Great Lakes Fishery Trust Project Completion Report

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Magnitude and potential causes of mortality in four lake whitefish populations in Lakes Michigan and Huron: a multidisciplinary approach

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INTRODUCTION

Estimates of natural mortality are essential for most fishery models that are used to determine harvest limits. Natural mortality, defined here as all fish deaths from causes other than fishing, is difficult to measure, especially in exploited populations. Further, there often is little that is known about factors that cause natural mortality, particularly in larger, older fish. Natural mortality rates can be readily measured in unexploited populations using stock-assessment techniques applied to age composition data (e.g., catch curves), but in exploited populations fishing and natural mortality can be difficult to separate. For many fisheries, natural mortality rates are simply assumed to have a constant, "typical" value, or are inferred from general models that relate mortality rates to life history-environment interactions (e.g., Pauly 1980). These assumed or inferred values have important implications for setting allowable harvest rates because sustainable management of fish populations requires that total mortality – the sum of natural and fishing mortality – remains below a target level. If natural mortality rates are underestimated, allowable harvest rates may be overestimated.

Within the Great Lakes, population models are used to manage lake whitefish (*Coregonus clupeaformis*) in 1836 treaty-ceded waters as mandated by the 2000 Consent Decree (U.S. v. Michigan 2000). The natural mortality rates used in these models were derived indirectly (Bence and Ebener 2002) using the Pauly (1980) regression model, which predicts natural morality rates based on fish growth parameters and environmental temperatures. These inferred rates imply that large numbers of whitefish die each year from unexplained causes (36% of total mortality in one Lake Huron stock). The fact that these rates have never been empirically verified is a significant concern for fishery managers. Additionally, widespread declines in *Diporeia* abundance in the Great Lakes, which is a primary diet item of lake whitefish, have led to concerns that natural mortality rates in lake whitefish may be increasing. *Diporeia* have high lipid content and are rich in many essential fatty acids (see below). Lake whitefish management would benefit greatly from a confirmation of existing models used to estimate natural mortality, as well as from insights into possible explanations (e.g. lipids, fatty acids, disease) for among-stock or among-year variations in natural mortality. The study described in this completion report had as its core objectives: (1) the estimation of natural mortality rates for four lake whitefish stocks in northern lakes Huron and Michigan, and (2) the determination of potential causes and indicators of differences in natural mortality rates among these whitefish stocks.

Study overview

We conducted an intensive, multi-year tagging study of four putative lake whitefish stocks in northern lakes Michigan and Huron. The recovery and reporting of tagged fish by commercial fishermen were used to estimate and compare instantaneous natural mortality rates for these stocks. Concurrently, we assessed fish health for the stocks using samples of adult lake whitefish collected seasonally late 2003 to early 2006. For the collected lake whitefish, we evaluated basic biological attributes (e.g., length, weight, sex), assessed total lipids and water content of whole fish, assessed fatty acid (FA) composition for several fish tissues, and completed a comprehensive pathological assessment including identification of fish pathogenic microbes and parasites.

We measured body composition of lake whitefish as an overall assessment of its nutritional status and well-being. Body stores of lipids serve multiple functions. Lipids are a source of metabolic energy and provide indications as to the quality and availability of food for fish. Lipids found in a cell's membrane affect structural integrity or its function as a physical,

chemical and biological barrier, for example for osmoregulation. The omega-3 and omega-6 fatty acids components of lipid are precursors of immune regulators referred to as eicosanoids. Eicosanoid is a collective term for prostaglandins, thromboxanes, leukotrienes and lipoxins that regulate immune function and disease resistance (Balfry and Higgs 2001).

Over the last four decades, many epizootics resulting in widespread mortalities in fish stocks have been attributed to infectious pathogens (Baumann 1998; Hansen and Olafson 1999; Kent et al. 2001; Tully and Nolan 2002; Siwicki et al. 2003). A number of viruses, bacteria, or parasites have been isolated from fish and Koch's postulates, confirming that these pathogens are the main cause of mortality, have been fulfilled (Follet et al. 1995; Rangdale et al. 1999). Multiple factors have been identified that contribute to or aggravate pathogen-caused mortalities, such as adverse environmental conditions and nutritional deficiencies. It is probable that several factors, not a single factor, contribute to the natural mortality observed in lake whitefish stocks in lakes Michigan and Huron. Thus, a multidisciplinary approach that includes a variety of indicators of fish health is essential for a comprehensive and holistic assessment of the causes of natural mortality in lake whitefish stocks.

Our approach allowed us to examine two sets of hypotheses. First, the direct measurement of natural mortality rates for four whitefish stocks allowed us to test the hypothesis that the predictive model currently used by fishery managers is appropriate for estimating natural mortality in lake whitefish stocks in the Great Lakes. The Pauly (1980) regression model was developed through a global analysis of fish populations from a wide variety of taxa; its applicability to individual populations of a single species has received limited attention. A test of its accuracy for Great Lakes whitefish stocks would be very valuable. Second, assessment of a variety of fish health indicators in these whitefish stocks enabled an exploration of possible hypotheses concerning the causes of natural mortality in these stocks.

At the outset of this study we recognized that our approach would enable us to formulate, more than test, hypotheses about causes of whitefish natural mortality. In our current state of ignorance, we argue that it is prudent to explore a variety of possible mechanisms, instead of narrowing the focus to test a small number specific hypotheses developed *a priori*. In general, however, our goal was to examine measures of whitefish health associated with diseases and nutritional stress, and to examine variation in these measures among stocks and over time.

Organization of this report

This completion report is divided into four sections, each with their own methods and results, followed by a short section containing a summary and recommendations for the future. The first section presents the methods and results of the tagging component of the study, including the field methods for tagging and recovering fish, assessment of tagging mortality, tag loss and reporting rates, and statistical models used in estimating mortality rates. The second section describes the nutritional component of the study, including whole fish assessments of lipids and water content, and fatty acid analysis of muscle, eye and liver tissue samples. The third section presents the assessment of pathogens and parasites for each whitefish stock. The fourth section contains an integrated statistical analysis of the entire dataset, in which we examine patterns of variation in fish health measures among stocks and over time, and relate these patterns to our pathological data and to our estimates of stock-specific natural mortality rates. Also included as a supplement to the report is the description of a simulation study that was conducted to evaluate the sensitivity of mortality estimates to target tagging levels and to inaccuracies in tag reporting, tag shedding, and handling mortality rates. This simulation study

was based on the tagging protocol employed in this research and was intended to provide insight as to potential biases in our mortality rate estimates.

Study Area

As previously stated, the lake whitefish stocks that were used in this research were from northern lakes Huron and Michigan. For simplicity, we reference these stocks by the names of their closest fishing port: Big Bay de Noc, Cheboygan, Detour (also referred to as Detour Village or Detour/Cedarville), and Naubinway. The Big Bay de Noc and Naubinway stocks are located in northern Lake Michigan, while the Cheboygan and Detour stocks are located in northern Lake Huron (Figure 1). Each of these areas are known to have 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 spawning season (Ebener and Copes 1985). These stocks are located in different lake whitefish management units (Big Bay de Noc - WFM-01; Naubinway – WFM-03; Cheboygan – WFH-01; Detour – WFH-02), which are managed through individual assessment models. Thus, we felt justified to treat these as separate lake whitefish stocks even though fish from these stocks can be mixed at other times of the year.

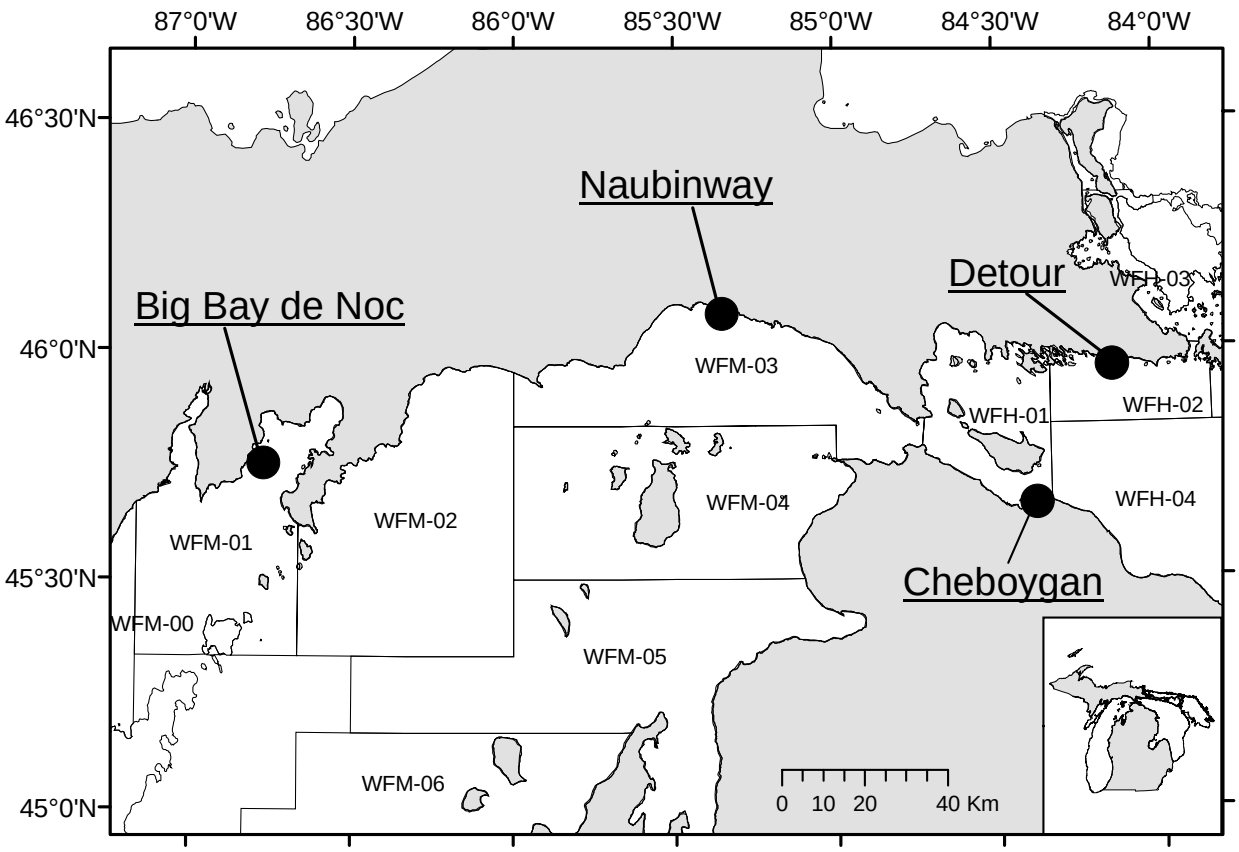

Figure 1. Map of northern lakes Huron and Michigan indicating the relative locations of the Big Bay de Noc, Cheboygan, Detour, and Naubinway lake whitefish stocks upon which this research was based.

PART 1 – TAGGING STUDY AND MORTALITY ASSESSMENT

Methods

Tagging

We tagged and released lake whitefish from the Big Bay de Noc, Cheboygan, Detour, and Naubinway stocks at 12 individual locations during early- to mid-November beginning in 2003. (Figure 1.1). Tagging locations were typically chosen by contract fishermen based on prior commercial catches, proximity of tagging locations to docking facilities, and through consultation with project investigators. Distances between tagging sites for a stock ranged from approximately 5 to 26 km (Big Bay de Noc = 5 km; Detour = 18 km; Naubinway = 21 km; Cheboygan = 26 km). Prior to beginning this study, we calculated a yearly target tagging goal of 2,000 fish per stock using formula presented in Brownie et al. (1985). This target tagging level was calculated using an assumed annual exploitation rate of 15% and a desired coefficient of variation for average survival of 5%. We were unable to meet this tagging goal for all stocks in all years due to various reasons, such as inclement weather conditions, broken tags, and inadequate fish catch. We initially had sought to tag fish in 2003, 2004, and 2005. However, we were unable to tag fish from the Cheboygan stock in 2005. As a result, additional tagging of lake whitefish from the Cheboygan stock was conducted in 2006. Additional tagging of lake whitefish was also conducted for the Detour stock in 2006 as part of another project.

Commercial-sized trap nets were used to capture all lake whitefish that were tagged as part of this project. Trap nets ranged from 300 to 364 m in length, 4 to 9 m in height, and had a maximum stretched mesh size of 114-mm on the lifting pot. Trap net material consisted of either polypropylene or multifilament nylon. Trap nets were set across depth contours in water ranging from 1 to 18 m deep ($\bar{x}$ = 7 m). Nets were typically lifted after 2 to 3 nights of fishing, although individual set times ranged from 1 to 7 nights. Dip nets were used to remove lake whitefish from the trap net lifting pots and fish were placed into 100-gallon holding tanks filled with lake water. Oxygen and a 1.36-kg block of salt were added to each tank to alleviate fish stress.

Lake whitefish were tagged with individually numbered t-bar anchor tags (Floy Tag, Inc., Seattle, Washington; Model FD-94), which were inserted near the anterior base of the dorsal fin. Total length (to the nearest mm), sex, stage of maturity, number of sea lamprey marks (see King 1980; Ebener et al. 2006), and presence of external abnormalities were recorded for each tagged fish. Each tag indicated that a $5 (US) reward would be given for the recovery of a tagged fish. Each tag also listed the phone number for the Inter-Tribal Fisheries and Assessment Program. We initially believed that a $5 reward would promote the return of tags because in most cases the reward would exceed a fish's market value. To encourage tag returns, Chippewa-Ottawa Resource Authority (CORA) commercial fishermen were mailed information regarding this project and the offer of a reward for the return of tagged fish. Additionally, at the end of each fishing year we contacted commercial fishermen in Lake Michigan and Huron regarding tags that had not yet been returned and provided these fishermen with self-addressed stamped envelopes to facilitate the recovery of tags. In 2007, we increased the reward for the return of tags to $10 because of an apparent decline in reporting rates.

Biological characteristics of tagged fish

A sample of lake whitefish captured during that the tagging process were sacrificed in order to collect biological information about the stocks. We typically sacrificed approximately 50 kg of lake whitefish per tagging day, which was equivalent to approximately 40 to 55 fish. Total length (to the nearest mm), weight (to the nearest 5 grams), sex, stage of maturity,

spawning condition, sea lamprey marks, presence of external abnormalities, and visceral fat index were recorded for each sacrificed fish. The visceral fat index (VFI) is a subjective evaluation of the total area of a fish's pylorus and cecae that is covered by fat. VFI values were assigned based on the following criteria: 0 = no fat covering the pylorus; 1 = less than 25% of the pylorus and cecae covered by fat; 2 = 50% of the pylorus and cecae covered by fat; 3 = 75% of the pylorus and cecae covered by fat; 4 = 100% of the pylorus and cecae covered by fat.

Short-term tag loss and handling mortality

Short-term tag loss and handling mortality of tagged lake whitefish were estimated by withholding samples of tagged fish in a 1.2×1.8 ×1 m (width×length×depth) cage that floated alongside the commercial fishing vessel. Approximately 1 out of every 50 tagged fish was placed within the holding cage. Tagged fish remained in the cage until every trap net was emptied, which typically took between one-half to four hours to complete. After the trap nets were emptied, the number of live and dead lake whitefish and the number of lake whitefish that had retained their tags were enumerated. Fish were considered "live" if they were swimming freely upright in the holding cage; fish were considered "dead" if they were floating upside down or were lying on the bottom of the holding cage.

Long-term tag loss

To estimate long-term tag loss in tagged lake whitefish, we supplementally marked most tagged specimens with a fin clip. Further, a sample of lake whitefish were double tagged. Clipped fins differed by year of tagging. In 2003, the left pectoral fin of tagged fish was clipped. In 2004, the adipose fin was clipped. In 2005 and 2006, the left pelvic fin was clipped. Double tags were given to every 25th tagged lake whitefish. The second tag was inserted in the same general location of the first tag but on the side opposite that of the first tag. Double tagged fish were not fin clipped.

Based on the observed pattern of tag loss (see Results below), we chose to model long-term tag loss using a sigmoidal function. The equation for this model was

$$Q_t = \frac{\alpha}{1+\exp(\beta - t)},$$

where Q_t was the tag shedding rate, t was the time in months since tagging, and α and β were model parameters that identified the maximum tag loss rate and the point of inflection for tag losses, respectively. Both double tagged and tagged-clipped datasets were used to fit the tag loss model. Each dataset was given equal weight when fitting the model. The long-term tag loss model was fit using AD Model Builder software (Otter Research Limited 2005).

Reporting rate

Tag reporting rates by commercial fishermen were measured by using observers to monitor commercial fishery harvest both on commercial fishing vessels and dockside. Monitoring of commercial fishery harvest consisted of sub-sampling a portion of the daily total catch of individual fishermen. We recorded the total number of 50-kg tubs of landed lake whitefish from each trap net or gang of gill nets and counted the number of fish in a single tub from each net or gang. Fishing effort and fishing locations were also recorded from the monitored commercial fishery harvests. The total catch of lake whitefish from each monitored harvest was estimated by multiplying the average number of lake whitefish in a 50-kg tub by the total number of tubs from that catch.

We estimated yearly reporting rates for the stocks based on the numbers of recovered tags and the total catch from both the monitored and unmonitored fishery harvests. The equation for estimating tag reporting rates was

$$\lambda = \frac{R_n \delta_o}{R_o \left(1 - \delta_o\right)},$$

where λ is the tag reporting rate, R_o is the number of tags returned from the monitored catch, R_n is the number of tags returned from the unmonitored catch, and δ_o is the proportion of the total catch from the monitored commercial fishery harvest (Pollock et al. 2002). This approach to calculating tag reporting rates assumes that 100% of the tags from the monitored commercial fishery harvest are reported. If the tag reporting rate for the monitored harvest is not 100%, then positive biases in tag reporting rates and negative biases in exploitation rate estimates can result (Pollock et al. 2002). Additional assumptions are that tags are sufficiently mixed such that the tag returns by the monitored or unmonitored component are reflective of the catch from those components, and that the catch data for the monitored and unmonitored harvests are accurate (Pollock et al. 2002). Tag reporting rates for the Detour stock were estimated from the commercial fishery harvest from lake whitefish management units H-01, H-02, and H-03. For the Cheboygan stock, tag reporting rates were calculated from the H-01 and H-04 lake whitefish management units. Data from the M-03 lake whitefish management unit were used to estimate reporting rate for the Naubinway stock. For the Big Bay de Noc stock tag reporting rates were estimated from the commercial fishery harvest from lake whitefish management units H-01, H-02, H-03, H-04, H-05, M-01, M-02, and M-03.

Fishing and natural mortality estimation

Instantaneous fishing and natural mortality rates for the lake whitefish stocks based on the numbers of recovered and reported tags were estimated using the Hoenig et al. (1998) re-parameterization of the Brownie et al. (1985) tag-recovery models for a Type-II fishery. We divided the year into 3 seasons that differed in duration and enumerated the total number of tag returns that were reported for each year and season. Season 1 was a 5-month period that began in December and lasted until the end of April (fraction of year = 0.417). Season 2 was a 4-month period that began in May and lasted until the end of August (fraction of year = 0.333). Season 3 was a 3-month period that began in September and lasted through the end of November (fraction of year = 0.25). Because the amount of fishing harvest likely differed among seasons, we apportioned fishing mortality by season within our tagging model based on the yield of lake whitefish harvest reported by Michigan, Wisconsin, and tribal commercial fishermen for the management units from which tagged fish were recovered (Hoenig et al. 1998). We chose to apportion fishing mortality according to catch rather than fishing effort because of the large number of gear types used by commercial lake whitefish fishermen in lakes Huron and Michigan, which we felt would make estimating the relative catchability of the different gear types difficult.

Using the modeling framework described above, the expected number of tag recoveries by season for the first year of tag recoveries can be predicted by the following equations:

$$r_{111} = \frac{N_1 \phi \gamma_{11} \lambda_1 \xi_{11} F_1}{\xi_{11} F_1 + \Delta_1 M_1} \left(1 - e^{-\xi_{11} F_1 - \Delta_1 M_1}\right)$$

$$r_{112} = \frac{N_1 \phi \gamma_{12} \lambda_1 \xi_{12} F_1}{\xi_{12} F_1 + \Delta_2 M_1} \left(1 - e^{-\xi_{12} F_1 - \Delta_2 M_1}\right) e^{-\xi_{11} F_1 - \Delta_1 M_1}$$

$$r_{113} = \frac{N_1 \phi \gamma_{13} \lambda_1 \xi_{13} F_1}{\xi_{13} F_1 + \Delta_3 M_1} \left(1 - e^{-\xi_{13} F_1 - \Delta_3 M_1}\right) e^{-(\xi_{11} + \xi_{12}) F_1 - (\Delta_1 + \Delta_2) M_1}$$

where r_{ijk} is the number of recovered tags from tagging cohort i in recovery period j and season k, N_i is the number of tagged fish in cohort i, λ_j is the tag reporting rate in period j, ϕ is the immediate tag loss and tagging mortality rate, γ_{jk} is the long-term tag loss rate for recovery period j and season k, ξ_{jk} is the fraction of catch for season k in recovery period j, Δ_k is the length of season k expressed as a fraction of the year, and M_j and F_j are the instantaneous fishing and natural mortality rates for period j. For the second year of recoveries, the expected number of tag recoveries can be predicted by the following equations

$$r_{121} = \frac{N_1 \phi \gamma_{21} \lambda_2 \xi_{21} F_2}{\xi_{21} F_2 + \Delta_1 M_2} \left(1 - e^{-\xi_{21} F_2 - \Delta_1 M_2}\right) e^{-F_1 - M_1}$$

$$r_{122} = \frac{N_1 \phi \gamma_{22} \lambda_2 \xi_{22} F_2}{\xi_{22} F_2 + \Delta_2 M_2} \left(1 - e^{-\xi_{22} F_2 - \Delta_2 M_2}\right) e^{-F_1 - \xi_{21} F_2 - M_1 - \Delta_1 M_2}$$

$$r_{123} = \frac{N_1 \phi \gamma_{23} \lambda_2 \xi_{23} F_2}{\xi_{23} F_2 + \Delta_3 M_2} \left(1 - e^{-\xi_{23} F_2 - \Delta_3 M_2}\right) e^{-F_1 - (\xi_{21} + \xi_{22}) F_2 - M_1 - (\Delta_1 + \Delta_2) M_2}$$

where all variables are as previously defined.

We evaluated 9 tag-recovery models that differed with respect to whether F and/or M varied among stocks, lakes, or years. For all models, we assumed that M for the stocks was constant over time. We made this assumption because the number of tagging events limited the number of yearly estimates of M that were obtainable. Additionally, the differences among stocks as to when fish were tagged (2003 to 2005 for Big Bay de Noc and Naubinway; 2003, 2004, and 2006 for Cheboygan; 2003-2006 for Detour) limited comparisons of yearly natural morality rates among the stocks. As our global model, we considered a model with fishing mortality rates that differed among years and stocks and natural mortality rates that differed among stocks. For the other evaluated models, yearly F's and/or M were considered similar among all stocks or for Lake Huron and Michigan stocks. Instantaneous fishing and natural mortality rates for the evaluated models were estimated by maximum likelihood estimation using AD Model Builder. The objective function, which consisted of the summed multinomial negative log likelihoods corresponding to the tagged cohorts for each stock, was minimized using a quasi-Newton optimization algorithm with termination criteria set to the software defaults. When specifying the recovery probabilities for the recovery periods for the tagged cohorts, we used the estimates of λ_j,ϕ, γ_{jk}, and ξ_{jk} that we calculated from the auxiliary information collected for this project, rather then trying to estimate these quantities as part of the model fitting process.

We tested for overdispersion in our tag-recovery data by calculating the variance inflation factor for our global model using the equation

$$\hat{c} = \chi^2 / \mathrm{df},$$

where $\hat{c}$ is the variance inflation factor estimate, χ^2 is the goodness-of-fit chi-square statistic and df is the degrees of freedom for the global model (Burnham and Anderson 2002). If the global model was overdispersed (i.e., if $\hat{c} > 1$), we estimated model parameters using an overdispersion corrected log likelihood (Burnham and Anderson 2002). Performance of our evaluated models was compared using Akaike information criteria (accounting for overdispersion if necessary) and Akaike weights. We did not correct the AIC values for small sample sizes as the ratio of the total number of tagged fish to the total number of model parameters was substantially greater then 40, which is the rule-of-thumb given by Burnham and Anderson for choosing between AIC and the small sample-size correction AIC. If the AIC differences (Δ_i) for the evaluated models indicated that there was substantial evidence for more then one model ($\Delta_i < 2.0$ for more then one model), then we calculated model average estimates and standard errors of *F* and *M* for the lake whitefish stocks using the following formulae

$$\bar{\theta} = \sum_{i=1}^{R} w_i \hat{\theta}_i$$

$$\widehat{\mathrm{se}}\left(\bar{\theta}\right) = \sqrt{\widehat{\mathrm{var}}\left(\bar{\theta}\right)} = \left[\sum_{i=1}^{R} w_i \sqrt{\widehat{\mathrm{var}}\left(\hat{\theta}_i \mid g_i\right) + \left(\hat{\theta}_i - \bar{\theta}\right)^2}\right],$$

where $\hat{\theta}_i$ is the natural or fishing mortality estimate for model g_i (i=1…R), R is the number of models with Δ_i less then 2.0, $\bar{\theta}$ is the model averaged mortality estimate, and $\widehat{\mathrm{var}}\left(\hat{\theta}_i \mid g_i\right)$ is the estimated conditional variance of the mortality estimate for model g_i (Burnham and Anderson 2002). Ninety-five percent confidence intervals for the model averaged estimates of *F* and *M* were calculated using the equation

$$\bar{\theta} \pm 1.96 \cdot \widehat{\mathrm{se}}\left(\bar{\theta}\right)$$

where 1.96 is the critical value from a standard normal distribution for an $\alpha = 0.05$.

Results

Biological characteristics of tagged fish

Altogether, we captured 30,341 lake whitefish in 55 commercial trap net lifts. We tagged approximately 74% (n = 22,416) of the captured fish with anchor tags and sacrificed approximately 6% (n =1,841) for biological data (Table 1.1). The remaining 20% (n = 9,766) of captured lake whitefish were released. The actual number of lake whitefish that were tagged each year ranged from 1,853 to 2,034 for the Big Bay de Noc stock, 1,722 to 1,914 for the Naubinway stock, 981 to 2,004 year for the Detour stock, and 1,431 to 1,838 for the Cheboygan stock (Table 1.2). Approximately 54% (n = 12,918) of tagged fish were sexually mature females and 42% (n = 9,418) were sexually mature males. Sex was indeterminable for approximately 4% (n = 798) of the tagged fish. The proportion of tagged lake whitefish that were sexually mature females ranged from 49% in both Lake Huron stocks to 67% in the Naubinway stock (Table 1.2).

Tagged lake whitefish ranged in total length from 318 to 675 mm total length, with an overall mean length of 486 mm (SE = 0.27 mm). Tagged female lake whitefish ($\bar{x}$ = 490 mm; SE = 0.37 mm) were slightly larger in size compared to tagged males ($\bar{x}$ =484 mm; SE = 0.40 mm).

Tagged lake whitefish from the Big Bay de Noc stock tended to be slightly larger than fish from the other stocks (Table 1.3). Overall mean length of lake whitefish from Big Bay de Noc was 509 mm (SE = 0.47 mm). In comparison, overall mean length of lake whitefish was 499 mm (SE = 0.49 mm) for the Cheboygan stock, 471 mm (SE = 0.50 mm) for the Detour stock, and 468 mm (SE = 0.50 mm) for the Naubinway stock. There was considerable variability in length distributions of tagged fish among stocks, within stocks among years, and among sites within stocks (Table 1.3; Figure 1.2).

Abnormalities observed on tagged fish

Of the lake whitefish that were tagged, 5% (n = 1,223) had external abnormalities. The most common abnormality was sea lamprey marks (Table 1.4). Sea lamprey marks were observed on 4% (n = 854) of tagged fish. The incidence of sea lamprey marks on tagged lake whitefish for the four stocks ranged from 1% (n = 67) for Naubinway to 6% (n = 286) for Cheboygan. The second most commonly observed abnormality on lake whitefish was the presence of red blotches (Table 1.4). These blotches occurred on approximately 1% (n = 170) of examined specimens. However, only one person recorded presence of red blotches on lake whitefish, so this rate of incidence is likely underestimated. Red blotches occurred on all lake whitefish external surfaces, except for fins, and were observed on fish from all stocks, except for Cheboygan. The next most commonly observed abnormality were scars from cormorant attacks (Table 1.4). Overall, the incidence of cormorant scars was low (< 1% of all tagged lake whitefish). Scars were observed on lake whitefish from the Big Bay de Noc, Detour, and Naubinway stocks, but not the Cheboygan stock.

More type-A sea lamprey marks were observed than type-B marks, and more stage-2 and stage-3 marks were observed than stage-1 and stage-4 marks (Table 1.5). Type-A lamprey marks, where marks penetrate the skin and muscle of lake whitefish, made up 71% (n = 606) of all sea lamprey marks observed. Partially healed type-A marks (A2 and A3) made up 39% (n = 337) of observed marks (Table 1.5). Fresh sea lamprey marks (A1) were the most common marks observed in Detour, whereas partially healed marks (A2 and A3) were more common for the other three stocks.

