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[Article]

Can Diet-Dependent Factors Help Explain Fish-to-Fish Variation in Thiamine-Dependent Early Mortality Syndrome?

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Abstract.—To provide insight into the reasons why offspring of certain salmonine females exhibit early mortality syndrome (EMS) in the Great Lakes whereas others do not, we measured the egg concentrations of potential biochemical markers (stable isotopes of nitrogen and carbon, fatty acid signatures, and lipid-soluble carotenoids and vitamins) that are indicative of differing food web and trophic structure. To corroborate the presence of EMS, we also measured the egg content of thiamine vitamers. For all the stocks of coho salmon *Oncorhynchus kisutch* and Chinook salmon *O. tshawytscha* we studied, there was a very high correspondence between EMS and low concentrations of unphosphorylated thiamine in unfertilized eggs. For salmonine stocks in the Platte River, Thompson Creek, and the Swan River, Michigan, small but significant shifts occurred in measures of egg carotenoids, retinoids, $\delta^{15}N$ depletion, and fatty acid profiles of fish producing normal offspring relative to those exhibiting EMS. Egg thiamine concentrations in Chinook salmon from the Little Manistee River, Michigan, in the low-EMS group were only marginally above the threshold for EMS induction. Along with this small thiamine differential, there was no evidence of differing food web or dietary factors between EMS-positive and normal Chinook salmon from the Little Manistee River. Further investigations are required to determine the potential dietary sources for the observed differences in biochemical markers between EMS-positive and normal fish. These findings are generally consistent with the hypothesis that a more diverse forage base may help to limit overall dietary content of species that contain thiaminase, such as alewives *Alosa pseudoharengus*, and may lead to improved embryonic survival for feral salmonids.

In the Great Lakes, there is mounting evidence implicating alewives *Alosa pseudoharengus* and possibly rainbow smelt *Osmerus mordax* in salmonine diets as primary factors producing thiamine-deficient parents (Fitzsimons et al. 1999). The resultant low egg concentration of thiamine is associated with the presence of EMS during embryo development (Brown et al. 1998). Alewives and rainbow smelt are known to contain a thiamine-degrading enzyme, thiaminase (enzyme number 2.5.1.2; IUBMB 1992) (Gnaedinger 1964; Anglesea and Jackson 1985; Ji and Adelman 1998). However, despite evidence that alewives represent a major dietary component for salmonines in the Great Lakes basin (Miller and Holey 1992; Jones et al. 1993; Fisher et al. 1996), fry losses due to EMS are not always tightly associ-

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ated with alewife presence in the diet, which suggests that other factors are also involved (Brown et al. 1998; Wolgamood et al. 2005, this issue). One possible explanation for these differences may be that salmonines whose offspring do not exhibit EMS may exploit alternative prey items that lack thiaminase activity. Because thiamine deficiency and EMS are expressed in developing embryos well after yolk deposition and oogenesis, identification of differences in the diets of fish producing these offspring is not a straightforward task. One possible approach is to identify potential biomarkers associated with differing dietary constituents and then to track these markers to their ecosystem sources.

Stable isotopes have been used increasingly to elucidate food webs and trophic structure in aquatic ecosystems (Fry 1991; France 1995; Vander Zanden et al. 1997). Analysis of $\delta^{13}C$ has been used to differentiate pelagic and littoral organisms (France 1995), and $\delta^{15}N$ has been used to differentiate trophic structure and assign trophic position in food chains (Vander Zanden et al. 1997). Fatty acid (FA) signatures can also be used to distinguish and characterize fish and invertebrate species in a given ecosystem, as well as to study finer-scale trophic interactions. For example, palmitoleic acid (16:1[n-7], where 16 is the number of carbon atoms, 1 is the number of double bonds, and 7 is the position of the first double bond from the methyl end) is thought to be strongly associated with diatoms, some cyanobacteria, and some sulfate-reducing bacteria (Napolitano 1999). The sum of fatty acids 15:0, 17:0, 17:1, and 18:1 is an index of the bacterial contribution (Napolitano 1999) to overall FA composition. Diatoms are known to be a good source of eicosapentaenoic acid (EPA; 20:5[n-3]) but are not generally a good source of docosahexaenoic acid (DHA; 22:6[n-3]). Certain cryptophytes are rich in EPA (Weers and Gulati 1997; von Elert and Stampel 2000). Dinoflagellates are generally associated with DHA (Napolitano 1999). Moreover, certain polyunsaturated FAs (PUFAs; e.g., α-linolenic acid [18:3{n-3}], EPA, DHA, and arachidonic acid [20:4{n-6}]) are thought to be crucial to the physiological competencies and reproductive success of fish and zooplankton in both marine and freshwater ecosystems (Arts 1997, 1999; Arts et al. 2001). Carotenoids and other antioxidants like tocopherol (vitamin E) are correlated with the presence of another thiamine-dependent syndrome, called M74, in Baltic salmon *Salmo salar* (Börjeson and Norrgren 1997; Pettersson and Lignell 1998). Carotenoids are synthesized by primary producers and passed up the food chain. In fish, certain carotenoids may be metabolized to retinoids (Scheidt et al. 1985, 1986; Guillou et al. 1989). Moreover, carotenoid pigment profiles have proven useful for distinguishing anadromous from nonanadromous brown trout *S. trutta* stocks (Youngson et al. 1997). Tocopherol generally plays a role in preventing oxidative damage (Packer 1991) and is important in fish owing to their high concentrations of PUFAs, compounds that are susceptible to lipid peroxidation (Singh et al. 1992).

