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Spatial and temporal dynamics of lake whitefish (*Coregonus clupeaformis*) health indicators: Linking individual-based indicators to a management-relevant endpoint

Tyler Wagner [a,*], Michael L. Jones [a], Mark P. Ebener [b], Michael T. Arts [c], Travis O. Brenden [a], Dale C. Honeyfield [d], Gregory M. Wright [e], Mohamed Faisal [f,g]

[a] *Quantitative Fisheries Center, Department of Fisheries and Wildlife, Michigan State University, 153 Giltner Hall, East Lansing, MI 48824-1101, USA*
[b] *Chippewa Ottawa Resource Authority, 179 West Three Mile Road, Sault Ste. Marie, MI 49783, USA*
[c] *Environment Canada, 867 Lakeshore Rd. Burlington, ON, Canada L7R 4A6*
[d] *US Geological Survey, Northern Appalachian Research Laboratory, 176 Straight Run Road, Wellsboro, PA 16901, USA*
[e] *Nunns Creek Fishery Enhancement Facility, HC 47, Box 8100, Hessel, MI 49745, USA*
[f] *Department of Pathobiology and Diagnostic Investigation College of Veterinary Medicine, Michigan State University S-110 Plant Biology Building East Lansing, Michigan 48824, USA*
[g] *Department of Fisheries and Wildlife College of Agriculture and Natural Resources, Michigan State University S-110 Plant Biology Building East Lansing, Michigan 48824, USA*

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ABSTRACT

We examined the spatial and temporal dynamics of health indicators in four lake whitefish (*Coregonus clupeaformis*) stocks located in northern lakes Michigan and Huron from 2003 to 2006. The specific objectives were to (1) quantify spatial and temporal variability in health indicators; (2) examine relationships among nutritional indicators and stock-specific spatial and temporal dynamics of pathogen prevalence and intensity of infection; and (3) examine relationships between indicators measured on individual fish and stock-specific estimates of natural mortality. The percent of the total variation attributed to spatial and temporal sources varied greatly depending on the health indicator examined. The most notable pattern was a downward trend in the concentration of highly unsaturated fatty acids (HUFAs), observed in all stocks, in the polar lipid fraction of lake whitefish dorsal muscle tissue over the three study years. Variation among stocks and years for some indicators were correlated with the prevalence and intensity of the swimbladder nematode *Cystidicola farionis*, suggesting that our measures of fish health were related, at some level, with disease dynamics. We did not find relationships between spatial patterns in fish health indicators and estimates of natural mortality rates for the stocks. Our research highlights the complexity of the interactions between fish nutritional status, disease dynamics, and natural mortality in wild fish populations. Additional research that identifies thresholds of health indicators, below (or above) which survival may be reduced, will greatly help in understanding the relationship between indicators measured on individual fish and potential population-level effects.

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Introduction

Fisheries management agencies routinely monitor fish health indicators with the goal of assessing responses to perturbations resulting from serious pathogens, malnutrition, management actions, and/or environmental change. For example, it has become commonplace to assess fish nutritional health using direct measures of energy reserves such as total lipid content and/or concentrations of fatty acids (Morton and Routledge 2006; Peters et al., 2007; Cai et al., 2007; Arts and Kohler 2009), used alone or in combination with other fish health indicators, such as condition indices and indicators of disease and infection such as hematological and blood protein measures (Lloret et al., 2002; Adams et al., 2003; Hartman and Margraf 2006; Islam and Tanaka 2006; DeBruyne et al., 2008; Butterworth et al., 2008). These measures become increasingly valuable when they reliably inform managers to take preventative actions to avoid overexploitation or other anthropogenic stresses on economically valuable fish stocks. However, to use health indicators in this way, the links between these indicators and population dynamics need to be better understood. This is important since nutritionally stressed and diseased individuals may experience poor growth and low survival combined with reduced foraging capacity (Thompson et al., 1991; Sheldon and Blazer 1991; Sutton et al., 2000a; Nakayama et al., 2003; Peters et al., 2007), which can in turn affect sustainable harvest rates. Relating changes in health indicators to changes in natural mortality rates for a species would be especially valuable because natural mortality estimates can have a strong influence on stock assessments and harvest policy. For example, sustainable management of exploited fish populations

* Corresponding author. Current address: US Geological Survey, Pennsylvania Cooperative Fish and Wildlife Research Unit, Pennsylvania State University, University Park, PA 16802, USA.

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requires that total mortality remains below a level that allows maintenance of adequate biomass of spawning fish. If natural mortality rates are underestimated, allowable harvest rates may be overestimated.

Natural mortality rates are very difficult to measure in fish populations. Conventional stock assessment methods, such as surplus production modeling or statistical catch-at-age analyses (Quinn and Deriso 1999), either do not incorporate natural mortality as an explicit parameter or tend to confound natural mortality with other demographic parameters. Direct estimation of natural mortality in a fish population usually requires a tagging study (Ebener et al., 2010a). Measuring natural mortality in a fish stock creates an opportunity to relate this critical demographic parameter to fish health indicators. If associations between readily measured health indicators and natural mortality can be identified, these health indicators could be exploited in future assessments to provide an indication of possible changes in natural mortality rates for a stock. In this study we have combined a tagging study to assess natural mortality rates with a survey of several fish health indicators for four lake whitefish (*Coregonus clupeaformis*) stocks in lakes Michigan and Huron to explore these potential linkages.

Lake whitefish are a benthivorous, coldwater fish, generally associated with deepwater habitats (Ebener et al., 2008), but which provide an important trophic link between benthic and pelagic food webs. Lake whitefish are also the basis of an important commercial fishery in the Great Lakes (Ebener et al., 2008). Recent unexplained declines in lake whitefish condition and growth have prompted concerns among ecologists and fishery managers as to the status of several populations (Nalepa et al., 2005). Hypotheses to explain the declines include; declining abundance of their lipid-rich prey resource (the benthic amphipod spp.), colonization by dreissenid mussels (*Dreissena polymorpha*, and *D. bugensis*), and density-dependent effects (McNickle et al., 2006; Kratzer et al., 2007; Pothoven and Madenjian 2008). Thus, there is a general need to better understand the dynamics of lake whitefish health indicators, and a specific need to determine if the variability in health indicators can be related to natural mortality rates.

Before health indicators can be related to population dynamics it is necessary to understand how variation in health indicators is partitioned spatially and temporally. This is necessary because of the substantial variability frequently observed in fish health indicators. Understanding variation in fish health indicators is particularly important when attempting to relate indicators measured at one level of organization (i.e., individual fish) to a management-relevant endpoint measured at a higher-level of organization (i.e., population level). In addition, relationships among health indicators and between these indicators and natural mortality rates will assist in generating hypotheses with respect to cause–effect relationships and help identify future research needs.

To this end, this study was designed with the following objectives: (1) to quantify spatial and temporal variability in health indicators for four stocks of lake whitefish; (2) to examine relationships between nutritional indicators and stock-specific spatial and temporal dynamics of pathogen prevalence and intensity of infection; and (3) to examine relationships between indicators measured at the individual fish-scale to the management-relevant endpoint of stock-specific natural mortality rates.

Methods and materials

Study area

We studied four lake whitefish stocks, two located in northern Lake Huron and two from northern Lake Michigan. For simplicity, we reference these stocks by the names of their closest fishing port: Big Bay de Noc, Naubinway, Cheboygan, and Detour Village. The Big Bay de Noc and Naubinway stocks are located in northern Lake Michigan, while the Cheboygan and Detour Village stocks are located in north-western Lake Huron (Fig. 1). Each of these areas has large spawning aggregations of lake whitefish, and although less than 50 km separates some of these locations, individuals have been found to display strong fidelity to these areas during the spawning season (Ebener and Copes 1985; Ebener et al. 2010b). These stocks spawn in different lake whitefish management units (Big Bay de Noc — WFM-01; Naubinway —

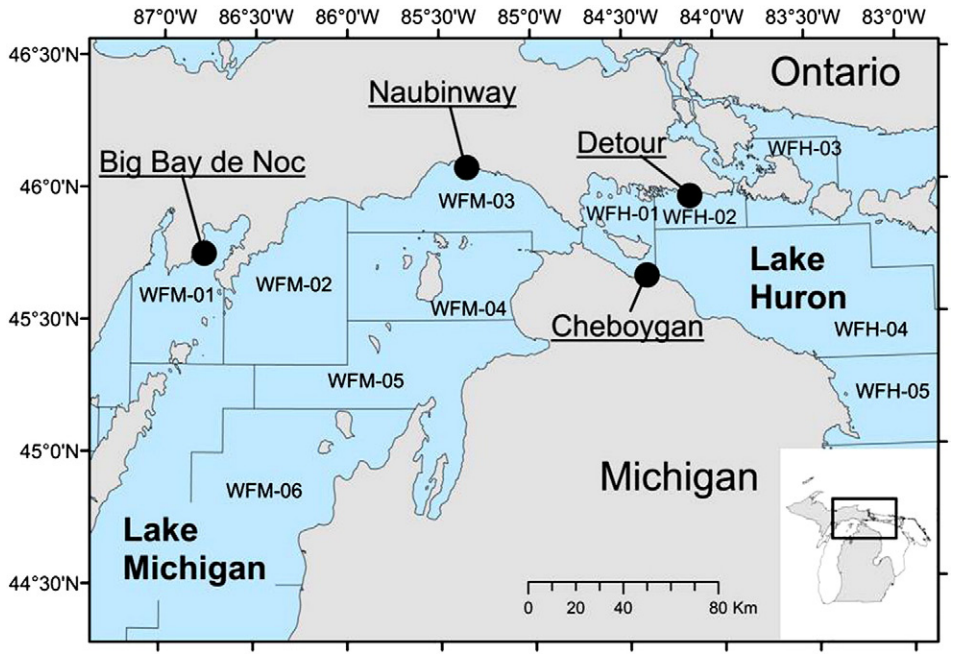

Fig. 1. Map of northern lakes Huron and Michigan indicating the locations of the Big Bay de Noc, Cheboygan, Detour, and Naubinway lake whitefish stocks upon which this research was based. The locations of whitefish management units (WFM-xx) which are managed through individual assessment models are shown.

WFM-03; Cheboygan — WFH-01; Detour — WFH-02; Fig. 1), which are partially managed using independent stock assessment models, and thus we treated them separately even though there is evidence of movement out of these management units at other times of the year (Ebener et al. 2010b).

Fish sampling

We sampled lake whitefish from each stock during each of the four seasons: fall (October–December), winter (January–March), spring (April–June), and summer (July–September) during 2003–2006. Throughout this study, sample years "one", "two" and "three" refer to fall 2003–summer 2004, fall 2004–summer 2005, fall 2005–summer 2006, respectively. Sampling locations were typically chosen by commercial fishermen based on their fishing practices and through consultation with project investigators.

Trap nets were used to capture lake whitefish in the fall, spring, and summer, whereas gill nets were used to capture fish in the winter. Lake whitefish were collected on 17 occasions from the Big Bay de Noc stock every sample year and in every season. Seven of eight samples collected in the winter from Big Bay de Noc were made with gill nets, the other nine collections were with trap nets. Fish were collected on 11 occasions from the Naubinway stock every sample year and every season except winter 2004; trap nets were used in the spring, summer, and fall, and gill nets in the winter. Fish were collected on 12 occasions from the Detour stock in every sample year and every season using trap nets. Fish were collected on 17 occasions from the Cheboygan stock in every sample year and every season except fall 2005; gill nets were used in the winter and trap nets were used in all other seasons. Trap nets were typically lifted after 1–5 nights, whereas gill nets were usually lifted after 1–2 nights. Trap nets were fished at depths of 2–46 m depending upon season; i.e. shallow in fall and deep in summer, whereas gill nets were typically fished at depths of 9–60 m. We sought to collect approximately equal numbers of male and female fish during each sampling occasion, but it was only possible to distinguish between sexes in the fall.