Tagged lake whitefish with sea lamprey marks were recovered at a rate similar to tagged fish without sea lamprey marks, suggesting that if a lake whitefish survived the initial sea lamprey attack, there was little to no latent mortality. Of the 854 lake whitefish with sea lamprey marks were tagged and released, approximately 8% were subsequently recovered. In comparison, 9% of tagged and released lake whitefish without sea lamprey marks were subsequently recovered. Differences in these recovery rates were not statistically significant ($X^2 = 0.921$, $P > 0.05$, v=2). Individual tests for differences in recovery rates among lamprey marked and unmarked lake whitefish also were not statistically significant for individual stocks ($P > 0.05$). There also was no statistically significant difference in recovery rates of lake whitefish with different types of sea lamprey marks ($P > 0.05$).

Using a previously developed relationship between the number of A1 and A2 sea lamprey marks per fish and the probability of surviving a sea lamprey attack (Ebener et al. 2005), we estimated that sea lamprey were a greater source of mortality for Lake Huron stocks than for Lake Michigan stocks. Mean estimates of sea lamprey-induced mortality for lake whitefish were 0.073/yr for Detour, 0.0527/yr for Cheboygan, 0.019/yr for Big Bay de Noc, and 0.0127/yr for Naubinway. Sea lamprey-induced mortality for all stocks was generally the greatest for fish larger than 500 mm in total length, particularly for lake whitefish from Detour. Estimated sea

lamprey-induced mortality for fish greater then 550 mm exceeded 0.25/yr for the Detour stock (Table 1.6).

Visceral fat index of sacrificed fish

Altogether, we evaluated the visceral fat index for 1,841 lake whitefish that were sacrificed from those fish caught during tagging operations. Of these fish, 948 were females and 753 were males. Visceral fat content varied among stocks, across years within stocks, and between sexes. The greatest VFI scores were found in male fish from the Big Bay de Noc stock; more than 70% of these fish had VFI scores of 2 or greater. For most stocks, VFI scores were generally less then 2 (Table 1.7). VFI score tended to increase through time for most stocks with the exception of the Detour stock. For males, the percentage of fish with a VFI score of 0 increased from 9% in 2003 to 68% in 2006. For female, the percentage of fish with a VFI score of 0 increased from 50% in 2003 to 88% in 2006. For all stocks, males generally had greater VFI scores then females.

Short-term survival and tag-loss

During tagging operations, we conducted 49 trials in which short-term survival and tag loss of tagged lake whitefish was evaluated. Altogether, 496 fish were withheld while tagging operations were completed. Of these fish, 485 (98%) were considered live when the tagging operation was completed and 11 (2%) were considered dead. There was slight variation in short-term survival for the stocks (Table 1.8). For the Big Bay de Noc stock, survival ranged from 96 to 98%. For the Cheboygan stock, survival ranged from 98 to 100%. For the Detour stock, survival ranged from 97 to 100%. For the Naubinway stock, survival ranged from 95 to 98%. There was no apparent pattern in survival to suggest that survival of tagged fish was related to anything but random spatial and temporal variability in tagging conditions. All withheld fish retained their tags, thus it would appear that short-term tag retention was near 100% (Table 1.8).

Long-term tag loss

Double tags were given to approximately 4% (n = 912) of tagged lake whitefish. Proportion of fish that were double tagged for the stocks was 4.0% for Detour, 4.0% in Naubinway, 4.1% in Cheboygan, and 4.3% in Big Bay de Noc. Females made up 57.0% of all double-tagged lake whitefish, males 40.0%, and fish of unknown sex 3.0%.

From the observed pattern of monthly tag retention based on both fin clipped and double tagged fish, tag retention in lake whitefish for the first few months after tagging was initially high (Figure 1.3). After approximately 5 or 6 month post-tagging, however, tag shedding became increasingly pervasive (Figure 1.3). There were very few observations of clipped or double tagged fish after approximately 16 to 18 months of tagging, which made fitting a model to describe long-term tag loss by time somewhat difficult. Our estimated sigmoidal relationship of tag shedding with respect to time was

$$Q_t = \frac{0.39}{1+\exp(10.08-t)},$$

where Q_t is the estimate tag shedding rate at t months post tagging. According to this model, tag shedding rate stabilized at approximately 40% at around 14 to 16 months post tagging.

Tag reporting rate

Dockside observers monitored commercial fishery harvest on 182 occasions. Onboard observers monitored commercial fishery harvest on 90 occasions. Annual reporting rates by commercial fishermen were surprisingly low throughout this study. We observed 61 tags caught out of 66,347 lake whitefish caught during onboard monitoring of commercial trap net and gill net harvests in H-01 through H-05 and M-01 through M-04 (Figure 1.1) during 2004-2007. During the same time, the commercial fishery returned 1,759 tags out of a harvest of 5.26 million lake whitefish during 2004-2007. This resulted in an overall tag reporting rate of 36.4%. Annual reporting rates were estimated to be 42.2% in 2004, 44.0% in 2005, 56.2% in 2006, and 17.8% in 2007. Tag reporting rates calculated for the individual stocks were quite variable (Table 1.9). For the Cheboygan stock, annual reporting rates ranged from 22 to 68%. For the Detour/Cedarville stock, tag reporting rates random from 24 to 77%. For the Naubinway stock, tag reporting rates ranged from 26 to 100%.

Tag recoveries

Between December 2003 and December 2007, we recovered tags from 1,952 lake whitefish. The majority of tags were recovered by commercial trap net fisheries (85%). Tagged lake whitefish were also recovered by commercial gill net (12%) and pound-net (0.2%) fisheries, recreational anglers (0.1%), and survey fisheries (0.1%). Approximately 3% of recovered tags were returned by wholesale buyers or retail shops and could not be attributed to a particular commercial fishery component. Three tags were recovered in retail shops in Philadelphia, Chicago, and Los Angles. One tag was recovered from a fish that had already been smoked.

Recovery of lake whitefish by sex occurred in nearly the same proportions that fish were originally tagged. Female lake whitefish made up 52.5% of all tag recoveries and 54.4% of all fish tagged, while males made up 43.4% of all tag recoveries and 42.0% of all fish tagged. For the Cheboygan stock, male lake whitefish were captured at a somewhat greater proportion than originally tagged (54% vs 46%), while females were captured at a lower rate then originally tagged (42 vs. 49%). For the other stocks, proportions of tagged and recovered lake whitefish by sex were nearly equal.

Based on our own recoveries of tagged fish during subsequent tagging operations, it was apparent that lake whitefish had strong fidelity to the areas where they were original released. During tagging operations, we captured 24 lake whitefish that had been previously tagged. Ninety-two percent ($n = 22$) of the recaptured individuals were within 3 km of their original release sites. In 2004, we recaptured 11 lake whitefish at Duncan Bay (Cheboygan stock) that had been tagged at this location in 2003. Also in 2004, we recaptured four tagged lake whitefish at Biddle Point (Naubinway stock), three of which were tagged at Naubinway Reef in 2003 and one which was tagged at Epoufette Island in 2003. In 2005, we recaptured two lake whitefish at Bay de Noc Shoal north (Big Bay de Noc stock) that had been tagged at Bay de Noc Shoals in 2003 and 2004. Also in 2005, we recaptured three lake whitefish at Epoufette Island (Naubinway stock) that had been tagged at Epoufette Island in 2004. In 2006, we recaptured three lake whitefish at La Salle Island (Detour stock) that had been tagged at La Salle Island in 2004 and 2005. Also in 2006, we recaptured one lake whitefish in Hammond Bay Harbor (Cheboygan stock) that had been tagged and released at Duncan Bay in 2004.

Although lake whitefish appeared to exhibit strong site fidelity during spawning season, at other times of the year tagged lake whitefish from some of the stock moved extensively throughout lakes Michigan and Huron. In general, lake whitefish from the Naubinway and

Detour stocks were more sedentary than fish from the Big Bay de Noc and Cheboygan stocks. Lake whitefish from the Big Bay de Noc stock exhibited the most extensive movements and a considerable portion of this stock appeared to inhabit Wisconsin waters of Lake Michigan during the non-spawning period (December-September). Over one-third of the lake whitefish tagged from the Big Bay de Noc stock were recovered from the Wisconsin portion of Lake Michigan, with most of these recoveries coming from the main basin waters along the east side of the Door Peninsula as far south as Sheboygan, Wisconsin. One of the tagged lake whitefish from the Big Bay de Noc tagged stock was captured off Ludington, Michigan on the east side of the main basin of Lake Michigan. Six tagged fish from the Big Bay de Noc stock were recovered in Lake Huron (Table 1.10). Within several weeks of being tagged lake whitefish from the Big Bay de Noc stock were being caught along the east side of the Door Peninsula in both 2003 and 2004 and all the Big Bay de Noc tag recaptures in W-5 were made in April through June. Slightly less than 50% of the Big Bay de Noc tag recaptures took place in the management unit of tagging (M-01), but 60% of these took place in October and November. Eighty-four percent of the Big Bay de Noc tag recoveries in Wisconsin waters took place during January through July.

Lake whitefish from Cheboygan were the second most migratory stock. Nearly 72% of the lake whitefish tagged at Cheboygan were recaptured in the management unit of tagging (H-01) or the adjacent management unit (H-04). Another 12% were captured north of the tagging site in H-02 and 8% were captured south of the tagging site in management units H-05, H-06, and H-07. One lake whitefish tagged at Cheboygan was caught in the North Channel (Figure 1.1). Less than 2% of the lake whitefish tagged at Cheboygan were recaptured in Lake Michigan. Lake whitefish from Cheboygan inhabit mainly the area along the east and north sides of Bois Blanc Island south to Alpena.

Lake whitefish from the Naubinway stock were both highly migratory and highly sedentary, depending upon spawning location. While 87% of the lake whitefish tagged in Naubinway were recaptured in the management unit of tagging (M-03), fish from Naubinway were recaptured in more management units (14) than fish from other stocks (Table 1.10). Naubinway stock tagged fish were recaptured in Green Bay, at the Fox Islands, in Georgian Bay, and off Alpena (H-05). All except 1% of the lake whitefish tagged at Naubinway Reef and Biddle Point were recaptured in the management unit of tagging (M-03). Conversely, 82% of lake whitefish tagged at Epoufette Island were recaptured in M-03 and 14% were recaptured in Lake Huron as far south as H-05. It was lake whitefish tagged at Epoufette Island that were recovered in Green Bay, at the Fox Island, and in Georgian Bay.

Lake whitefish from the Detour stock were the least migratory of the four stocks we studied. About 90% of the lake whitefish tagged at Detour were recaptured in the management unit of tagging (H-02) or the adjacent management unit (H-01). A small proportion (2%) of lake whitefish tagged at Detour moved south to H-04, H-05, and H-06, five fish were recaptured in Lake Michigan, and one tagged fish was recaptured in Wisconsin waters of Green Bay. Lake whitefish from Detour appeared to exhibit mainly a east-west movement through H-01 and H-02 and few fish leave this area as do fish from Big Bay de Noc and Cheboygan.

Fishing and natural mortality estimation

The variance inflation factor for our global tag-recovery model (yearly and stock specific estimates of F; stock specific estimates of M) equaled 6.12, indicating that our tag-recovery data were overdispersed. Because our global model was overdispersed, we used the overdispersion-adjusted AIC (QAIC) to evaluate our different models. Based on QAIC, our best performing

model had yearly and stock specific estimates of *F* but a constant estimate of *M* (QAIC = 3,116.3) (Table 1.11). The yearly estimates of *F* for the stocks from this model ranged from 0.04 to 0.07 for Big Bay de Noc, 0.12 to 0.20 for Cheboygan, 0.23 to 0.69 for Detour, and 0.15 to 0.23 for Naubinway. The estimate of *M* from this model was 0.47. The second best-performing model had yearly and stock specific estimates of *F* and stock-specific estimates of *M* (QAIC = 3117.3). The QAIC difference for this second best-performing model was 1.0, suggesting that there was substantial evidence for this model as the best model. The yearly estimates of *F* for the stocks from this second model ranged from 0.04 to 0.07 for Big Bay de Noc, 0.11 to 0.17 for Cheboygan, 0.25 to 0.83 for Detour, and 0.09 to 0.22 for Naubinway. The estimates of *M* for the stocks were 0.50, 0.36, 0.62, and 0.26 for Big Bay de Noc, Cheboygan, Detour, and Naubinway, respectively. All other evaluated models had QAIC differences greater then 10, indicating that there was very little evidentiary support for these models.

Because of the levels of support for the two best performing models, we calculated model-averaged estimates of *F* and *M* for each lake whitefish stock using the QAIC weights for the respective models. The QAIC weight for the best performing model was 0.625, while the QAIC weight for the second best performing model was 0.375. Thus, mortality estimates from the best performing model had a somewhat larger influence on the model-averaged mortality estimates. The model-averages estimates of *F* for the stocks ranged from 0.04 to 0.07 for Big Bay de Noc, 0.12 to 0.19 for Cheboygan, 0.24 to 0.74 for Detour, and 0.13 to 0.22 for Naubinway (Table 1.12). The estimates of *M* for the stocks equaled 0.48 (SE = 0.18) for Big Bay de Noc, 0.43 (SE = 0.12) for Cheboygan, 0.53 (SE = 0.11) for Detour, and 0.39 (SE = 0.14) for Naubinway (Table 1.12)

Table 1.1. Number of lake whitefish tagged and released at specific sites within each stock during 1-18 November of 2003-2006.

Stock	Site	Latitude	Longitude	Date	Number tagged
Big Bay de Noc	Ripley Shoal	4546.30	8645.10	3-Nov-2003	508
				5-Nov-2003	508
	Bay de Noc Shoal north	4547.50	8642.50	3-Nov-2003	218
				5-Nov-2003	636
				1-Nov-2004	761
				2-Nov-2004	1,092
				2-Nov-2005	706
				3-Nov-2005	483
				4-Nov-2005	71
	Bay de Noc Shoal south	4545.17	8642.09	2-Nov-2005	250
				3-Nov-2005	271
				4-Nov-2005	253
Naubinway	Naubinway Reef	4603.30	8525.50	8-Nov-2003	675
				17-Nov-2003	484
	Biddle Point	4604.40	8522.50	8-Nov-2004	925
	Epoufette Island	4602.36	8512.17	12-Nov-2003	755
				12-Nov-2004	883
				8-Nov-2005	1,116
				11-Nov-2005	606
Cheboygan	Duncan Bay	4520.24	8426.30	10-Nov-2003	766
				11-Nov-2003	272
				12-Nov-2003	269
				18-Nov-2003	429
				12-Nov-2004	802
				13-Nov-2004	485
				16-Nov-2004	551
	Hammond Bay harbor	4536.31	8410.60	7-Nov-2006	882
				8-Nov-2006	549
Detour/Cedarville	Stevenson Bay	4558.30	8408.27	8-Nov-2003	425
				12-Nov-2003	464
				15-Nov-2003	286
	Beavertail Point	4557.71	8410.11	8-Nov-2003	189
				12-Nov-2003	139
	Boot Island	4557.03	8416.41	6-Nov-2006	381
				9-Nov-2006	295
	La Salle Island	4556.70	8418.43	2-Nov-2005	557
				5-Nov-2005	1,158
				8-Nov-2005	289
				6-Nov-2006	104
				9-Nov-2006	201
	La Salle Island Reef	4556.97	8419.12	1-Nov-2004	896
				2-Nov-2004	352
				9-Nov-2004	474

Table 1.2. Number of female, male, and unknown sex lake whitefish tagged annually in each stock during November 2003-2006.

		Year of tagging				
Stock	Sex	2003	2004	2005	2006	Total
Big Bay de Noc	female	769	1,341	981		3,091
	male	1,067	490	1,019		2,576
	unknown	34	22	34		90
	subtotal	1,870	1,853	2,034		5,757
Naubinway	female	1,070	1,494	1,064		3,628
	male	681	311	641		1,633
	unknown	163	3	17		182
	subtotal	1,914	1,808	1,722		5,444
Cheboygan	female	702	829		926	2,457
	male	933	923		433	2,289
	unknown	101	86		72	259
	subtotal	1,736	1,838		1,431	5,005
Detour	female	598	886	1,219	319	3,022
	male	860	809	658	593	2,920
	unknown	45	27	127	69	267
	subtotal	1,503	1,722	2,004	981	6,210

Table 1.3. Mean total length (mm), standard deviation (std), and number (n) of female and male lake whitefish tagged in four stocks of northern Lake Michigan and Huron during 1-18 November 2003-2006. Stocks abbreviations are: BBN=Big Bay de Noc, NAB=Naubinway, DET=Detour, and CHB=Cheboygan.

		----------Female----------			----------Male----------			----------Total----------		
Stock	Year	n	mean	std	n	mean	std	n	mean	std
BBN	2003	769	505	36	1067	490	34	1,870	495	36
	2004	1,341	523	32	489	510	30	1,852	520	33
	2005	981	519	33	1019	510	33	2,034	513	34
	subtotal	3,091	517	34	2,575	501	34	5,756	509	36
NAB	2003	1,069	465	41	680	472	35	1,911	464	41
	2004	1,491	467	33	311	467	38	1,805	467	34
	2005	1,064	476	31	641	468	37	1,722	473	34
	subtotal	3,624	469	35	1,632	470	36	5,438	468	37
DET	2003	597	460	40	859	452	38	1,500	454	40
	2004	885	487	35	809	478	40	1,720	481	38
	2005	1,218	477	36	656	472	37	1,999	473	38
	2006	319	477	36	592	477	33	980	477	35
	subtotal	3,019	475	38	2,916	469	39	6,199	471	39
CHB	2003	702	493	35	929	489	35	1,732	490	36
	2004	829	499	34	923	497	35	1,838	497	34
	2006	926	516	30	433	502	28	1,431	511	30
	subtotal	2,457	504	34	2,285	494	34	5,001	499	35
Total	2003	3,137	480	43	3,535	477	39	7,013	477	42
	2004	4,546	493	40	2,532	490	39	7,215	491	40
	2005	3,263	489	39	2,316	487	41	5,755	487	40
	2006	1,245	506	36	1,025	487	34	2,411	497	36
	subtotal	12,191	490	41	9,408	484	39	22,394	486	41

Table 1.4. Number of tagged lake whitefish observed with external abnormalities in four stocks during 1-18 November 2003-2006.

Abnormality	Naubinway	Detour	Big Bay de Noc	Cheboygan
Bad caudal fin	1	1	0	0
Bent spine	0	3	2	1
Black	1	0	0	0
Both ventral fins missing	1	0	0	1
Bulging eyes	0	0	1	0
Contusion	0	0	1	0
Cormorant scars	5	3	19	0
Excessively thin	7	1	1	0
External growth	1	0	0	0
Leech attached	1	0	0	0
Left pelvic fin missing	0	0	1	0
Left ventral fin missing	0	0	2	0
Net abrasions	0	5	0	1
No caudal peduncle	5	0	0	0
No gill plate	0	0	1	1
Poor physical condition	0	2	0	0
Pseudomonas	3	1	0	0
Red blotches	101	12	157	0
Right ventral fin missing	0	0	1	0
Scar	0	0	2	0
Sea lamprey marks	67	342	159	286
Two mouths	0	0	0	1

Table 1.5. Number of tagged lake whitefish with various types and stages of sea lamprey marks and number of these marked fish subsequently recovered.

Stock	Recovery statistic	Sea lamprey mark type and stage								Total
		A1	A2	A3	A4	B1	B2	B3	B4	
BBN	marked	7	29	43	46	6	11	10	7	159
	recovered	0	0	1	2	0	1	0	0	4
NAB	marked	5	17	14	14	5	4	5	3	67
	recovered	0	2	0	3	0	0	2	0	7
CHB	marked	15	71	56	59	32	20	18	15	286
	recovered	1	4	5	4	4	1	1	0	20
DET	marked	91	55	52	32	49	27	23	13	342
	recovered	7	7	6	5	5	3	1	1	35

Table 1.6. Estimated sea lamprey-induced mortality rate of six length classes of lake whitefish based on marking rates of tagged fish in four stocks during 1-18 November 2003-2006.

	Whitefish	Sea lamprey mortality rate				
Stock	length class	2003	2004	2005	2006	Mean
Big Bay de Noc	<400	0.0000	0.0000	0.0000		0.0000
	400-449	0.0000	0.0000	0.0000		0.0000
	450-499	0.0182	0.0355	0.0000		0.0169
	500-549	0.0389	0.0164	0.0052		0.0179
	550-599	0.0561	0.0530	0.0118		0.0372
	>599	0.0000	0.0000	0.0000		0.0000
Naubinway	<400	0.0000	0.0000	0.0000		0.0000
	400-449	0.0103	0.0053	0.0077		0.0078
	450-499	0.0035	0.0202	0.0033		0.0091
	500-549	0.0333	0.0497	0.0158		0.0317
	550-599	0.0000	0.0000	0.0000		0.0000
	>599	0.0000	0.0000	0.0000		0.0000
Detour	<400	0.0000	0.0000	0.0500	0.0000	0.0135
	400-449	0.0424	0.0208	0.0137	0.0323	0.0284
	450-499	0.0823	0.0723	0.0500	0.0841	0.0689
	500-549	0.2919	0.1026	0.0542	0.1441	0.1200
	550-599	0.2308	0.3529	0.0000	0.7500	0.2821
	>599	0.0000	0.3000	0.5000	0.0000	0.2857
Cheboygan	<400	0.0000	0.0000			0.0000
	400-449	0.0000	0.0000		0.0000	0.0000
	450-499	0.0204	0.0247		0.0609	0.0310
	500-549	0.0412	0.0574		0.1037	0.0702
	550-599	0.2308	0.0545		0.1615	0.1377
	>599	0.0000	0.3000		0.3333	0.1875

Table 1.7. Proportion of sacrificed male and female lake whitefish with visceral fax index scores of 0 to 4 in four stocks during 1-18 November 2003-2006.

			Number	Visceral fat index				
Location	Sex	Year	fish	0	1	2	3	4
BBN	Male	2003	99	0.111	0.364	0.374	0.152	0.000
		2004	11	0.000	0.091	0.545	0.364	0.000
		2005	47	0.021	0.085	0.404	0.383	0.106
	Female	2003	70	0.500	0.357	0.100	0.043	0.000
		2004	65	0.231	0.538	0.200	0.031	0.000
		2005	42	0.095	0.595	0.310	0.000	0.000
NAB	Male	2003	108	0.685	0.194	0.083	0.037	0.000
		2004	13	0.692	0.308	0.000	0.000	0.000
		2005	57	0.491	0.263	0.211	0.035	0.000
	Female	2003	161	0.901	0.043	0.031	0.025	0.000
		2004	92	0.880	0.098	0.011	0.011	0.000
		2005	109	0.651	0.321	0.028	0.000	0.000
DET	Male	2003	54	0.093	0.556	0.278	0.074	0.000
		2004	39	0.205	0.564	0.154	0.051	0.026
		2005	45	0.444	0.289	0.222	0.044	0.000
		2006	31	0.677	0.323	0.000	0.000	0.000
	Female	2003	24	0.500	0.417	0.083	0.000	0.000
		2004	52	0.423	0.288	0.173	0.115	0.000
		2005	94	0.713	0.160	0.064	0.064	0.000
		2006	24	0.875	0.042	0.042	0.042	0.000
CHB	Male	2003	117	0.154	0.521	0.299	0.026	0.000
		2004	101	0.238	0.297	0.406	0.059	0.000
		2006	31	0.355	0.226	0.355	0.032	0.032
	Female	2003	78	0.564	0.397	0.038	0.000	0.000
		2004	49	0.776	0.204	0.020	0.000	0.000
		2006	88	0.455	0.375	0.136	0.034	0.000

Table 1.8. Estimated mortality and tag loss of lake whitefish associated with the tagging process in four stocks during 1-18 November 2003-2006. Sample size represents the number of times tagged fish were placed into a live cage alongside a commercial fishing vessel.

Year	Stock	Sample size	Number fish	Number Live	Number Dead	Survival	Tag loss rate
2003	Big Bay de Noc	4	54	52	2	0.963	0.000
	Naubinway	5	39	37	2	0.949	0.000
	Cheboygan	3	31	31	0	1.000	0.000
	Detour	4	24	24	0	1.000	0.000
	subtotal	16	148	144	4	0.973	0.000
2004	Big Bay de Noc	4	22	21	1	0.955	0.000
	Naubinway	2	42	41	1	0.976	0.000
	Cheboygan	2	9	9	0	1.000	0.000
	Detour	6	70	69	1	0.986	0.000
	subtotal	14	143	140	3	0.979	0.000
2005	Big Bay de Noc	4	33	32	1	0.970	0.000
	Naubinway	3	23	22	1	0.957	0.000
	Cheboygan						
	Detour	6	69	69	0	1.000	0.000
	subtotal	13	125	123	2	0.984	0.000
2006	Cheboygan	2	44	43	1	0.977	0.000
	Detour	4	36	35	1	0.972	0.000
	subtotal	6	80	78	2	0.975	0.000

Table 1.9. Numbers of recovered tags and catches from the monitored and unmonitored commercial fishery catches, which were used to estimate tag reporting rates as described in Pollock et al. (2002).

		Monitored catch		Non-monitored catch		Reporting
Stock	Year	fish	tags	fish	tags	rate
BBN	2004	25,687	22	1,239,234	448	0.42
	2005	15,859	13	1,361,617	425	0.38
	2006	13,516	12	1,439,406	616	0.48
	2007	11,285	14	1,336,633	252	**≈0.43**
NAB	2004	6,216	5	256,829	241	1.17
	2005	5,509	6	250,305	139	0.51
	2006	2,175	2	248,107	202	0.89
	2007	782	0	115,714	13	**≈0.20**
DET	2004	10,050	14	280,181	166	0.43
	2005	4,651	5	334,487	217	0.60
	2006	5,987	8	341,065	350	0.77
	2007	3,645	12	241,110	192	0.24
CHB	2004	2,951	4	174,966	53	0.22
	2005	2,457	3	288,958	197	0.56
	2006	5,171	6	289,010	228	0.68
	2007	2,463	6	190,499	128	0.28

Table 1.10. Number of tagged lake whitefish from each stock captured in management units of lakes Michigan and Huron from December 2003 through December 2007. See Figure 1.1 for locations of the whitefish management units.

	Mgt	Stock				
Lake	unit	BBN	CHB	DET	NAB	Total
Huron	5-1				1	1
	6-1		1			1
	H-01	2	86	351	50	489
	H-02	4	35	398	3	440
	H-03		3	30	1	34
	H-04		126	9	1	136
	H-05		22	7	2	31
	H-06		2	3		5
	H-07		1			1
Michigan	M-00	5			1	6
	M-01	66			4	70
	M-02	6			5	11
	M-03	1	3	4	600	608
	M-04		1	1	1	3
	M-05				1	1
	M-07	1				1
	W-1	3				3
	W-2	9		1	1	11
	W-3	21			1	22
	W-4	3				3
	W-5	14				14
	Unknown		16	26	19	61

Table 1.11. Total number of parameter, overdispersion adjusted negative log likelihood (Neg. LL), overdispersion adjusted AIC(QAIC) value, and QAIC differences (Δ_i) for the tag-recovery models evaluated for estimating fishing and natural mortality for four lake whitefish stocks. The total number of parameters listed for each model includes the estimation of the variance inflation factor for the assessment of overdispersion for the global model. Models are sorted ascending according to Δ_i.

Model[†]	Total # parameters	Neg. LL	QAIC	Δ_i
F(S×Y), *M*(•)	18	1,540.16	3,116.30	0
F(S×Y), *M*(S)	21	1,537.67	3,117.30	1
F(L×Y), *M*(S)	13	1,552.96	3,131.90	15.6
F (Y), *M*(S)	9	1,562.49	3,143.00	26.7
F(L×Y), *M*(L)	11	1,566.23	3,154.50	38.1
F(L×Y), *M*(•)	10	1,567.75	3,155.50	39.2
F(S×Y), *M*(L)	19	1,566.23	3,170.50	54.1
F (Y), *M*(L)	7	1,579.63	3,173.30	56.9
F (Y), *M*(•)	6	1,585.86	3,183.70	67.4

[†]*F*(S×Y) = yearly *Fs* differing for the 4 stocks
F(Y) = yearly *Fs* the same across the 4 stocks
F(L×Y) = yearly *Fs* differing for Lake Huron and Michigan
M(S) = *M* differing for the 4 stocks
M(•) = *M* the same across the 4 stocks
M(L) = *M* differing for Lake Huron and Michigan

Table 1.12. AIC model averaged estimates, standard errors, and upper and lower 95% confidence limits of F and M for the Big Bay de Noc, Cheboygan, Detour, and Naubinway stocks based on t-bar anchor tag recoveries. Model averaging was conducted using the parameter estimates from the two best performing models of those that we evaluated (F1 = *F* in 2004, F2 = *F* in 2005, F3 = *F* in 2006; F4 = *F* in 2007).