Because of their association with differing dietary items, stable isotope and FA signatures, along with carotenoids and retinoids, could provide better insight to dietary preferences of salmonines than that achievable from routine stomach analysis. Moreover, these dietary biomarkers are potentially more integrative of diet over the time of yolk deposition than stomach content analysis, which represents a short period prior to capture. As an initial step towards assessing their potential utility as dietary markers to follow-up for source tracking of EMS, we compared egg content of stable isotopes ($\delta^{13}C$ and $\delta^{15}N$), lipid-soluble antioxidants (carotenoids, retinoids, tocopherol), and FA between families of coho salmon *Oncorhynchus kisutch* and Chinook salmon *O. tshawytscha* that expressed EMS (+EMS) and those that exhibited little or no EMS (−EMS). We assessed the general utility of these measurements between species and locations by examining two different stocks of coho salmon from Lake Michigan tributaries and Chinook salmon from lakes Michigan and Huron.

Methods

Source of eggs and EMS monitoring.—Eggs were collected as part of the Michigan Department of Natural Resources' annual coho salmon and Chinook salmon rearing and stocking programs for lakes Michigan and Huron (see Wolgamood et al. 2005 for details). In 1996–1999, eggs from individual coho salmon were collected from the Platte River and Thompson Creek spawning runs. In 1998 and 1999, sampling included female Chinook salmon from spawning runs on the Little Manistee River (Lake Michigan) and Swan River (Lake Huron). Maternal size (length and weight) and egg weight measurements were taken. Prior to fertilization, a sample (10 g) of eggs was quick-frozen on dry ice and stored frozen (−80°C) until analysis. On the same day, remaining eggs were transported to the Wolf Lake Fish Quality Laboratory,

where eggs of individual females were incubated separately in 10.6°C spring water until just prior to swim-up (see Wolgamood et al. 2005). For analysis, eggs from specific female coho salmon and Chinook salmon were randomly chosen from the different years and locations based on whether they had high EMS (>20% mortality between hatch and first-feeding fry) or low EMS (<20% mortality between hatch and first-feeding fry) (Wolgamood et al. 2005). To ensure that the subset of fish selected for analysis of dietary biomarkers were generally representative of the fish reared and evaluated for EMS (see Wolgamood et al. 2005), we included measures of egg size (weight), maternal size (fork length and weight), and maternal condition between −EMS and +EMS fish. Fulton's condition factor (CF) was calculated as 100 × (total fish weight—expressed egg weight)/ length^3.

Measurements of thiamine vitamers.—Concentrations of thiamine were determined by a specific high-performance liquid chromatography (HPLC) procedure that separated thiamine pyrophosphate (TPP), thiamine monophosphate (TMP), and unphosphorylated thiamine (Th) forms (Brown et al. 1998).

Carotenoids, retinoids, and tocopherol.—Concentrations of carotenoids, retinoids, and tocopherol were measured by means of a specific HPLC procedure (Palace and Brown 1994; Brown and Vandenbyllaardt 1996). Tissue extraction procedures were similar to those reported in Brown and Vandenbyllaardt (1996) and were conducted under subdued light. Prior to analysis, any extract storage was at −80°C and extracts were protected from light. A Waters HPLC system (717 Autosampler, 610 Fluid Unit, and 600E System Controller equipped with a 996 Photodiode Array Detector) was used to analyze the extracts. Samples and standards were eluted isocratically with acetonitrile: methylene chloride: methanol: water: propionic acid (71:22:4:2:1) delivered at a flow rate of 1.5 mL/min. The column was a 5-μm bead size Adsorbosphere C_{18} (4.6-mm internal diameter [ID], 250-mm length; Alltech Associates, Deerfield, Illinois) with an attached Adsorbosphere guard column. There were three major carotenoid peaks (astaxanthin, carotenoid A, and carotenoid B) present on most chromatograms. Although carotenoids A and B were unidentified, they were characterized by their absorbance spectra (maximum near 450 nm). Because we did not have authentic standards for unidentified carotenoids, we provided only a preliminary quantification relative to authentic β-carotene. Major retinoids found in salmonid eggs included dehydroretinal, retinyl palmitate, and an unidentified dehydroretinyl ester. We categorized the dehydroretinyl ester based on characteristic absorbance spectra similar to those of dehydroretinal, which exhibits absorption maxima at 280 and 340 nm. We estimated the concentration of this dehydroretinyl ester relative to authentic retinyl palmitate.

Stable isotopes.—Approximately 0.5 g of each egg sample was accurately weighed and placed in a test tube. Lipid was extracted by adding 5.0 mL of ethyl acetate: hexane (3:2) to eggs. The mixture was vortex-mixed for several minutes. The organic phase was removed, and the process was repeated with another 2.0 mL of ethyl acetate: hexane. Organic phases were combined and dried under a gentle stream of N_2. The test tube was re-weighed to determine total lipid content of the eggs. The egg residual remaining after lipid extraction was freeze-dried and then ground with a glass pestle. Egg $\delta^{13}C$ and ^{15}N were determined on the egg residual (protein) and $\delta^{13}C$ was determined on the lipid fraction by use of a continuous-flow isotope ratio mass spectrometer (Beaudoin et al. 2001). The $\delta^{13}C$ and $\delta^{15}N$ are the per-mille (‰) deviations of the sample from the recognized isotope standards: Pee Dee Belemnite limestone for $\delta^{13}C$ and atmospheric N_2 for $\delta^{15}N$.

Fatty acid analysis.—Fatty acids were quantified in a three-step process: (a) gravimetric extraction for determination of total lipid (Bligh and Dyer 1959), (b) derivitization of FA methyl esters (FAMEs) based on the Morrison and Smith (1964) boron trifluoride (BF_3–methanol; 10% weight/weight) method, and (c) quantification of FAMEs on a Hewlett-Packard 6890 Series gas chromatograph (GC). A synthetic lipid (cholestane) was added to all samples to provide an estimate of extraction efficiency (Sigurgisladottir et al. 1992). Three pure, individual FA standards, *cis*-11,14-eicosadienoic acid (20:2), EPA, and DHA were used to estimate derivitization efficiency (mean = 76%). Briefly, the GC was configured as follows: splitless injection; Supelco column (Model SP-2560; 100 m × 0.25 mm ID × 0.20-μm-thick film); oven set at 100°C (hold 1 min) and increased to 240°C at a rate of 5°C/min (hold for 38 min); the carrier gas was H at 20 cm/s; the detector was a flame ionization detector set at 260°C; the injector was set at 260°C; and total run time was 67 min/sample. A 37-component FAME standard (Supelco number 47885) was used to produce four-point calibration curves and establish the identity of unknown sam-

TABLE 1.—Gaschromatograph output of the fatty acid methyl ester (FAME) analyses.