For compositional analysis (whole-body percent lipids and water, and fatty acid methyl esters [FAMEs]) we sought to collect 40 fish per stock during each sampling occasion. Simultaneously, we attempted to collect an additional 30 fish from each stock and sampling period for pathological analysis. Separate fish were collected for pathological analysis because of contamination and logistic concerns involved in processing the same fish for both pathogens and compositional analysis. Collecting separate fish for pathological and compositional analyses necessitated a novel approach to statistical analyses aimed at modeling the relationship among these health indicators (see Statistical analysis).

Laboratory analyses

Whole-body lipids and percent water

Gross compositional analyses of whitefish whole-body water and total lipid content were conducted at Michigan State University. The entire carcass was homogenized using a tabletop grinder. A 5 g sub-sample was freeze-dried and subsequently weighed to obtain a measure of water content. The total lipid content of the freeze-dried sample was determined using the Soxtec solvent extraction method (Soxtec System HT6; Tecator, Sweden; AOAC, 1995), and is reported on a percent of dry weight basis.

Fatty acid analysis

We measured selected fatty acids as FAMEs in the polar lipid fraction of skinless dorsal muscle tissue (landmarked to either side of the dorsal fin). We focused on the polar lipid fraction because it is mainly comprised of phospholipids, the main structurally and physiologically important constituents of cell membranes, and as such is expected to reflect longer-term adaptations to more sustained changes in dietary fatty acid (FA) supply.

Selected FAs included α-linoleic acid (18:3*n*−3; ALA), linoleic acid (18:2*n*−6; LIN), palmitoleic acid (16:1*n*−7), arachidonic acid (20:4*n*−6; ARA), eicosapentaenoic acid (20:5*n*−3; EPA), and docosahexaenoic acid (22:6*n*−3; DHA). We focused on these FAs because they are known to be associated with a wide variety of physiological competencies in fish including cold adaptation, immune response, growth, and reproduction (Bell et al., 1995; Sargent et al., 1999; Arts and Kohler 2009). We also examined the DHA/ARA ratio and an unsaturation index (UI). The UI was calculated using the formula:

$$\mathrm{UI} = \sum_{i-j}(\text{proportion of fatty acid}_i \times \text{number of double bonds of fatty acids}_i). \quad (1)$$

Although the UI has not yet been definitively associated with fish health and growth, it does provide a weighted metric of unsaturated FAs, and is particularly weighted towards the highly unsaturated fatty acids (HUFA; e.g. ARA, EPA and DHA) which are known to be associated with growth and health of fish (Balfry and Higgs 2001, Bogut et al., 2002; Arts and Kohler 2009).

Muscle tissue biopsies were quickly collected from anesthetized lake whitefish, cryogenically-frozen (dry ice), and then shipped on dry ice to the National Water Research Institute where they remained under cryogenic conditions (−85 °C) until they could be freeze-dried in preparation for total lipid and FAME analyses. Analysis included three procedures: gravimetric extraction, derivatization, and quantification on a gas chromatograph (GC) following the methods described in Zellmer et al. (2004) with the exception of the following. The bulk lipid extracts were applied to pre-conditioned Sep-Paks (Waters Silica cartridges, #WAT023537) and, following a series of pre-extractions, the phospholipid fraction was eluted and collected in the final wash and then dried under nitrogen gas for later GC analyses. FAMEs were identified using Supelco's 37 component FAME mix (#47885-U). An internal standard (5 α-cholestane; Sigma-Aldrich; #C8003) was added to the tissue before extraction to estimate the percent recovery for the entire procedure. The FA results are reported as μg FA/mg dry mass of tissue and as μg FA/mg polar lipid.

Pathological analysis

We focused on two major pathogens; the swimbladder nematode *Cystidicola farionis* and the gram-positive diplobacillus *Renibacterium salmoninarum* (the causative agent of bacterial kidney disease, BKD). We focused on these pathogens because both are widespread in the Laurentian Great Lakes (Faisal and Hnath 2005) and represent two potential sources of natural mortality of lake whitefish.

C. farionis is a relatively long-lived nematode, like other species of *Cystidicola*, living up to several years in the swimbladder of infected fish (Black and Lankester 1980), with no apparent movement of adult worms out of the swimbladder. The long life-span and lack of movement out of the swimbladder provide the opportunity for large numbers of parasites to accumulate over a fish's lifetime causing damage of the swimbladder membranes (Faisal et al., 2010). In this study, nematode collection, processing, and identification were performed as detailed in and Faisal et al. (2010). Prevalence of *C. farionis* was calculated as the number of infected fish divided by the total number of fish. Intensity of infection was calculated as the total number of *C. farionis* worms (larval and adult stages combined) divided by the number of infected fish.

BKD is a serious disease of salmonines and is very difficult to control (Faisal and Hnath 2005). The disease is characterized by the excessive formation of granulomatous responses in hematopoietic

organs, kidney damage, accumulation of harmful inflammatory products, and immunosuppression (Bruno 1986; Olsen et al., 1992). In the present study, *R. salmoninarum* prevalence and intensity was ascertained using enzyme-linked immunosorbent assay (ELISA). ELISA was performed as described in Eissa (2005). We cultured *R. salmoninarum* on Modified Kidney Disease Medium (MKDM) and all colonies were investigated for their conformance with colony and bacterial morphological criteria of *R. salmoninarum* as well as its biochemical characteristics as detailed in Sanders and Fryer (1980), Austin and Austin (1999), and Bruno and Munro (1986).

Statistical analysis

Partitioning individual variation in fish health indicators

We used mixed models to examine how the total variation in whole-fish water and lipid content, and selected tissue FAMEs were partitioned spatially and temporally. The variance partitioning was restricted to the indicators from the compositional analyses because the pathological data were collected on different fish. However, the pathological data were subsequently integrated into the statistical analysis (see Fish, stock, and annual correlates of fish health). The spatial–temporal variance components included; stock-to-stock variation, year-to-year variation, stock-by-season variation, stock-by-year variation, year-by-season variation, and residual variation. We were particularly interested in the amount of variation among stocks and years, because this addressed questions of whether fish within stocks were more similar to one another compared to fish among stocks, or if all stocks demonstrated similar dynamics over time. However, we also included higher-order interactions to quantify the proportion of the total variation due to independent seasonal or annual variation among stocks, seasons, and years. The mixed model used to partition the total variation is given by:

$$y_{i,j,k,l} = u + \alpha_j + \upsilon_l + \eta_{j,k} + \pi_{j,l} + \tau_{k,l} + e_{i,j,k,l} \quad (2)$$

where y is a measure of fish health for fish i, $i=1\ldots,n$ and n is the total number of fish sampled in stock j, $j=1\ldots,4$, with stocks corresponding to Big Bay de Noc, Naubinway, Cheboygan, and Detour Village, in season k, $k=1\ldots,4$, where seasons correspond to season of sampling and include fall, winter, spring, and summer, and in year l, $l=1\ldots,3$. The fixed intercept in the model is u and represents the grand mean of the response variable y. The random effect α_j is a random effect for stock j, representing stock-to-stock variability, independent and identically distributed (iid) as $N(0,\sigma^2_\alpha)$; υ_l is a random effect for the lth year, iid as $N(0,\sigma^2_\upsilon)$; $\eta_{j,k}$ is a random effect for stock j in season k, iid as $N(0,\sigma^2_\eta)$; $\pi_{j,l}$ is a random effect for stock j in year l, iid as $N(\sigma^2_\pi)$; $\tau_{k,l}$ is a random effect for season k in year l, iid as $N(0,\sigma^2_\tau)$; and $e_{i,j,k,l}$ is the residual variation, iid as $N(0,\sigma^2_e)$. The residual variation, or unexplained error, includes variation among individual fish. A random effect for the season of sampling was not estimated because we viewed season as a fixed rather than as a random effect. We estimated variance components using restricted maximum likelihood and assessed the significance of random effects using a likelihood ratio test (Self and Liang 1987; Littell et al., 1996). We considered all variance components significant at $P<0.10$. We used $P<0.10$ rather than the typical 0.05 because of the small number of stocks and years in our study.

Fish, stock, and annual correlates of fish health

After partitioning total variability in total carcass water contents, total carcass lipid contents, total muscle lipid contents and concentrations of selected FAs in muscle tissue, we used mixed models that included fixed effects to explain variation that was partitioned into different spatial and temporal components. Specifically, we attempted to explain variation in nutritional indicators among individual fish, stocks, and years by modeling factors such as season, sex, weight, and pathogen prevalence and intensity as fixed effects (Table 1). The analyses were performed using the following steps. First, variance components, estimated using restricted maximum likelihood as described above, were identified and significant effects were retained in the model. Second, we estimated and tested the significance of fixed effects using maximum likelihood (Yang 2004). The general form of the mixed model used was:

$$y_{i,j,k,l} = u + \sum_{k=1}^{3} \beta_k season + \sum_{r=0}^{R} \varphi + \sum_{f=0}^{F} \theta_{i,j,k,l} + \sum_{s=0}^{S} \lambda_i + \sum_{b=0}^{B} \xi_l + e_{i,j,k,l} \quad (3)$$

where y and u are as defined above, β_k is the estimated fixed effect for season k, $k=0\ldots,3$, φ is a random effect described in Eq. (2), with the number of random effects in the model ranging from $r=0\ldots,R$ with $R\leq 5$. The fixed fish-level covariates, θ, range from $f=0\ldots,F$ with $F\leq 6$ (see Table 1). The fixed effects for the stock and year-level covariates are defined as λ and ζ, respectively and range from $s=0\ldots,S$ with $S\leq 3$ for stock-level covariates and from $b=1\ldots,B$ with $B\leq 3$ for year-level covariates. The residual error is defined as $e_{i,j,k,l}$. We considered all fixed effects significant at $P<0.05$. All values are presented as means ± SE unless otherwise noted.

Table 1
Fixed effects used to explain variation among individual fish, stocks, and years in selected fish health indicators for lake whitefish from Lakes Michigan and Huron.

Fish-level covariates	Stock-level covariates	Annual covariates
Season	*Cystidicola farionis* prevalence[a]	*Cystidicola farionis* prevalence[a]
Sex	*Cystidicola farionis* intensity[b]	*Cystidicola farionis* intensity[b]
Weight (g)	*Renibacterium salmoninarum* prevalence[a]	*Renibacterium salmoninarum* prevalence[a]
Percent lipids (whole fish)		
Percent water (whole fish)		
Percent lipids (muscle)		

[a] Prevalence was calculated as the number of infected fish divided by the total number of fish.
[b] Intensity was calculated as the total number of *C. farionis* divided by the number of infected fish.

Natural mortality and fish health indicators

To examine patterns between natural mortality estimates for each stock (see Ebener et al., 2010a) and health indicators, we plotted natural mortality estimates, along with 95% confidence intervals, versus the best linear unbiased predictors (BLUPs) of the stock effects for FA, percent lipid, and percent water from among those that exhibited significant variation among stocks. Because very little is known about the relationship between stock-level estimates of natural mortality and stock-average measures in fish health, these plots were constructed to assist visualizing patterns rather than to test specific hypothesis.