Parameter	Estimate	SE	95% LCL	95% UCL
Bay de Noc F1	0.07	0.033	0.01	0.14
Bay de Noc F2	0.04	0.021	0.00	0.08
Bay de Noc F3	0.04	0.018	0.01	0.08
Bay de Noc F4	0.04	0.033	0.00	0.11
Bay de Noc M	0.48	0.177	0.13	0.83
Cheboygan F1	0.18	0.072	0.03	0.32
Cheboygan F2	0.12	0.035	0.05	0.19
Cheboygan F3	0.19	0.074	0.04	0.33
Cheboygan F4	0.19	0.076	0.04	0.34
Cheboygan M	0.43	0.123	0.19	0.67
Detour F1	0.48	0.118	0.25	0.71
Detour F2	0.24	0.053	0.14	0.35
Detour F3	0.24	0.043	0.16	0.33
Detour F4	0.74	0.189	0.37	1.11
Detour M	0.53	0.113	0.31	0.75
Naubinway F1	0.24	0.042	0.15	0.32
Naubinway F2	0.22	0.049	0.13	0.32
Naubinway F3	0.14	0.033	0.08	0.21
Naubinway F4	0.13	0.078	0.00	0.28
Naubinway M	0.39	0.136	0.12	0.66

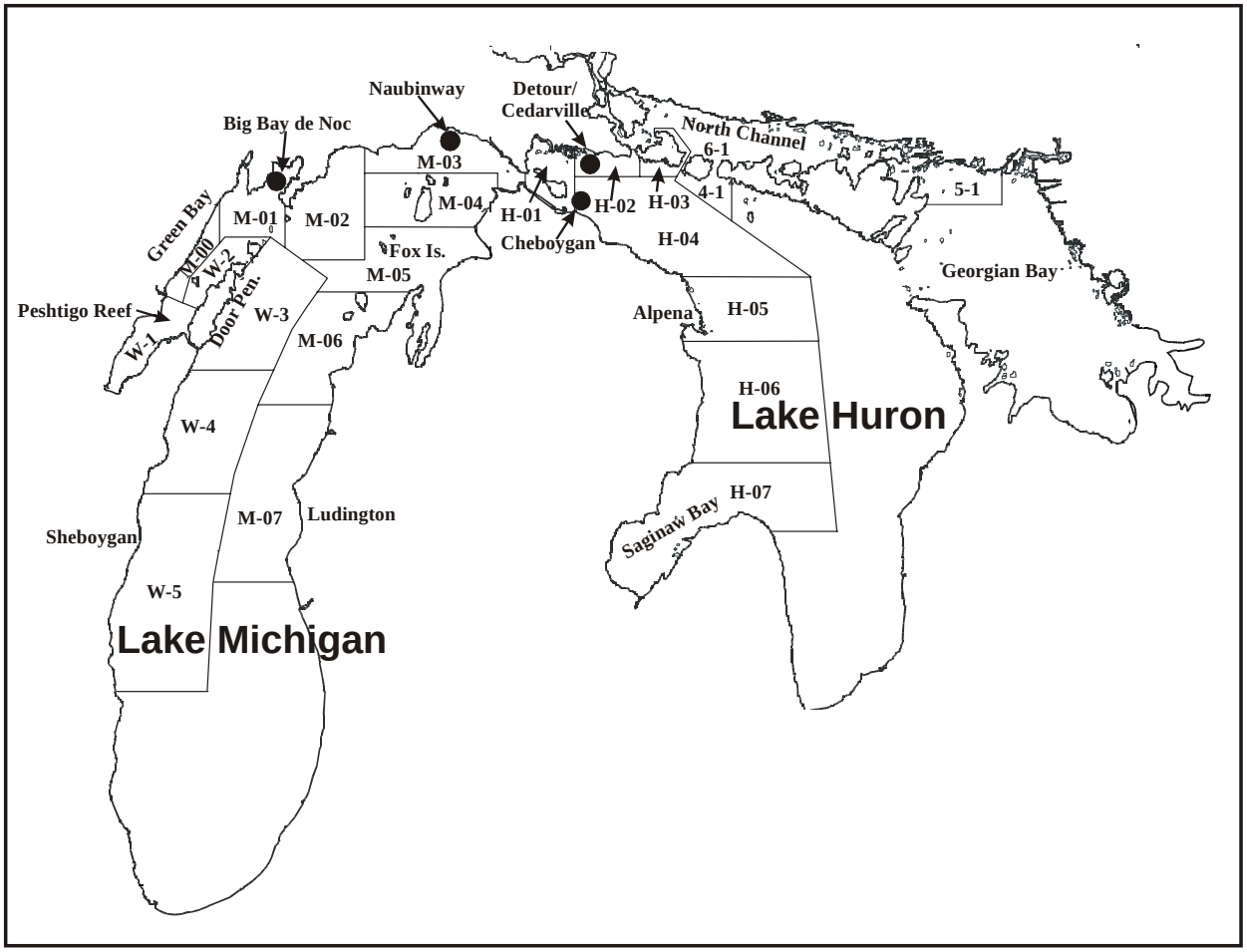

Figure 1.1. General locations of sites (black circle) within the four stocks of lake whitefish (M-01, M-03, H-01/H-04, and H-02) where tagging occurred during November 2003-2007, and selected whitefish management units in lakes Michigan and Huron.

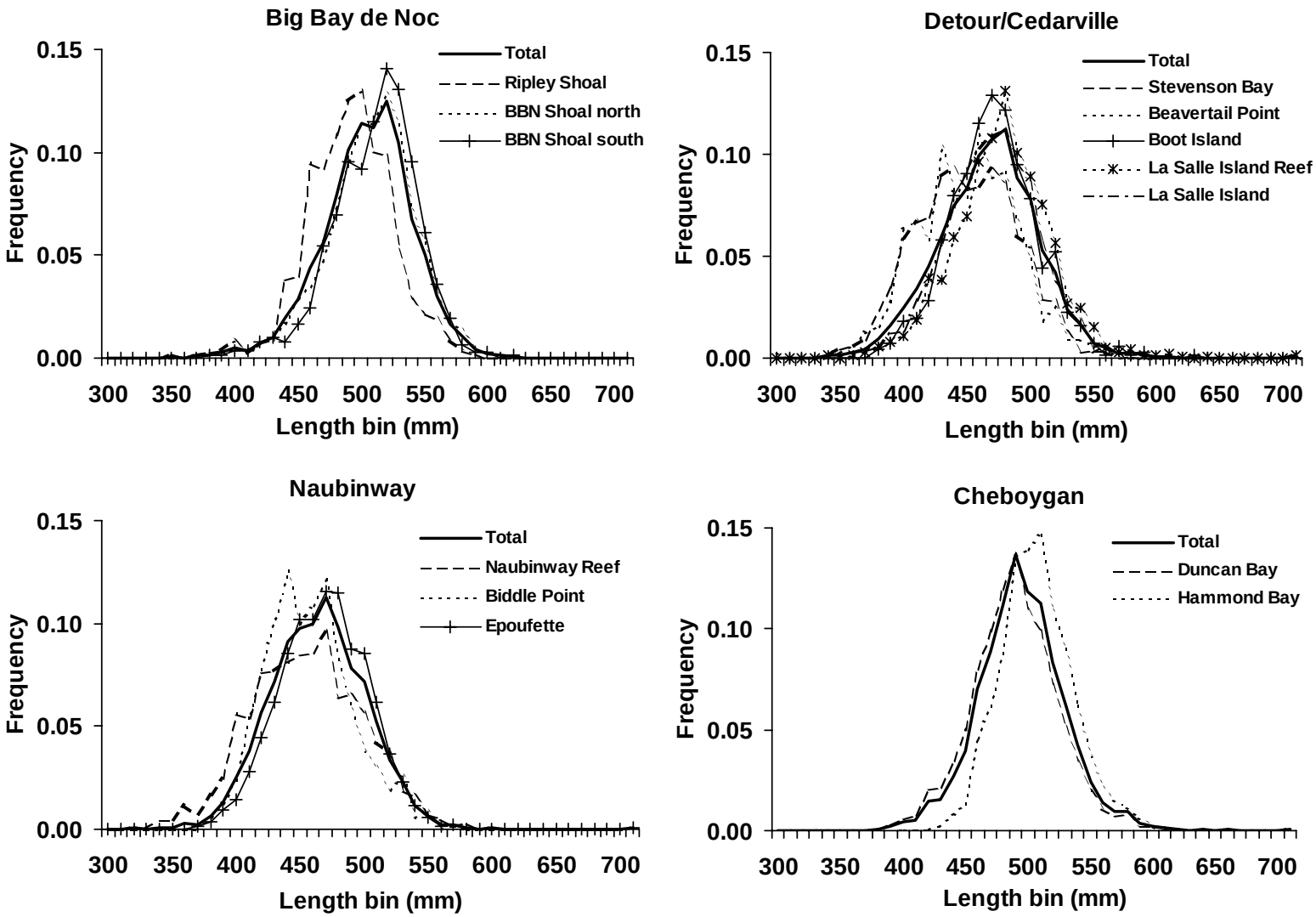

Figure 1.2. Length-frequency distribution of lake whitefish tagged at various sites in four stocks during 1-18 November 2003-2006. The abbreviation BBN stands for Bay de Noc Shoal.

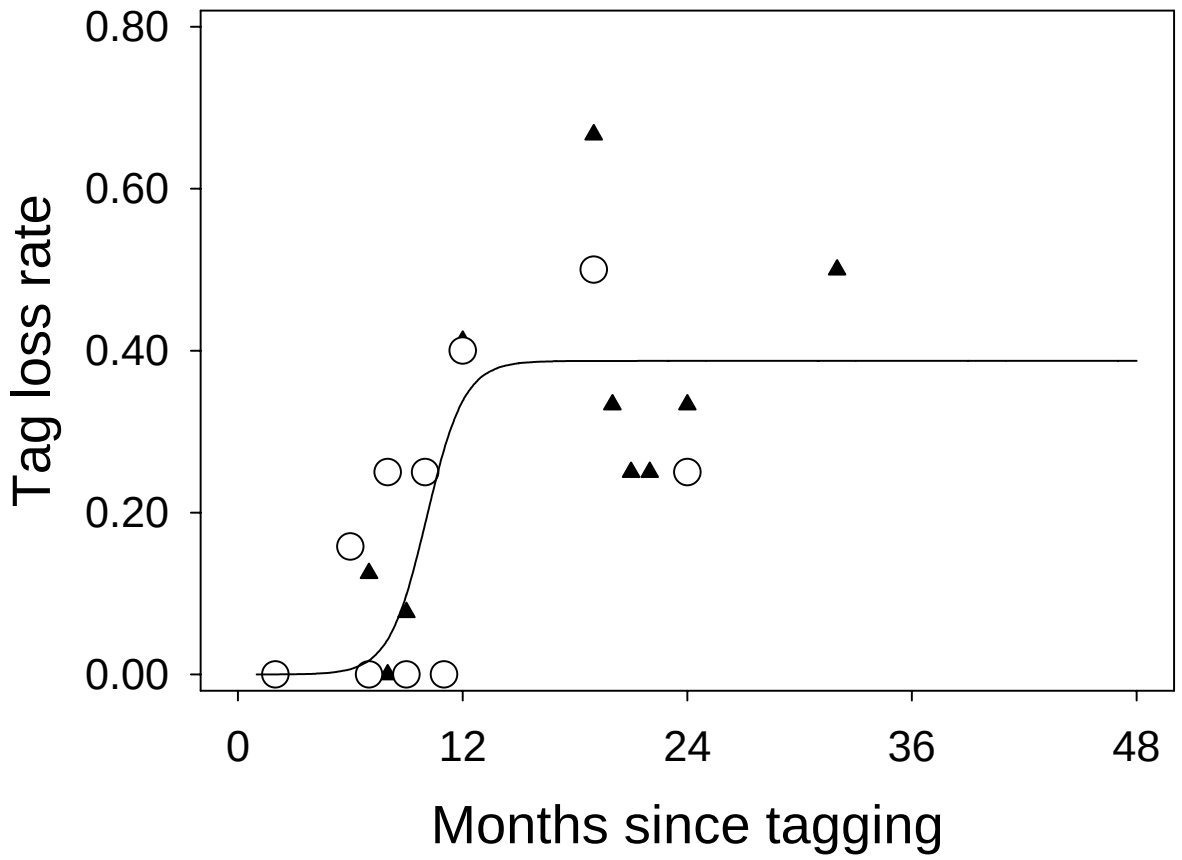

Figure 1.3. Predicted rate of long-term tag-shedding based on the sigmoidal function that was fit to the double tagged and tagged-clipped datasets. (Prediction = solid line; ± 1 SE = dashed lines)

PART 2 – NUTRITIONAL ANALYSIS

Methods

Gross composition analysis

Whitefish samples from the Big Bay de Noc, Cheboygan, Detour, and Naubinway stocks were collected seasonally from fall 2003 to summer 2006 for gross composition analysis. We were unable to collect samples from the Naubinway stock during winter 2004, nor were we able to collect samples from the Cheboygan stock during fall 2005. Our target was to collect 40 fish per stock on each sampling occasion. We also sought to collect approximately equal numbers of male and female fish during each sampling occasion.

Gross compositional analyses of whitefish whole-carcasses water and total lipid content were conducted at Michigan State University. Each carcass was homogenized using a tabletop grinder after which individual fish homogenates were pooled into composites of up to 5 fish of the same sex to yield 4 replicate pooled samples for each fish. 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 estimated using the Soxtec solvent extraction method.

Fatty acid analysis

Fish for fatty acid analyses were collected mainly by trap net (live fish). The tissue biopsies were quickly collected and cryogenically-frozen (dry ice) and then shipped on dry ice to CCIW (Arts laboratory) whereupon they remained under cryogenic conditions (-85ºC) until analyses. Samples were collected for three full years starting with the fall of 2003 and ending with summer 2006 (Tables 2.2). At the onset of the study we collected four different types of tissues for fatty acid analyses (muscle, liver, retina, gill). However, after the first summer, it was decided to drop the gill tissues mainly because of the dissection component of the gill tissues. The gills are composed of soft epithelial tissue laid down over bony/cartilaginous rakers. Once this material was freeze-dried it proved to be exceedingly difficult to achieve consistency in the make-up of the ground materials. Some technologists were able to scrap off mostly skin whereas in other cases (and on other days) there was some bony material in the mix. This resulted in problems in that the gravimetric results (% total lipid) as well as the absolute FA results (expressed on a per mg DW basis) were affected by the relative amounts of bony tissue in individual samples. It was also decided at this time not to pool muscle samples from groups of five fish to create composite samples but instead to use the time gained (by not analyzing the gill samples) to increase the number of individual fish assayed for fish muscle fatty acids.

Results

Abbreviations used in the summary below
ALA = α-linoleic acid (18:3n-3)
LIN = linolenic acid (18:2n-6)
EPA = eicosapentaenoic acid (20:5n-3)
ARA = arachidonic acid (20:4n-6)
DHA = docosahexaenoic acid (22:6n-3)
FAME = fatty acid methyl esters

Gross compositional analysis

Although we sought to collect 40 per stock during each sampling occasion, we were unable to meet this goal for all stocks and seasons. The average number of fish sampled from each stock on each sampling occasion was 38, 40, 39, and 40 for Big Bay de Noc, Naubinway, Cheboygan, and Detour, respectively. Overall, 47% of the fish we collected during the reporting period were female, although the sex ratio varied from 35-58% females among individual sampling occasions, primarily due to limited availability of fish on a few occasions.

Whole fish water content exhibited a strong inverse correlation with whole fish lipid levels. However, the slope and intercept of the relationship differed among lakes (analysis of covariance (ANCOVA), percent water × stock interaction $P < 0.001$; Figure 2.1). Because of the significant interaction in the ANCOVA, regression models that related whole fish water content to whole fish lipid levels were fit for each lake separately. For Lake Huron fish (Cheboygan and Detour stocks), the model explained 77% of the total variation, while the model for Lake Michigan fish (Big Bay de Noc and Naubinway stocks) explained 67% of the total variation. The relationship between water content and lipids for Lake Huron fish had a significantly larger intercept ($\hat{\beta}_0 = 196.1 \pm 4.9$) and steeper slope ($\hat{\beta}_1 = -2.4 \pm 0.06$) compared to Lake Michigan stocks ($\hat{\beta}_0 = 173.4 \pm 4.6$; $\hat{\beta}_1 = -2.1 \pm 0.06$). However, the relationship between percent lipids and water did not differ between stocks located in the same lake ($P > 0.20$). Percent total lipids were often lowest and percent water highest during the winter; however this was dependent on the stock and year of sampling (Table 2.1; refer to Part 4 (Integrated Data Analysis) and Appendix 4.1 for models examining seasonal and sex differences in whole fish water content and whole fish percent lipid levels).

Fatty acid analysis

Fatty acids in dorsal muscle tissue
(μg FAME · mg DW^{-1} and/or μg FAME · mg polar $lipid^{-1}$)

Muscle tissue represents the largest tissue mass of any tissue in the fish's body. Fatty fish such as lake whitefish contain significant amounts of lipid in their muscle tissues. The fats in the muscle tissues are partitioned into various lipid fractions or pools, the two most dominant of these being the triacylglycerol (energy reserves) and the polar lipid fractions. The triacylglycerol fraction is known to be quite responsive (plastic) in that it responds relatively quickly to short-term changes to the supply of fatty acids in the diet. The polar lipid fraction is mainly comprised of phospholipids; the main structurally and physiologically important constituents of cell membranes. In contrast to the triacylglycerol fraction, polar lipid (phospholipid) fractions are expected to reflect longer term adaptations to more sustained changes in the fatty acids supplied by a fish's diet. We chose to investigate the fatty acid profiles of the polar lipid fraction because we were interested to see if there had been any long-term systemic changes to the diet of specific stocks or to the diets of lake whitefish in general from the samples locations in Lakes Huron in Michigan.

Essential omega-3 fatty acids

ALA (Figures 2.2 & 2.3) - Although there was some variation from year to year and from stock to stock, in general, the data suggested that concentrations of this fatty acid generally began to increase in the polar lipid fraction in late summer and peak in the fall. Concentrations of ALA then declined in the winter, reaching new lows by the spring-summer period of each following year. ALA is abundant in green algae and crosses the plant-animal interface when algae are

grazed on by zooplankton and other herbivores. The high levels of ALA in lake whitefish dorsal muscle tissue in the fall suggested that lake whitefish were either eating zooplankton directly or were eating organisms that had eaten zooplankton at that time. There did not appear to be a consistent pattern of one stock having higher levels of ALA than any other stock with the possible exception of apparently higher concentrations in Big Bay de Noc fish in the second and third year of the study.

EPA (Figures 2.4 & 2.5) - As with ALA concentrations, levels of EPA in the polar lipid fraction generally peaked in the fall within each of the three fall-to-summer cycles. No one stock appeared to have consistently higher levels of EPA than the other stocks. Over the three-year study period there appeared to have been an overall decline in EPA concentrations in dorsal muscle tissue of lake whitefish.

DHA (Figures 2.6 & 2.7) - As with EPA concentrations, levels of DHA in the polar lipid fraction generally peaked in the fall within each of the three fall-to-summer cycles, although this was much less evident when the fatty acid concentration data is presented on a per mg polar lipid basis. There was a marked decline in the concentrations of this long chain omega-3 fatty acid over the three-year study period. This may have important implications, from a biochemical perspective, for the health/condition of lake whitefish since DHA is known to positively enhance/participate in, a wide range of physiologically important processes including cold tolerance (Arts and Kohler 2008), vision (Bell et al.1995; and see below) and schooling behavior (Masuda et al. 1998).

Essential omega-6 fatty acids

LIN (Figures 2.8 & 2.9) - Concentrations of LIN in the polar lipid fraction of lake whitefish dorsal muscle tissue were generally highest in the winter-spring period and lowest in the summer. Mussels, including exotic invaders such as zebra and quagga mussels, have high levels of omega-6 fatty acids including LIN and especially ARA. Thus, it is interesting to note that the highest levels of this fatty acid recorded during this study came from the Cheyboygan and Naubinway stocks and the lowest levels were generally associated with fish from the Big Bay de Noc stock.

ARA (Figures 2.10 & 2.11) - As with LIN, fish from the Cheboygan and Naubinway stocks had the highest concentrations of ARA in the polar lipid fraction of dorsal muscle tissue, while fish from the Big Bay de Noc stock and, occasionally, the Detour stock had the lowest concentrations. There appeared to be a declining trend in the concentrations of ARA over the course of the study (although not as extreme as with the long chain omega-3 fatty acids EPA and DHA).

Unsaturation Index (Figure 2.12)

Individual FA were summed based on their degree of saturation, including the sum of the; saturated fatty acids (ΣSAFA), monounsaturated fatty acids (ΣMUFA) and polyunsaturated fatty acids (ΣPUFA). An unsaturation index (UI) was calculated using the formula:

$$UI = \Sigma_{i\text{-}j}(\text{proportion of fatty acid}_i \cdot \text{number of double bonds of fatty acid}_i)$$

Although the UI has not yet been definitively associated with fish health and growth, it does provide a weighted metric of unsaturated FA, particularly the polyunsaturated fatty acids (PUFA) which are known to be associated with growth and health of fish (Balfry and Higgs

2001, Bogut et al., 2002; Arts and Kohler 2008). The UI metric clearly indicated an overall decline in the degree of unsaturation of fatty acids in the polar lipid fraction. There was roughly a 13% decline (50/375 * 100) in the UI during the course of the study. This could have important implications for the health of lake whitefish as the degree of unsaturation of membrane lipids (phospholipids) has long been associated with increased membrane "fluidity" (decreased order); a vital adaptive response to cold temperature challenge (Arts and Kohler 2008).

Fatty acids in retinal tissue
(µg FAME · mg polar lipid)

Note: data from fall 2003 (Year 1) were not included because it was suspected that poor dissection technique resulted in too low a yield of retinal tissue (inclusion of too much surrounding tissue in the biopsy). Furthermore, analyses is restricted to data presented on a µg FAME · mg polar lipid basis to help control for any potential differences between technicians with regard to their efficiency at removing relatively pure samples of retinal tissue. This is because long chain omega-3 fatty acids are known to be especially concentrated in the phospholipid fraction of retina (usually about 10X higher than in surrounding tissues). Thus, by focusing on the polar lipid fraction we more accurately homed in on the main differences in physiologically important retinal FA concentrations amongst stocks or seasons.

Essential omega-3 fatty acids

ALA (Figure 2.13) - This fatty acid is the precursor for EPA. Concentrations of ALA in the retina appeared to follow a cyclical pattern over the study period, with high points in different seasons in different years suggesting that there is differential access to foods rich in EPA. The value for Naubinway for spring of Year 2 is probably an error since, a) it is exceptionally high and, b) females do not demonstrate the same pattern. Ignoring this value one can observe that during the study the majority of the highest retinal polar lipid ALA concentrations were from fish from Big Bay de Noc suggesting that fish from this stock had the best access to easily digestible and available prey containing significant concentrations of ALA (e.g. *Diporeia* and/or mysids)

EPA (Figure 2.14) – This fatty acid is the precursor for DHA. Concentrations of EPA in the retina appear to follow cyclical patterns over the study period with high points in different seasons in different years suggesting that there is differential access to foods rich in EPA. During the first 2 years of the study the highest retinal polar lipid EPA concentrations were from fish from Big Bay de Noc. As with muscle EPA, retinal EPA concentrations appear to be in general decline over the study period.

DHA (Figure 2.15) - This fatty acid has been shown to be important in visual acuity in vertebrates (SanGiovanni and Chew 2005). Furthermore, dietary DHA supply can affect DHA levels in the cones of the retina (Birch et al. 1992). Shortages in dietary DHA supply can affect vision of fish, especially at low light levels (Bell et al. 1995). DHA levels in lake whitefish change over time confirming that, as in other species, dietary supply likely affects retinal DHA concentrations. The highest DHA levels in the polar lipid fraction of retina where observed in the first year of the study (i.e. spring 2004). As with muscle tissue there is a suggestion of overall decreasing concentrations of DHA with time in all of the stocks surveyed. It is not possible without performing carefully controlled laboratory experiments, to determine which effects, if

any, such observed declines might have on visual acuity of lake whitefish particularly at low light levels.

Essential omega-6 fatty acids

LIN (Figure 2.16) - This fatty acid is the precursor for ARA. LIN concentrations were highly variable, both among stocks and seasons. The highest levels of LIN usually were observed in fish from Naubinway and/or Cheboygan and the lowest levels were typically associated with fish from Big Bay de Noc and Naubinway.

ARA (Figure 2.17) – Concentrations of this fatty acid in the polar lipid fraction of retina were cyclical with the highest values recorded (for all stocks) during the first spring period sampled. Peaks and troughs in retinal ARA concentrations followed the pattern seen for EPA. This fatty acid occurs in high concentration in mussels suggesting that the observed peaks (perhaps with some time lag) may be indicated periods of high consumption of zebra and/or quagga mussels by lake whitefish.

Fatty acids in liver tissue
($\mu g\ FAME \cdot mg\ DW^{-1}$)

Fatty acid profiles present in liver tissue represent a more recent dietary influence than fatty acid profiles from muscle tissue and therefore give some indication therefore of recent feeding success on specific food items. We chose to analyze fatty acids in the total lipid pool from liver. This differed from muscle tissue and retinal tissue where we intentionally focused on fatty acids in the polar lipid pool. We did this because we wanted to include the energy reserve lipids (triacylglycerols) in the fatty acid profile analyses. Triacylglycerols are a ubiquitous class of lipids that are commonly acquired in the diet. Thus, by including this lipid class in the analyses we hoped to gain insights on the recent feeding choices of lake whitefish.

Essential omega-3 fatty acids

ALA (Figure 2.18) – Concentrations of this 18 carbon omega-3 fatty acid were generally highest for fish from the Big Bay de Noc stock and lowest for fish from the Naubinway stock; both for males and females. Typically, lake whitefish collected in winter or spring had the lowest concentrations of this essential fatty acid suggesting reduced feeding on easily captured, readily digestible, prey enriched with ALA (e.g. *Diporeia*, *Mysis*) during that time of year.

EPA (Figure 2.19) – No particular stock emerged as having consistently higher or lower levels of this fatty acid however there was a trend (females) for an overall decline in EPA liver concentrations with time suggesting a more systemic degradation in the quality of the diet with respect to this essential fatty acid. *Diporeia* and *Mysis* are known to rich in EPA.

DHA (Figure 2.20) – As with EPA, no particular stock emerged as having consistently higher or lower levels of DHA however there was a trend (females) for an overall decline in DHA liver concentrations with time suggesting a more systemic degradation in the quality of the diet with respect to this essential fatty acid. *Diporeia* and *Mysis* are known to especially rich in DHA.

Essential omega-6 fatty acids

LIN (Figure 2.21) – Liver concentrations of LIN were lowest in spring for all stocks in the first two years of the study. There was no consistent temporal decline in this fatty acid in any of the stocks nor did any one stock emerge as having consistently higher or lower levels of this fatty acid suggesting that fish from the 4 stocks all consumed prey containing LIN in roughly the same quantity when average out over time.

ARA (Figure 2.22) – No one season emerged as having either the highest or lowest levels of ARA. As with LIN, concentrations of ARA did not demonstrate a consistent temporal decline in any of the stocks nor did any one stock emerge as having consistently higher or lower levels of this fatty acid suggesting that fish from the 4 stocks all consumed prey containing ARA in roughly the same quantity when averaged out over time.

Table 2.1. Mean concentrations of whole-fish total percent lipids and water of lake whitefish from four stocks sampled in Lakes Michigan and Huron in 2003 – 2005. Means are followed by standard errors in parentheses. 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. Sample year one was from fall 2003 – summer 2004, sample year two was from fall 2004 – summer 2005, and sample year three was from fall 2005 – summer 2006. NS = not sampled.

	Year 1				Year 2				Year 3			
	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer
Big Bay de Noc												
Lipids	20.2	14.9	23.1	22.9	21.3	18.8	21.5	16.4	23.2	18.2	15.6	16.2
	(0.94)	(0.90)	(1.4)	(0.73)	(0.80)	(1.4)	(1.1)	(1.0)	(0.77)	(0.91)	(1.1)	(0.82)
Water	73.1	75.5	72.8	73.0	72.0	74.9	71.9	74.2	72.1	75.9	74.3	73.9
	(0.32)	(0.35)	(0.49)	(0.36)	(0.29)	(0.40)	(0.43)	(0.36)	(0.29)	(0.33)	(0.42)	(0.37)
Naubinway												
Lipids	17.1	NS	21.7	19.7	18.0	18.5	20.3	20.3	18.4	18.5	19.3	18.2
	(0.69)		(0.97)	(0.67)	(0.68)	(0.97)	(0.82)	(0.53)	(0.69)	(0.97)	(0.84)	(1.2)
Water	74.2	NS	72.8	73.3	73.7	75.1	72.6	73.1	73.3	75.5	74.5	74.8
	(0.23)		(0.37)	(0.27)	(0.30)	(0.36)	(0.29)	(0.22)	(0.24)	(0.30)	(0.29)	(0.46)
Cheboygan												
Lipids	15.7	21.2	17.7	18.1	14.8	14.0	16.3	14.3	NS	15.0	20.3	11.9
	(0.80)	(1.3)	(1.3)	(1.2)	(0.86)	(1.1)	(0.97)	(1.0)		(1.0)	(0.92)	(1.1)
Water	75.3	75.0	74.9	74.5	75.5	76.9	75.1	75.6	NS	76.4	74.5	77.3
	(0.34)	(0.48)	(0.40)	(0.47)	(0.32)	(0.31)	(0.38)	(0.40)		(0.39)	(0.30)	(0.43)
Detour												
Lipids	16.9	13.7	22.9	18.8	15.5	13.7	17.4	15.7	22.2	17.4	20.8	10.1
	(0.93)	(1.1)	(0.93)	(0.95)	(0.89)	(1.1)	(1.2)	(1.2)	(0.89)	(1.1)	(1.0)	(0.67)
Water	75.3	75.4	73.1	74.9	75.0	76.1	74.0	75.7	72.4	74.9	73.2	76.5
	(0.29)	(0.38)	(0.30)	(0.38)	(0.32)	(0.45)	(0.64)	(0.41)	(0.34)	(0.39)	(0.45)	(0.33)

Table 2.2. Number of lake whitefish analyzed for total lipids (gravimetric analysis) and individual fatty acids (gas chromatography analysis) from 2003 – 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. Sample year one was from fall 2003 – summer 2004, sample year two was from fall 2004 – summer 2005, and sample year three was from fall 2005 – summer 2006.

