purpose. n = 5–8. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

PUFAs did not. All of omega-6 PUFAs such as C20:3n6, C20:4n6 and C22:4n6 significantly increased. However, minor omega-3 PUFAs C20:3n3 and C22:5n3 significantly increased while the major C20:5n3 and C22:6n3 did not change. These results could mean that dietary retention of omega-3 LC-PUFAs may be limited in this species or that the process of elongation was not inhibited for both omega-3 and 6 fatty acids while desaturation capacity may be limited in the downstream production of omega-3 LC-PUFAs. The *in vivo* synthetic pathway for omega-3 and omega-6 fatty acids has been well established, and is hypothesized to be almost identical across all vertebrates (Li et al., 2010; Park et al., 2009; Sprecher, 2000; Tocher, 2010). Omega-3 or omega-6 LC-PUFAs could be synthesized from either C18:3n3 or C18:2n6. Downstream conversion is facilitated through a series of biochemical reactions with Δ 5 or 6 desaturases, fatty acid elongase (Elovl2 and Elovl5) and a peroxisomal β-oxidation for shortening fatty acid chains. Δ5 and 6 desaturases are promiscuous with both omega-3 and omega-6 fatty acids (Guillou et al., 2010; Sprecher, 2000). For example, Δ6

desaturase can bind with one of two potential substrates - C18:3n3 or C18:2n6 (Park, 2018). Thus, despite a higher binding affinity to omega-3 fatty acids (Berger and German, 1990), relative substrate availability of these fatty acids may compete for Δ6 desaturase, which will significantly influence downstream production of AA (C20:4n6), EPA (C20:5n3) and DHA (C22:6n3) in mammals (De Antueno et al., 2001; Emken et al., 1994). In fish, effects of substrate availability of C18:3n3 and C18:2n6 on omega 3 and 6 fatty acid metabolism have shown to be equivocal. A previous study in rainbow trout (*Oncorhynchus mykiss*) demonstrated that substrate availability between C18:3n3 and C18:2n6 did not influence conversion to C18:4n3 and C18:3n6 (Emery et al., 2013). In contrast, in Atlantic salmon (*Salmo salar*), when fed the same ratio between C18:3n3 and C18:2n6, synthesis of omega-3 LC-PUFAs was 9 fold greater than omega-6 LC-PUFAs (Sprague et al., 2019). In the present study, it is hypothesized that the significant increase in AA, but not in EPA when fish were exposed to cold temperatures could be due to the relative amount of dietary C18:3n3 and C18:2n6. Future studies are suggested to understand how availability of C18:3n3 and C18:2n6 might influence LC-PUFA metabolism in sturgeon species during cold adaptation. We also saw a significant increase in AA in phospholipids when temperature decreased. In marine teleosts, it has been suggested that production of EPA and AA may be limited due to low activity levels of Δ5 desaturase, which converts C20:4n3 and C20:3n6 to EPA (C20:5n3) and AA (C20:4n6) respectively (Nakamura and Nara, 2004; Tocher, 2003; Tocher and Ghioni, 1999). It remains unclear if the increase in AA in cold adapted fish from the present study is due to increased activity and/or gene expression of Δ5 desaturase as similar to Δ9 desaturase described above or simply selective retention of AA from the diet.

We observed a similar trend in changes of saturated and unsaturated fatty acids in triglycerides. Specifically, we reported increases in both MUFAs and PUFAs of triglycerides in response to adaptation to decreased environmental temperature in age-0 lake sturgeon. These findings are, however, in disagreement with previous studies conducted in freshwater alewife (Snyder et al., 2012; Snyder and Hennessey, 2003). It is hypothesized that HVA may be less important in triglycerides because it is considered as a mechanism for storage of fatty acids where less PUFAs are found (Tocher, 2003; Tocher and Sargent, 1990). However, it was suggested that mobilization of fatty acids in triglycerides at low temperatures may be influenced by a fluid state of fatty acids that vary with fatty acid composition (Florant, 1998; Frank, 1992), suggesting substantial relevance of HVA in triglyceride metabolism. For example, in both blue mussels (*Mytilus edulis*) and oyster (*Crassostrea virginica*), exposure to cold temperatures resulted in significant increases of PUFAs such as EPA in triglycerides (Pernet et al., 2007). It is important to note that Pernet et al. (2007) used algae (*Chaetoceros muelleri* and *Isochrysis galbana*) that contained high levels of PUFAs, which both bivalves may have accumulated from their diet during winter.