Results

Although we sought to collect 40 fish per stock during each sampling occasion, we were unable to meet this goal for all stocks and seasons. Due to harsh weather conditions, we were not able to collect samples from the Naubinway stock during winter 2004 or the Cheboygan stock during fall 2005. The average number of fish sampled from each stock on each successful sampling occasion was 38, 40, 39, and 40 for Big Bay de Noc, Naubinway, Cheboygan, and Detour Village, respectively. Total sample sizes by stock, over all three

years, for FAs ranged from 291 for Big Bay de Noc to 302 for Detour Village. Total sample sizes for gross compositional analyses ranged from 425 for Cheboygan to 479 for Detour Village (see Appendix A for raw means and sample sizes of health indicators by stock, season, and year). Overall, 47% of the fish were female, although the sex ratio varied from 35 to 58% females among individual sampling occasions, primarily due to limited availability of fish on a few occasions.

Partitioning variation in fish health indicators

We focused on health indicators that had significant stock and/or year variance components. Of the 18 nutritional indicators examined, 7 exhibited significant variation among stocks and 9 demonstrated significant temporal variation (Table 2). Indicators with significant stock (spatial) and year (temporal) effects included whole-fish percent water and several FAs. Even when significant stock effects were found, however, the variation among stocks was small and ranged from 7.4% of the total variation for percent water to 12.9% of the total variation for palmitoleic acid (16:1$n-7$) concentrations measured as μg/mg dry weight of tissue extracted (Figs. 2–4). For significant year effects, the proportion of the total variation attributed to annual variation ranged from 7.8% for the DHA/ARA ratio (on both a μg/mg dry weight of tissue extracted and μg/mg polar lipid basis) and palmitoleic acid (μg/mg polar lipid) to 52% of the total variation for DHA in muscle (μg/mg dry weight of tissue extracted). For most nutritional indicators, residual variation comprised a majority of the total variation ranging from 46 to 85% of the total variation (Figs. 2–4).

Stock effect

Percent water in whole-fish homogenates exhibited significant stock variation (Fig. 2). Patterns in percent water suggested a lake-effect where Big Bay de Noc and Naubinway fish from Lake Michigan tended to have lower percent water than fish from Cheboygan and Detour Village in Lake Huron. Stock effects for FAs revealed that Naubinway fish tended to differ from the other three stocks in terms of the selected FA concentrations examined. This pattern was evident for most FAs on both a per mg dry weight basis and on a per mg polar lipid basis. For example, Naubinway fish tended to have higher palmitoleic acid concentrations and a lower DHA/ARA ratio compared to the other stocks.

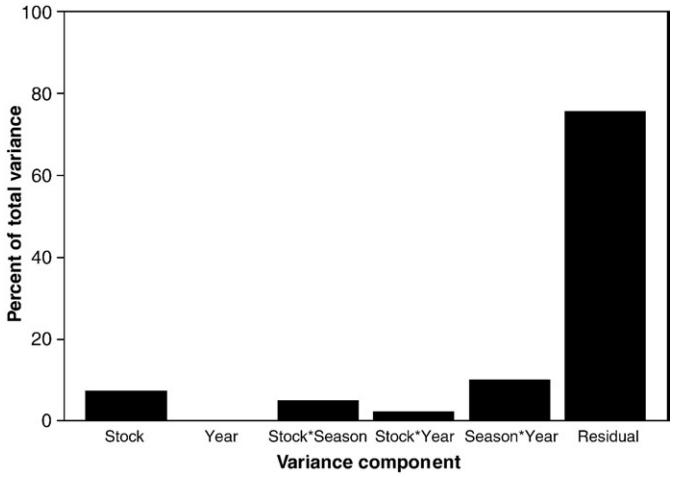

Fig. 2. The proportion of the total variance in percent water for lake whitefish due to spatial and temporal factors. Variances were estimated using linear mixed models.

Table 2
P values for spatial and temporal components of variance for selected fatty acids, muscle and whole-fish percent lipids, whole-fish percent water for four lake whitefish stocks in Lakes Huron and Michigan.

Response variable	Variance component				
	Stock	Year	Stock × season	Stock × year	Season × year
% lipids (whole fish)	0.15	0.5	<**0.0001**	**0.0095**	<**0.0001**
% lipids (muscle)	0.5	0.29	<0.0001	0.003	<0.0001
% water (whole fish)	**0.038**	0.5	<**0.0001**	<**0.0001**	<**0.0001**
Per mg dry weight of tissue extracted					
DHA	0.5	<**0.0001**	<**0.0001**	**0.001**	<**0.0001**
EPA	0.49	**0.016**	<**0.0001**	**0.0005**	**0.0028**
DHA/ARA	**0.028**	**0.089**	<**0.0001**	<**0.0001**	<**0.0001**
Palmitoleic acid	**0.003**	**0.009**	<**0.0001**	**0.024**	**0.027**
ARA	0.18	0.23	<**0.0001**	<**0.0001**	<**0.0001**
Linoleic acid	**0.09**	0.5	<**0.0001**	<**0.0001**	**0.0028**
ALA	0.5	0.5	<**0.0001**	**0.003**	<**0.0001**
Per mg polar lipid					
Unsaturation index	**0.01**	**0.0002**	**0.008**	**0.048**	<**0.0001**
DHA	0.38	<**0.0001**	<**0.0001**	<**0.0001**	<**0.0001**
EPA	0.5	**0.0009**	<**0.0001**	**0.001**	0.082
DHA/ARA	**0.028**	**0.09**	<**0.0001**	<**0.0001**	<**0.0001**
Palmitoleic acid	**0.005**	**0.003**	<**0.0001**	**0.009**	**0.035**
ARA	0.45	0.5	<**0.0001**	**0.004**	<**0.0001**
Linoleic acid	0.16	0.4	<**0.0001**	**0.0009**	<**0.0001**
ALA	0.24	0.17	<**0.0001**	<**0.0001**	<**0.0001**

Variance components were estimated using restricted maximum likelihood and *P* values using a likelihood ratio test (Self and Liang 1987; Littell et al., 1996). Total variance was partitioned into stock, annual, stock × season, stock × year, season × year, and residual variation. *P* values for residual variation are always significant and not included in the table. Analyses were performed on natural log-transformed FAMEs from muscle tissue, measured as μg FAME/mg dry weight of tissue extracted and μg FAME/mg polar lipid. Significant *P* values ($P<0.10$) are shown in bold.

Year effect

Year effects were evident for several FAs, including DHA, EPA, DHA/ARA ratio, and palmitoleic acid measured as μg/per mg dry weight tissue extracted (Fig. 5). Several FAs demonstrated a declining trend over time. For example, mean levels of the highly unsaturated fatty acid (HUFA) DHA declined from 10.6 ± 0.12 in year 1 to 7.1 ± 0.07 in year 3. Trends for the DHA/ARA ratio and palmitoleic acid were less pronounced.

For FAs measured from the polar lipid fraction (per mg polar lipid), those that demonstrated significant year effects included DHA, EPA, the DHA/ARA ratio, the UI, and palmitoleic acid. DHA, EPA and the UI demonstrated declining trends over the three-year study period (Fig. 6). For example, mean DHA declined from 368.3 ± 8.06 in year 1 to 233.5 ± 2.2 in year 3, while mean values of the UI declined from 367 ± 1.14 in year 1 to 321 ± 0.99 in year 3.

Fish, stock, and annual correlates of fish health

The percentage of the total variation explained by mixed models that contained both individual fish-level covariates and stock and year-level covariates ranged from 1 to 66% for LIN and EPA measured as μg FA/mg polar lipid. There were few consistent relationships among fish-level covariates and health indicators; however, several indicators were positively correlated to the weight of individual fish. Overall, the covariates that explained variation among fish, stocks, and years, and the direction of the effects varied among health indicators (Appendix B).

Seasonal patterns

Seasonal patterns were evident for whole-fish measures of percent lipids and water and for three FAs. The FAs included EPA measured as both μg/mg dry weight muscle and μg/mg polar lipid in muscle, ALA measured both as μg/mg dry weight of tissue extracted and μg/mg polar lipid, and LIN measured as μg/mg dry weight of tissue extracted (Appendix B). As expected, seasonal

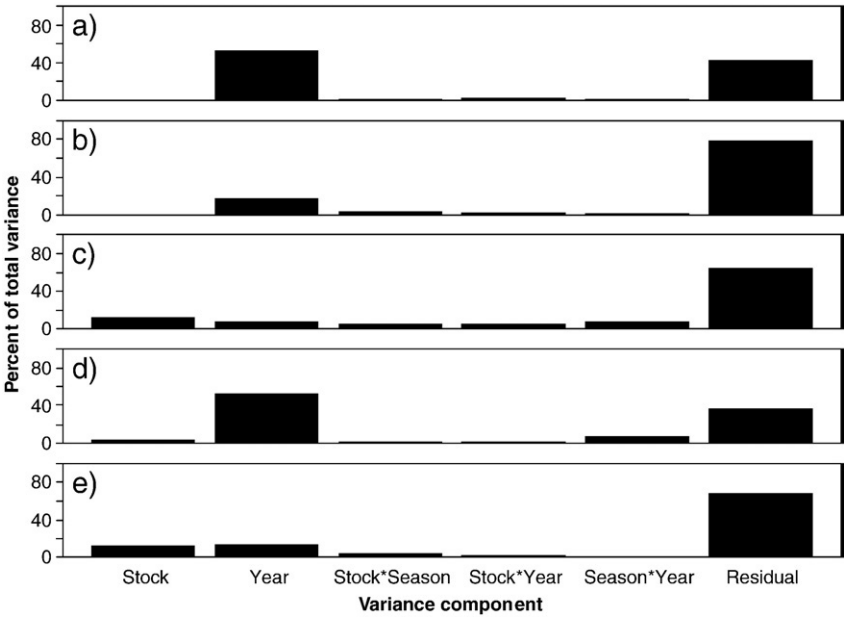

Fig. 3. The proportion of the total variance in whitefish health indicators due to spatial and temporal factors. (a) DHA; (b) EPA; (c) DHA/ARA; (d); unsaturation index; (e) palmitoleic acid. Figures are for fatty acids with significant stock and/or year effects. Fatty acids are for muscle samples measured as μg fatty acid/mg polar lipid. Variances were estimated using linear mixed models.

patterns of percent lipids and water were characterized by seasonal lows of percent lipid levels and corresponding seasonal highs of percent water levels in the winter (winter percent lipid least-squares means [LSM] = 16.3% ± 0.97 and winter percent water LSM = 75.6% ± 0.32). Highest percent lipid levels and lowest percent water levels were observed in the spring, with a LSM for percent lipids of 20.3% ± 0.97 and for percent water of 73.3% ± 0.32. Intermediate levels of percent lipids and water were observed in the summer and fall (LSM = 17.1% ± 0.97 in summer 17.8% ± 0.98 in fall for percent lipids and 74.6% ± 0.32 in summer and 74.1% ± 0.32 in fall for percent water).