	Year 1				Year 2				Year 3			
Tissue	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer	Fall	Winter	Spring	Summer
Muscle	67	50	70	67	120	104	118	120	90	117	120	118
Liver	27	25	30	27	120	104	118	120	90	117	120	118
Retina (eye)	27	25	30	27	120	104	118	120	90	117	120	118
Gill	27	25	30	27	NA	NA	NA	NA	NA	NA	NA	NA

NA = No data available. The project stopped analyzing gill tissue (see text for explanation).

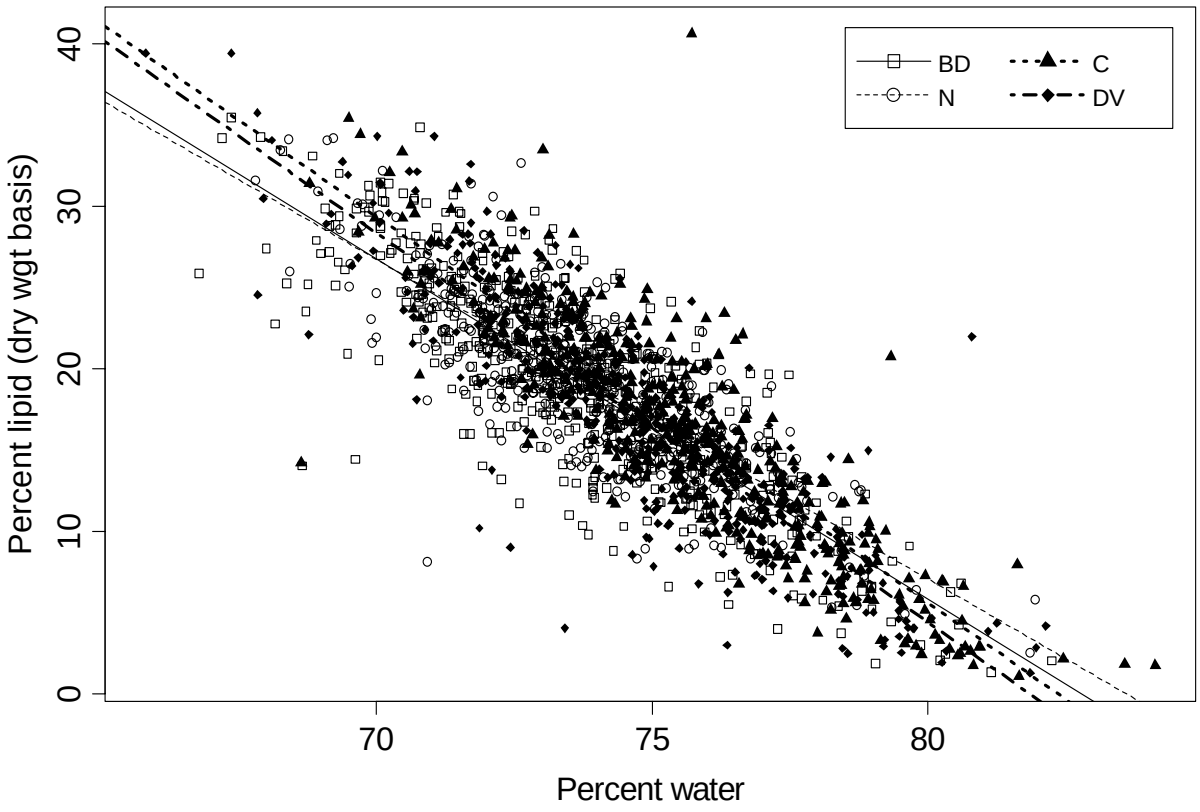

Figure 2.1. Relationship between whole fish water and lipid (dry weight basis) content for lake whitefish from four stocks in Lakes Michigan and Huron during 2003-2006. Lake Michigan stocks are Big Bay de Noc (BD) and Naubinway (N). Lake Huron stocks are Cheboygan (C) and Detour (DV). Lines represent linear regression model fits for each stock.

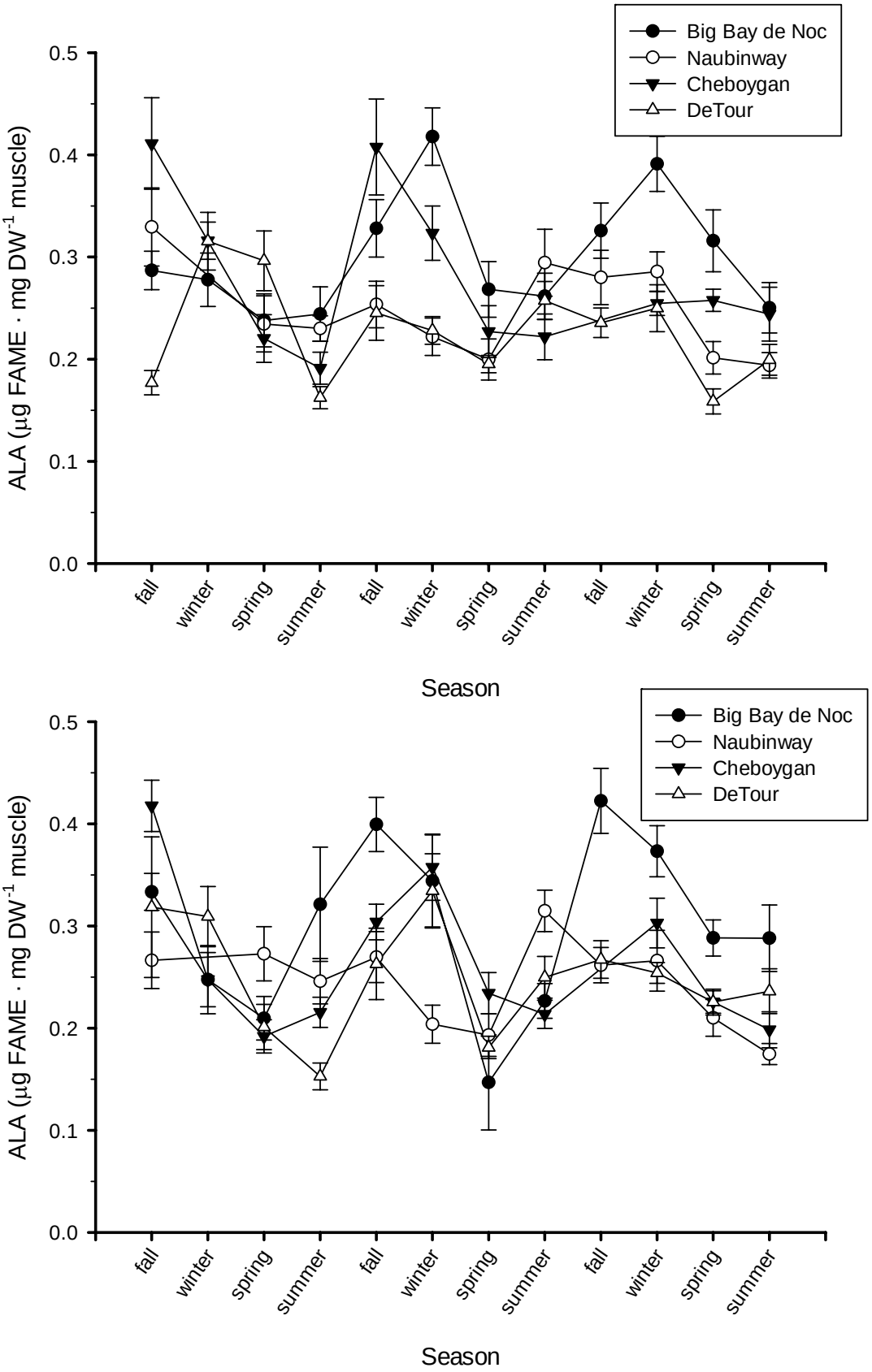

Figure 2.2. ALA in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg^{-1} DW basis. Top panel = male fish, bottom pane = female fish.

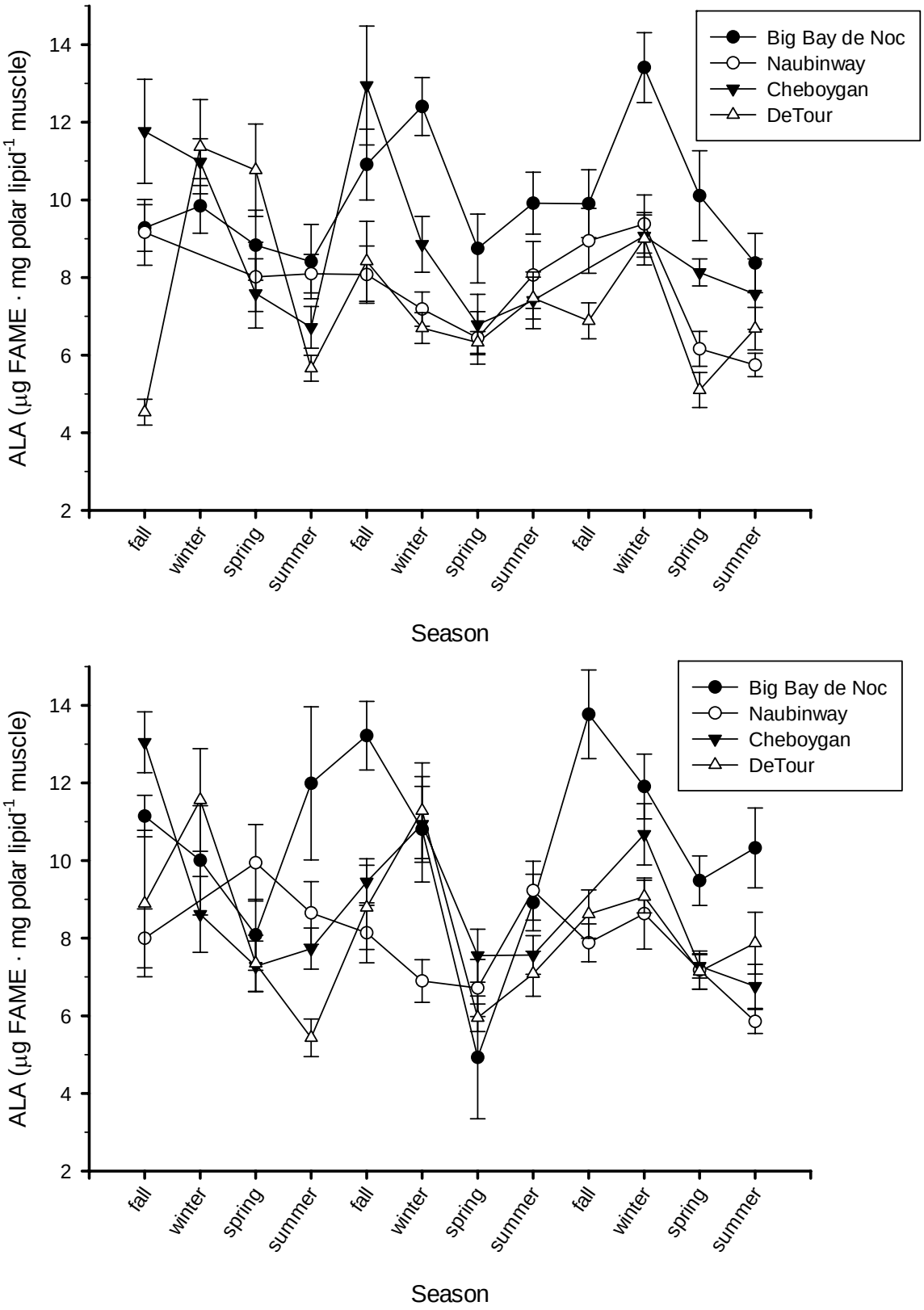

Figure 2.3. ALA in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

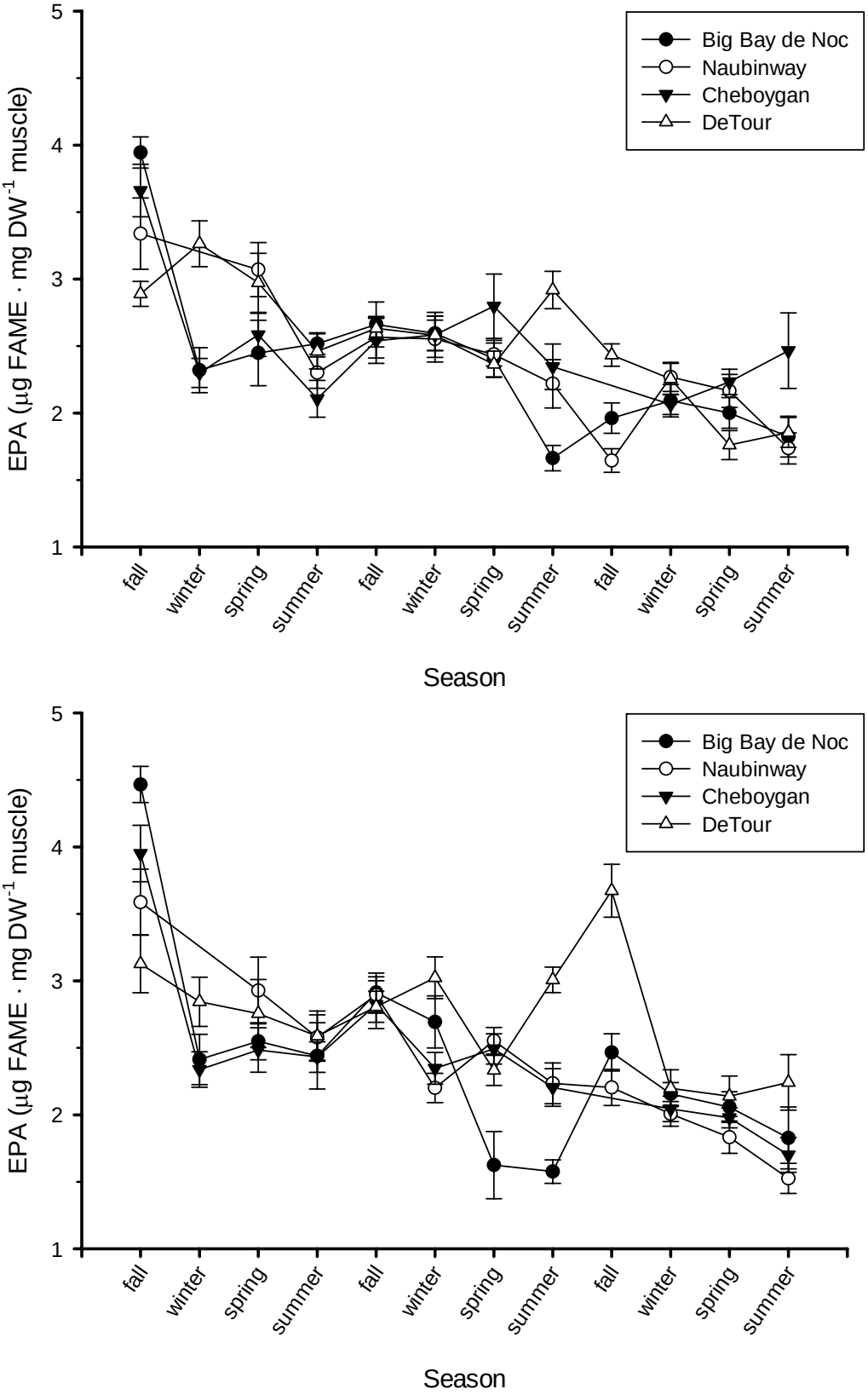

Figure 2.4. EPA in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a μg FAME · mg^{-1} DW basis. Top panel = male fish, bottom pane = female fish.

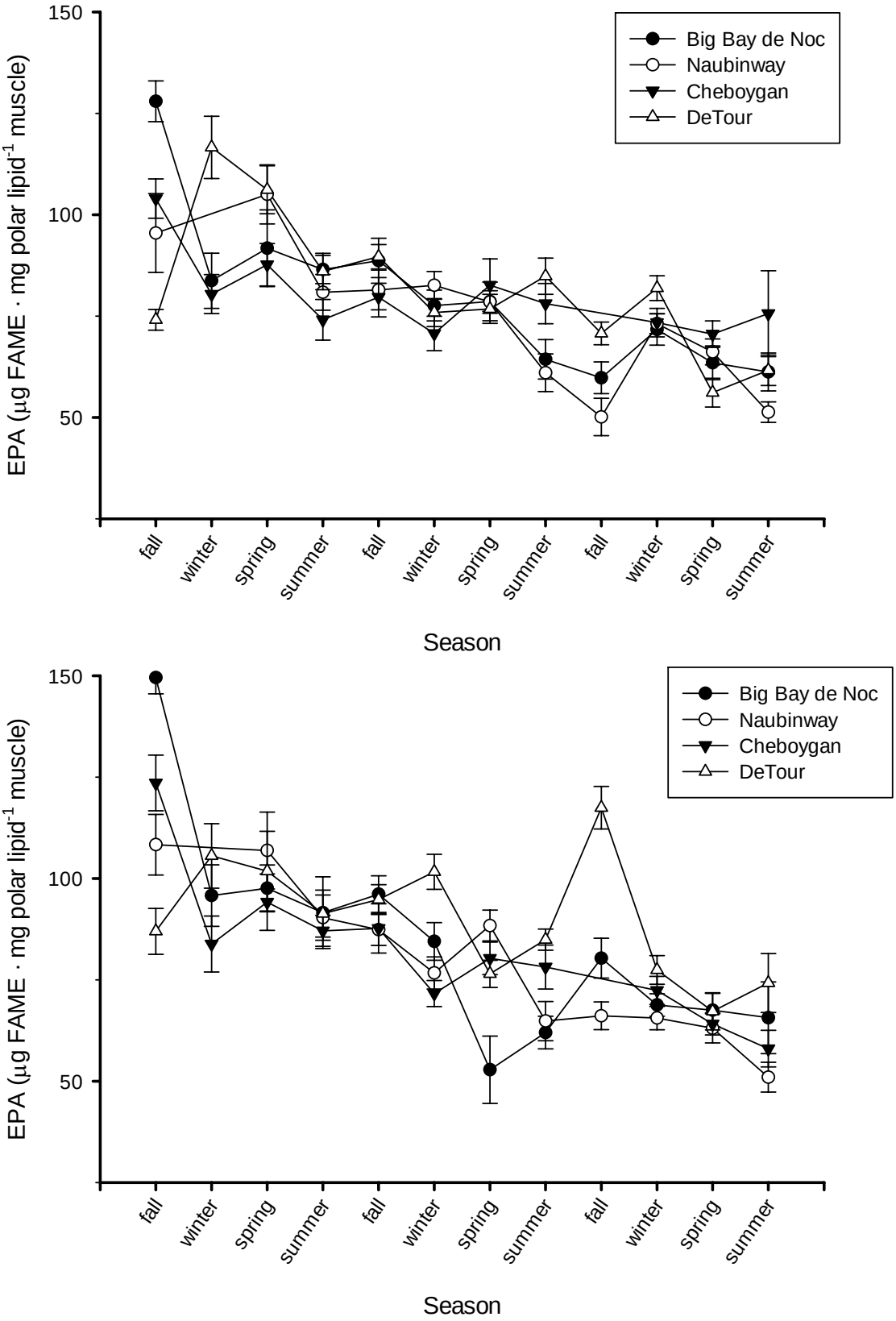

Figure 2.5. EPA in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

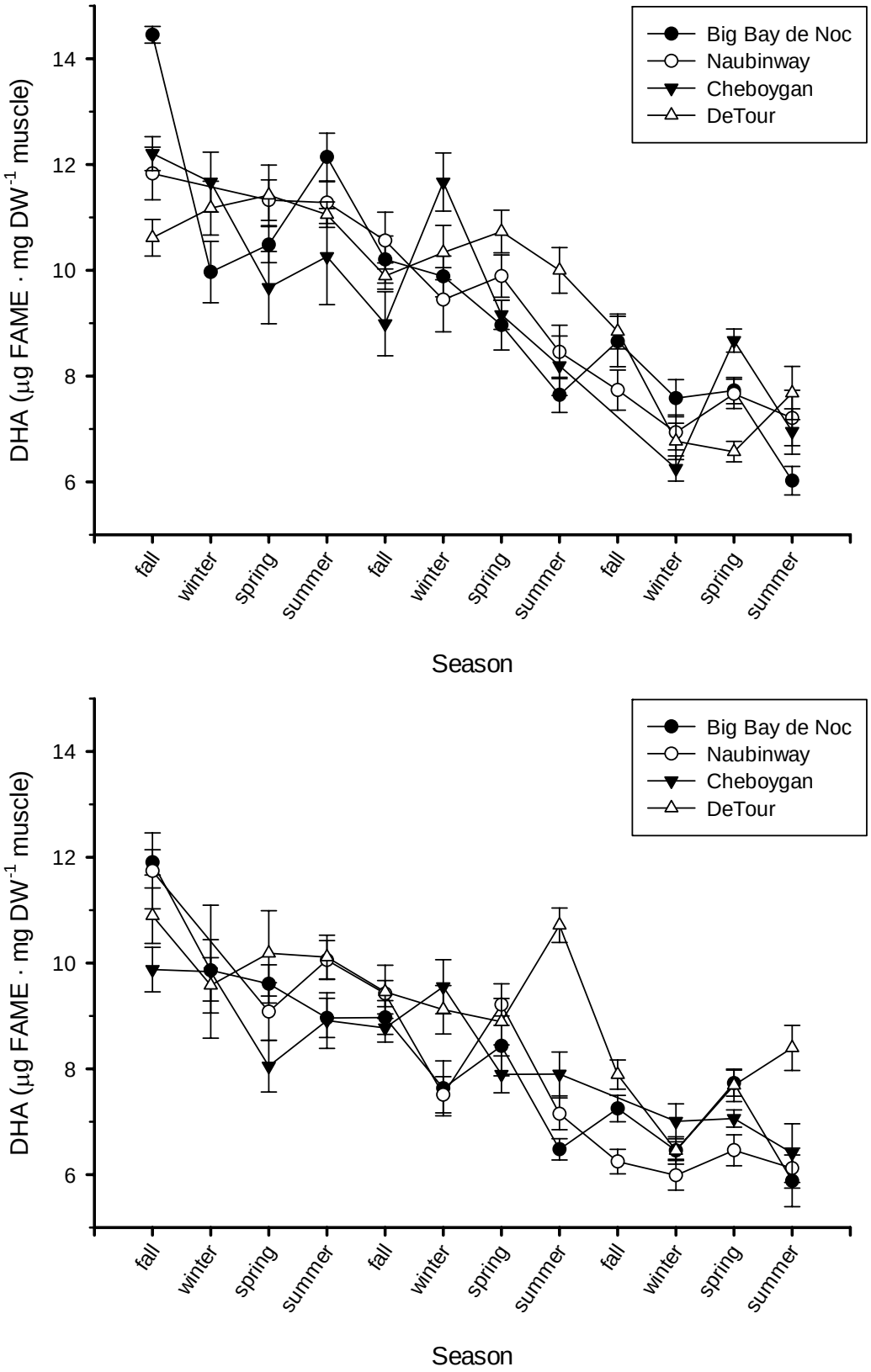

Figure 2.6. DHA in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg^{-1} DW basis. Top panel = male fish, bottom pane = female fish.

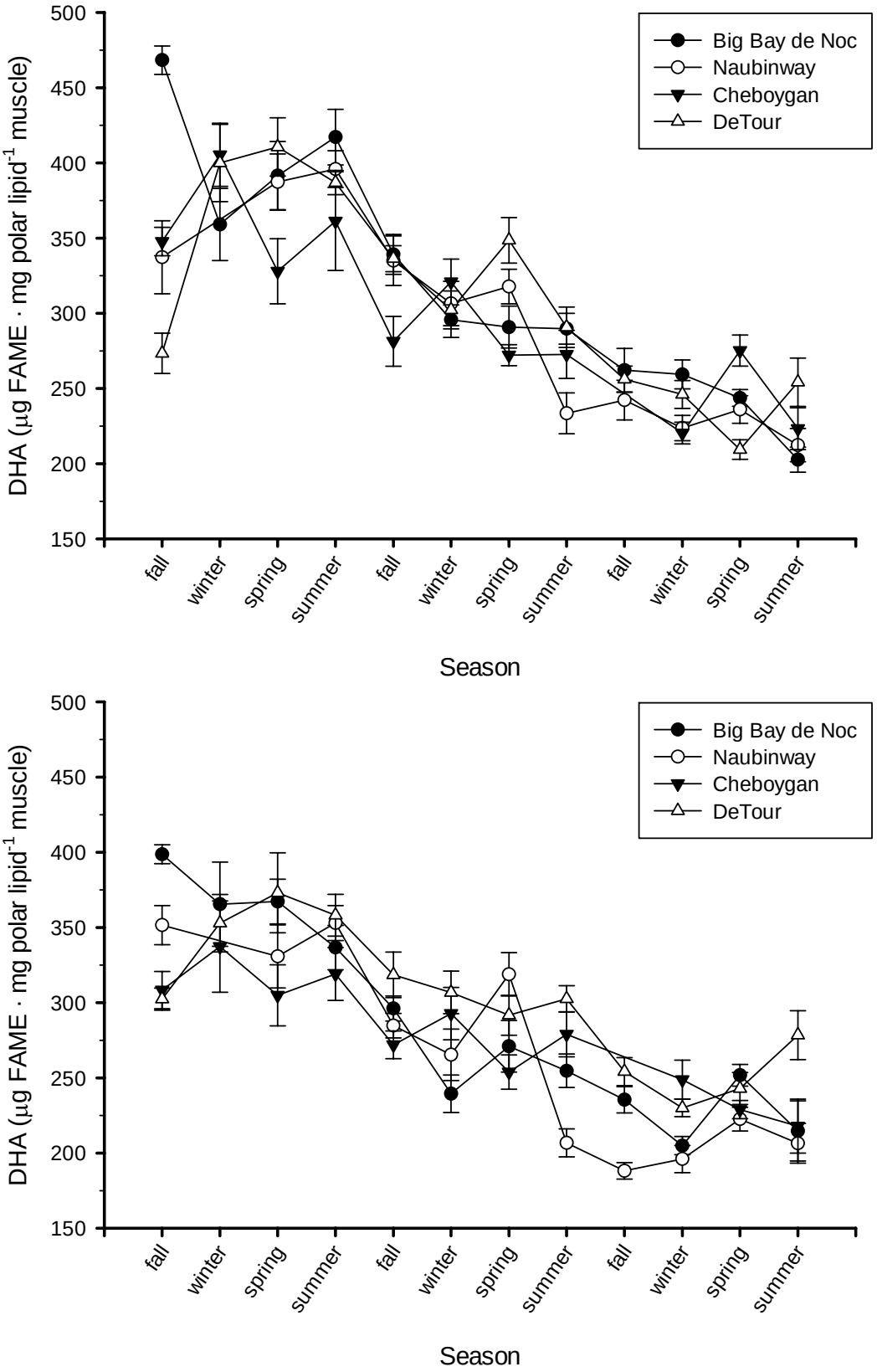

Figure 2.7. DHA in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

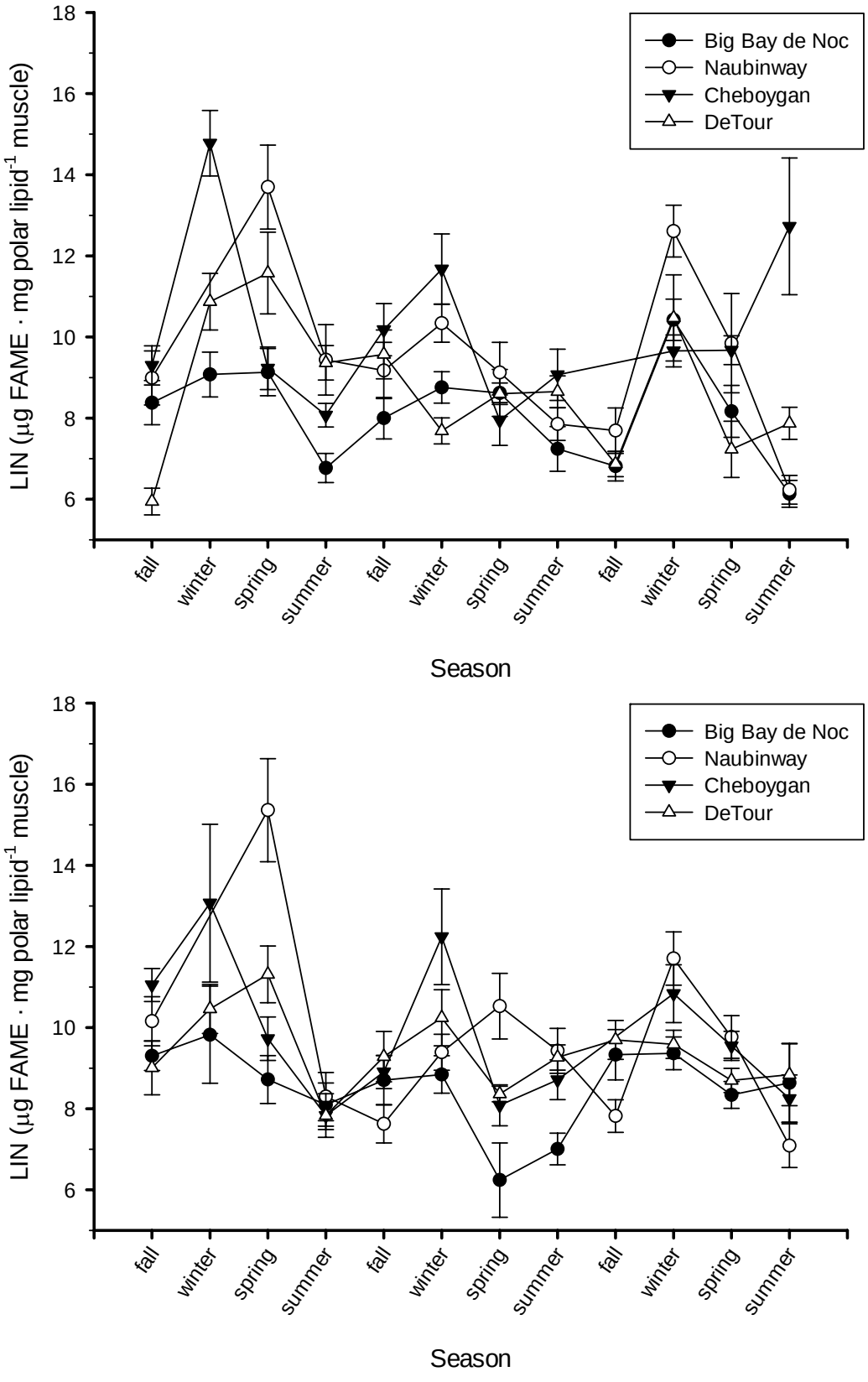

Figure 2.8. LIN in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg^{-1} DW basis. Top panel = male fish, bottom pane = female fish.