Peak	Molecular formula	Chemical name	Common name	Detection limit (μg/mL)
6	12:0	Dodecanoic acid	Lauric acid	0.20
7	13:0	Tridecanoic acid	Tridecanoic acid	0.10
8	14:0	Tetradecanoic acid	Myristic acid	0.20
9	14:1(n-5)	9-tetradecenoic acid	Myristoleic acid	0.10
10	15:0	Pentadecanoic acid	Pentadecanoic acid	0.10
11	15:1	*Cis*-10-pentadecanoic acid	*Cis*-10-pentadecanoic acid	0.10
12	16:0	Hexadecanoic acid	Palmitic acid	0.30
13	16:1(n-7)	9-hexadecenoic acid	Palmitoleic acid	0.10
14	17:0	Heptadecanoic acid	Heptadecanoic acid	0.10
15	17:1	*Cis*-10-heptadecanoic acid	*Cis*-10-heptadecanoic acid	0.10
16	18:0	Octadecanoic acid	Stearic acid	0.20
17	18:1(n-9t)	*Trans*-9-octadenoic acid	Elaidic acid	0.10
18	18:1(n-9c)	*Cis*-9-octadenoic acid	Oleic acid	0.20
19	18:2(n-6t)	*Trans*-9, 12-octadecadienoic acid	Linolelaidic acid	0.10
20	18:2(n-6c)	*Cis*-9, 12-octadecadienoic acid	Linoleic acid	0.10
21	20:0	Eicosanoic acid	Arachidic acid	0.20
22	18:3(n-6)	6, 9, 12-octadecatrienoic acid	γ-linolenic acid	0.10
23	20:1(n-9)	*Cis*-11-eicosenoic acid	Eicosenoic acid	0.10
24	18:3(n-3)	9, 12, 15-octadecatrienoic acid	α-linolenic acid	0.10
25	21:0	Heneicosanoic acid	Heneicosanoic acid	0.10
26	20:2	*Cis*-11, 14-eicosadienoic acid	*Cis*-11, 14-eicosadienoic acid	0.10
27	22:0	Docosanoic acid	Behenic acid	0.20
28	20:3(n-6)	8, 11, 14-eicosatrienoic acid	Homo-γ-linolenic acid	0.10
29	22:1(n-9)	13-docosenoic acid	Crucic acid	0.10
30	20:3(n-3)	11, 14, 17-eicosatrienoic acid	Eicosatrienoic acid	0.10
31	20:4(n-6)	5, 8, 11, 14-eicosatetraenoic acid	Arachidonic acid	0.10
32	23:0	Tricosanoic acid	Tricosanoic acid	0.10
33	22:2	*Cis*-13, 16-docosadienoic acid	*Cis*-13, 16-docosadienoic acid	0.10
34	24:0	Tetracosanoic acid	Lignoceric acid	0.20
35	20:5(n-3)	5, 8, 11, 14, 17-eicosapentaenoic acid	Eicosapentaenoic acid	0.10
36	24:1(n-9)	15-tetracosanoic acid	Nervonic acid	0.10
37	22:5(n-3c)	7, 10, 13, 16, 19-docosapentaenoic acid	Docosapentaenoic acid	0.25
38	22:6(n-3)	4, 7, 10, 13, 16, 19-docosahexaenoic acid	Docosahexaenoic acid	0.10

ple peaks by comparing their retention times to those of the FAME standard. An additional FA, docosapentaenoic acid (22:5[n-3]), was added to the 37-component mix (Table 1). All FA results were reported as milligrams of FAME per milligram dry mass of tissue, and only the FAMEs consistently detected in all egg samples are reported in the tables. Further details on the extraction, methylation, and GC quantification processes can be found in Zellmer et al. (2004).

Statistics.—For coho salmon data, all biochemical measures except for TMP, $\delta^{13}C$, and DHA were $\log_e$ transformed. Thiamine monophosphate was square-root transformed, while $\delta^{13}C$ and DHA values were normally distributed and were therefore left untransformed. The Chinook salmon biochemical measures were $\log_e$ transformed except for TMP, palmitic acid, linoleic acid, and eicosatrienoic acid, which were left untransformed. The effect of sample location and EMS presence was tested on the group means in a split-block design by use of a general linear model in SYSTAT (SPSS 1999) with sample year as the replicate. Thus, to test for main effect differences, we used the replicate-by-effect cross product as an error term, thereby avoiding a pseudoreplicated design. Because none of the measured parameters exhibited a significant among-year difference, data for all years were grouped together to simplify presentation in tables and figures. We found that astaxanthin was correlated with egg lipid content, and therefore it was corrected relative to lipid content for each egg sample and the corrected values are presented. No other measures were correlated with egg lipid content. Coho salmon and Chinook salmon maternal body sizes were assessed for covariance with EMS and collection location. Maternal size was correlated to some parameters; in such cases, we tested the parameters by use of size as a covariate. Pairwise comparisons were conducted by applying the least-significant-difference test to the least-squares means produced by the analysis of variance. Linear relationships between variables were examined by Pearson's product-moment cor-

relation. For all tests, a probability level less than 0.05 was considered significant. For clarity of presentation, arithmetic means with standard errors are used in tables and figures.

Results

Coho Salmon

Fish and egg characteristics.—Hatch-to-feeding-fry mortality averaged 80.4% in +EMS coho salmon from the Platte River and 95.2% in +EMS fish from Thompson Creek (Table 2). For coho salmon collected in 1998 and 1999, females that produced −EMS offspring tended to weigh less and to exhibit higher CFs than females that produced +EMS offspring. Egg size was positively correlated with maternal size ($r^2 = 0.394$, $P < 0.01$). Egg moisture content was slightly higher in Thompson Creek fish than in Platte River fish, and total egg lipid was slightly lower in −EMS fish than in +EMS fish.