Stock covariates

Of the health indicators with significant variation among stocks (i.e., a significant stock random effect; Table 2), two were significantly correlated with stock intensity of infection or prevalence of *C. farionis*, while none were significantly correlated with *R. salmoninarum* prevalence (Appendix B). It is important to consider that although these covariates explained variation among stocks, the total variation explained was a small proportion of the total variation (i.e., stock variation ranged from 7–13% of the total variation). Variation in percent water among stocks was positively correlated with *C. farionis* intensity of infection, with Lake Michigan

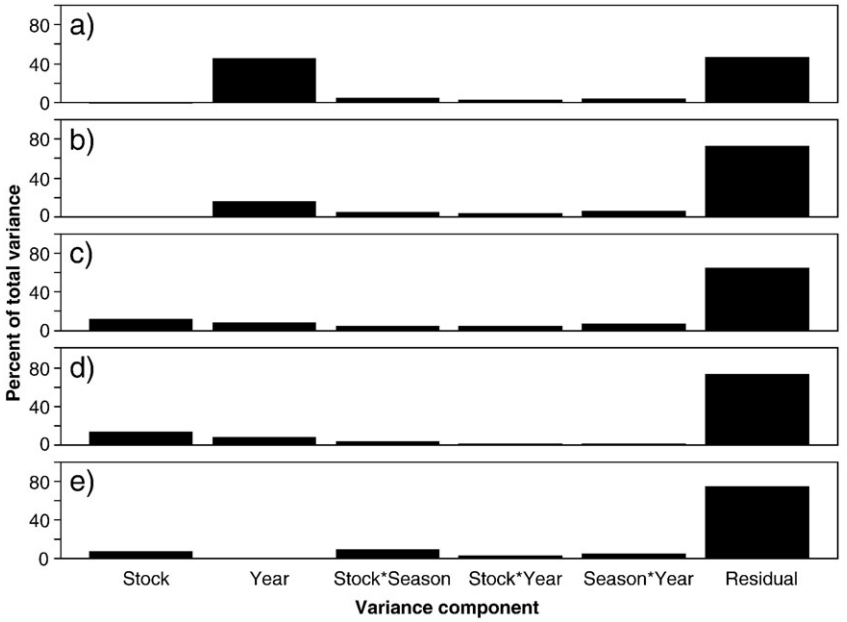

Fig. 4. The proportion of the total variance in whitefish health indicators due to spatial and temporal factors. (a) DHA; (b) EPA; (c) DHA/ARA; (d) palmitoleic acid; (e) linoleic acid. Figures are for fatty acids with significant stock and/or year effects. Fatty acids are for muscle samples measured as μg fatty acid/mg dry weight of tissue extracted. Variances were estimated using linear mixed models.

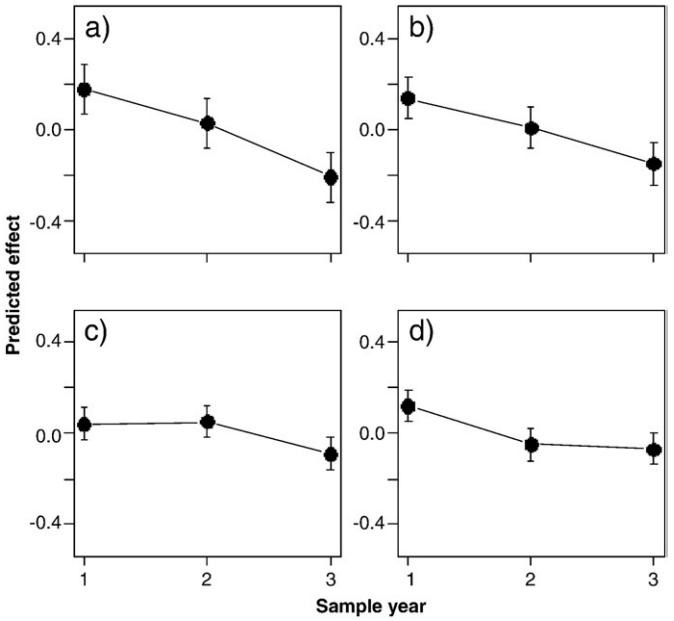

Fig. 5. Predicted year effects (±SE) for select fatty acids measured in muscle tissue samples for four lake whitefish stocks. (a) DHA; (b) EPA; (c) DHA/ARA; (d) palmitoleic acid. Fatty acids were measured as μg fatty acid/mg dry weight of tissue extracted. Predicted effects are best linear unbiased predictors for significant year effects from linear mixed models. Year of sampling is indicated by numbers 1–3. Sample year one was from fall 2003 to summer 2004, sample year two was from fall 2004 to summer 2005, and sample year three was from fall 2005 to summer 2006.

stocks (Big Bay de Noc and Naubinway) having lower percent water and lower *C. farionis* intensity of infection, while Lake Huron stocks (Detour village and Cheboygan) were characterized by higher percent water and higher *C. farionis* intensity of infection. Stock effects for palmitoleic acid were negatively correlated with *C. farionis* prevalence (both on a μg/mg dry weight muscle tissue and on a μg/mg polar lipid basis). The relationship between palmitoleic acid and *C. farionis* prevalence also highlights the differences among lakes, with Lake Michigan stocks having higher palmitoleic acid concentrations and lower *C. farionis* prevalence rates compared to Lake Huron stocks.

Year covariates

Variation among years in four health indicators was correlated with either *C. farionis* intensity or prevalence. Year effects for the DHA/ARA ratio (measured as both μg/mg dry weight muscle tissue and μg/mg polar lipid) were positively correlated with average annual *C. farionis* infection intensity (Fig. 7). The third year was associated with the lowest DHA/ARA ratio, on average, and the lowest *C. farionis* intensity. Annual concentrations of the HUFAs DHA and EPA demonstrated negative trends over time, and these year effects were negatively correlated with annual *C. farionis* prevalence (Fig. 8). DHA measured as μg/mg polar lipid demonstrated a similar relationship with *C. farionis* prevalence as DHA measured as μg/mg dry weight of tissue extracted, but was not statistically significant at $\alpha = 0.05$ ($P = 0.07$). The inter-annual decline in palmitoleic acid, measured as μg/mg polar lipid, showed a similar negative correlation with *C. farionis* prevalence to that observed with DHA and EPA. Variation among years in health indicators was not correlated to *R. salmoninarum* prevalence.

Natural mortality and fish health indicators

Few patterns emerged from examining plots of natural mortality estimates versus BLUPs for stock effects of fish health indicators (Figs. 9–10). The large amount of uncertainty in natural mortality rates and BLUPs (95% confidence intervals overlapped in all cases) made it difficult to identify relationships.

Discussion

Variation in fish health indicators

Fish variation

A large proportion of the total variation in health indicators could not be attributed to spatial or temporal sources; rather a majority of variation was contained in the residual error term, which mainly comprises variation among individual fish. Depending on the health indicator examined, some of the among-fish variation was explained by fish sex, weight, or variability in whole-body lipids or percent water. However, for most health indicators we were able to explain relatively little of the variation among individual fish, suggesting that differences in other aspects of diet, behavior, or physiology contributed to the observed among-fish variance.

Stock variation

Because lake whitefish from the different stocks should have similar abilities for FA synthesis and modifications, any observed

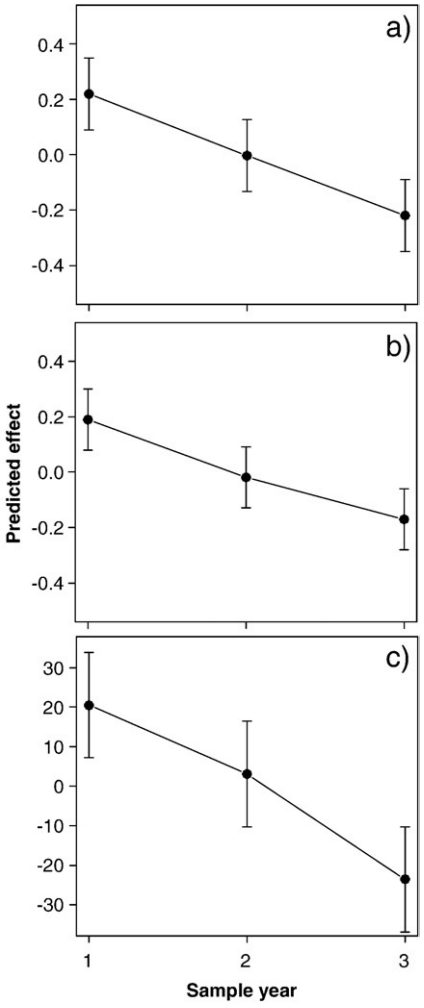

Fig. 6. Predicted year effects (±SE) for select fatty acids measured in muscle tissue samples for four lake whitefish stocks. (a) DHA; (b) EPA; (c) unsaturation index. Fatty acids were measured as μg fatty acid/mg polar lipid. Predicted effects are best linear unbiased predictors for significant year effects from linear mixed models. Year of sampling is indicated by numbers 1–3. Sample year one was from fall 2003 to summer 2004, sample year two was from fall 2004 to summer 2005, and sample year three was from fall 2005 to summer 2006.

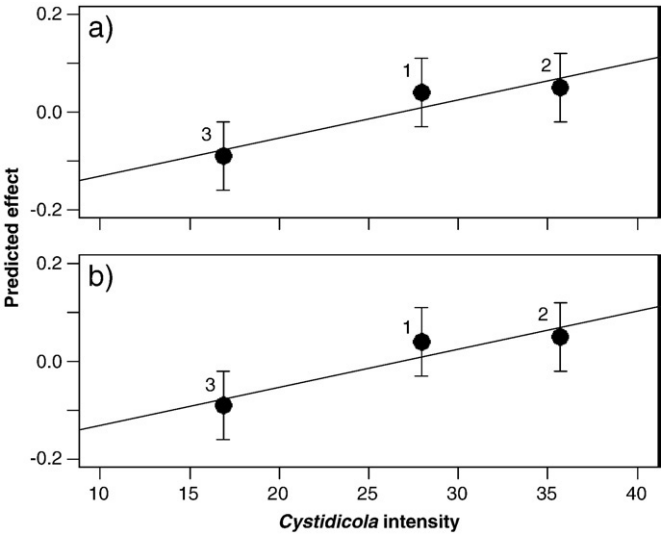

Fig. 7. Relationship between predicted year effects (±SE) for the DHA/ARA ratio in muscle tissue sampled from four lake whitefish stocks and average annual *Cystidicola farionis* intensity of infection. The DHA/ARA ratio was measured as μg/mg dry weight of tissue extracted (a), as μg/mg polar lipid (b). Predicted effects are best linear unbiased predictors for significant year effects from a linear mixed model. Year of sampling is indicated by numbers 1–3. Sample year one was from fall 2003 to summer 2004, sample year two was from fall 2004 to summer 2005, and sample year three was from fall 2005 to summer 2006.

differences in FA signatures are likely to reflect differences in foraging patterns (Thiemann et al., 2008). On average, fish from the Naubinway stock tended to differ in the concentrations of several FAs compared to fish from other stocks. For example, Naubinway fish tended to have lower DHA/ARA ratio, suggesting that those fish may be consuming relatively more bivalves (e.g. native and nonnative mussels and clams which tend to have higher ARA and lower DHA concentrations compared to prey such as *Diporeia*; M. Arts; unpublished data), compared to other stocks. Overall, however, we observed low variation among stocks in health indicators. Two non-mutually exclusive hypotheses that may explain the low variation among stocks are (1) ecological and environmental conditions were similar among stocks such that feeding conditions and diets were similar; and (2) there was mixing of fish among the four stocks resulting in a weak stock 'signature'. The first hypothesis is possible, as the geographic distance among stocks was relatively small. The second hypothesis is supported by a tagging study on these same stocks (Ebener et al. 2010b), which found fish from individual stocks to be segregated during the spawning season, which lasts through the fall and early winter, but during the remainder of the year the stocks were more widely distributed and mixed. Because of this mixing, it is possible that fish from different stocks experienced similar levels of resource availability and exposure to parasites and pathogens throughout much of the year.