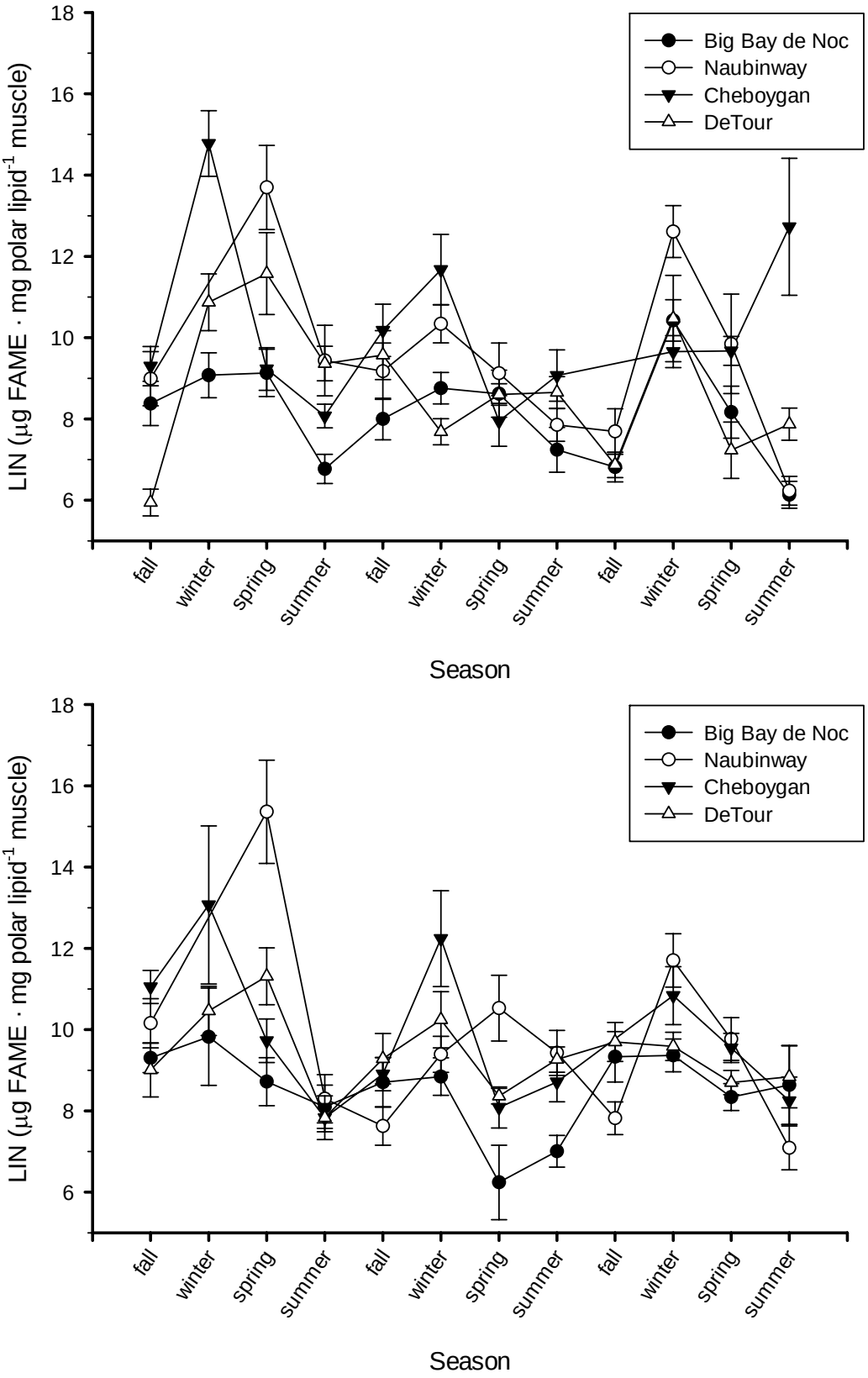

Figure 2.9. LIN in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis.

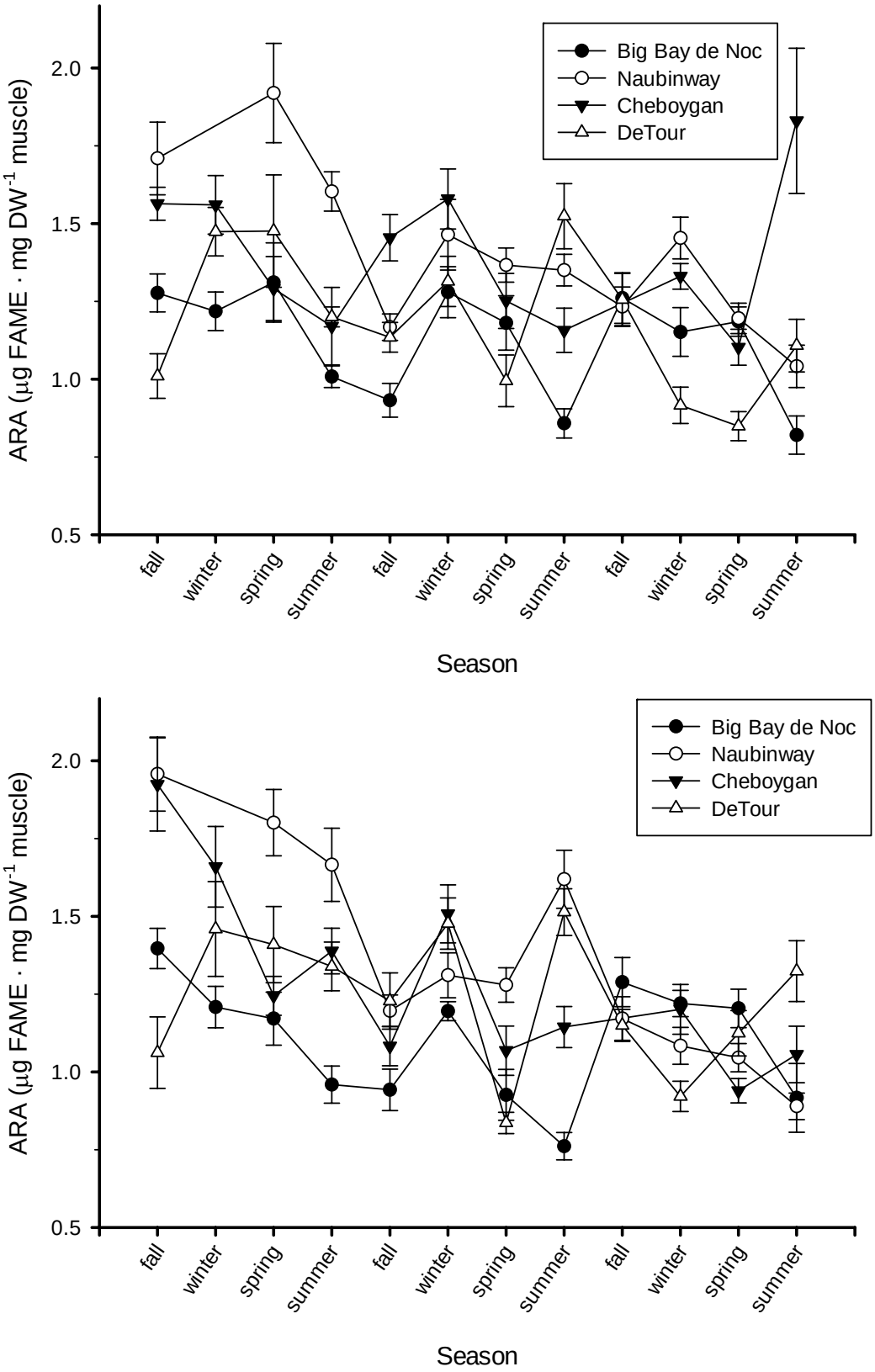

Figure 2.10. ARA in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg^{-1} DW basis. Top panel = male fish, bottom pane = female fish.

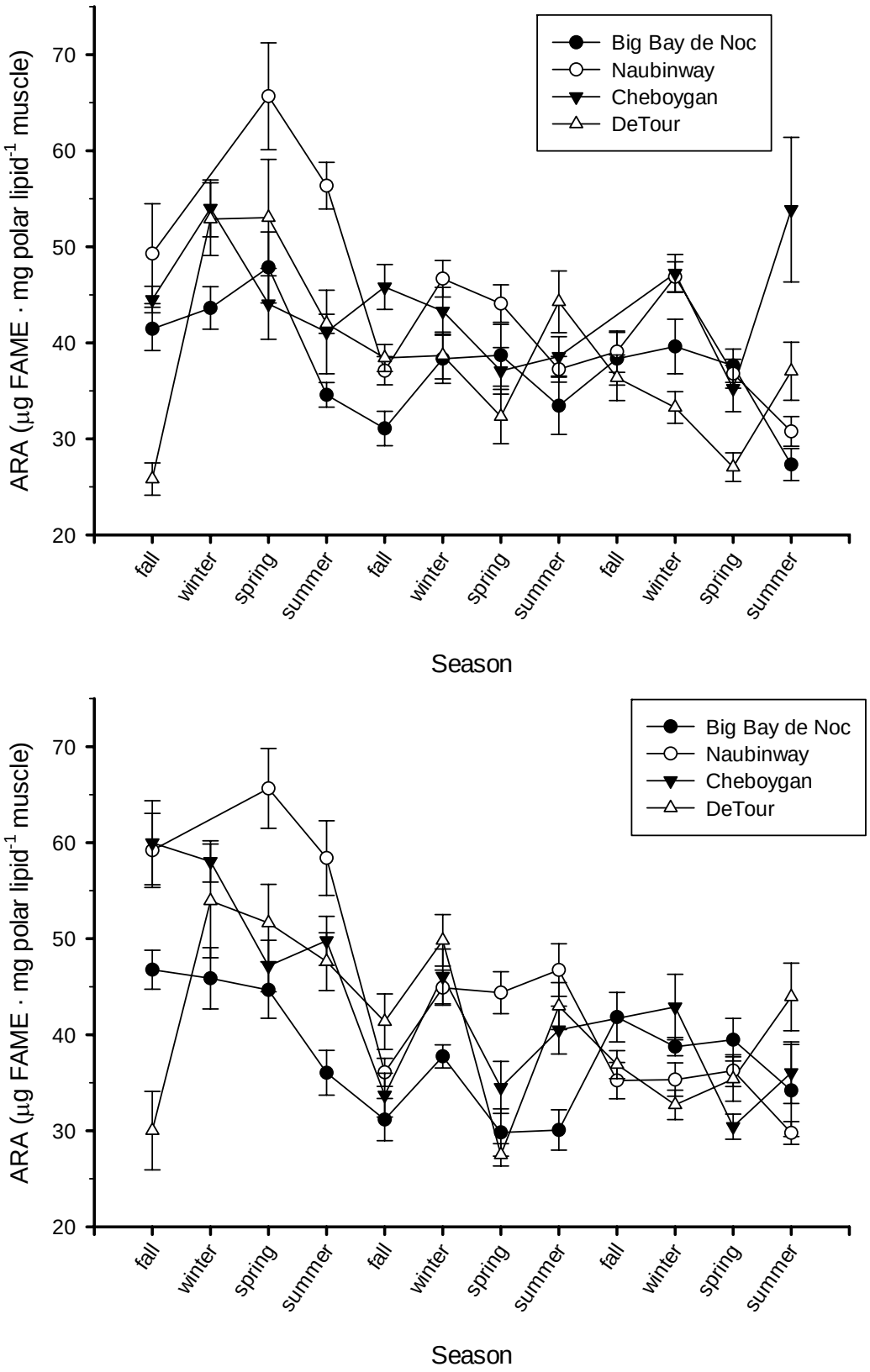

Figure 2.11. ARA in the polar lipid fraction of dorsal muscle tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

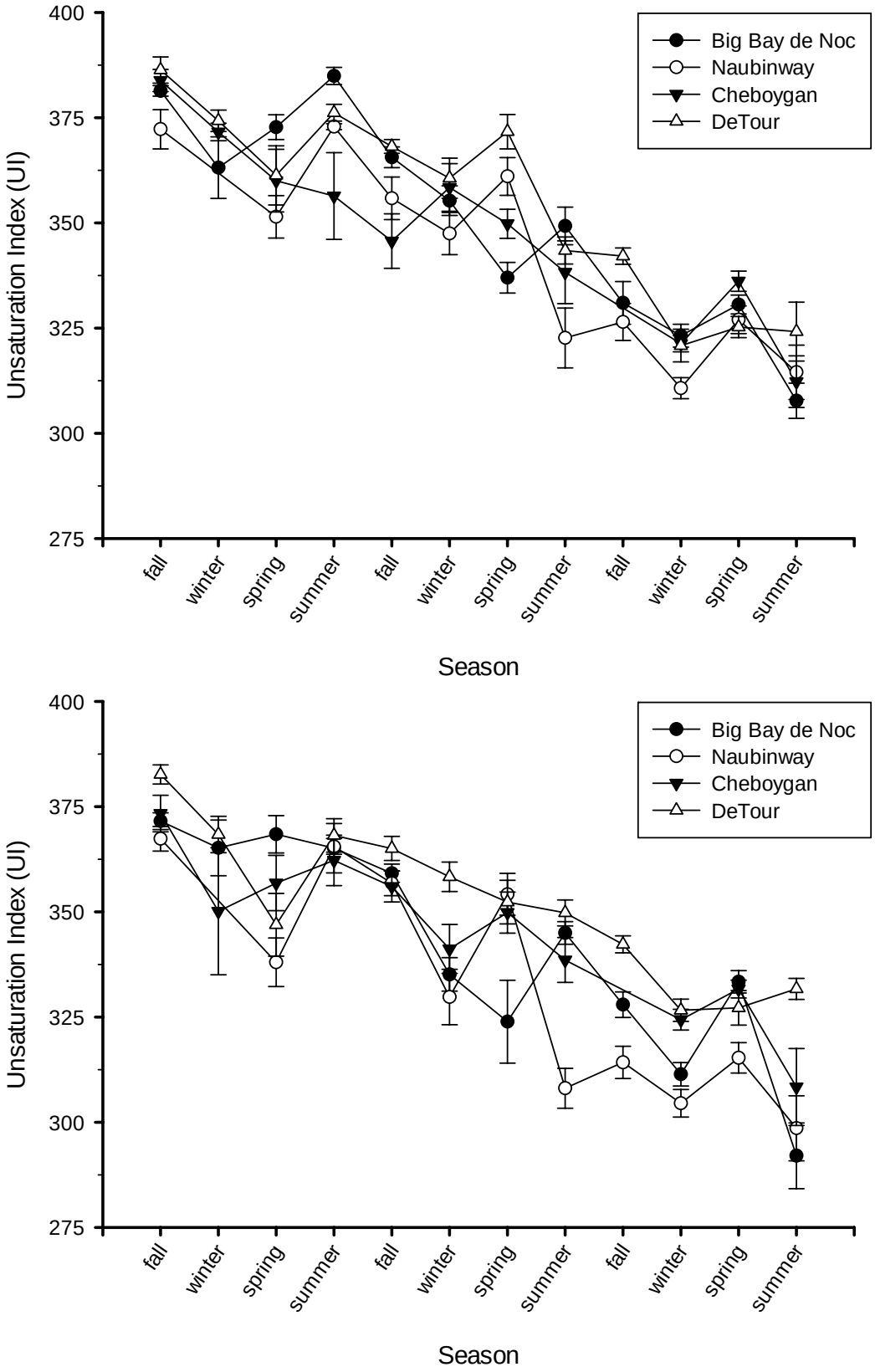

Figure 2.12. Unsaturation Index (UI) in the polar lipid fraction of dorsal muscle tissue of lake whitefish (see text for details). Top panel = male fish, bottom pane = female fish.

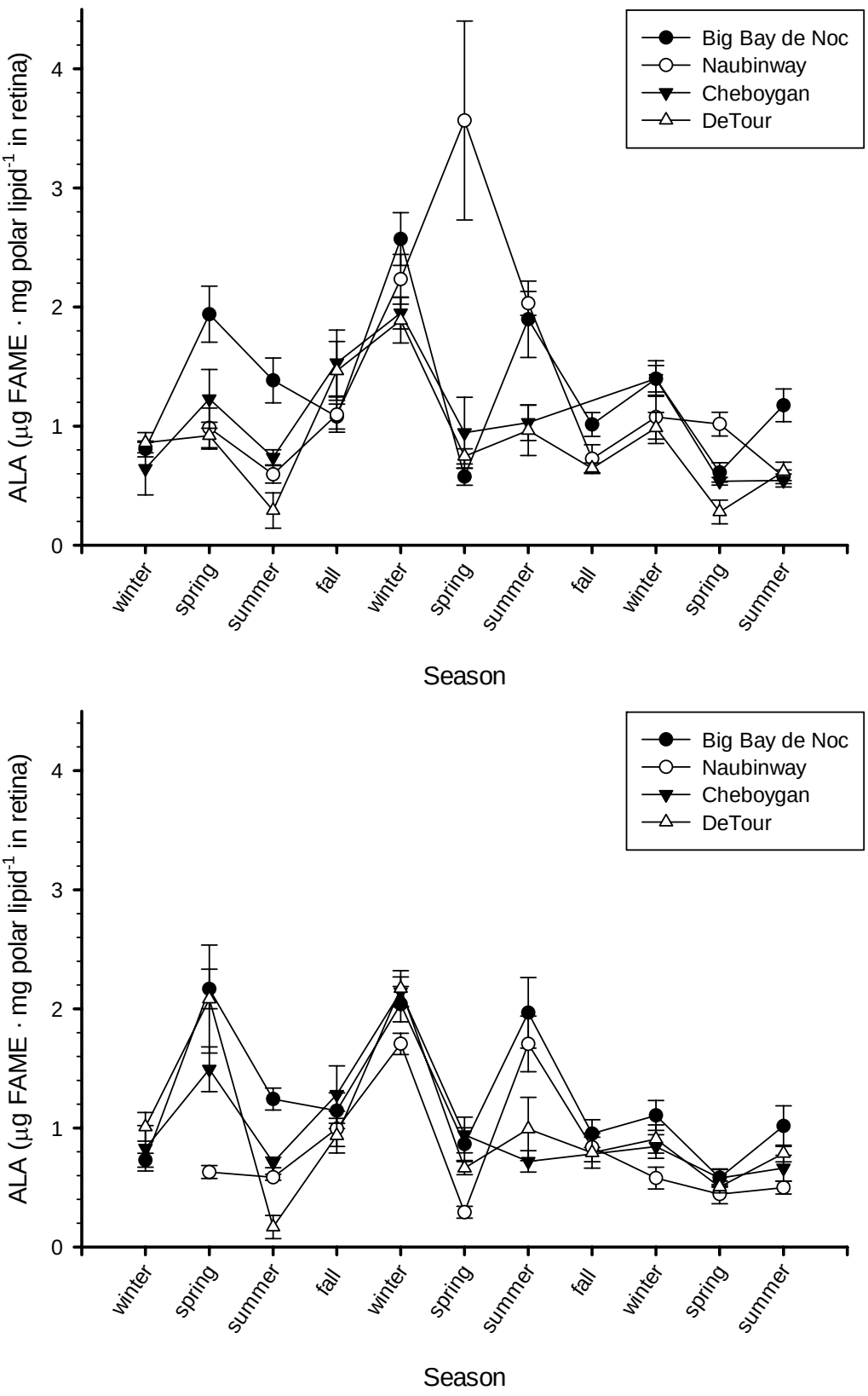

Figure 2.13. ALA in the polar lipid fraction of retinal tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

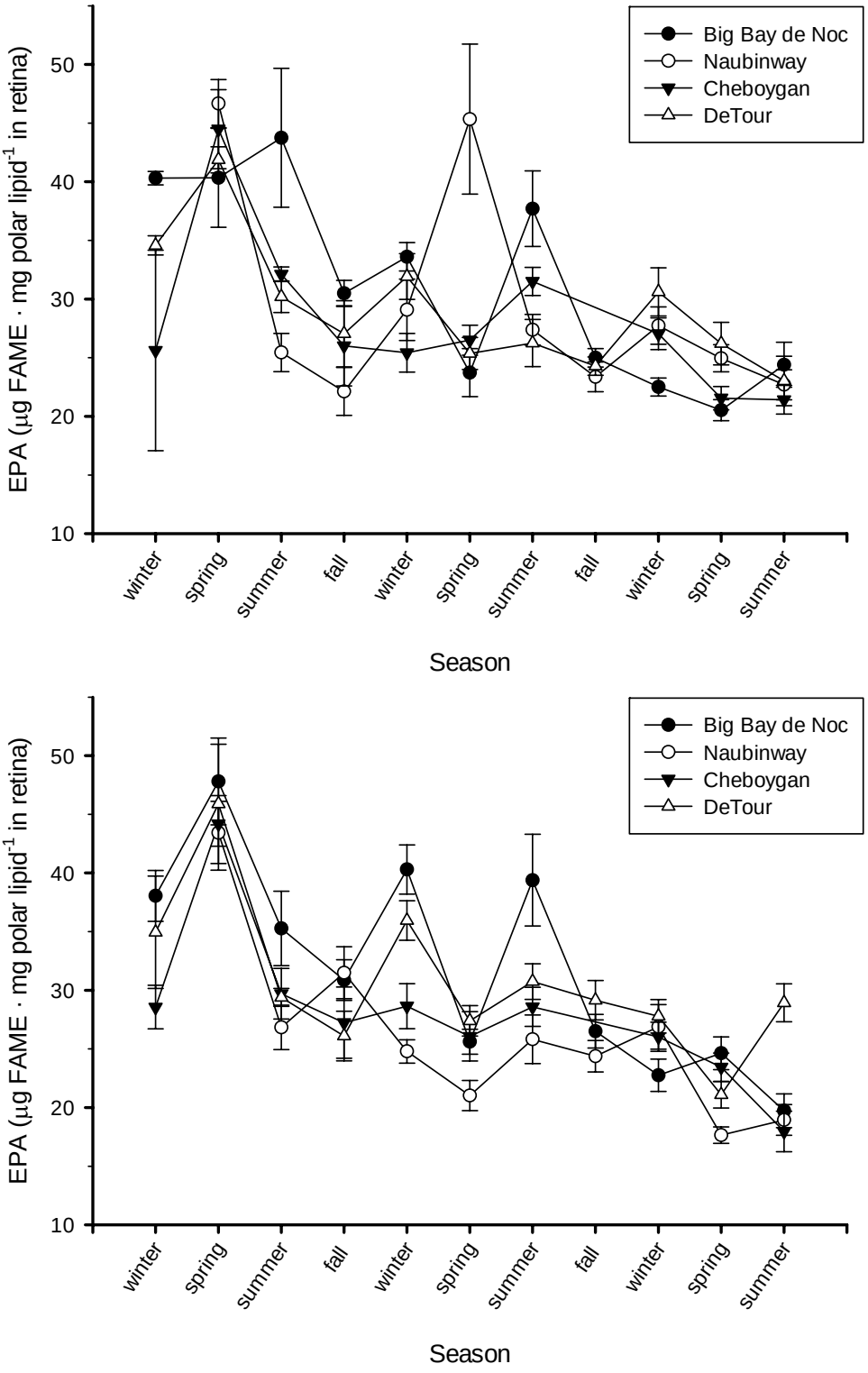

Figure 2.14. EPA in the polar lipid fraction of retinal tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

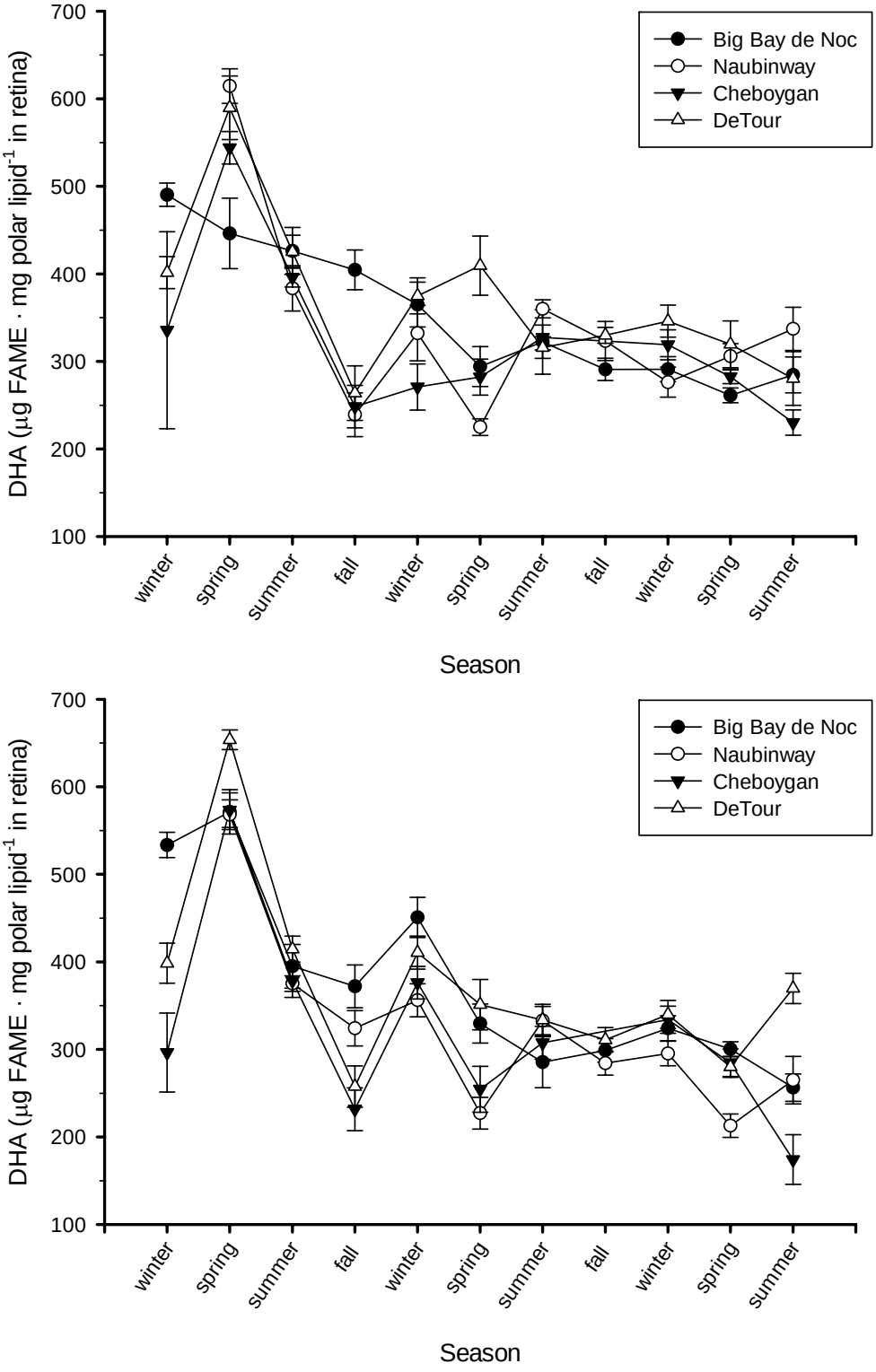

Figure 2.15. DHA in the polar lipid fraction of retinal tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