Thiamine.—Total thiamine concentrations were lower in +EMS family groups of Lake Michigan coho salmon than in −EMS family groups. This was mostly attributable to lower levels of Th (Table 2). Thresholds for presence of EMS were about 0.4 nmol Th/g (Figure 1).

Carotenoids, retinoids, and tocopherol.—In Lake Michigan coho salmon collected from the Platte River and Thompson Creek, −EMS eggs contained higher total levels of carotenoids (astaxanthin, carotenoid A, and carotenoid B) than did +EMS eggs (Table 2). While dehydroretinal was lower in the −EMS groups than in the +EMS groups, retinyl palmitate stores were higher in −EMS fish. In the −EMS groups from both the Platte River and Thompson Creek, egg concentrations of tocopherol were 12–20% lower than those in +EMS groups.

Stable isotopes.—When stable isotopes in eggs of fish exhibiting +EMS were compared to those of −EMS eggs, there were no differences in $\delta^{13}C$ for either protein or lipid fractions (Table 2). We found a slight but consistent depletion of $\delta^{15}N$ content in eggs from the +EMS group.

Fatty acids.—In coho salmon, there were some minor differences in egg FA profile between the −EMS and +EMS groups. Specifically, myristic, palmitoleic, and eicosenoic acids were 10–20% lower in the −EMS fish than in +EMS fish, while nervonic acid (24:1[n-9]) was 30–40% higher in the −EMS group (Table 2).

Chinook Salmon

Fish and egg characteristics.—In Chinook salmon, hatch-to-feeding-fry mortality averaged 83.8% in the +EMS group from the Swan River and 70.4% in the +EMS group from the Little Manistee River (Table 3). Females producing −EMS offspring tended to be smaller than +EMS fish in the Swan River stock, but no such difference was evident for Little Manistee River Chinook salmon. In contrast to the results in coho salmon stocks, maternal CF did not differ between −EMS and +EMS Chinook salmon. Similar to results in coho salmon, egg size was positively correlated with maternal size ($r^2 = 0.47$, $P < 0.01$), but egg size was not related to the presence of EMS. Also, egg moisture and lipid content did not differ between the Swan River and Little Manistee River groups.

Thiamine.—The threshold for presence of EMS in Chinook salmon was about 0.6 nmol/g for Th (Figure 1). Total thiamine concentrations were lower in the family groups of Chinook salmon that exhibited EMS. As was observed for +EMS coho salmon, this was mostly associated with lower levels of Th in +EMS fish (Table 3). The average Th concentration (1.06 nmol/g) in −EMS Chinook salmon from the Little Manistee River was fairly close to the threshold for EMS and was about 34% of the level found in the −EMS group from the Swan River.

Carotenoids, retinoids, and tocopherol.—Fewer carotenoid and retinoid differences associated with the presence of EMS were seen in Chinook salmon than in coho salmon. Carotenoid B was generally higher in the Swan River stock than in fish from the Little Manistee River. Carotenoid B and retinyl palmitate levels were higher in −EMS fish from the Swan River than in +EMS fish (Table 3). There were no significant differences in carotenoids or retinoids relative to EMS status for Little Manistee River Chinook salmon. Tocopherol concentration showed considerable variability and did not differ relative to location or to the presence of EMS.

Stable isotopes.—In Swan River Chinook salmon, the $\delta^{15}N$ content of eggs from the +EMS group was significantly depleted relative to the −EMS group. However, in Little Manistee River fish, $\delta^{15}N$ content of eggs did not differ with respect to EMS, and the values were similar to those of the +EMS group in the Swan River. There were no differences in $\delta^{13}C$ from either the protein or lipid fraction in eggs between +EMS and −EMS Chinook salmon (Table 3) for either location.

Fatty acids.—Egg contents of palmitic acid, *cis*-

TABLE 2.—Incidence of early mortality syndrome (EMS), fish size, egg characteristics, and egg concentrations of thiamine, retinoids, carotenoids, tocopherol, stable isotopes and those fatty acids consistently detected in coho salmon eggs from two Lake Michigan stocks. Values represent means, with SEs in parentheses. Means in a table row with differing letters are significantly different ($P < 0.05$).