Year variation

Variation among years in health indicators was more common and of larger magnitude compared to stock-to-stock variation. We observed temporal trends in several FA and these trends were common to all four stocks. For fish health indicators with a relatively large temporal variation component, it was often due to a linear decrease in the value of the indicator over the three-year study period. Although our study period only spanned three years, declines in HUFAs may have important implications, from a biochemical perspective, for the health and condition of lake whitefish. For example, the decreasing trend observed for the UI may be important because the degree of unsaturation of membrane lipids (phospholipids) has often been implicated, at least at some level, with increased

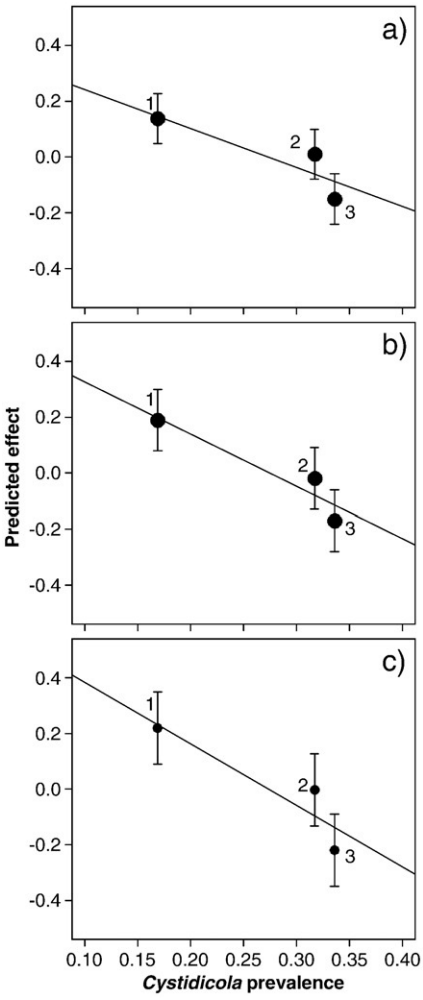

Fig. 8. Relationship between predicted year effects (±SE) for EPA (a and b) and DHA (c) in tissues from four lake whitefish stocks, and average annual *Cystidicola farionis* prevalence. EPA was measured as μg/mg dry weight of tissue extracted for muscle samples in (a), and as μg/mg polar lipid (b). DHA was measured as μg/mg polar lipid. Predicted effects are best linear unbiased predictors for significant year effects from a linear mixed model. Year of sampling is indicated by numbers 1–3. Sample year one was from fall 2003 to summer 2004, sample year two was from fall 2004 to summer 2005, and sample year three was from fall 2005 to summer 2006.

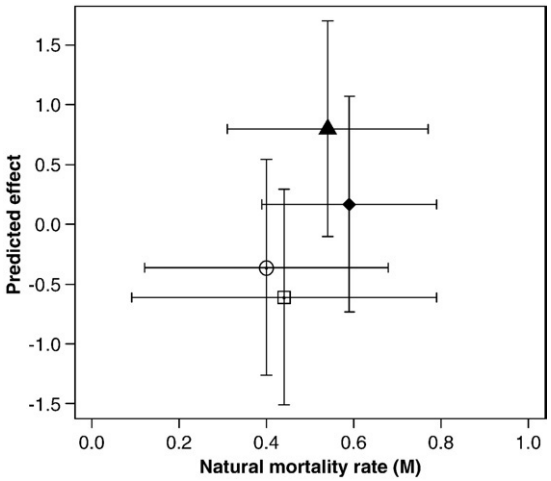

Fig. 9. Relationship between predicted stock effects for percent water from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour Village (◆), and natural mortality rates. Error bars are 95% confidence intervals. Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model.

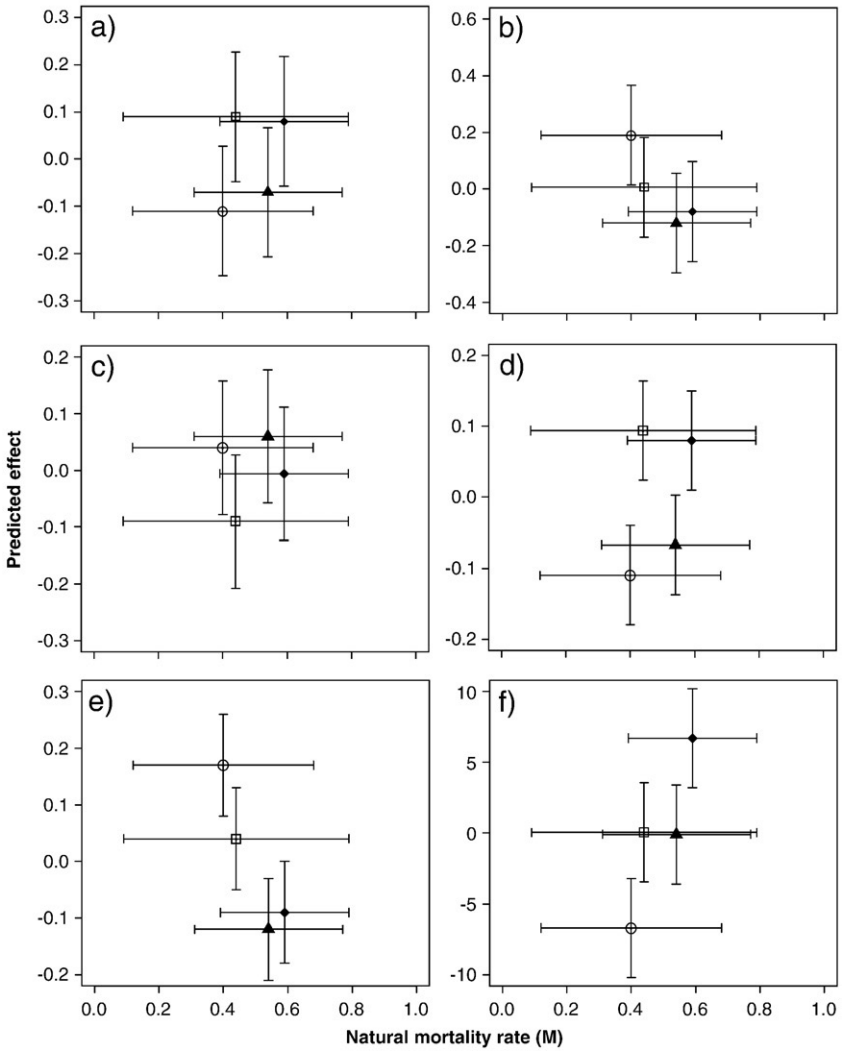

Fig. 10. Relationship between predicted stock effects for DHA/ARA ratio (a) palmitoleic acid (b), and linoleic acid (c) in muscle tissue measured as μg/mg dry weight of tissue extracted, and DHA/ARA ratio (d), palmitoleic acid (e) and the unsaturation index (f) in muscle tissue measured as μg/mg polar lipid and natural mortality rates. Fish were sampled from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour Village (♦). Error bars are 95% confidence intervals. Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model. Note differences in *y*-axis scales.

membrane "fluidity"; a vital adaptive response to cold temperature challenge (Arts and Kohler, 2009). In addition, long-chain HUFAs, such as DHA and EPA, are required for normal development and reproduction in fish (Sargent et al., 1999; Tocher 2003). HUFAs are also important for neural development and as precursors for eicosanoids (specifically ARA and EPA; Tocher 2003; Arts and Kohler, 2009): biochemicals involved in a wide-range of physiological processes, including egg production, spawning and hatching, schooling behavior, and in immune responses (Brett and Müller-Navarra 1997; Masuda et al., 1998). There is also evidence, from other species of fish, that deficiencies in HUFAs can limit growth (Ballantyne et al., 2003), impair visual acuity (Benitez-Santana et al., 2007) leading to decreased ability to feed at low light intensities (Bell et al., 1995), and increase susceptibility to predators (Nakayama et al., 2003).

The decreasing trends observed in several HUFAs reflect changes in lake whitefish diets over time. Historically, lake whitefish diets in lakes Michigan and Huron were dominated by HUFA-rich macroinvertebrates such as *Diporeia* spp. and *Mysis* spp. (Pothoven and Madenjian 2008). However, recent changes to the benthic food web in the Great Lakes, potentially related to dreissenid mussel colonization, have resulted in lake whitefish diets being dominated by relatively HUFA-poor prey items such as dreissenid mussels and gastropods. In fact, Pothoven and Madenjian (2008) determined that consumption of non-mollusk macroinvertebrates by an average lake whitefish was 46–96% lower post-dreissenid mussel colonization compared to pre-dreissenid colonization. Although there is evidence that density-dependent mechanisms are involved in observed decreases in condition of lake whitefish in southern and mid Lake Michigan, observed decreases in northern Lake Michigan (where our Lake Michigan study stocks were located) are largely due to food web changes and not regulated by density-dependent effects (DeBruyne et al., 2008).

Acknowledging the fact that our study was not designed to elucidate the effects of food web changes such as declines in *Diporeia* densities on lake whitefish FA composition, the temporal decreases in HUFAs observed in our study, in addition to the recent declines in HUFA-rich prey in the Great Lakes, suggest that a better understanding of food web changes on health dynamics is warranted. In addition, we currently do not know the implications of decreased HUFA levels on the physiological and behavioral functioning of lake whitefish, and thus the potential effects on natural mortality or other demographic rates. For example, Naubinway fish tended to have a lower DHA/ARA ratio and UI; however, this was not reflected in differences in natural mortality rates among stocks. Identifying critical levels (thresholds) of

important FA and other health indicators, below (or above) which survival may be reduced, will greatly improve the interpretability of future studies with respect to potential population-level effects.

Fish health indicators and pathogens

The spatial and temporal patterns of HUFAs may also have implications for mediating the effects of pathogens on lake whitefish. It is well documented that nutritional stress (e.g., deficiencies in essential nutrients) can increase a fish's susceptibility to pathogens (Eya and Lovell 1998; Lim and Klesius 2003), and that certain pathogens can induce mortality in fishes. Of the seven health indicators with significant variation among stocks, two were significantly correlated with stock intensity of infection or prevalence of *C. farionis*: a positive relationship between percent water and *C. farionis* intensity of infection and a negative relationship between *C. farionis* prevalence and palmitoleic acid concentrations. In addition, variation among years in health indicators was correlated with either *C. farionis* intensity or prevalence. Although the direction of effects for these relationships suggests that, on average, decreased nutritional status is associated with increased pathogens at the stock-level, it is impossible to determine cause-and-effect relationships. However, if lake whitefish immune systems become compromised due to HUFA or lipid deficiencies, their susceptibility to pathogens would be expected to increase over time.

Natural mortality and fish health indicators

We did not find relationships between observed spatial patterns in fish health indicators and the natural mortality rates of lake whitefish stocks. The relatively low amount of variation among stocks in fish health indicators suggests that these fish were experiencing similar ecological and environmental conditions, at least with respect to physical and biological conditions that would be reflected in whole body composition and FA profiles. If the lake whitefish health indicators we examined were sensitive indicators of natural mortality, we would then predict, based on the low variation among stocks in health indicators, that natural mortality rates among stocks would also be similar. This prediction is supported by the estimates of natural mortality rates (Ebener et al. 2010a). However, our ability to elucidate relationships was influenced by the limited number of stocks used in the analysis, their geographical proximity, and large amounts of uncertainty in both the natural mortality estimates and the estimated stock effects for the various health indicators. To obtain a better understanding of the link between health indicators measured on individual fish and natural mortality at the stock-level, future research needs to either (1) study stocks that exhibit larger among-stock variation with respect to health indicators, or (2) obtain more precise estimates of natural mortality. Because precise estimates of natural mortality are difficult to obtain in many cases, we suggest research designed to maximize among-stock differences.

We were limited in this study to examining spatial patterns between natural mortality and health indicators. Although we did observe relatively large annual variation in some health indicators, we were unable to examine relationships between annual variability of indicators and temporal patterns in natural mortality because the number of tagging events limited the number of yearly estimates of natural mortality obtainable (Ebener et al. 2010a). However, if a downward trend in HUFAs continues, research that examines temporal trends in natural mortality will help determine if observed trends in health indicators ultimately translate into population-level effects.