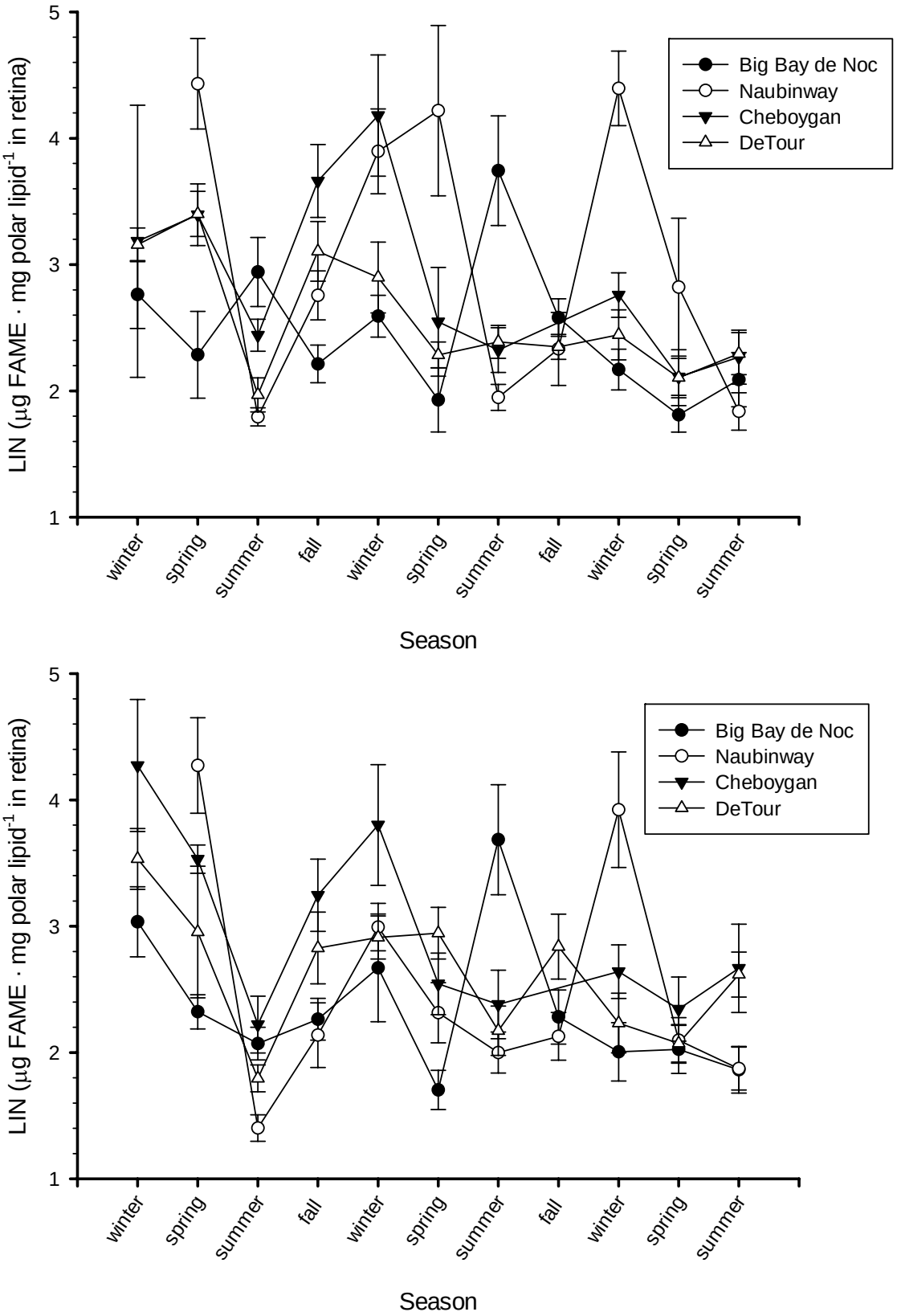

Figure 2.16. LIN in the polar lipid fraction of retinal tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

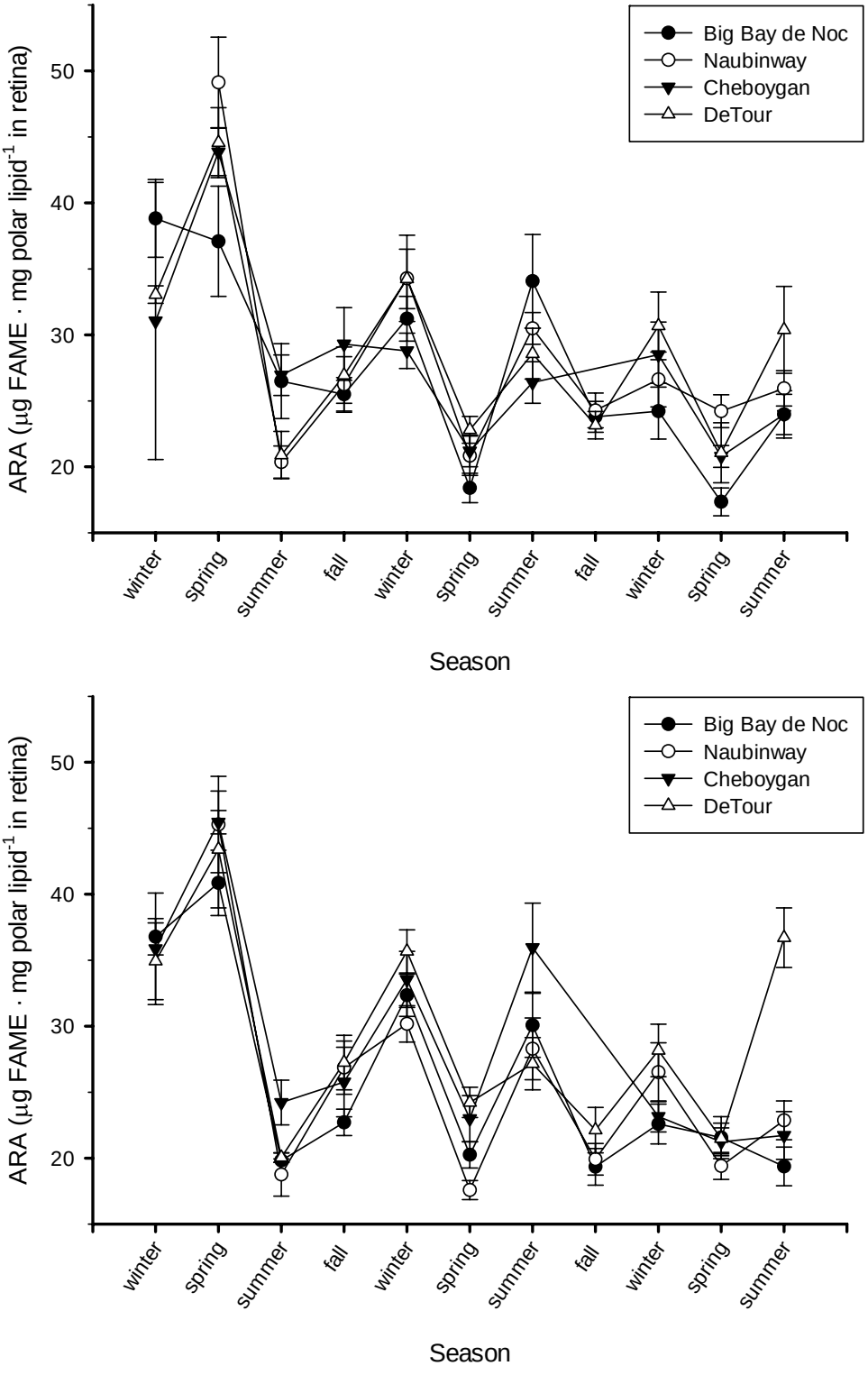

Figure 2.17. ARA in the polar lipid fraction of retinal tissue of lake whitefish expressed on a µg FAME · mg polar lipid^{-1} basis. Top panel = male fish, bottom pane = female fish.

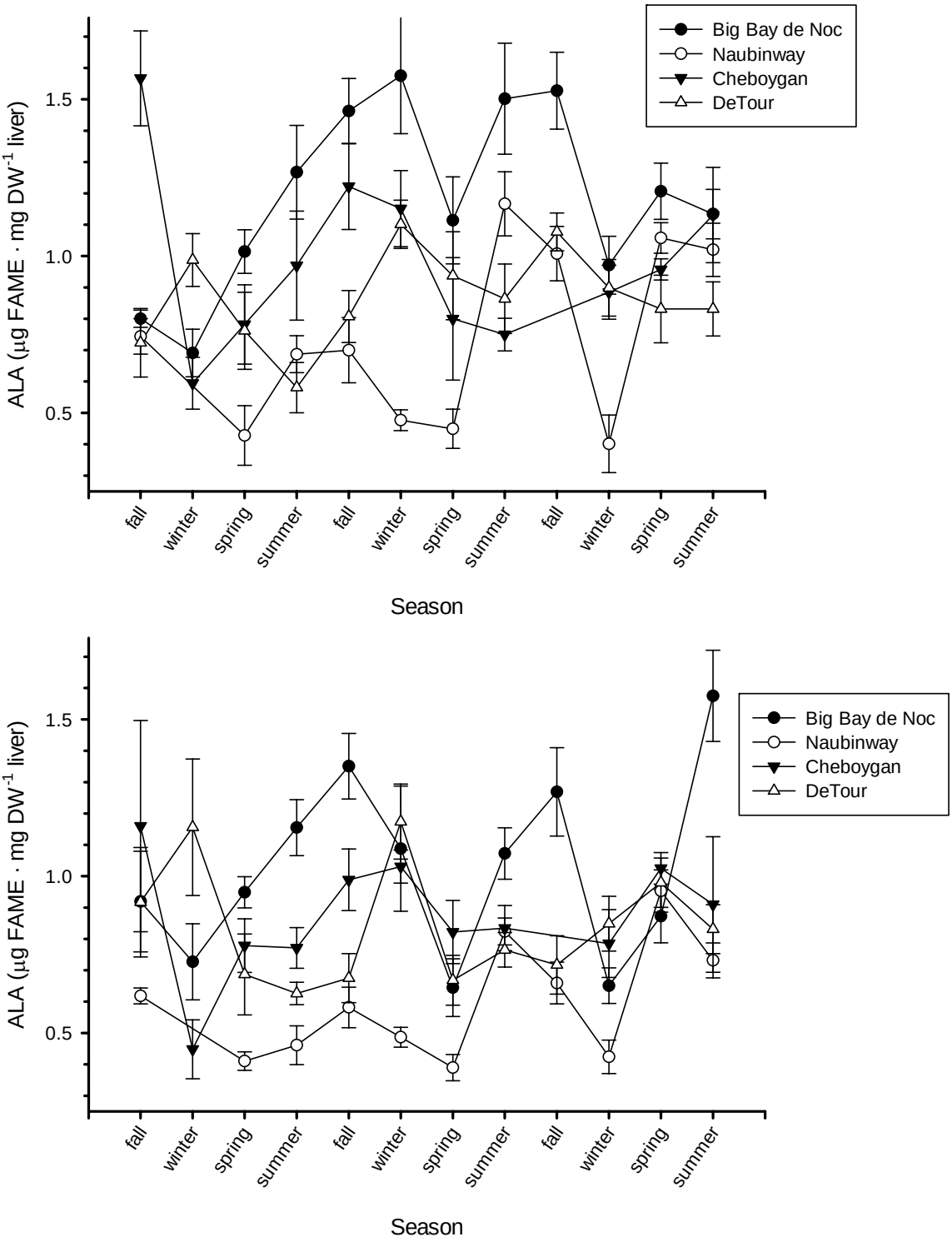

Figure 2.18. ALA in the total lipid fraction of liver tissue of lake whitefish expressed on a µg FAME • mg-1 DW basis. Top panel = male fish, bottom pane = female fish.

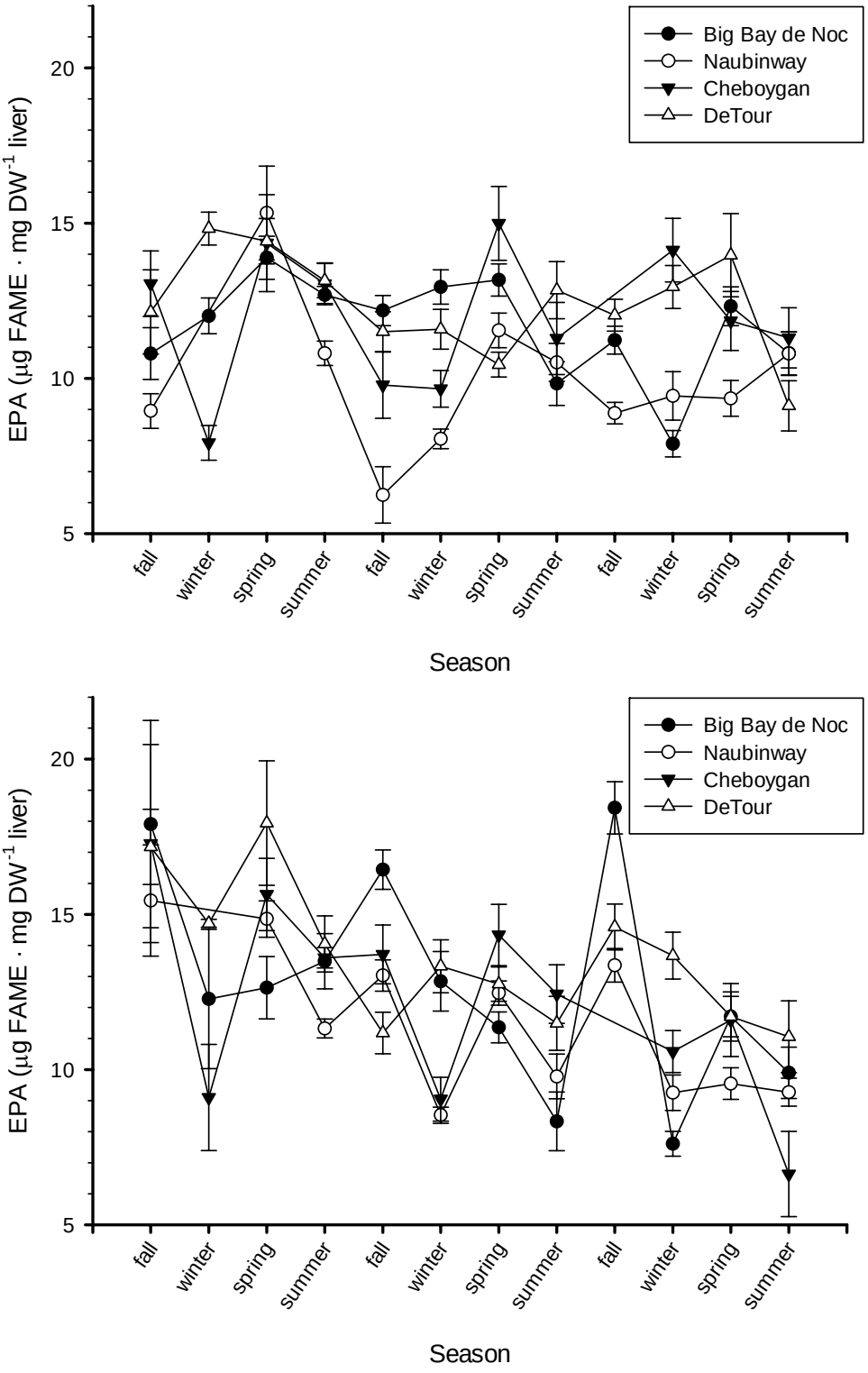

Figure 2.19. EPA in the total lipid fraction of liver tissue of lake whitefish expressed on a µg FAME • mg-1 DW basis. Top panel = male fish, bottom pane = female fish.

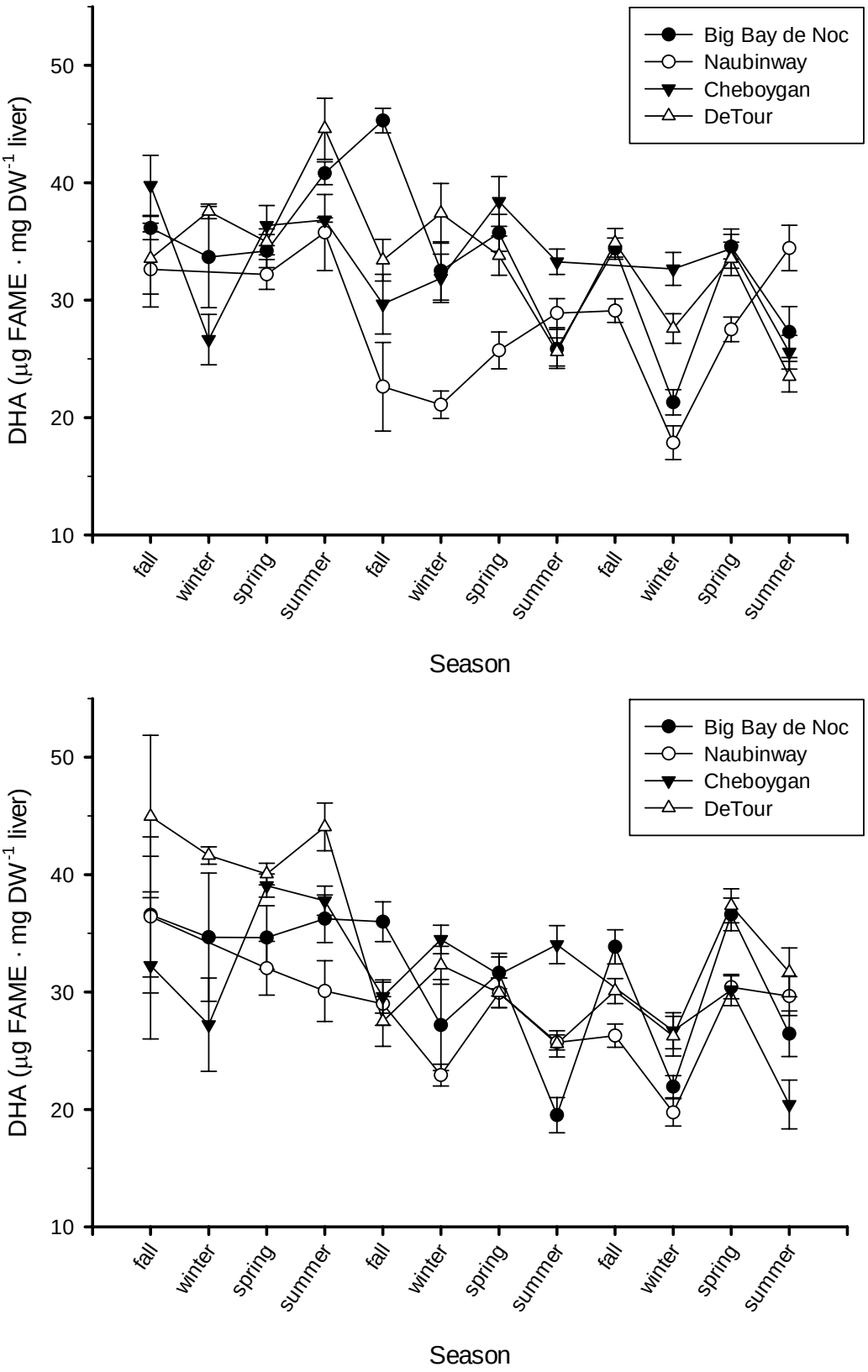

Figure 2.20. DHA in the total lipid fraction of liver tissue of lake whitefish expressed on a µg FAME • mg-1 DW basis. Top panel = male fish, bottom pane = female fish.

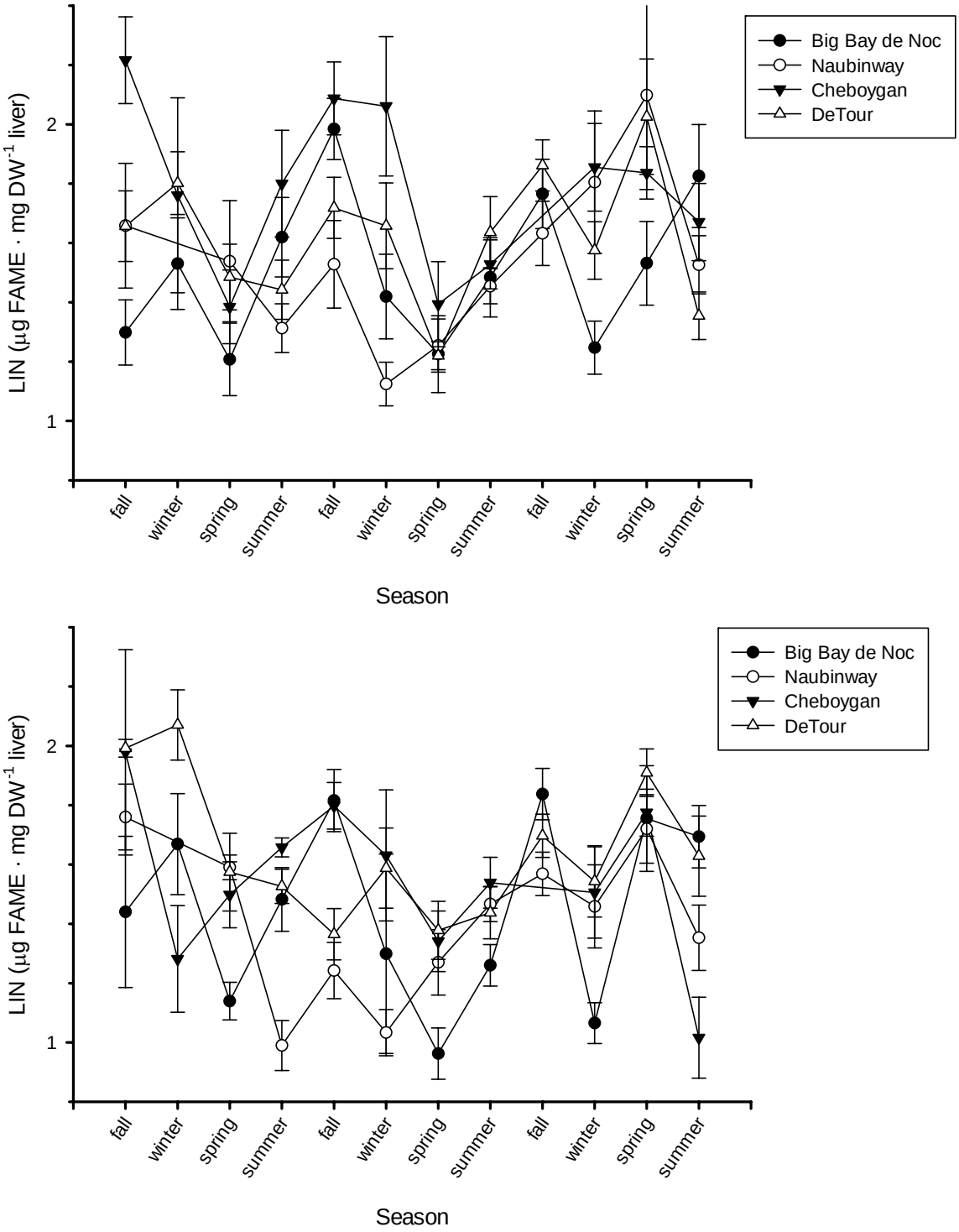

Figure 2.21. LIN in the total lipid fraction of liver tissue of lake whitefish expressed on a µg FAME • mg-1 DW basis. Top panel = male fish, bottom pane = female fish.

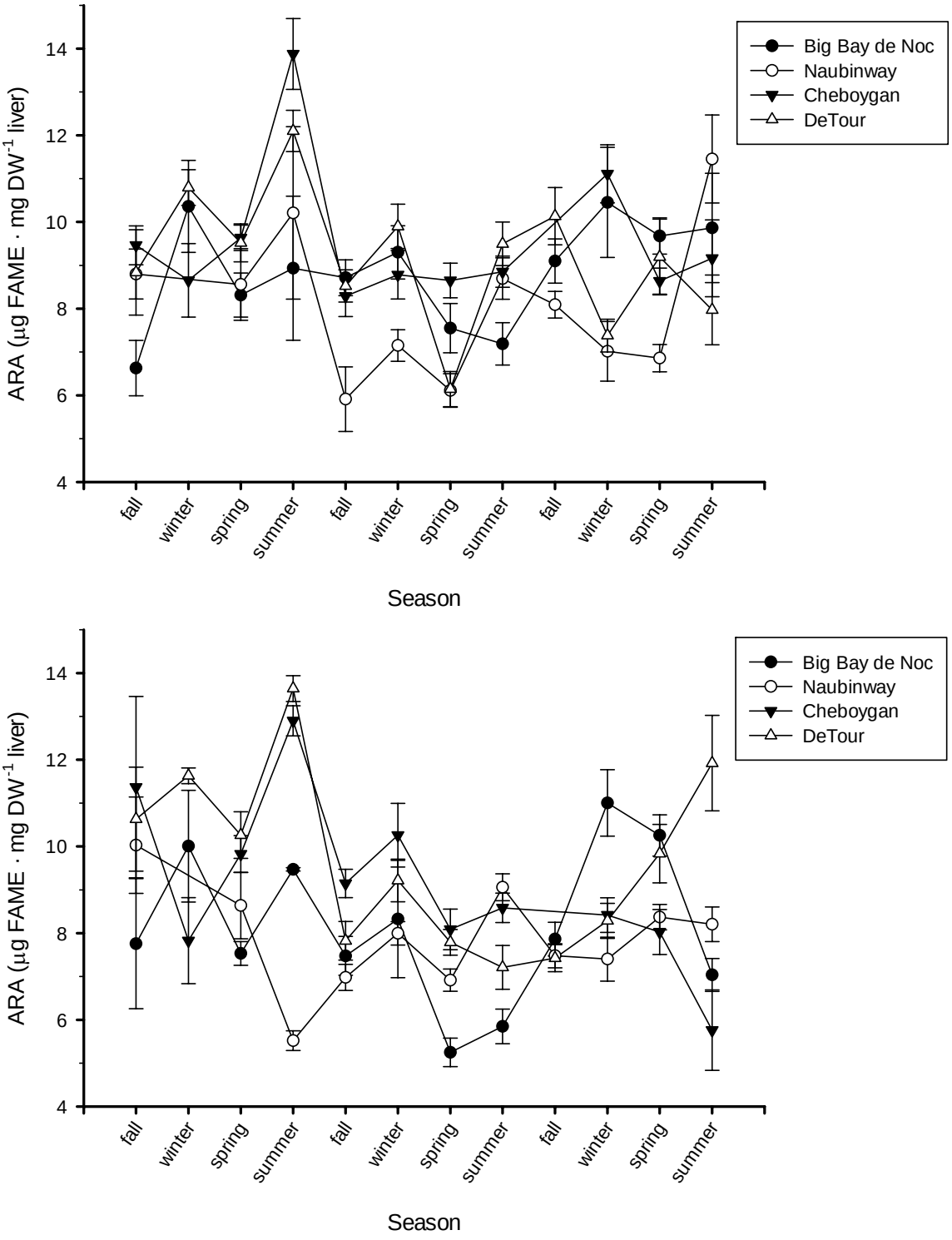

Figure 2.22. ARA in the total lipid fraction of liver tissue of lake whitefish expressed on a µg FAME • mg-1 DW basis. Top panel = male fish, bottom pane = female fish.

PART 3 – PATHOLOGICAL ANALYSIS

Methods

Field collection

Lake whitefish samples were obtained for pathological analysis at the same time as the fish collected for nutritional analysis. We attempted to collect at least 30 adult fish from each of the stocks during each of the sampling period. Fish were transported alive or on ice to the Aquatic Animal Health laboratory (AAHL) at Michigan State University for pathological analysis.

Clinical examination

Upon arrival at the AAHL, lake whitefish were immediately subjected to a comprehensive clinical examination, which included length and weight measurements, blood film/serum collection from the hemal arch, skin/gill scrapping, and thorough external examination for gross lesions, such as hemorrhage, exophthalmia, and skin ulcerations. Fish that arrived alive at the AAHL were sacrificed with an overdose of Tricaine Methanesulfonate (MS-222, Argent Chemicals, Redmond, and WA). External surfaces of lake whitefish were disinfected with 70% ethanol. Under aseptic conditions, peritoneal cavities were opened and all internal organs were examined for gross abnormalities, such as enlarged visceral organs, granulomatous lesions, hemorrhage, and the presence of parasitic cysts. In cases where gross pathological changes were evident, tissue samples were preserved in 10% buffered formalin for histopathological analyses. Histopathological analysis consisted of fixing affected tissues in 10% buffered formalin; tissues were then dehydrated, paraffin-embedded, sectioned at 5 μm thickness, and then stained with haematoxylin and eosin as described by Willers et al. (1991). The degree to which abnormal tissues were affected was graded and recorded (i.e., mild congestion, moderate congestion, severe congestion). Clinical signs that were of particular interest were those that are considered indicative of a bacteremia/septicemia/viremia, namely petechial/ecchmotic hemorrhage, exophthalmia, and abdominal distension/ascites accumulation. As the spleen and kidneys are the major sites containing lymphomyeloid tissue in fish, samples from the anterior and posterior portions of the kidney, as well as the spleen, were collected from every lake whitefish for bacteriological and viral analyses. All skin, gill, and fin scrapings were examined under a light microscope to detect the presence of any protozoa, helminthes, filamentous bacteria, and/or fungi. Gastrointestinal tracts were examined for parasitological evaluation, as were swimbladders.