Variable	Platte River		Thompson Creek	
	+EMS[a]	−EMS[b]	+EMS[a]	−EMS[b]
N	14	10	18	9
Hatch–feeding fry mortality (%)	80.39 (2.06) z	4.90 (0.62) y	95.16 (0.83) z	13.10 (0.88) y
Maternal weight (kg)	1.96 (0.10) z	1.22 (0.05) y	2.77 (0.12) z	1.46 (0.05) y
Maternal length (cm)	70.54 (1.65) z	51.32 (0.92) y	69.65 (0.76) z	51.83 (0.42) y
Maternal condition factor	0.55 (0.03) x	0.89 (0.01) y	0.83 (0.03) y	1.05 (0.02) z
Egg weight	0.22 (0.01)	0.16 (0.004)	0.26 (0.01)	0.20 (0.01)
Egg moisture (%)	41.27 (0.31) yx	42.42 (0.38) zy	40.31 (0.31) x	45.43 (0.55) z
Egg dry weight lipid (%)	31.66 (0.56) z	26.63 (0.34) y	30.69 (0.22) z	28.20 (0.54) y
TPP[c] (nmol/g)	0.76 (0.03) x	1.12 (0.05) y	0.95 (0.03) x	2.18 (0.11) z
TMP[d] (nmol/g)	0.51 (0.02) y	0.81 (0.05) z	0.39 (0.02) y	1.17 (0.06) z
Th[e] (nmol/g)	0.41 (0.04) y	4.01 (0.38) z	0.13 (0.005) y	3.12 (0.37) z
Astaxanthia (μg/g)	24.59 (1.12) x	52.24 (3.04) z	20.97 (0.85) x	33.04 (1.97) y
Carotenoid A (μg/g)	2.82 (0.17) y	6.69 (0.47) z	1.71 (0.09) x	3.02 (0.18) z
Carotenoid B (μg/g)	0.81 (0.08) x	3.55 (0.31) z	0.89 (0.07) x	1.80 (0.14) y
Dehydroretinol (μg/g)	0.52 (0.02) z	0.33 (0.02) y	0.53 (0.01) z	0.31 (0.03) y
Dehydroretinol ester (μg/g)	3.07 (0.11)	2.67 (0.08)	3.71 (0.12)	4.09 (0.26)
Retinyl palmitate (μg/g)	1.26 (0.06) y	2.39 (0.15) z	1.29 (0.05) y	2.60 (0.19) z
Tocopherol (μg/g)	180.30 (4.75) z	159.10 (2.81) yx	176.36 (2.11) z	137 94 (3.50) x
δ^{15}N protein (‰)	13.03 (0.03) z	12.59 (0.04) y	13.03 (0.01) z	12.75 (0.05) y
δ^{13}C protein (‰)	−25.09 (0.02)	−24.82 (0.03)	−24.87 (0.02)	−24.83 (0.04)
δ^{13}C lipid (‰)	−31.07 (0.03)	−21.00 (0.02)	−30.96 (0.02)	−30.94 (0.09)
Fatty acids (mg/g)				
Myristic	0.86 (0.01) z	0.78 (0.01) y	0.84 (0.01) z	0.67 (0.03) y
Pentadecanoic	0.20 (0.003)	0.22 (0.004)	0.19 (0.002)	0.17 (0.01)
Palmitic	10.35 (0.18)	12.50 (0.23)	10.47 (0.10)	10.89 (0.30)
Palmitoleic	1.75 (0.02) z	1.49 (0.03) y	1.71 (0.02) z	1.56 (0.04) y
Heptadecanoic	0.24 (0.005)	0.34 (0.01)	0.26 (0.003)	0.28 (0.02)
Stearic	5.33 (0.11)	6.26 (0.11)	5.68 (0.05)	6.13 (0.14)
Elaidic	0.08 (0.002)	0.09 (0.004)	0.08 (0.003)	0.05 (0.01)
Oleic	6.71 (0.07)	6.24 (0.11)	6.53 (0.06)	6.06 (0.15)
Linoleic	1.40 (0.02)	1.37 (0.03)	1.34 (0.01)	1.42 (0.03)
Arachidic	0.16 (0.003)	0.16 (0.003)	0.15 (0.003)	0.14 (0.01)
γ-linolenic	0.06 (0.002)	0.08 (0.002)	0.06 (0.001)	0.07 (0.004)
Eicosenoic	0.95 (0.02) z	0.76 (0.01) y	0.92 (0.01) z	0.66 (0.03) y
α-linolenic (ALA)	1.20 (0.02)	1.24 (0.02)	1.21 (0.02)	1.33 (0.02)
Cis-11, 14-eicosadienoic	0.88 (0.01)	0.81 (0.02)	0.82 (0.01)	0.75 (0.03)
Homo-γ-linolenic	0.26 (0.02)	0.26 (0.006)	0.28 (0.002)	0.26 (0.01)
Arachidonic	6.62 (0.07)	7.18 (0.13)	6.66 (0.06)	6.75 (0.23)
Cis-13, 16-docosadienoic	0.16 (0.02)	0.23 (0.04)	0.28 (0.02)	0.53 (0.04)
Nervonic	0.47 (0.01) y	0.68 (0.03) z	0.45 (0.01) y	0.60 (0.03) z
Docosapentaenoic	6.46 (0.10)	5.78 (0.08)	5.97 (0.06)	5.41 (0.08)
Eicosatrienoic	0.65 (0.01)	0.58 (0.01)	0.62 (0.01)	0.62 (0.01)
Eicosapentaenoic (EPA)	6.73 (0.09)	7.45 (0.08)	7.03 (0.07)	7.40 (0.15)
Docosahexaenoic (DHA)	18.09 (0.18)	19.18 (0.34)	16.88 (0.15)	16.72 (0.49)
Σω3[f]	33.14 (0.35)	34.23 (0.48)	31.70 (0.25)	31.48 (0.69)
Σω6[g]	8.34 (0.09)	8.90 (0.16)	8.35 (0.08)	8.50 (0.26)
SAFA[h]	17.15 (0.30)	20.28 (0.36)	17.58 (0.15)	18.28 (0.48)
MUFA[i]	9.97 (0.11)	9.25 (0.15)	9.68 (0.08)	8.93 (0.21)
PUFA[j]	42.52 (0.43)	44.18 (0.65)	41.15 (0.32)	41.25 (0.93)
TFA[k]	69.63 (0.69)	73.71 (1.14)	68.41 (0.53)	68.46 (1.55)
EPA/ARA ratio	1.02 (0.01)	1.06 (0.01)	1.06 (0.01)	1.15 (0.03)
ΣALA, EPA, DHA	26.03 (0.25)	27.88 (0.41)	25.12 (0.21)	25.45 (0.62)
ΣC15:0, C17:0, C17:1, C18:1[l]	7.23 (0.08)	6.89 (0.12)	7.06 (0.06)	6.56 (0.16)
Σω3:Σω6 ratio	3.98 (0.02)	3.89 (0.04)	3.82 (0.02)	3.79 (0.05)

[a] Swim-up fry with EMS.
[b] Swim-up fry with no excess mortality from EMS.
[c] Thiamine pyrophosphate.
[d] Thiamine monophosphate.
[e] Unphosphorylated thiamine.
[f] Sum of omega-3 fatty acids.
[g] Sum of omega-6 fatty acids.
[h] Sum of saturated fatty acids.
[i] Sum of monounsaturated fatty acids.
[j] Sum of polyunsaturated fatty acids.
[k] Total fatty acid sum.
[l] Bacterial-source fatty acids.