Acknowledgments

We thank all the contracted fishermen who participated in this study. Without their participation fish collection would not have been possible. We also thank Hilary Ahman, Jerry Chao and Martina Drebenstedt (Arts lab) and all current and past members of Michigan State University-Aquatic Animal Health Laboratory who assisted in this multi-year effort. Nathan Nye and Kendra Porath assisted with the collection of samples and the gross compositional analysis. Funding was provided by the Great Lakes Fishery Trust Project 2003–06 and by Environment Canada to M.T. Arts. Use of trade names does not imply endorsement by the federal government. This is manuscript 2009-14 of the Quantitative Fisheries Center.

Appendix A

Means followed by standard errors/sample size in parentheses for selected fatty acid methyl esters (FAMEs) and percent lipids and water for four lake whitefish stocks in Lakes Huron and Michigan. Samples were measured as μg FAME/mg dry weight of tissue extracted and μg FAME/mg polar lipid from muscle tissue. Year of sampling is indicated by numbers 1–3. Sample year one was from fall 2003 to summer 2004, sample year two was from fall 2004 to summer 2005, and sample year three was from fall 2005 to summer 2006.

Winter was categorized to include the months of January, February, and March; spring included April, May, and June; summer included July, August, and September; and fall included October, November, and December. Stocks are defined as BD = Big Bay de Noc, N = Naubinway, C = Cheboygan, and DV = Detour Village. The BD and N stocks are located in northern Lake Michigan and C and DV are located in northern Lake Huron. NS = not sampled.

Appendix B

Parameter estimates followed by standard errors in parentheses for mixed models for selected fatty acid methyl esters (FAMEs) and percent lipids and water for four lake whitefish stocks in Lakes Huron and Michigan. Analyses were performed on natural log-transformed FAMEs from muscle tissue. Samples were measured as μg FAME/mg dry weight of tissue extracted and μg FAME/mg polar lipid. When season of sampling was significant, parameter estimates for spring, summer, and fall are given (winter is the reference category contained in the intercept). Winter was categorized to include the months of January, February, and March; spring included April, May, and June; summer included July, August, and September; and fall included October, November, and December. For significant differences among sexes, parameter estimates are given for female fish and male fish are the reference category. All fixed effect parameter estimates are significant at $P<0.05$. See Methods and materials for selection process for random effects, and see Table 1 for complete description of covariates. Cys (s in) = *Cystidicola farionis* intensity, a stock-level covariate; Cys (s) = *C. farionis* prevalence, a stock-level covariate; Cys (y) = *C. farionis* prevalence, a year covariate; Cys (y in) = *C. farionis* intensity, a year-level covariate; Rs (s) = *Renibacterium salmoninarum* prevalence, a stock-level covariate. The percent variation explained by the model is in parentheses below the response variable.

Appendix A

Health indicator	Year 1				Year 2				Year 3			
	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
BD												
% lipids (whole fish)	23.1 (1.4/40)	22.9 (0.7/33)	20.2 (0.9/40)	14.9 (0.9/40)	21.5 (1.1/40)	16.4 (1.0/40)	21.3 (0.8/40)	18.8 (1.4/25)	15.6 (1.1/38)	16.2 (0.8/40)	23.2 (0.8/40)	18.2 (0.9/40)
% lipids (muscle)	5.8 (0.5/17)	5.6 (0.2/16)	6.0 (0.4/17)	5.1 (0.2/18)	7.0 (0.3/30)	7.6 (0.5/30)	7.0 (0.4/30)	7.2 (0.6/22)	6.2 (0.4/30)	6.0 (0.2/30)	7.6 (0.4/30)	6.2 (0.2/30)
% water (whole fish)	72.8 (0.5/40)	73.0 (0.4/33)	73.1 (0.3/40)	75.5 (0.4/40)	71.9 (0.4/40)	74.2 (0.4/40)	72.0 (0.3/40)	74.9 (0.4/25)	74.3 (0.4/38)	73.9 (0.4/40)	72.1 (0.3/40)	75.9 (0.3/40)
Per mg dry weight of tissue extracted												
DHA	10.07 (0.26/17)	10.75 (0.50/16)	13.13 (0.38/17)	9.91 (0.40/18)	8.72 (0.36/30)	7.06 (0.22/30)	9.59 (0.29/30)	9.17 (0.38/22)	7.73 (0.17/30)	6.00 (0.27/30)	7.95 (0.29/30)	7.02 (0.24/30)
EPA	2.50 (0.14/17)	2.48 (0.11/16)	4.25 (0.11/17)	2.37 (0.12/18)	2.04 (0.15/30)	1.62 (0.06/30)	2.79 (0.11/30)	2.63 (0.11/22)	2.03 (0.08/30)	1.79 (0.13/30)	2.21 (0.10/30)	2.12 (0.07/30)
DHA/ARA	8.48 (0.46/17)	10.97 (0.52/16)	10.09 (0.50/16)	8.40 (0.50/18)	8.87 (0.54/30)	9.14 (0.45/30)	10.74 (0.54/30)	7.61 (0.47/22)	6.64 (0.24/30)	7.08 (0.38/30)	6.50 (0.32/30)	6.19 (0.34/30)
Palmitoleic acid	0.47 (0.03/17)	0.45 (0.02/16)	0.81 (0.06/17)	0.58 (0.04/18)	0.47 (0.03/30)	0.33 (0.02/30)	0.43 (0.04/30)	0.40 (0.04/22)	0.41 (0.02/30)	0.40 (0.05/30)	0.44 (0.04/30)	0.44 (0.02/30)
ARA	1.25 (0.08/17)	0.99 (0.03/16)	1.32 (0.05/16)	1.21 (0.04/18)	1.06 (0.06/30)	0.81 (0.03/30)	0.94 (0.04/30)	1.25 (0.06/22)	1.19 (0.04/30)	0.94 (0.09/30)	1.27 (0.06/30)	1.19 (0.04/30)
LIN	0.24 (0.01/17)	0.21 (0.01/16)	0.28 (0.01/17)	0.25 (0.01/18)	0.23 (0.02/30)	0.18 (0.01/30)	0.25 (0.01/30)	0.29 (0.01/22)	0.26 (0.01/30)	0.21 (0.01/30)	0.26 (0.01/30)	0.30 (0.01/30)
ALA	0.22 (0.02/17)	0.28 (0.03/16)	0.32 (0.01/17)	0.26 (0.02/18)	0.30 (0.02/21)	0.24 (0.01/30)	0.36 (0.02/30)	0.39 (0.02/22)	0.30 (0.02/30)	0.26 (0.02/30)	0.37 (0.02/30)	0.38 (0.02/30)
Per mg polar lipid												
Unsaturation index	370.7 (2.6/17)	376.3 (3.7/16)	375.0 (2.0/17)	364.2 (4.8/18)	330.9 (5.0/30)	347.1 (2.6/30)	362.4 (1.7/30)	348.9 (3.4/22)	332.0 (1.7/30)	330.4 (4.3/29)	329.5 (2.9/30)	317.3 (2.2/30)
DHA	380.0 (13.9/17)	381.9 (15.7/16)	428.2 (11.7/17)	362.1 (17.7/17)	281.5 (10.9/30)	272.2 (8.1/30)	317.6 (8.7/30)	277.8 (10.5/22)	247.7 (4.5/30)	212.6 (10.9/30)	248.8 (8.7/30)	232.1 (7.5/30)
EPA	94.5 (5.6/17)	88.7 (4.2/16)	138.8 (3.8/17)	99.5 (11.2/18)	66.6 (5.1/30)	63.2 (3.1/30)	92.4 (3.6/30)	79.8 (3.0/22)	65.5 (2.9/30)	63.0 (4.7/30)	70.1 (3.6/30)	70.3 (2.4/30)