Parasites and metazoa

Parasitological examination and metazoan identification

The gastrointestinal tract (GIT), comprising everything from the esophagus to the anus, was removed from each fish, covered with tap water and kept at 4ºC for approximately 24 to 48 hours to allow for parasite relaxation. GIT endoparasites were retrieved manually and preserved in 70 % ethanol for subsequent enumeration. To facilitate the identification of internal structures, collected parasites were cleared in a mixture of glycerol and 70% ethanol (1:1 v/v) and then examined microscopically (Petrochenko, 1956). Species identification was achieved using morphological criteria following the description and identification keys of Amin (1985), Aliff et al. (1977), Howard and Aliff (1980), Moravec (1980b), Ingham and Dronen (1982), and Hoffman (1999). Morphological criteria used to speciate acanthocephalans were the shape and

size of the proboscis, shape of the body, absence or presence of spines on the body trunk, number of hooks on the proboscis, number of rows of hooks, length of the neck, length of lemnisci, the absence or presence of the bulbous expansion, and number of cement glands. Criteria used to speciate cestodes were the length and segmentation of the body, shape of scolex, and organs of attachments. Scanning Electron Microscopy (SEM) was used to speciate swimbladder nematodes. The anterior parts of the worms as well as extruded eggs were dehydrated through an ethanol gradient (35%, 50%, 70%, 85%, and 95%) followed by three washes of 100% ethanol. During the dehydration process the eggs were pelleted by centrifugation at 2000 rpm for 5 minutes. Dehydrated worms were critical point-dried with carbon dioxide in a blazer union mounted on aluminum stubs, and gold coated in a Nanotech SEM prep II sputter coater (Miscampbell et al. 2004).

Description of parasite community structure

To describe and compare parasite community structure among the four stocks, a number of community metrics as described by Bush et al. (1997) were calculated. Prevalence was calculated as the number of infected hosts divided by the total possible number of hosts. It is expressed as a percentage when used descriptively and as a proportion when incorporated into mathematical models. Mean intensity was calculated by dividing the total number of parasites of a certain species by the total number of infected fish. Mean parasite abundance was calculated as the total number of individual parasites of a certain species found in a sample that include both infected and non-infected hosts. Mean abundance is an indication of the dispersion of the parasites within the sample.

There is a number of indices that are used to describe and compare parasite communities such as richness, defined as the number of parasite species in a community, and diversity, which describes the composition of a community in terms of number of species and the evenness of their distribution. Parasite diversity was summarized using Shannon's and Simpson's diversity indices. Similarity in endoparasite communities among the four lake whitefish stocks was calculated using the Jaccard similarity index (Cheetham and Hazel 1969). Dominance of a particular parasite species was expressed as the Berger-Parker Dominance Index (*d*), which measures the proportion of the total number of parasites in relation to the dominant parasite species (Berger and Parker, 1970). Cluster analysis, as described by Aldenderfer and Blashenfield (1984), was used to determine the association between parasites and differences within sites and lakes.

The significance of the relationship between the parasite species was tested using the Pearson correlation coefficient (r). Differences among the four stocks in prevalence of endoparasites were tested using Chi square (χ^2) tests. Differences in mean abundance, species richness, diversity indices, and Berger-Parker Dominance index, were tested using one-way analysis of variance (data were log transformed when necessary) or the Kruskal-Wallis test for data that were not distributed normally. Statistical significance was evaluated with an alpha of 0.05 and 0.01. Pairwise multiple comparison were conducted using Tukey's procedure. All calculations were performed using the Sigma Stat (Jandel Scientific Inc, San Rafael, CA) and Minitab software (Minitab Inc., State College, Pennsylvania) packages. The cluster analysis was conducted using Euclidean distance as the dissimilarity coefficient and the weighted pair group means averaging method as the linkage method (Aldenderfer and Blashenfield 1984).

Cystidicola

The swimbladder from every lake whitefish was examined for the presence of swimbladder nematodes. When nematodes were detected, swimbladders were excised and the worms extracted out of their lumen. The worms were then counted, cleared using a 1:1 mixture of glycerol and 70% ethanol at room temperature, and examined microscopically for identification. Eggs were extracted from gravid female *C. farionis* and their morphology examined as described by Dextrase (1987). Briefly, the mid-section of female nematodes was excised, covered with a drop of glycerin, and then mounted with a cover slip with gentle pressure to permit the extrusion of eggs from uteri. Nematode larval stages and adults were identified according to the morphological criteria described by Smith and Lankester (1979) and Black and Lankester (1980) using light microscopy.

Differences among the four stocks in the prevalence of the *Cystidicola farionis* were tested using a Chi square (χ^2) tests. Differences in intensity and mean abundance were tested using one-way analysis of variance or the Kruskal-Wallis test (for data that were not normally distributed). The Kruskal-Wallis test was used to test for differences in stages and sites based on seasons. Statistical significance was evaluated according to an alpha (α) of 0.05. Two-way analyses of variance (ANOVA) were used to test for differences in infection intensity among stocks, stages, and seasons. Additionally, analysis of variance for stages and seasons without replications in each site was used to test the effect of the different stages and different seasons on the intensity of infection in each site. Multiple regressions were used to examine the effect stages of the parasite, site location, and sampling season on the prevalence and intensity of *Cystidicola farionis* infection. Pearson's correlation coefficient (r) was used as an indication of the relationship among swimbladder parasite species found in each site collected between fall of 2003 and winter of 2006. Dunn's multiple comparison method was used to conduct pairwise comparisons among stocks.

Bacteria

Bacteriological Examination

Bacterial samples retrieved from lake whitefish were collected from kidneys and visible lesions. 10 µl loops were stabbed throughout anterior and posterior kidneys and plated directly onto culture media known to support a number of bacterial pathogens, such as Trypticase Soy Agar (TSA; Remel, Lenexa, KS), Cresol Red Thallium Acetate Sucrose Inulin (CTSI) agar (all ingredients are from Sigma Chemical Co., St. Louis, MO), a selective and differential medium for *Carnobacterium* spp. (Wasney et al. 2001), Coomassie Brilliant Blue Agar (CBBA) (Udey 1982), a differential medium for distinguishing strains of *A. salmoncida salmonicida* that possess an additional layer (poly-A layer) associated with the external cellular membrane, Hsu-Shotts agar (Shotts 1991), a selective medium for members of the fastidious genus *Flavobacterium*, as well as Modified Kidney Disease Medium (MKDM; Eissa 2005), a selective medium for *Renibacterium salmoninarum*, the etiological agent of bacterial kidney disease. In instances where bacterial cultures were taken from external lesions, the area was disinfected with 70% ethanol, the periphery of the lesion was incised, and a 10 µl loop was inserted and used to plate the inoculum onto relevant bacterial media. incubated at 22 °C for up to 72 hr. Periodic examination of bacterial growth was recorded, and individual colonies were sub-cultured onto TSA and then incubated for 24 hours at 22°C.

Isolated bacteria were initially identified using a battery of morphological and biochemical tests, including Gram reaction, cytochrome oxidase, catalase reaction (3% H_2O_2), presence of the pigment flexirubin using the 3% Potassium Hydroxide test, motility, indole

production, hydrogen sulfide production, oxidation/fermentation reaction (BD Scientific, Sparks, MD), methyl red, 2,3-butanediol production from glucose (Voges-Proskauer), production of gas from glucose, nitrate reduction, citrate utilization, TSI reaction, ONPG (*o*-nitrophenyl-*β*-D-galactopyranoside), lysine decarboxylase, ornithine decarboxylase, arginine dihydrolase, esculin hydrolysis, phenylalanine deaminase (BD Scientific), growth on acetate agar (pH 5.4), and resistance to the vibriostatic agent 0/129 (2,4-diamino,6,7-di-isopropyl pteridine). Production of acid from the following carbohydrates was examined in phenol red broth base at a final concentration of 1%: adonitol, arabinose, cellobiose, dextrose, galactose, glycerol, inositol, innulin, lactose, malonate, maltose, mannitol, mannose, melibiose, raffinose, rhamnose, salicin, sorbitol, sucrose, trehalose, and xylose. Results were recorded up to 7 days post-inoculation with the following exceptions: methyl red, Voges-Proskauer, indole production, Simmons citrate, and TSI reactions were read at 2 days. All materials and reagents were purchased from Remel Inc. (Lenexa, KS) unless specified otherwise.

For culturing *Renibacterium salmoninarum,* 100 µl aliquots of stomached kidney tissues were spread onto modified Kidney Disease Medium (MKDM), which consists of peptone (1% w/v), yeast extract (0.05% w/v), L-cysteine HCl (0.1% w/v) and Cycloheximide (0.005 % w/v) dissolved in distilled water. The medium's pH was adjusted to 6.8 and then agar (1.5 % w/v) was added. Following autoclaving at 121°C for 15 min, the medium was left to cool to 48°C and then new born calf serum (10% v/v), 0.22 µm filter-sterilized *R. salmoninarum* spent broth (1% v/v), Oxolinic acid [b] (0.00025% w/v), Polymyxin B sulfate (0.0025% w/v) and D-cycloserine (0.00125 % w/v) were added. Inoculated plates were incubated for up to 20 days at 15 °C. All colonies were investigated for their conformance with colony and bacterial morphological criteria of *R. salmoninarum*, as previously detailed in Sander and Fryer (1980) and Austin and Austin (1999). A number of biochemical tests on each isolate were performed including motility, using motility test medium (DIFCO- BD and Company Sparks, MD, USA), cytochrome oxidase with Pathotec strips (Remel), catalase test with 3 % hydrogen peroxide, hydrolysis of esculin using bile esculin agar (Remel), and DNAse test using DNAse test medium (Remel). Carbohydrate utilization was performed using basal media (DIFCO-BD). The basal medium was prepared according to manufacturer instructions prior to the addition of individual sugars. Ten ml of 0.45 µm filter sterilized 10 % sugar solution was added to autoclaved and cooled (48 °C) basal media to obtain a final concentration of 1 % with the exception of salicin which was made as 5 % solution to reach 0.5 % final concentration. Each one of the following sugars was added individually to the basal medium to test for the utilization of each sugar: arabinose, glucose, lactose, maltose, rhamnose, salicin, sucrose, sorbitol, xylose. All sugars were from Sigma. Results of biochemical tests were matched against standard *R. salmoninarum* biochemical characters described by Bruno and Munro (1986).

<u>Measurements of R. salmoninarum antigen using the Quantitative Enzyme-linked Immunosorbent Assay (Q-ELISA)</u>

Kidney samples representing the anterior, posterior, and middle sections of the kidney were transferred in sterile 7.5 cm x 18.5 cm Whirl Pak® bags kept on ice, and were softened as much as possible through multiple cycles of physical pressure. The homogenized kidney tissues were diluted in 1:4 (w/v) Hank's Balanced Salt Solution (HBSS) and then stomached for 2 minutes at high-speed using the Biomaster Stomacher-80.

Aliquots (250 µl) of each sample were transferred into 1.5 ml safe lock microfuge tubes, to which an equal volume of 0.01 M Phospate Buffered Saline Tween 20 (0.05 %, PBS-T20) with 5% natural goat serum and 50 µl CitriSolv solution were added. The solution was then thoroughly mixed via vortexing, incubated at 100°C on heat blocks with a rotary shaker for 15 minutes, followed by 2 hours of incubation at 4 ºC. After incubation, the mixture was centrifuged at 6000g for 15 minutes at 4 ºC. The aqueous supernatant of each sample was carefully collected and then transferred to a 1.5 ml microfuge tube for Q-ELISA testing. The positive–negative cutoff absorbance for the kidney homogenate was 0.10. The tested positive samples were assigned the following antigen level categories: low (0.10 to 0.19), medium (0.20-0.99) and high (1.000 or more).

Nested PCR for confirmation of R. salmoninarum isolates

Bacterial DNA was extracted using the DNeasy tissue extraction kit. DNA was extracted from 100 µl aliquots of kidney tissue homogenates according to manufacturer's instructions. The tissue pellets were obtained by centrifugation at 6000 g for 20 minutes at 4 °C and then incubated with lysozyme buffer consisting of 180 µl of 20 mg lysozyme , 20mM Tris-HCl (pH 8.0), 2 mM EDTA and 1.2 % (v/v) Triton X100 at 37 °C for 1 hour. The nPCR method used primers recommended by Pascho et al. (1999) with slight modifications to the volume of DNA (5µl for the first round and 2µl for the second round nPCR) and master mixes (45µl for the first round and 48µl for the second round nPCR). The controls were composed of a PCR mixture containing no DNA template (reagent negative control), positive *R. salmoninarum* and positive tissue control. A volume of 10 µl of the nPCR product and controls were mixed with 2 µl of 6X loading dye and used on a 2 % agarose gel. Each electrophoresis gel included a 1kbp DNA ladder with 100 bp increments. Gels were run in 1 X Tris Acetate (1 X TAE) Buffer . Gels were visualized under the KODAK EDAS Camera System and UV Trans-illuminator. Samples were considered positive when a 320 bp band was detected.

Viruses

Virological Examination

Virus isolation was performed according to the standard protocols published by the American Fisheries Society (2004) and the Office International des Epizooties (2003). Samples from kidneys, spleen and swim bladder lesions were excised aseptically from individual fish, weighed, diluted with nine volumes of antibiotic-free minimum essential medium (MEM), and then homogenized using a Biomaster Stomacher-80 at the high-speed setting for 2 min. Homogenates were allowed to settle, while kept on ice, for 15 min and an aliquot of cell culture medium was added to produce 10^{-2} and 10^{-3} dilutions (w/v) of the original tissues. For the initial isolation, the fathead minnow (FHM) and the chinook salmon embryo (CHSE-214) cell lines were used. Tissue culture flasks were inoculated with dilutions of the homogenate (two flasks/dilution). Inoculated cells were incubated at 20 and 15 °C for FHM and CHSE-214, respectively, and examined for the appearance of cytopathic effects (CPE) for 21–28 days post-inoculation. Growth of viral isolates and additional studies were performed using FHM and the 'Epithelioma papulosum cyprini' (EPC) cell line grown at 25 °C. Medium from cell cultures showing CPE was stored in aliquots at −80 °C for use as stock virus.

To determine virus morphology, medium from cell cultures showing cytopathic effects was replaced with 5 mL of 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2). Following fixation for 2 h at 4°C, cells were harvested and centrifuged at 1500 *g* for 10 min. The cell pellet

was then collected, post-fixed in 1% osmium tetroxide for 90 min, and embedded in epoxy resin according to the protocols of Chang et al. (2002). Ultrathin sections were examined with a JEOL JEM-100 electron microscope.

Virus isolates were identified using PCR. Total RNA was extracted from suspected tissue culture or fish tissues using the TRI Reagent. Pellets of RNA were resuspended in 50 μL of deionized water, heated at 60 °C for 10 min, and cooled on ice until added to reverse transcriptase (RT)-PCR reaction mixtures. Reverse transcription and PCR of the central region of the glycoprotein (G) gene of VHSV was performed following the procedures outlined in Hedrick et al. (2003) and Winton and Einer-Jensen (2002). Thirty PCR cycles amplified a 914-bp region using the central G primers. In order to amplify the entire G gene (1609 nt) and N gene (1386 nt) of the lake whitefish isolate, additional primers were used as described in Elsayed et al. (2006).

The PCR products were then purified with a StrataPrep PCR purification kit and sequenced with a fluorescent dye terminator cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) in individual reactions using the primers listed Elsayed et al. (2006).

Results

External examination

External clinical examinations of lake whitefish collected as part of this study revealed the presence of a variety of external and internal lesions. The highest incidence of lesions were found on fish from the Cheboygan stock, while the lowest incidences of lesions were found on fish from the Naubinway stock. External lesions included petechial and/or ecchymotic hemorrhages that can be associated with bacteremias, viremias, as well as external parasitic infections (Figure 3.1a), ulcerations (Figure 3.1b and 3.1c), furuncle-like lesions (Figure 3.1d), lacerations, and numerous wounds corresponding to probable trauma. Additional abnormalities observed during external examination included degraded fins, exophthalmia, abdominal distension, and generalized anemia.

Parasitological examination

Skin and gill parasites

A number of skin parasites have been found parasitizing skin and gills including Trichodina spp., Epistylis spp., and monogeneans. Overall, prevalence and abundance of skin and gill parasites were low.

Gastrointestinal tract parasites

A total of 21,023 worms were retrieved from the gastrointestinal tract (GIT) of 1,286 lake whitefish collected from the four sites. Acanthocephalans constituted the majority of gastrointestinal helminths (54%) while cestodes constituted 46% of the total metazoan endoparasite community. Three acanthocephalans and two cestode species were identified: *Acanthocephalus dirus* Van Cleave 1931, *Neoechinorhynchus tumidus* Van Cleave and Bangham 1949, *Echinorhynchus salmonis* Muller 1784, *Bothriocephalus* sp., and *Cyathocephalus truncatus* Pallus 1781. Our collections were new geographic locations for these five species of parasites. Representative specimens were deposited at the Michigan State University Museum of Natural History. Among acanthocephalans, *A. dirus* exhibited the highest prevalence among lake whitefish GIT (63%) followed by *N. tumidus* (17%), and *E. salmonis* (6%). *Bothriocephalus* sp. parasitized 8% of examined fish, while *C. truncatus* were found in 10% of the examined fish and was found exclusively in pyloric caeca.

There were site and seasonal differences in prevalence, intensity, and abundance of GIT parasites (Table 3.2). Fish from the Naubinway stock had the lowest prevalence of GIT parasites, while fish from the Cheboygan stock had significantly higher prevalence and intensity of parasite infection rates then fish from the other stocks ($P < 0.05$). Fish caught from the Big Bay De Noc and Detour stocks exhibited a medium range of GIT parasitism.

E. salmonis was the GIT worm with the lowest prevalence, intensity, and abundance in the stocks. In earlier samples, it was found in BBN samples only, but then later found in Cheboygan and Detour. *Acanthocephalus dirus* was significantly higher than other parasite species in terms of prevalence ($P<0.05$), intensity ($P<0.05$) and mean abundance ($P<0.05$) for both Lake Michigan and Lake Huron sites.

Cluster analysis revealed the presence of two main clusters of GIT parasites: one contained the Naubinway stock, while the second contained the Big Bay de Noc, Detour, and Cheboygan stocks (Figure 3.3). Detour and Cheboygan stocks were more similar to each other than the Big Bay De Noc stock. This observation may be of interest as it suggests that it might be possible to discriminate fish among the four stocks based on GIS parasites.

We observed substantial seasonal shifts in GIT community structure over the course of this study. Studying the community structure of GIT parasites over a 3-year period demonstrated that these communities are indeed dynamic with substantial shifts in certain seasons (Table 3.3). In the Cheboygan stock, GIT communities during the spring of 2004, 2005, and 2006 were significantly different from the GIT communities in the other seasons (P<0.05). GIT parasite community was found dominated primarily by *A. dirus* and, to a lesser extent, by *C. truncates*.

GIT endoparasite species richness was the highest in fish from the Cheboygan stock and lowest in fish from the Naubinway stock (P<0.05). Diversity indices demonstrated that the parasite community in a given site is more diverse in some season than others. For example, diversity in Cheboygan was higher in the spring 2005, spring 2006 and winter 2004 seasons than others. Analyzing the community structure of all fish examined in this study, a significant positive correlation was found between *A. dirus* and *C. truncatus* (P=0.021), while there was no significant correlation between all other parasites. This relationship, however, did not exist among the community structure of individual sites.

Swimbladder parasites

During this study, we recovered 9,064 *Cystidicola farionis* individuals from the swimbladders of lake whitefish. Based on morphological criteria revealed by light and electron microscopy, *Cystidicola farionis* was the only swimbladder nematode observed in the four lake whitefish stocks. A number of larval stages were identified among the *C. farionis* infrapopulations. According to identification features listed by Black and Lankester (1980), Lankester and Smith (1980) and Dextrase (1987), five stages were identified: the third larval stage (L3), fourth larval stage (L4), sub-adult immature stage (SA), mature adult male (M) with specula, caudal or postanal papillae and sperms in the vas deferens and mature adult female (F) with shelled eggs in the uterus. Histopathological examination of swimbladders from individuals infected with *C. farionis* infection indicated severe erosions and wall thickening of the swim bladder, both of which can result in abnormal swimming behavior.

The average count of worms per fish was very low ($\bar{x} = 5$) in the first year of the study, but increased ($\bar{x} = 36$) during the second year of the study. The average count of worms then decreased to approximate year one levels ($\bar{x} = 4.5$) during the third year of the study. The prevalence of swimbladder nematode infection ranged from 100% in spring collection of 2005 in

Cheboygan to 0.0% in the fall of 2003 for Detour, and 0.0% in the spring of 2004, summer of 2004, winter of 2005, spring of 2005, summer of 2005 and summer of 2006 for NB.

The analysis for stages and seasons showed significant differences among seasons (*P=0.006*), with the spring being the highest of *C. farionis* prevalence (*P<0.01*). However, the multiple regression analysis for the intensity of infection using the sites, stages and seasons, revealed that the site is the only significant aspect in the regression model (*P= 0.024*), while stages and seasons were not significant (*P>0.05*). The prevalence of infection tended to peak during the spring for all sites except for Naubinway (Figure 3.4), with L3 and L4 being the predominant stages in infected individuals. From the analysis it was concluded that the prevalence of infection of lake whitefish with *C. farionis* in the swimbladder was significantly higher for Lake Huron than Lake Michigan during all the collection incidences from fall of 2003 through the summer of 2006. The highest prevalence of the parasite was 90% and 100% in the spring of 2005 for Detour and Cheboygan samples, respectively.

Two-way analysis of variance indicated that there was a significant among sites in terms of *C. farionis* intensity ($P < 0.01$), but there were not significant differences in stages ($P = 0.31$). The intensity of the swimbladder nematode infection peaked in the spring of 2004, 2005 and 2006 with a mean of 70.3, 85.2, 30.6 worm per infected fish for Cheboygan. Two-way analysis of variance also indicated that was used to test the significant difference between seasons and sites based on intensity. The difference in the mean intensity among the sites was statistically significant ($P < 0.001$), but we did not find significant differences in intensity among seasons ($P = 0.11$).

Bacteriology

A number of bacterial pathogens were identified from lake whitefish from the four stocks. These bacteria were isolated primarily from the internal organs, particularly in the kidneys. A synopsis on each of the major bacterial species isolated is given below. In addition to the bacteria mentioned below, several additional species were recovered during the course of this study. Among these were *Shewanella putrifaciens*, a Gram negative saprophyte that is sometimes associated with various infections in humans, various *Flavobacterium* spp., and a few members of the Family Enterobacteriaceae.

Renibacterium salmoninarum

Renibacterium salmoninarum is a gram positive bacterium that is the causative agent of Bacterial Kidney Disease (BKD), which is a serious disease of salmonid fish species. *R. salmoninarum* elicits the formation of granulomatous tissues, primarily in hematopoietic organs (Bruno, 1986) and the production of harmful inflammatory mediators (Evelyn et al., 1981, Olsen et al., 1992). During this study, 1,284 lake whitefish were subjected to multiple assays to assess the spread of *R. salmoninarum* among the four stocks. As far as we are aware, this is the largest study ever performed on wild *Coregonus* spp.

In the first year of this study, a number of *R. salmoninarum* isolates were retrieved from lake whitefish from each of the four stocks. These isolates were confirmed using PCR and gene sequencing assays. Quantitative ELISA indicated that both prevalence and intensity of *R. salmoninarum* was much higher in lake whitefish from the Lake Huron stocks then in fish from the Lake Michigan stocks (P<0.001 for both values). Lake whitefish from the Cheboygan stock had the highest prevalence of *R. salmoninarum* infection. Prevalence of *R. salmoninarum* infection in the Cheboygan stock peaked during the fall and winter sampling

occasions, and often was as high as 100% prevalence. In the Detour stock, peaks in prevalence were observed slightly later then those observed in the Cheboygan stock. The cyclic pattern of *R. salmoninarum* prevalence in lake whitefish was similar in shape to that of a propogated epidemic curve, suggesting that there was a permanent pathogen source for the stocks (Figure 3.6).

Motile aeromonads

Approximately 14% of examined fish were found to be infected with a motile *Aeromonas* sp., the majority of which were recovered from the kidneys. Additional sites of recovery included exudate present within the lumen of the swimbladder and external ulcerations. In-depth analysis of isolates recovered during the third year of this study revealed that lake whitefish were infected with members from all three complexes within the genus *Aeromonas*, the *hydrophila, sobria,* and *caviae* complexes. Infections varied by season and site and were caused by four species in particular; *A. hydrophila sensu stricto*, *A. jandaei, A. veronii* bv *sobria,* and *A. eucrenophila. A. hydrophila sensu stricto* had the highest prevalence of the aeromonads, and accounted for the highest proportion of infections among all seasons and sites. The prevalence of this bacterium was at least double that of the next most predominant species in all sampled sites. According to Noga (2000), *A. hydrophila* is considered one of the most important fish pathogen and is perhaps the most well described.

The next most prevalent motile aeromonad species was *A. jandaei*, which was detected in over 2% of the lake whitefish sampled from 3 of the 4 stocks. An interesting aspect of these infections was that they were most prevalent during winter. This in contrast to all other detected motile aeromonad species, which without exception were most prevalent during the summer. Reasons for this seasonal difference in *A. jandaei* prevalence are currently unknown. It is also interesting to note that this bacterium was always recovered from fish concurrently infected with another bacterial species, although the mixed flora was not consistent.

Aeromonas veronii bv *sobria* was also isolated in this study, although its presence was limited only to lake whitefish from the Big Bay de Noc stock during summer sampling. Additionally, two of the three *A. veronii* bv *sobria* isolates were recovered in pure culture, and the infected individuals presented with clinical signs of disease, including renal mottling and pallor, multi-focal necrotic foci within the liver, and mild hemorrhagic enteritis in the posterior portion of the intestine. As such, the relatively high prevalence of this bacterium in lake whitefish collected from Big Bay de Noc in the summer, along with the observed gross pathological effects, suggested that an *A. veronii* bv *sobria*-derived widespread infection may have occurred.

Two isolates, identified as *A. eucrenophila,* were recovered from Big Bay de Noc and Detour whitefish in the summer. These isolates were recovered from individuals with mixed infections exhibiting hemorrhagic and prolapsed vents, generalized erythema within the peritoneum, including the internal lateral and ventral musculature, a small amount of serosanguinous fluid within the peritoneum, multi-focal hemorrhage within the liver, moderate splenomegaly, a friable kidney, and gastroeneteritis/hemorrhagic enteritis.

Carnobacterium maltaromaticum

A *Carnobacterium maltaromaticum*-like bacterium, which is the causative agent of Pseudokidney Disease, was isolated from kidneys and swim-bladders of lake whitefish caught from all four stocks. Isolates were Gram-positive, nonmotile, facultatively anaerobic,

asporogenous rods arranged in palisades that did not produce catalase, cytochrome oxidase, or H_2S, and did not grow on acetate agar. Except for carbohydrate fermentation, many phenotypic characteristics of the lake whitefish isolates coincided with those of *C. maltaromaticum*. Partial sequencing of the 16S and 23S rRNA genes, as well as the piscicolin 126 precursor gene, yielded 97% and 98% nucleotide match with *C. maltaromaticum,* respectively (sequences were deposited in the GenBank under accession numbers EU546836 & EU546837). Phylogenetic analysis indicated that the lake whitefish isolates were highly related to each other, and were similar, but fully identical to *C. maltaromaticum* type strain AF374295 and *C. gallinarum* (Figure 3-7). Additionally, the lake whitefish isolated formed a phylogenetic cluster with *Tetragenococcus halophilus*, *Streptococcus thermophilus*, and a number of *Enterococcus* spp.

The revalence of *C. maltaromaticum*-like infections in the four lake whitefish stocks varied among seasons and stocks (Table 3.4). The presence of the *C. maltaromaticum*-like bacterium was associated with splenomegaly, renal and splenic congestion, and thickening of the swim bladder wall with accumulation of a mucoid exudate. Examination of stained tissue sections revealed the presence of kidney and spleen congestion, vacuolation and bile stasis within the liver, and hyperplasia within the epithelial lining of the swim bladder. These pathological changes associated with *C. maltaromaticum*-like bacteria suggest that this bacterium may be harmful to lake whitefish populations.

Pseudomonas fluorescens

Pseudomonas fluorescens was recovered from lake whitefish at a prevalence of 0.093% in this study, with all infections occurring in lake whitefish from either the Big Bay de Noc or Cheboygan stocks during Fall sampling. The overall prevalence of infection for lake whitefish collected from Big Bay de Noc and Cheboygan were 2.66% and 0.997%, respectively. Clinical signs associated with the infections included pallor in the liver, spleen, and kidney, hemorrhagic enteritis, unilateral exophthalmia, splenomegaly, and external ulceration. All isolates were Gram negative non-fermentative bacilli that produced the pigment fluorescein.