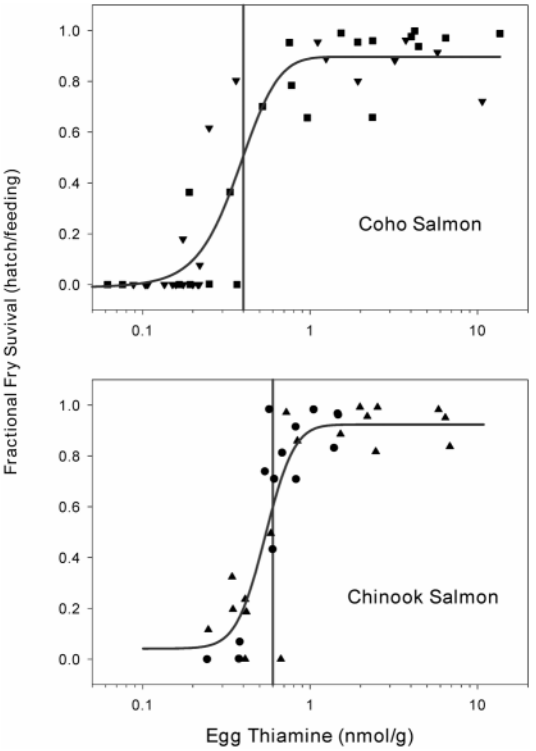

FIGURE 1.—Relationships between hatch-to-feeding-fry survival in offspring and egg levels of free thiamine. The upper panel shows the relationship for coho salmon from the Platte River (squares) and Thompson Creek (inverted triangles), the lower panel that for Chinook salmon from the Little Manistee River (circles) and the Swan River (upright triangles). Vertical lines indicate the estimated threshold egg concentration of unphosphorylated thiamine for the presence of early mortality syndrome.

13,16-docosadienoic acid, nervonic acid, and the sum of saturated fatty acids (SAFA) were all higher in Little Manistee River Chinook salmon than in fish from the Swan River. However, we found no FA profile differences associated with the presence or absence of EMS in Little Manistee River Chinook salmon. In Chinook salmon from the Swan River, the presence of EMS was associated with slightly lower egg concentrations of oleic acid (18:1[n-9]), DHA, sum of monounsaturated FAs (MUFA), and bacterial-source FAs and slightly higher egg concentrations of eicosatrienoic acid (20:3[n-3]).

Discussion

The expression of EMS and associated low Th in eggs of larger-sized Chinook salmon and coho salmon from the Platte River, Thompson Creek, and the Swan River was similar to that reported by Wolgamood et al. (2005). These observations, coupled with the evidence for maternal size differences between the +EMS and the –EMS groups, suggest that the subset of fish we used for analysis of dietary markers was generally representative of the fish monitored for EMS in Lake Michigan (Wolgamood et al. 2005). A size difference was also reported in Atlantic salmon *Salmo salar* whose offspring exhibited Th deficiency and expressed early life stage mortality (Fisher et al. 1998). The general applicability of the lower CF in +EMS fish remains to be established because Chinook salmon exhibiting EMS showed no evidence of poorer condition than −EMS fish. However, anorexia and weight loss are symptoms of thiamine deficiency in experimental fish (Saunders and Henderson 1974; Morito et al. 1986). As previously observed in lake trout *Salvelinus namaycush* (Fitzsimons et al. 1995; Brown et al. 1998), egg size in coho salmon and Chinook salmon, while directly related to maternal size, was unrelated to the presence of EMS in offspring.

The low levels of Th and threshold concentrations associated with EMS in coho salmon were consistent with values reported by Wolgamood et al. (2005) and in previous studies for this species (Honeyfield et al. 1998; Hornung et al. 1998). The lower carotenoids in +EMS coho salmon from the Platte River and Thompson Creek are similar to changes in carotenoids, especially low astaxanthin, that have been related to the presence of M74 in Baltic salmon (Pettersson and Lignell 1998, 1999; Pickova et al. 1998; Lundström et al. 1999). In lake trout from Lake Ontario, eggs that developed EMS had significantly lower total carotenoid stores than eggs that did not exhibit the syndrome (Palace et al. 1998). In Chinook salmon from the Swan and Little Manistee rivers, there were no significant differences in carotenoids, suggesting that carotenoid content is not directly associated with the presence of EMS. In coho salmon and Chinook salmon eggs, higher concentrations of retinyl palmitate occurred in −EMS groups from the Platte River, Thompson Creek, and the Swan River. Christiansen and Torrissen (1997) investigated the role of dietary astaxanthin in Atlantic salmon by maintaining fish on diets with 0 and 100 mg of astaxanthin per kilogram during the entire period of seawater rearing (2.5 and 3.5 years). These fish produced eggs that contained 0–15 mg astaxanthin/kg, but there were no relationships between astaxanthin content of eggs and subsequent survival and growth of yolk sac fry. Moreover, the

TABLE 3.—Incidence of early mortality syndrome (EMS), fish size, egg characteristics, and egg concentrations of thiamine, retinoids, carotenoids, tocopherol, stable isotopes, and those fatty acids consistently detected in Chinook salmon eggs. Values represent means, with SEs in parentheses. Means in a table row with differing letters are significantly different ($P < 0.05$).