DHA/ARA	8.49 (0.46/17)	10.98 (0.52/16)	10.10 (0.50/16)	8.41 (0.50/18)	8.88 (0.54/30)	9.15 (0.45/30)	10.76 (0.54/30)	7.61 (0.47/22)	6.64 (0.24/30)	7.09 (0.38/30)	6.51 (0.32/30)	6.20 (0.34/30)
Palmitoleic acid	18.1 (1.2/17)	16.4 (0.9/16)	26.5 (1.9/17)	23.8 (2.6/18)	15.1 (0.9/30)	12.9 (0.6/30)	14.3 (1.2/30)	12.0 (1.2/22)	13.2 (0.6/30)	14.4 (1.8/30)	14.1 (1.4/30)	14.5 (0.7/30)
ARA	46.4 (2.4/17)	35.2 (1.2/16)	43.4 (1.6/16)	53.8 (9.3/18)	34.6 (2.2/30)	31.8 (1.8/30)	31.1 (1.4/30)	38.2 (1.8/22)	38.6 (1.4/30)	33.4 (3.7/30)	40.1 (1.9/30)	39.2 (1.5/30)
LIN	8.94 (0.40/17)	7.35 (0.34/16)	9.05 (0.37/17)	10.57 (1.28/18)	7.51 (0.56/30)	7.12 (0.33/30)	8.35 (0.40/30)	8.79 (0.30/22)	8.25 (0.35/30)	7.60 (0.59/30)	8.07 (0.42/30)	9.90 (0.33/30)
ALA	8.48 (0.63/17)	9.98 (1.08/16)	10.28 (0.45/17)	10.95 (1.24/18)	7.73 (0.92/27)	9.42 (0.54/30)	12.06 (0.66/30)	11.90 (0.67/22)	9.80 (0.65/30)	9.22 (0.63/30)	11.84 (0.79/30)	12.66 (0.62/30)
N												
% lipids (whole fish)	21.7 (1.0/40)	19.7 (0.7/40)	17.1 (0.7/40)	NS	20.3 (0.8/40)	20.3 (0.5./40)	18.0 (0.7/40)	18.5 (1.0/40)	19.3 (0.8/40)	18.2 (1.2/40)	18.4 (0.7/40)	18.5 (0.8/40)
% lipids (muscle)	7.5 (0.6/18)	6.6 (0.2/17)	6.8 (0.4/17)	NS	7.3 (0.4/28)	12.1 (0.9/27)	6.4 (0.2/30)	5.9 (0.2/30)	5.9 (0.2/30)	5.3 (0.3/30)	6.3 (0.2/30)	5.7 (0.2/29)
% water (whole fish)	72.8 (0.4/40)	73.2 (0.3/40)	74.2 (0.2/40)	NS	72.6 (0.3/40)	73.1 (0.2/40)	73.7 (0.3/40)	75.1 (0.4/40)	74.5 (0.3/40)	74.8 (0.5/40)	73.3 (0.2/40)	75.5 (0.3/40)
Per mg dry weight of tissue extracted												
DHA	10.21 (0.42/18)	10.63 (0.30/17)	11.78 (0.43/17)	NS	9.58 (0.28/28)	7.80 (0.31/30)	9.99 (0.31/30)	8.35 (0.37/30)	7.06 (0.23/30)	6.67 (0.33/30)	6.83 (0.28/30)	6.48 (0.23/29)
EPA	3.00 (0.16/18)	2.45 (0.11/17)	3.47 (0.18/17)	NS	2.49 (0.06/28)	2.23 (0.12/30)	2.73 (0.11/30)	2.35 (0.10/30)	2.00 (0.09/30)	1.63 (0.08/30)	1.87 (0.11/30)	2.14 (0.07/29)
DHA/ARA	5.60 (0.27/18)	6.62 (0.26/17)	6.61 (0.38/17)	NS	7.35 (0.27/28)	5.43 (0.27/30)	8.61 (0.33/30)	6.27 (0.29/30)	6.37 (0.19/30)	7.01 (0.31/30)	5.92 (0.24/30)	5.26 (0.23/29)
Palmitoleic acid	0.69 (0.03/18)	0.51 (0.03/17)	0.63 (0.04/17)	NS	0.49 (0.03/28)	0.61 (0.02/30)	0.46 (0.02/30)	0.51 (0.02/30)	0.52 (0.02/30)	0.46 (0.03/30)	0.45 (0.02/30)	0.64 (0.02/29)
ARA	1.86 (0.09/18)	1.64 (0.07/17)	1.84 (0.09/17)	NS	1.33 (0.04/28)	1.48 (0.06/30)	1.18 (0.03/30)	1.38 (0.06/30)	1.12 (0.04/30)	0.97 (0.04/30)	1.18 (0.05/30)	1.28 (0.06/29)
LIN	0.41 (0.02/18)	0.25 (0.01/17)	0.33 (0.01/17)	NS	0.29 (0.02/28)	0.30 (0.01/30)	0.27 (0.01/30)	0.29 (0.01/30)	0.30 (0.02/30)	0.21 (0.01/30)	0.24 (0.01/30)	0.37 (0.01/29)
ALA	0.25 (0.02/18)	0.24 (0.01/17)	0.30 (0.02/17)	NS	0.20 (0.01/28)	0.30 (0.02/30)	0.26 (0.02/30)	0.21 (0.01/30)	0.21 (0.01/30)	0.18 (0.01/30)	0.27 (0.02/30)	0.28 (0.02/29)
Per mg polar lipid												
Unsaturation index	344.7 (4.1/18)	369.0 (1.4/17)	369.7 (2.7/17)	NS	357.9 (3.3/28)	315.4 (4.4/30)	356.3 (2.9/30)	337.5 (4.6/30)	321.1 (2.6/30)	306.6 (5.2/30)	320.1 (3.1/29)	307.7 (2.1/29)
DHA	359.1 (15.2/18)	373.1 (9.8/17)	344.8 (13.0/17)	NS	318.3 (8.9/28)	220.1 (8.5/30)	309.9 (10.2/30)	283.2 (12.0/30)	229.3 (6.1/30)	209.4 (8.5/30)	215.2 (8.6/30)	210.3 (6.6/29)
EPA	106.0 (5.8/18)	85.6 (3.7/17)	102.3 (6.1/17)	NS	83.1 (2.5/28)	62.9 (3.3/30)	84.4 (3.1/30)	79.2 (2.7/30)	64.6 (2.4/30)	51.2 (2.2/30)	60.1 (2.6/29)	69.5 (2.0/29)
DHA/ARA	5.61 (0.27/18)	6.63 (0.26/17)	6.61 (0.38/17)	NS	7.35 (0.27/28)	5.43 (0.27/30)	8.62 (0.34/30)	6.28 (0.29/30)	6.38 (0.19/30)	7.02 (0.31/30)	5.92 (0.24/30)	5.27 (0.23/29)
Palmitoleic acid	24.5 (1.1/18)	17.9 (0.9/17)	18.3 (1.2/17)	NS	16.5 (1.0/28)	17.3 (0.5/30)	14.2 (0.6/30)	17.2 (0.6/30)	16.7 (0.5/30)	14.6 (0.8/30)	14.2 (0.7/30)	20.6 (0.6/29)
ARA	65.7 (3.4/18)	57.4 (2.3/17)	54.5 (3.3/17)	NS	44.2 (1.4/28)	42.0 (1.7/30)	36.6 (1.0/30)	45.7 (1.3/30)	36.5 (1.1/30)	30.3 (1.0/30)	37.2 (1.5/30)	41.3 (1.6/29)
LIN	14.53 (0.82/18)	8.84 (0.52/17)	9.61 (0.46/17)	NS	9.78 (0.55/28)	8.64 (0.37/30)	8.40 (0.44/30)	9.80 (0.33/30)	9.81 (0.65/30)	6.66 (0.33/30)	7.76 (0.34/30)	12.17 (0.46/29)
ALA	8.98 (0.69/18)	8.39 (0.48/17)	8.55 (0.57/17)	NS	6.57 (0.49/28)	8.64 (0.58/30)	8.10 (0.52/30)	7.02 (0.36/30)	6.67 (0.34/30)	5.80 (0.21/30)	8.41 (0.49/30)	9.02 (0.58/29)
C												
% lipids (whole fish)	17.7 (1.3/40)	18.1 (1.2/40)	15.7 (0.8/40)	21.2 (1.3/33)	16.3 (1.0/40)	14.3 (1.0/40)	14.8 (0.9/40)	14.0 (1.1/32)	20.3 (0.9/40)	11.9 (1.1/40)	NS	15.0 (1.0/40)
% lipids (muscle)	6.2 (0.6/18)	6.4 (0.3/17)	4.9 (0.3/18)	6.1 (0.2/15)	6.8 (0.4/30)	6.3 (0.4/30)	6.2 (0.2/30)	5.7 (0.2/24)	5.7 (0.2/30)	5.3 (0.2/28)	NS	6.1 (0.2/30)
% water (whole fish)	74.9 (0.4/40)	74.5 (0.5/40)	75.3 (0.3/40)	75.0 (0.5/33)	75.1 (0.4/40)	75.6 (0.4/40)	75.5 (0.3/40)	76.9 (0.3/32)	74.5 (0.3/40)	77.3 (0.4/40)	NS	76.4 (0.4/40)
Per mg dry weight of tissue extracted												
DHA	8.86 (0.45/18)	9.55 (0.52/17)	11.04 (0.38/18)	10.93 (0.63/15)	8.55 (0.25/30)	8.05 (0.34/30)	8.82 (0.31/30)	10.87 (0.44/24)	7.87 (0.20/30)	6.71 (0.33/28)	NS	6.63 (0.21/30)
EPA	2.53 (0.11/18)	2.28 (0.09/17)	3.81 (0.14/18)	2.31 (0.08/15)	2.59 (0.13/30)	2.27 (0.11/30)	2.66 (0.11/30)	2.49 (0.11/24)	2.10 (0.06/30)	2.14 (0.17/28)	NS	2.05 (0.06/30)
DHA/ARA	7.13 (0.38/18)	7.66 (0.46/17)	6.65 (0.44/18)	6.83 (0.30/15)	8.08 (0.49/30)	7.20 (0.35/30)	7.30 (0.35/30)	7.19 (0.35/24)	7.93 (0.30/30)	5.40 (0.46/28)	NS	5.40 (0.22/30)
Palmitoleic acid	0.37 (0.02/18)	0.38 (0.03/17)	0.44 (0.03/18)	0.49 (0.03/15)	0.33 (0.02/30)	0.38 (0.03/30)	0.40 (0.02/30)	0.50 (0.02/24)	0.33 (0.02/30)	0.45 (0.05/28)	NS	0.36 (0.03/30)
ARA	1.27 (0.06/18)	1.29 (0.07/17)	1.74 (0.09/18)	1.60 (0.07/15)	1.15 (0.06/30)	1.15 (0.05/30)	1.27 (0.06/30)	1.55 (0.07/24)	1.02 (0.04/30)	1.54 (0.16/28)	NS	1.27 (0.05/30)
LIN	0.26 (0.01/18)	0.22 (0.01/17)	0.34 (0.01/18)	0.40 (0.02/15)	0.27 (0.01/30)	0.26 (0.01/30)	0.32 (0.02/30)	0.42 (0.02/24)	0.30 (0.01/30)	0.35 (0.04/28)	NS	0.29 (0.01/30)
ALA	0.21 (0.01/18)	0.20 (0.01/17)	0.41 (0.02/18)	0.29 (0.02/15)	0.26 (0.03/29)	0.22 (0.01/30)	0.42 (0.04/30)	0.34 (0.02/24)	0.24 (0.01/30)	0.23 (0.02/28)	NS	0.28 (0.01/30)