In the fed group, we saw that there was no clear pattern of changes in MUFAs or PUFAs at 225 and 245 dph. This result suggests that fish may have been adapted to a cold environment between 153 and 199 dph and required less to regulate MUFAs and PUFAs under 1 °C. A short term response to adjust biological membranes such as changes in head groups could occur within 24 h of temperature exposure, but a long-term response such as changes in LC-PUFAs could be completed within two weeks of exposure (Hazel, 1997; Hazel and William, 1990). Thus, 16 days of acclimation to 1 °C may have been sufficient for age-0 lake sturgeon to complete HVA and survive at 1 °C. Also, we did not observe any change of fatty acids in the fasted group at 225 and 245 dph, which could be due to a lack of changes in body mass that did not trigger any changes in fatty acids alongside plasma cortisol concentration during the simulated overwintering event (see below).

We observed higher baseline cortisol in the fed group at 153 and 245 dph. It remains unknown what drove higher baseline cortisol in the fed group. Although we did not quantify food intake during overwintering, we occasionally found food in the gut of fish from the fed group during dissection, suggesting fish were actively foraging at 1 °C. Thus, it is possible that lack of growth in the fed group despite presence of food in the gut during overwintering could be attributable to reduced digestive capacities at low environmental temperatures. It was suggested that increased plasma cortisol concentration may not necessarily indicate distressed individuals as cortisol plays numerous roles in homeostasis (MacDougall-Shackleton et al., 2019; Romero and Beattie, 2021) such as nutrient uptake across the intestine (Rosengren et al., 2018; Tanaka et al., 1995). Our baseline cortisol data in the fasted group disagrees with a previous study conducted in age-0 lake sturgeon where whole-body plasma cortisol concentration significantly increased after 30 days of fasting at 1 °C (Deslauriers et al., 2018). The discrepancy is likely due to reporting plasma cortisol in the present study compared to whole-body cortisol (Mommsen et al., 1999; Romero and Beattie, 2021). We also found that baseline and peak cortisol concentrations did not differ in the fasted group during the overwintering event. Although baseline and peak cortisol concentrations in sturgeon species are influenced by environmental temperature (Bates et al., 2014; Zubair et al., 2012), limited production capacity of cortisol in fasting may be detrimental for age-0 fish as cortisol plays an important role in energy mobilization that can be used to forage or escape predation (Bar, 2014; Barcellos et al., 2010; Piccinetti et al., 2015).

5. Conclusion

This research demonstrated that cold exposure resulted in significant increases in both MUFAs and PUFAs of phospholipids and triglycerides in age-0 lake sturgeon supporting homoeviscous adaptation in this species. We also observed upregulated omega-6 fatty acid synthesis but not omega-3 possibly due to substrate availability from the diet. We saw no difference between baseline and peak plasma cortisol concentrations in fasted fish, which may influence survival during winter months. The present study aids in our understanding of mechanisms needed to promote survival during the first winter of life in age-0 lake sturgeon, which would inform the current conservation aquaculture programs for lake sturgeon.

Credit author statement

Gwangseok R Yoon: conceptualization, methodology, validation, formal analysis, investigation, data curation, writing – original draft, funding acquisition; Madison Earhart: methodology, data curation, resources, writing- review & editing; Yidi Wang: methodology, data curation, writing- review & editing; Miyoung Suh: conceptualization, methodology, validation, investigation, resources, writing- review & editing, supervision; W. Gary Anderson: conceptualization, methodology, validation, investigation, writing- review & editing, supervision, funding acquisition

Declaration of Competing Interest

The authors declare no conflict of interest.

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Manitoba campuses is sourced from the Shoal Lake 40 First Nation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpa.2021.111056.

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