Variable	Swan River, Lake Huron		Little Manistee River, Lake Michigan	
	+EMS[a]	−EMS[b]	+EMS[a]	−EMS[b]
N	10	10	9	7
Hatch–feeding fry mortality (%)	83.84 (1.62) z	7.60 (0.68) y	70.40 (3.84) z	7.77 (1.03) y
Maternal weight (kg)	6.23 (0.13) y	4.46 (0.15) x	7.3 (0.19) z	6.95 (0.26) z
Maternal length (cm)	88.95 (0.51)	83.61 (0.57)	87.9 (0.74)	88.02 (0.86)
Maternal condition factor	0.97 (0.01)	0.85 (0.01)	1.07 (0.01)	1.00 (0.01)
Egg weight (g)	0.28 (0.00)	0.27 (0.01)	0.30 (0.00)	0.30 (0.01)
Egg moisture (%)	38.02 (0.48)	38.58 (0.48)	42.99 (0.24)	39.27 (0.74)
Egg dry weight lipid (%)	32.06 (0.63)	30.01 (0.56)	33.37 (0.88)	28.26 (0.62)
TPP[c] (nmol/g)	0.21 (0.01)	0.29 (0.01)	0.32 (0.01)	0.45 (0.03)
TMP[d] (nmol/g)	0.11 (0.01) y	0.21 (0.02) z	0.15 (0.004) y	0.25 (0.01) z
Th[e] (nmol/g)	0.52 (0.03) x	3.14 (0.23) z	0.61 (0.03) x	1.06 (0.05) y
Astaxanthin (μg/g)	13.31 (0.58)	15.68 (0.70)	20.04 (1.12)	16.55 (1.47)
Carotenoid A (μg/g)	1.23 (0.07)	2.00 (0.11)	1.98 (0.11)	1.93 (0.12)
Carotenoid B (μg/g)	0.91 (0.19) y	2.76 (0.28) z	0.51 (0.04) yx	0.19 (0.03) x
Dehydroretinol (μg/g)	0.64 (0.04)	0.66 (0.03)	0.83 (0.02)	0.84 (0.02)
Dehydroretinol ester (μg/g)	1.70 (0.14)	1.83 (0.11)	2.34 (0.17)	1.67 (0.23)
Retinyl palmitate (μg/g)	0.42 (0.04) x	2.22 (0.16) z	1.42 (0.09) zy	0.92 (0.12) zyx
Tocopherol (μg/g)	137.07 (4.27)	160.27 (3.82)	157.60 (7.33)	134.26 (3.00)
δ^{15}N protein (‰)	14.00 (0.04) z	12.84 (0.14) y	13.83 (0.04) z	13.89 (0.08) z
δ^{13}C protein (‰)	−25.05 (0.05)	−24.81 (0.13)	−25.55 (0.06)	−25.22 (0.08)
δ^{13}C lipid (‰)	−31.34 (0.03)	−31.41 (0.06)	−31.13 (0.03)	−31.51 (0.02)
Fatty acids (mg/g)				
Myristic	0.77 (0.01)	0.81 (0.01)	0.84 (0.03)	0.88 (0.02)
Pentadecanoic	0.20 (0.003)	0.19 (0.001)	0.20 (0.01)	0.20 (0.003)
Palmitic	10.28 (0.14) y	10.65 (0.10) y	11.2 (0.28) z	11.69 (0.27) z
Palmitoleic	1.70 (0.04)	2.14 (0.05)	1.8 (0.07)	2.05 (0.06)
Heptadecanoic	0.30 (0.01)	0.30 (0.01)	0.34 (0.01)	0.34 (0.01)
Stearic	6.54 (0.10)	6.62 (0.11)	7.28 (0.21)	7.67 (0.20)
Elaidic	0.08 (0.003)	0.10 (0.01)	0.08 (0.004)	0.07 (0.004)
Oleic	6.86 (0.12) y	7.22 (0.14) z	7.52 (0.18) z	7.44 (0.17) z
Linoleic	1.25 (0.02)	1.22 (0.02)	1.44 (0.04)	1.41 (0.04)
Arachidic	0.16 (0.003)	0.17 (0.003)	0.15 (0.01)	0.18 (0.01)
γ-linolenic	0.07 (0.001)	0.08 (0.001)	0.08 (0.003)	0.08 (0.002)
Eicosenoic	0.67 (0.01)	0.60 (0.02)	0.68 (0.02)	0.64 (0.02)
α-linolenic	0.94 (0.02)	0.82 (0.02)	1.06 (0.04)	1.10 (0.04)
Cis-11, 14-eicosadienoic	0.68 (0.01) y	0.56 (0.01) y	0.76 (0.03) z	0.79 (0.02) z
Homo-γ-linolenic	0.39 (0.01) y	0.42 (0.01) y	0.42 (0.01) z	0.42 (0.01) z
Arachidonic	6.93 (0.12)	7.16 (0.09)	6.97 (0.16)	6.68 (0.15)
Cis-13, 16-docosadienoic	0.28 (0.05)	0.20 (0.04)	0.67 (0.08)	0.82 (0.09)
Nervonic	0.54 (0.02)	0.46 (0.02)	0.67 (0.02)	0.61 (0.02)
Docosapentaenoic	4.78 (0.08)	4.41 (0.08)	4.57 (0.11)	4.77 (0.10)
Eicosatrienoic	0.66 (0.01) z	0.51 (0.01) y	0.60 (0.01) z	0.59 (0.02) zy
Eicosapentaenoic (EPA)	8.17 (0.11)	8.16 (0.10)	8.15 (0.23)	8.52 (0.20)
Docosahexaenoic (DHA)	18.61 (0.26) y	19.54 (0.33) z	19.73 (0.48) z	18.58 (0.42) zy
Σω3[f]	33.16 (0.43)	33.43 (0.42)	34.10 (0.82)	33.57 (0.71)
Σω6[g]	8.63 (0.14)	8.88 (0.11)	8.91 (0.22)	8.58 (0.19)
SAFA[h]	18.25 (0.25) y	18.74 (0.20) y	20.01 (0.52) z	20.97 (0.49) z
MUFA[i]	9.84 (0.16) x	10.53 (0.20) y	10.75 (0.28) y	10.81 (0.24) y
PUFA[j]	42.75 (0.54)	43.07 (0.52)	44.44 (1.09)	43.75 (0.90)
TFA[k]	70.85 (0.89)	72.33 (0.87)	75.20 (1.81)	75.54 (1.49)
EPA/ARA ratio	1.19 (0.01)	1.15 (0.01)	1.16 (0.01)	1.28 (0.01)
ΣALA, EPA, DHA	27.72 (0.36)	28.52 (0.36)	28.94 (0.71)	28.20 (0.63)
ΣC15:0, C17:0, C17:1, C18:1[l]	7.44 (0.13) x	7.81 (0.15) y	8.14 (0.20) y	8.05 (0.18) y
Σω3:Σω6	3.87 (0.03)	3.77 (0.02)	3.85 (0.03)	3.92 (0.03)