(continued on next page)

Appendix A (*continued*).

Health indicator	Year 1				Year 2				Year 3			
	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter
Per mg polar lipid												
Unsaturation index	358.4 (4.8/18)	359.5 (5.7/17)	378.6 (2.8/18)	363.0 (6.5/15)	350.0 (2.9/30)	338.4 (4.5/30)	349.4 (3.6/30)	352.0 (4.4/24)	333.9 (1.6/30)	310.3 (5.2/28)	NS	322.8 (1.6/30)
DHA	316.4 (14.7/18)	338.9 (18.2/17)	328.0 (8.9/18)	378.1 (19.0/15)	264.4 (6.9/30)	275.8 (10.8/30)	274.7 (9.0/30)	310.3 (11.7/24)	252.0 (7.3/30)	223.7 (10.3/28)	NS	234.6 (7.7/30)
EPA	90.9 (4.3/18)	81.0 (3.4/17)	113.8 (4.7/18)	81.8 (3.9/15)	80.1 (3.8/30)	78.1 (3.6/30)	83.0 (3.5/30)	71.1 (2.9/24)	67.3 (2.2/30)	71.9 (5.9/28)	NS	72.9 (2.4/30)
DHA/ARA	7.13 (0.38/18)	7.67 (0.46/17)	6.66 (0.44/18)	6.83 (0.30/15)	8.09 (0.49/30)	7.20 (0.35/30)	7.30 (0.35/30)	7.20 (0.35/24)	7.94 (0.30/30)	5.41 (0.46/28)	NS	5.41 (0.22/30)
Palmitoleic acid	13.4 (0.7/18)	13.3 (1.2/17)	13.1 (0.9/18)	17.6 (1.7/15)	10.1 (0.7/30)	13.1 (0.9/30)	12.5 (0.7/30)	14.4 (0.6/24)	10.4 (0.5/30)	15.0 (1.6/28)	NS	12.7 (0.9/30)
ARA	45.6 (2.2/18)	45.7 (2.6/17)	52.3 (2.9/18)	55.6 (2.0/15)	35.5 (1.8/30)	39.5 (1.6/30)	39.6 (1.8/30)	44.3 (1.9/24)	32.9 (1.4/30)	51.5 (5.6/28)	NS	45.1 (2.0/30)
LIN	9.48 (0.37/18)	7.94 (0.31/17)	10.18 (0.37/18)	14.09 (0.90/15)	8.24 (0.44/30)	8.89 (0.39/30)	10.12 (0.48/30)	11.89 (0.69/24)	9.61 (0.25/30)	11.45 (1.11/28)	NS	10.25 (0.41/30)
ALA	7.43 (0.54/18)	7.25 (0.39/17)	12.41 (0.77/18)	10.03 (0.60/15)	8.11 (0.82/29)	7.49 (0.43/30)	13.07 (1.32/30)	9.63 (0.61/24)	7.70 (0.24/30)	7.54 (0.53/28)	NS	9.87 (0.49/30)
DV												
% lipids (whole fish)	22.9 (0.9/40)	18.8 (1.0/40)	16.9 (0.9/39)	13.7 (1.1/40)	17.4 (1.2/40)	15.7 (1.2/40)	15.5 (0.9/40)	13.7 (1.1/40)	20.8 (1.0/40)	10.1 (0.7/40)	22.2 (0.9/40)	17.4 (1.1/40)
% lipids (muscle)	6.3 (0.3/17)	5.8 (0.4/17)	5.3 (0.3/15)	5.2 (0.2/17)	7.2 (0.5/30)	11.2 (0.7/28)	6.4 (0.3/30)	5.9 (0.5/28)	7.0 (0.4/30)	5.5 (0.2/30)	7.4 (0.3/30)	5.7 (0.3/28)
% water (whole fish)	73.1 (0.3/40)	74.5 (0.4/40)	75.3 (0.3/39)	75.4 (0.4/40)	74.0 (0.6/40)	75.7 (0.4/40)	75.0 (0.3/40)	76.1 (0.5/40)	73.2 (0.5/40)	76.5 (0.3/40)	72.4 (0.3/40)	74.9 (0.4/40)
Per mg dry weight of tissue extracted												
DHA	10.84 (0.49/17)	10.56 (0.27/17)	10.75 (0.30/15)	10.42 (0.41/17)	9.81 (0.34/30)	10.36 (0.27/30)	9.67 (0.28/30)	9.77 (0.36/28)	7.17 (0.21/30)	8.01 (0.33/30)	8.37 (0.23/30)	6.62 (0.20/28)
EPA	2.87 (0.16/17)	2.53 (0.11/17)	3.00 (0.11/15)	3.07 (0.13/17)	2.35 (0.07/30)	2.96 (0.08/30)	2.72 (0.07/30)	2.79 (0.10/28)	1.96 (0.10/30)	2.04 (0.12/30)	3.05 (0.16/30)	2.23 (0.09/28)
DHA/ARA	8.05 (0.70/17)	8.44 (0.33/17)	10.95 (0.73/15)	7.52 (0.51/17)	11.05 (0.39/30)	7.19 (0.36/30)	8.60 (0.43/30)	7.37 (0.41/28)	7.59 (0.34/30)	6.99 (0.36/30)	7.28 (0.35/30)	7.44 (0.29/28)
Palmitoleic acid	0.57 (0.03/17)	0.43 (0.02/17)	0.48 (0.04/15)	0.41 (0.04/17)	0.35 (0.02/30)	0.41 (0.03/30)	0.41 (0.02/30)	0.43 (0.03/28)	0.40 (0.03/30)	0.31 (0.02/30)	0.46 (0.03/30)	0.40 (0.03/28)
ARA	1.44 (0.11/17)	1.27 (0.05/17)	1.03 (0.06/15)	1.47 (0.08/17)	0.92 (0.05/30)	1.52 (0.06/30)	1.18 (0.05/30)	1.39 (0.06/28)	1.00 (0.05/30)	1.21 (0.07/30)	1.20 (0.05/30)	0.92 (0.04/28)
LIN	0.31 (0.02/17)	0.24 (0.01/17)	0.27 (0.02/15)	0.29 (0.01/17)	0.26 (0.01/30)	0.31 (0.01/30)	0.28 (0.01/30)	0.28 (0.01/28)	0.25 (0.01/30)	0.25 (0.01/30)	0.27 (0.01/30)	0.28 (0.01/28)
ALA	0.25 (0.02/17)	0.16 (0.01/17)	0.24 (0.04/15)	0.31 (0.02/17)	0.19 (0.01/30)	0.25 (0.01/30)	0.25 (0.02/30)	0.28 (0.02/28)	0.19 (0.01/30)	0.22 (0.01/30)	0.25 (0.01/30)	0.25 (0.01/28)
Per mg polar lipid												
Unsaturation index	354.5 (5.3/17)	371.9 (2.4/17)	384.6 (2.0/15)	371.5 (2.5/17)	362.0 (3.7/30)	346.6 (2.3/30)	366.6 (1.7/30)	359.6 (3.0/28)	326.3 (2.5/30)	327.7 (3.9/30)	342.2 (1.4/30)	323.5 (2.4/28)
DHA	392.9 (16.3/17)	371.7 (8.8/17)	286.9 (8.6/15)	377.8 (16.9/17)	320.1 (11.2/30)	296.6 (8.0/30)	327.4 (8.7/30)	304.4 (9.3/28)	227.2 (7.1/30)	265.5 (11.5/30)	255.2 (6.3/30)	238.6 (5.8/28)
EPA	104.1 (5.4/17)	88.8 (3.7/17)	80.1 (3.3/15)	111.4 (5.5/17)	76.6 (2.4/30)	84.9 (2.5/30)	92.2 (2.4/30)	87.8 (3.6/28)	62.1 (3.1/30)	67.5 (4.0/30)	94.1 (5.2/30)	79.8 (2.3/28)
DHA/ARA	8.06 (0.70/17)	8.44 (0.33/17)	10.96 (0.73/15)	7.53 (0.51/17)	11.06 (0.39/30)	7.20 (0.36/30)	8.61 (0.43/30)	7.37 (0.41/28)	7.60 (0.34/30)	7.00 (0.36/30)	7.28 (0.35/30)	7.45 (0.29/28)
Palmitoleic acid	21.1 (1.4/17)	14.9 (0.7/17)	12.9 (1.1/15)	14.7 (1.4/17)	11.4 (0.7/30)	11.5 (1.0/30)	13.8 (0.8/30)	13.3 (1.0/28)	12.6 (0.8/30)	10.4 (0.8/30)	14.3 (1.1/30)	14.4 (1.0/28)
ARA	52.4 (3.6/17)	45.0 (1.8/17)	27.8 (2.1/15)	53.4 (3.3/17)	29.9 (1.6/30)	43.6 (2.0/30)	39.9 (1.6/30)	43.9 (2.1/28)	31.5 (1.6/30)	40.3 (2.4/30)	36.6 (1.4/30)	33.0 (1.1/28)
LIN	11.45 (0.61/17)	8.55 (0.32/17)	7.37 (0.53/15)	10.68 (0.45/17)	8.48 (0.16/30)	8.96 (0.25/30)	9.43 (0.43/30)	8.87 (0.43/28)	8.01 (0.38/30)	8.32 (0.42/30)	8.28 (0.38/30)	10.06 (0.59/28)
ALA	9.16 (0.82/17)	5.54 (0.29/17)	6.56 (1.04/15)	11.46 (0.87/17)	6.14 (0.23/30)	7.27 (0.39/30)	8.61 (0.74/30)	8.83 (0.74/28)	6.19 (0.37/30)	7.24 (0.48/30)	7.75 (0.41/30)	9.03 (0.40/28)

Appendix B

Response variable	Fixed effects							Random effects					
% lipids (whole fish) (10.8%)	Intercept	Weight	Spring	Summer	Fall			Stock	Year	Stock × season	Stock × year	Season × year	Residual
	16.3 (0.98)	0.007 (0.0006)	4.0 (1.2)	0.79 (1.2)	1.5 (1.3)			–	–	0.95 (0.66)	2.1 (1.3)	1.3 (0.74)	35.1 (1.2)
% lipids (muscle) (24.1%)	Intercept	Weight	Percent lipids (whole fish)	Female									
	0.21 (0.006)	0.00002 (4.2 × 10–6)	0.002 (0.0001)	−0.006 (0.001)				–	–	0.00004 (0.00002)	0.00008 (0.00005)	0.0002 (0.0001)	0.001 (0.00004)
% water (whole fish) (27.5%)	Intercept	Spring	Summer	Fall	Female	Weight	Cys (s in)						
	75.0 (0.35)	−2.2 (0.43)	−1.0 (0.44)	−1.5 (0.43)	−0.58 (0.10)	−0.003 (0.0002)	0.05 (0.008)	0.0[b]	–	0.12 (0.06)	0.06 (0.05)	0.16 (0.09)	4.6 (0.16)
Per mg dry weight of tissue extracted													
DHA (16.9%)	Intercept	Female	Weight	Percent water									
	1.5 (0.23)	−0.10 (0.01)	0.0001 (0.00003)	0.009 (0.003)				–	0.03 (0.02)	0.003 (0.001)	0.002 (0.001)	0.003 (0.002)	0.04 (0.002)
EPA (51.4%)	Intercept	Spring	Summer	Fall	Cys (y)								
	1.3 (0.13)	−0.04 (0.06)	−0.13 (0.06)	0.16 (0.06)	−1.5 (0.43)			–	0.001 (0.003)	0.003 (0.002)	0.004 (0.002)	0.002 (0.001)	0.07 (0.002)
DHA/ARA (14.6%)	Intercept	Female	Percent lipids (whole fish)	Cys (y in)									
	1.6 (0.13)	−0.08 (0.2)	0.004 (0.001)	0.01 (0.004)				0.009 (0.009)	0.0[a]	0.005 (0.003)	0.005 (0.003)	0.007 (0.004)	0.07 (0.003)
Palmitoleic acid (19.0%)	Intercept	Female	Weight	Percent lipids (muscle)	Cys (s)								
	−0.83 (0.09)	0.06 (0.02)	0.0003 (0.00004)	0.02 (0.004)	−0.69 (0.14)			0.0[a]	0.01 (0.01)	0.004 (0.003)	0.005 (0.003)	0.005 (0.003)	0.11 (0.005)
ARA (33.7%)	Intercept	Percent lipids (whole fish)											
	0.30 (0.06)	−0.005 (0.001)						–	–	0.009 (0.005)	0.02 (0.009)	0.006 (0.004)	0.06 (0.003)
LIN (9.6%)	Intercept	Spring	Summer	Fall	Percent water	Weight							
	−1.8 (0.29)	−0.12 (0.07)	−0.25 (0.07)	−0.08 (0.07)	0.009 (0.004)	−0.0001 (0.00004)		0.005 (0.006)	–	0.005 (0.003)	0.003 (0.002)	0.002 (0.001)	0.07 (0.003)
ALA (47.5%)	Intercept	Spring	Summer	Fall									
	−1.3 (0.06)	−0.23 (0.79)	−0.24 (0.08)	0.07 (0.08)				–	–	0.009 (0.006)	0.006 (0.005)	0.008 (0.001)	0.12 (0.005)
Per mg polar lipid													
Unsaturation index (21.3%)	Intercept	Female	Percent lipids (whole fish)										
	355.6 (11.2)	−5.4 (1.1)	−0.41 (0.09)					27.5 (26.3)	326.9 (284.0)	10.7 (7.8)	4.0 (4.7)	73.9 (39.0)	317.8 (13.5)
DHA (51.9%)	Intercept	Female	Weight	Percent lipids (muscle)	Cys (y)								
	6.4 (0.18)	−0.08 (0.01)	0.00007 (0.00003)	−0.01 (0.002)	−2.2 (0.61)			–	0.005 (0.005)	0.002 (0.001)	0.002 (0.001)	0.0009 (0.0007)	0.04 (0.001)
EPA (66.2%)	Intercept	Spring	Summer	Fall	Female	Cys (y)							
	4.9 (0.10)	−0.07 (0.05)	−0.015 (0.05)	0.07 (0.05)	0.05 (0.02)	−1.9 (0.03)		–	0.0007 (0.001)	0.003 (0.002)	0.004 (0.002)	0.0002 (0.0005)	0.07 (0.003)
DHA/ARA (14.6%)	Intercept	Female	Percent lipids (muscle)	Cys (y in)				0.009 (0.008)	0.0[a]	0.005 (0.003)	0.005 (0.003)	0.007 (0.004)	0.07 (0.003)
Palmitoleic acid (31.8%)	Intercept	Female	Weight	Cys (y)	Cys (s)	Percent lipids (muscle)							
	3.2 (0.10)	0.10 (0.02)	0.0003 (0.00004)	−1.9 (0.31)	−0.72 (0.14)	0.01 (0.004)		0.0[a]	0.0[a]	0.006 (0.003)	0.003 (0.002)	0.002 (0.002)	0.11 (0.005)
ARA (51.2%)	Intercept	Percent lipids (whole fish)											
	3.8 (0.06)	−0.005 (0.001)						–	–	0.007 (0.003)	0.02 (0.01)	0.008 (0.005)	0.07 (0.003)
LIN (1.2%)	Intercept	Female	Weight										
	2.2 (0.04)	0.03 (0.02)	−0.0002 (0.00003)					–	–	0.01 (0.006)	0.004 (0.003)	0.008 (0.006)	0.07 (0.002)
ALA (49.4%)	Intercept	Spring	Summer	Fall									
	2.2 (0.07)	−0.30 (0.10)	−0.24 (0.09)	−0.02 (0.10)				–	–	0.01 (0.007)	0.005 (0.005)	0.004 (0.002)	0.15 (0.007)

[a]Variance estimated to be zero after accounting for stock or year covariate.

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