[a] Swim-up fry with EMS.
[b] Swim-up fry with no excess mortality from EMS.
[c] Thiamine pyrophosphate.
[d] Thiamine monophosphate.
[e] Unphosphorylated thiamine.
[f] Sum of omega-3 fatty acids.
[g] Sum of omega-6 fatty acids.
[h] Sum of saturated fatty acids.
[i] Sum of monounsaturated fatty acids.
[j] Sum of polyunsaturated fatty acids.
[k] Total fatty acid sum.
[l] Bacterial-source fatty acids.

addition of astaxanthin or β-carotene did not influence the occurrence of EMS in yolk sac fry of steelhead *O. mykiss* (Hornung et al. 1998). Given these findings and given the utility of carotenoid pigment profiles for distinguishing anadromous from nonanadromous brown trout stocks (Youngson et al. 1997), it seems most likely that differences in carotenoid and retinoid profiles between −EMS and +EMS fish from the Platte River, Thompson Creek, and the Swan River are indicative of differing dietary sources.

Tocopherol deficiencies cause growth suppression and liver histopathology in salmon (Taveekijakarn et al. 1996). Palace et al. (1996) correlated elevated oxidative stress and depleted tissue vitamin E in fish exposed to a coplanar polychlorinated biphenyl. However, owing to the high survival observed in −EMS coho salmon, it seems unlikely that their slightly lower concentration of tocopherol relative to the +EMS group had much biological significance. In support of this conclusion, tocopherol concentration was unrelated to the presence of EMS in Chinook salmon from the Swan and Little Manistee rivers.

Based on the similarity of $\delta^{13}C$ in protein and lipid in +EMS and EMS groups, there was little evidence for dietary shifts among sources of C (France 1995). The excretion of $\delta^{15}N$-depleted substances is a common feature of animals, such that N is often an effective predictor of trophic level position in aquatic environments (Fry 1991). Over successive trophic levels, there is normally a 2–5‰ increase in $\delta^{15}N$ (Cabana and Rasmussen 1994; Vander Zanden et al. 1997). Average $\delta^{15}N$ differences between −EMS and +EMS for salmon from the Platte River (0.4‰), Thompson Creek (0.3‰), and the Swan River (1.2‰) were less than that reported for complete trophic level steps. One hypothesis for these small but consistent differentials is that −EMS fish may be consuming more dietary items from lower trophic levels than +EMS fish do. However, because differences in feeding locations of the prey items themselves could also alter $\delta^{15}N$ signatures, further investigations of food web dynamics are required to conclusively identify the altered factors.

In coho salmon, there was little indication that concentrations of the essential FAs like EPA and DHA differed between +EMS and −EMS families. Although the evidence for dietary differences is stronger when altered levels of essential FAs are found, nonessential FAs do change among prey items and hence can also be used in elucidating dietary differences (Smith et al. 1997; Kirsch et al. 1998). The lower levels of some nonessential FAs (myristic, palmitoleic, and eicosenoic acids) in −EMS groups may also indicate dietary differences among groups. The most conspicuous difference for nonessential FAs between eggs of the +EMS and −EMS groups was the significantly higher concentration of nervonic acid in eggs from −EMS fish. Nervonic acid is rare in most tissues and lipid classes but is enriched in nervous tissue, especially in the sphingomyelins that occur in the myelin sheaths of nerve cells. They contain a phosphate group, a sphingosine moiety, and a FA chain (where nervonic acid is common). This sheathing increases the fidelity of the depolarization pulse as it traverses the length of the nerve. The suggestion that production or acquisition of this long-chain FA is somehow impaired in +EMS fish is supported by the suggestion that oleic acid, the precursor of nervonic acid, is found at slightly higher concentrations in their eggs. In Chinook salmon, differences in thiamine level between the −EMS and +EMS populations were not nearly as great as those found for coho salmon. Similarly, differences in the FA profile were less apparent. Of the two groups, the most extreme difference in thiamine level occurred in Swan River Chinook salmon. In this population, we also observed a significantly higher DHA concentration in eggs of the −EMS group than in +EMS eggs. This was associated with evidence of higher levels of bacterial-source FAs. Docosahexaenoic acid is critical in both the development and physiological competency of the nervous system (Horrobin 1998) and visual systems (Bell and Dick 1993) in vertebrates.

For all the salmon stocks studied, there was a very high correspondence between EMS and low concentrations of Th in unfertilized eggs. For three of the four salmon stocks, we found a high differential (sixfold to 20-fold) in Th concentration between −EMS and +EMS groups.

While studies of salmonid diets indicate that alewives form the major part of the diet in Lake Michigan (Miller and Holey 1992; Fitzsimons et al. 1999), measures of egg carotenoids and retinoids, $\delta^{15}N$ depletion, and FA profiles all imply that Platte River, Thompson Creek, and Swan River salmon that produce offspring with low levels of EMS may have more diverse diets than fish with +EMS offspring. Further work investigating the profiles of stable isotopes, carotenoids, and FAs in lower trophic levels will provide information about the potential dietary resources utilized by adult salmon that produce offspring without EMS.

Chinook salmon from the Little Manistee River had Th concentrations in the −EMS group that were only marginally above the threshold for induction of mortality, and there was little evidence of differing dietary components in these fish. Overall, our findings are consistent with the hypothesis that a forage base that limits the content of thiaminase-containing species like the alewife may lead to more thiamine-replete broodstocks and improved embryonic survival for feral salmonids.

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