Aeromonas salmonicida subspecies salmonicida

Aeromonas salmonicida subspecies *salmonicida* is the etiological agent of furunculosis, a disease that devastates salmonid fish species. Four *A. salmonicida* subspecies *salmonicida* isolates were recovered from the kidneys of infected lake whitefish. The isolates were Gram-negative, non-motile, coccobacilli that produced a brown diffusible pigment on Trypticase Soy Agar. All isolates produced deep blue colonies on Coomassie Brilliant Blue Agar. Amplification of selected 16S rRNA regions specific to *A. salmonicida* subspecies *salmonicida* via polymerase chain reactions and subsequent gel electrophoresis analyses of the retrieved isolates yielded amplicons of the expected size (512 bp). Clinical signs associated with infection included extensive external hemorrhaging, exopthalmia, varying degrees of splenomegaly, hepatic and renal pallor, spenic and renal congestion, friability of the kidney, fibrinous adhesions on the spleen and liver, as well as severe hemorrhagic enteritis. Histopathological examination of culture positive lake whitefish revealed multi-focal hemorrhage and mild to moderate infiltration of lymphocytes and histiocytes in the fat under the skin and in the muscultature, and massive diffuse congestion within the spleen. Histopathological examination of furuncle-like lesions showed ulceration, necrotizing dermatitis and myositis, along with an infiltration of mixed lymphocytes, macrophages, and infrequent heterophils wihin the skin and underlying musculature, as well as hemorrhage and fibrin deposition; however, no bacterial colonies were

observed. *A. salmonicida* subspecies *salmonicida* infections were limited to the Naubinway, Lake Michigan, and Detour, Lake Huron sites.

Virological examination

A virus was isolated in only one lake whitefish from the Cheboygan stock. This individual exhibited severe splenomegaly and internal hemorrhages. Extensive virological and molecular examination of the virus isolate revealed the following. Electron microscopy of infected cell lines revealed bullet-shaped viral particles having the characteristic morphology of members of the family Rhabdoviridae. Many virus particles were present in the intra- and extracellular spaces. The virions appeared to possess an envelope around a striated nucleocapsid. Enveloped virions measured 170–180 nm in length by 60–70 nm in width. The virus isolate was identified as viral hemorrhagic septicemia virus (VHSV) by the reverse transcriptase-polymerase chain reaction (RT-PCR). Nucleotide sequence analysis of the glycoprotein gene demonstrated that the 2005 virus isolate was most closely related to the Great Lakes strain of the North American genotype IVb of VHSV. Chronologically, this is the first VHSV strain to be isolated from Lake Huron. The emergence of VHSV in Lake Huron is unlikely a singular event. In 2003, VHSV was isolated from muskellunge in Lake St. Clair (Elsayed et al., 2006). During the spring/summer of 2005, a large fish kill occurred in eastern Lake Ontario, Canada among freshwater drum, *Aplodinotus grunniens.* In the spring of 2006, large mortalities were recorded among several additional species of fish in Lake St Clair, Lake Erie and Lake Ontario and VHSV was isolated from both moribund and normal-appearing fish. The absence of VHSV in 2003 and 2004 suggests that the virus may have been recently introduced into Lake Huron whitefish populations.

Table 3.1. Numbers of lake whitefish fish collected for clinical, pathological, bacteriological, and viral examination by sampling period and stock.

Sampling Period	Big Bay de Noc	Naubinway	Cheboygan	Detour
Fall 2003	35	30	30	34
Winter 2004	29	0	32	10
Spring 2004	30	30	20	30
Summer 2004	22	20	30	20
Fall 2004	26	30	26	30
Winter 2005	16	30	15	30
Spring 2005	30	30	30	30
Summer 2005	30	30	28	30
Fall 2005	30	30	0	30
Winter 2006	30	23	30	30
Spring 2006	30	30	30	30
Summer 2006	30	30	30	30

Table 3.2. Prevalence/intensity/ and abundance of GIT parasites from lake whitefish collected from the stocks from Fall 2003 to Summer 2006.

Season	Site	Date	Sample size	Acanthocephalus	Neoechinorhynchus	Echinorhynchus	Botheriocephalus sp.	Cystidicola
	BBN	11/05/03	35	40/17.2/6.9	17.1/10.8/1.9	0/0/0	0/0/0	8.6/3.3/0.3
	NB	11/18/03	30	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
	DV	11/19/03	34	8.8/7/0.6	2.9/35/1	0/0/0	0/0/0	0/0/0
Fall 03	CHEB	11/11/03	30	13.3/12.8/1.7	0/0/0	0/0/0	0/0/0	0/0/0
	BBN	04/05/04	29	17.2/14.8/2.6	0/0/0	0/0/0	17.2/4.2/0.7	6.9/2.0/0.1
	NB	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	DV	02/03/04	10	30/30/9	0/0/0	0/0/0	0/0/0	10.0/1.0/0.1
Winter 04	CHEB	02/26/04 & 03/30/04	32	9.4/29.7/2.8	3.3/9/0.3	0/0/0	3.1/7/0.2	18.8/6.5/1.2
	BBN	05/26/04	30	56.7/40.4/22.9	16.7/10/1.7	0/0/0	40/8.8/3.5	53.3/8.9/4.7
	NB	06/04/04	30	13.3/4.5/0.6	3.3/38/1.3	0/0/0	33.3/2.3/0.8	0/0/0
	DV	05/29/04	30	46.7/12.9/6	3.3/9/0.3	0/0/0	53.3/6.2/3.3	36.7/26.9/9.9
Spring 04	CHEB	06/15/04	20	75/47.2/35.4	0/0/0	0/0/0	65/7.5/4.9	85.0/72.5/61.7
	BBN	08/24/04	22	59.1/28.5/16.8	9.1/6/0.5	4.5/2/0.1	0/0/0	4.5/3.0/0.1
	NB	09/11/04	20	0/0/0	15/3/0.5	0/0/0	0/0/0	0/0/0
	DV	08/28/04	20	80/19.6/15.7	0/0/0	0/0/0	10/1.5/0.2	10.0/18.5/1.9
Summer 04	CHEB	09/24/04	30	60/13.9/8.4	3.3/6/0.2	0/0/0	6.7/1.5/0.1	23.3/11.4/2.7
	BBN	11/03/04	26	80.8/19.6/15.8	0/0/0	0/0/0	0/0/0	26.9/7.0/1.9
	NB	10/27/04	30	33.3/20.2/6.7	0/0/0	0/0/0	3.3/1/0.03	10.0/2.7/0.3
	DV	10/27/04	30	60/16.7/10	3.3/5/0.2	0/0/0	10/1.3/0.1	30.0/9.6/2.9
Fall 04	CHEB	11/12/04	26	0/0/0	0/0/0	0/0/0	0/0/0	11.5/27.3/3.2
	BBN	02/04/05	16	31.3/2.8/0.9	0/0/0	0/0/0	0/0/0	6.3/1.0/0.1
	NB	02/12/05	30	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
	DV	01/07/05	30	0/0/0	0/0/0	0/0/0	0/0/0	43.3/9.6/4.2
Winter 05	CHEB	03/20/05	15	0/0/0	0/0/0	0/0/0	13.3/2.5/0.3	0/0/0
	BBN	05/24/05	30	66.7/11.9/7.9	0/0/0	0/0/0	36.7/1.5/0.5	20.0/6.2/1.2
	NB	06/01/05	30	6.7/1/0.1	3.3/1/0.03	0/0/0	43.3/1.9/0.8	0/0/0
	DV	06/07/05	30	83.3/65.7/54.7	33.3/7.9/2.6	0/0/0	43.3/13/5.6	90.0/30.9/27.8
Spring 05	CHEB	06/02/05	30	83.3/70.3/58.6	0/0/0	0/0/0	26.7/5.9/1.6	100.0/85.2/85.2

Table 3.2. Cont.

Season	Site	Date	Sample size	Acanthocephalus	Neoechinorhynchus	Echinorhynchus	Botheriocephalus sp.	Cystidicola
	BBN	08/08/05	30	6.7/12.5/0.8	0/0/0	3.3/15/0.5	3.3/14/0.5	13.3/1.8/0.2
	NB	08/23/05	30	0/0/0	0/0/0	0/0/0	6.7/4.5/0.3	0/0/0
	DV	08/23/05	30	76.7/17.6/13.5	16.7/2.2/0.4	0/0/0	10/1.3/0.1	56.7/16.5/9.3
Summer 05	CHEB	08/26/05	28	67.9/71.7/48.7	3.6/6/0.2	28.6/5/1.4	10.7/1.7/0.2	71.4/46.8/33.4
	BBN	11/03/05	30	40/5.7/2.3	0/0/0	0/0/0	0/0/0	3.3/2.0/0.1
	NB	10/28/05	30	10/1.3/0.1	0/0/0	0/0/0	0/0/0	10.0/1.3/0.1
	DV	10/26/05	30	66.7/13.7/9.1	0/0/0	0/0/0	0/0/0	0/0/0
Fall 05	CHEB	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	BBN	01/31/06	30	0/0/0	0/0/0	0/0/0	0/0/0	10.0/1.7/0.2
	NB	03/28/06	23	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
	DV	01/12/06	30	0/0/0	0/0/0	0/0/0	0/0/0	46.7/5.5/2.6
Winter 06	CHEB	01/12/06	30	0/0/0	0/0/0	0/0/0	0/0/0	63.3/12.5/7.9
	BBN	05/24/05	30	23.2/19.4/4.5	33.3/7.5/2.5	20/4.2/0.8	33.3/8.9/3	10.0/5.3/0.5
	NB	06/01/05	30	0/0/0	23.3/4.9/1.1	0/0/0	10/10.3/1	3.3/1.0/0.0
	DV	06/07/05	30	26.7/6.8/1.8	23.3/12.7/3	10/8/0.8	13.3/15/2	66.7/13.1/8.7
Spring 06	CHEB	06/02/05	30	26.7/5.4/1.4	56.7/8.2/4.6	53.3/5.1/2.7	3.3/1/0.03	90.0/30.6/27.5
	BBN	08/08/05	30	0/0/0	16.7/2.8/0.5	3.3/3/0.1	0/0/0	6.7/2.0/0.1
	NB	08/23/05	30	0/0/0	0/0/0	0/0/0	0/0/0	0/0/0
	DV	08/23/05	30	23.3/1.3/0.3	66.7/9.8/6.5	63.3/4.5/2.8	6.7/2.5/0.2	80.0/30.3/24.2
Summer 06	CHEB	08/26/05	30	3.3/1/0.03	16.7/3.4/0.6	13.3/2.5/0.3	0/0/0	70.0/80.0/5.6

Table 3.3: Simpson index, Shannon's diversity index, Berger-Parker dominance index, richness, and dominant species of Lake Whitefish metazoan endoparasites from Lakes Michigan and Huron.

Season	Site	Sample	Simpson	Shannon	Richness	Berger-Parker	Dominant
Fall 2003	Bay De Noc	35	1.50	0.63	2	0.05	*A. dirus*
	Naubinway	34	1.88	0.93	2	0.01	*N. tumidus*
	De Tour village	31	0	0	0	0	-
	Cheboygan	30	1	0	1	0.011	*A. dirus*
Winter 2004	Bay De Noc	10	2	1	3	0.3	*C. truncates*
	Naubinway	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]
	De Tour village	8	1.97	0.98	2	0.63	*C. truncates*
	Cheboygan	24	1.9	0.81	4	0.05	*A. dirus*
Spring 2004	Bay De Noc	30	1.15	0.21	3	0.2	*A. dirus*
	Naubinway	30	1.77	0.85	3	0.012	*C. truncates*
	De Tour village	30	1.71	0.78	4	0.15	*C. truncates*
	Cheboygan	20	1.88	0.93	3	0.95	*C. truncates*
Summer 2004	Bay De Noc	22	1.1	0.11	4	0.2	*A. dirus*
	Naubinway	20	1	0	2	0.0045	*C. truncates*
	De Tour village	30	1.79	0.87	3	0.10	*A. dirus*
	Cheboygan	20	1.11	0.15	4	0.132	*A. dirus*
Fall 2004	Bay De Noc	30	1	0	1	0.1	*A. dirus*
	Naubinway	30	1.1	0.15	3	0.05	*A. dirus*
	De Tour village	26	1.15	0.20	4	0.1	*A. dirus*
	Cheboygan	26	0	0	0	0	-
Winter 2005	Bay De Noc	16	1	0	2	0.011	*C. truncates*
	Naubinway	30	0	0	1	0	*C. truncates*
	De Tour village	30	0	0	0	0	-
	Cheboygan	15	1	0	2	0.017	*C. truncates*
Spring 2005	Bay De Noc	30	1.6	0.71	3	0.07	*C. truncates*
	Naubinway	30	2.13	0.34	3	0.002	*C. truncates*
	De Tour village	30	2.04	0.95	4	0.63	*A. dirus*
	Cheboygan	30	1.99	0.99	3	0.86	*C. truncates*
Summer 2005	Bay De Noc	30	2.8	1.023	3	0.012	*A. dirus*
	Naubinway	30	0	0	0	0	-
	De Tour village	30	1.33	0.42	4	0.11	*A. dirus*
	Cheboygan	30	1.1	0.10	5	0.31	*A. dirus*
Fall 2005	Bay De Noc	30	1	0	1	0.02	*A. dirus*
	Naubinway	30	1.5	0.6	1	0.001	*A. dirus*
	De Tour village	30	1	0	1	0.061	*A. dirus*
	Cheboygan	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]	[redacted]

Table 3.3. Cont.

Season	Site	Sample size	Simpson Index	Shannon Index	Richness	Berger-Parker	Dominant species
Winter 2006	Bay De Noc	30	0	0	0	0	-
	Naubinway	30	0	0	0	0	-
	De Tour village	30	0	0	0	0	-
	Cheboygan	30	0	0	0	0	-
Spring 2006	Bay De Noc	30	1.36	0.46	5	0.135	*A. dirus*
	Naubinway	30	1.95	0.59	3	0.007	*B*. sp.
	De Tour village	30	1.88	0.87	5	0.39	*A. dirus*
	Cheboygan	30	1.94	0.96	4	0.905	*A. dirus*
Summer 2006	Bay De Noc	30	1.95	0.57	4	0.106	*A. dirus*
	Naubinway	30	0	0	0	0	-
	De Tour village	30	1.56	0.65	5	0.105	*A. dirus*
	Cheboygan	30	1.10	0.13	4	0.221	*A. dirus*

Table 3-4. Prevalence of *Carnobacterium maltaromaticum* infections in lake whitefish by site and season throughout the course of the study. (Nov. 2003-Aug. 2006).

Sampling Period	Big Bay de Noc	Naubinway	Cheboygan	Detour	Season Total
Fall 2003	0/35 (0%)	0/30 (0%)	0/30 (0%)	0/34 (0%)	
Fall 2004	0/26 (0%)	0/30 (0%)	0/26 (0%)	0/30 (0%)	0/331 (0%)
Fall 2005	0/30 (0%)	0/30 (0%)	0	0/30 (0%)	
Winter 2004	0/29 (0%)	0	0/32 (0%)	0/10 (0%)	
Winter 2005	0/16 (0%)	10/30 (33.33%)	0/15 (0%)	0/30 (0%)	14/275 (5.09%)
Winter 2006	0/30 (0%)	1/23 (4.35%)	0/30 (0%)	3/30 (10.0%)	
Spring 2004	0/30 (0%)	0/30 (0%)	0/20 (0%)	0/30 (0%)	
Spring 2005	0/30 (0%)	2/30 (6.67%)	0/30 (0%)	2/30 (6.67%)	8/350 (2.29%)
Spring 2006	1/30 (3.33%)	0/30 (0%)	0/30 (0%)	2/30 (6.67%)	
Summer 2004	0/22 (0%)	0/20 (0%)	0/30 (0%)	0/20 0%)	
Summer 2005	0/30 (0%)	0/30 (0%)	0/28 (0%)	1/30 (3.33%)	1/330 (0.30%)
Summer 2006	0/30 (0%)	0/30 (0%)	1/30 (3.33%)	0/30 (0%)	
Site Total	**1/338 (0.30%)**	**13/313 (4.15%)**	**1/301 (0.33%)**	**8/334 (2.40%)**	**23/1286 (1.79%)**

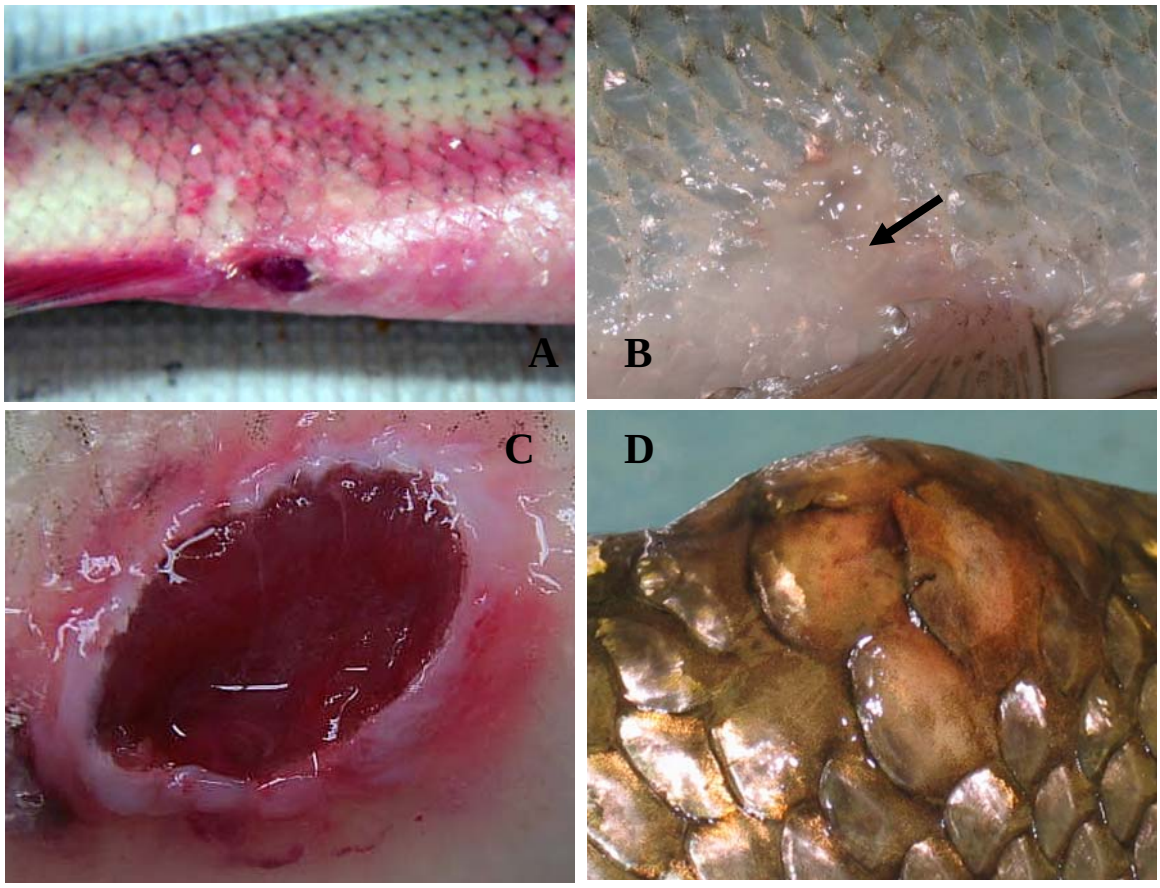

Figure 3.1. External lesions observed on lake whitefish collected from the four sites

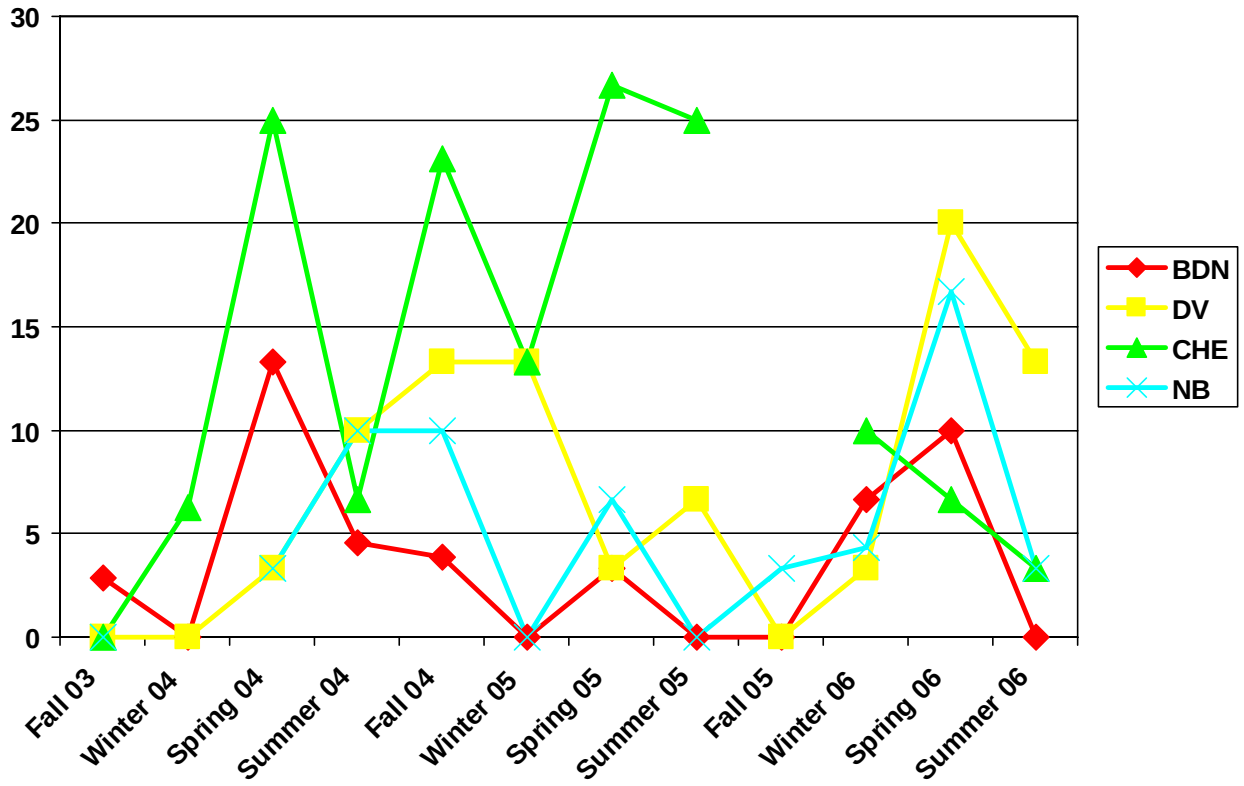

Figure 3.2. Incidence of external lesions (combined) in lake whitefish.

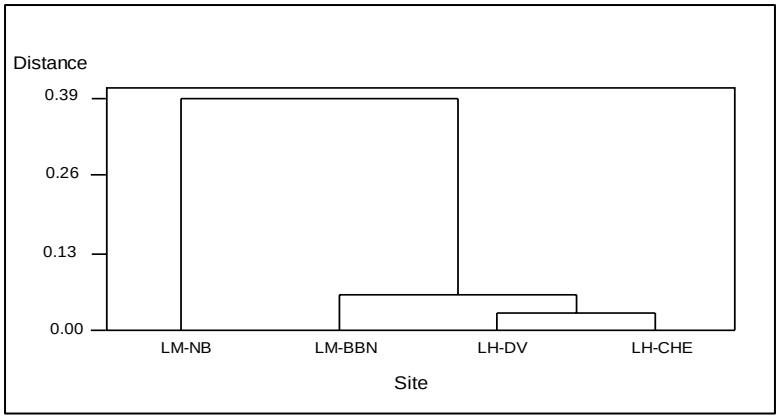

Figure 3.3. Dendogram of the cluster analysis (using the Euclidean distance and the weighted pair group mean average method) using the prevalence data of the five parasite species found in Lake Whitefish (2003-2006).

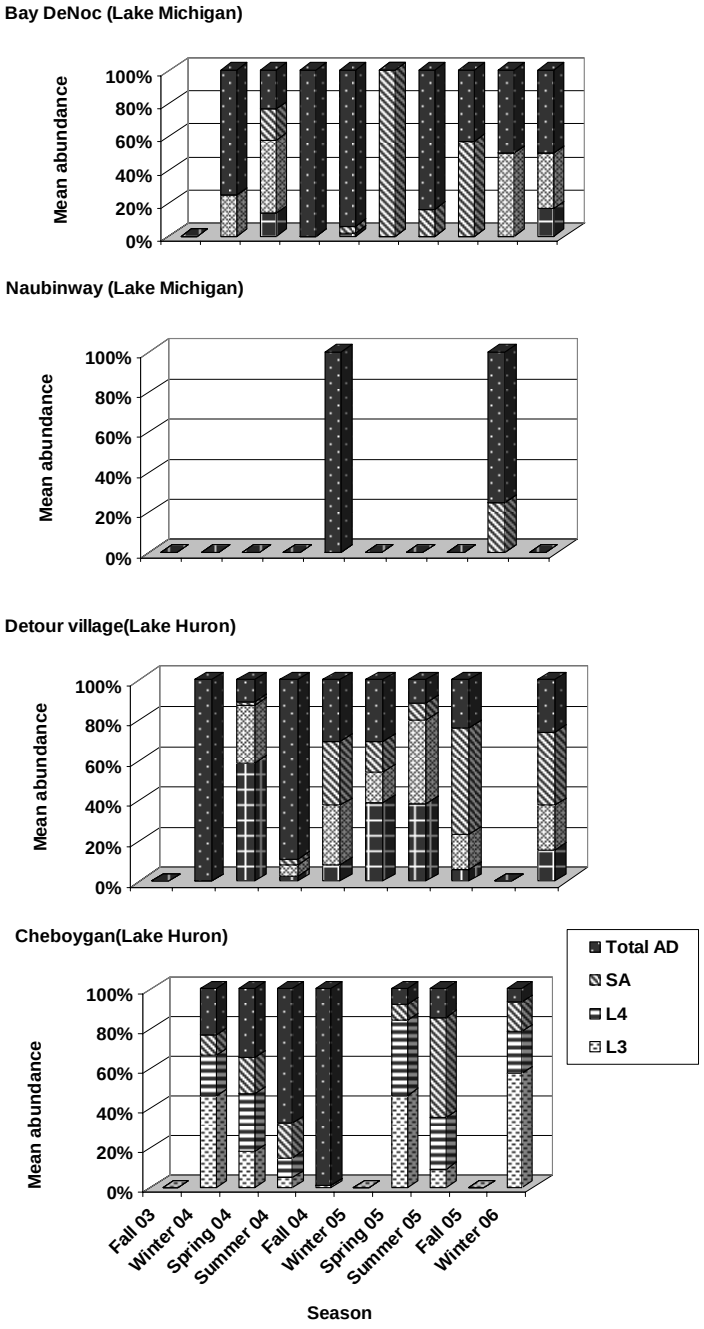

Figure 3.4. Fluctuations in the abundance of each of *Cystidicola farionis* larval stages: Third larval stage (L3); fourth larval stage(L4); sub adults(SA); and total adults (TA) per season and sampling site. *Cystidicola farionis* prevalence is in Table 3-2.

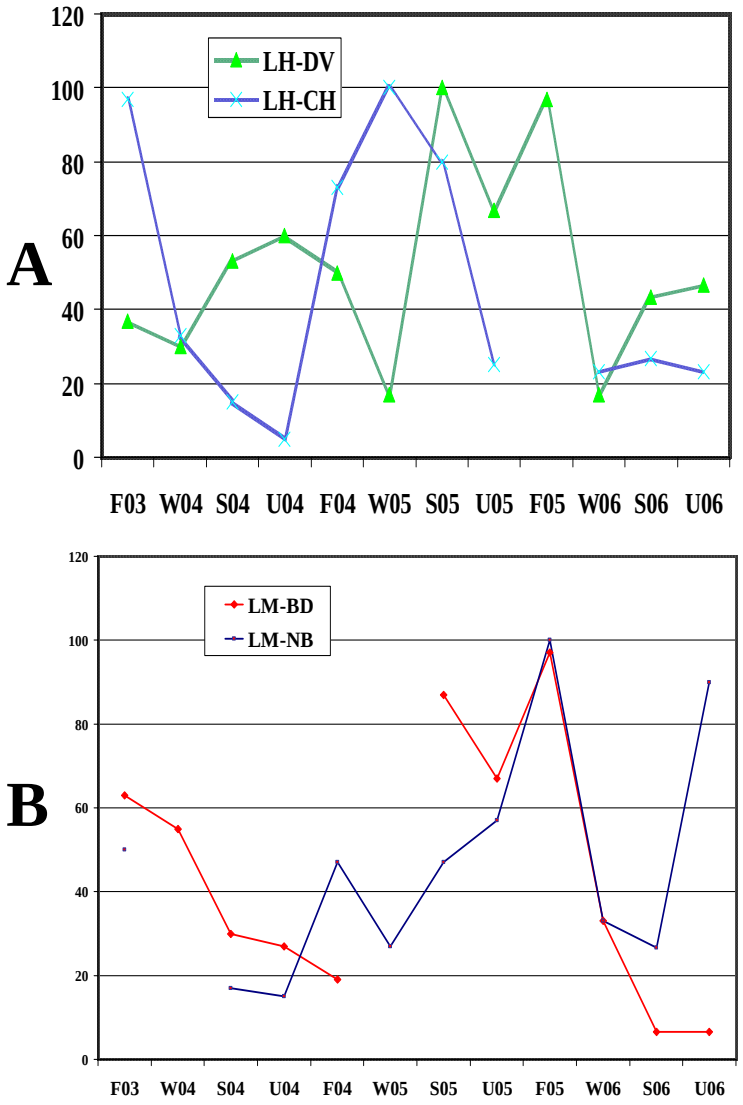

Figure 3.5. Prevalence *Renibacterium salmoninarum* in lake whitefish by quantitative ELISA.

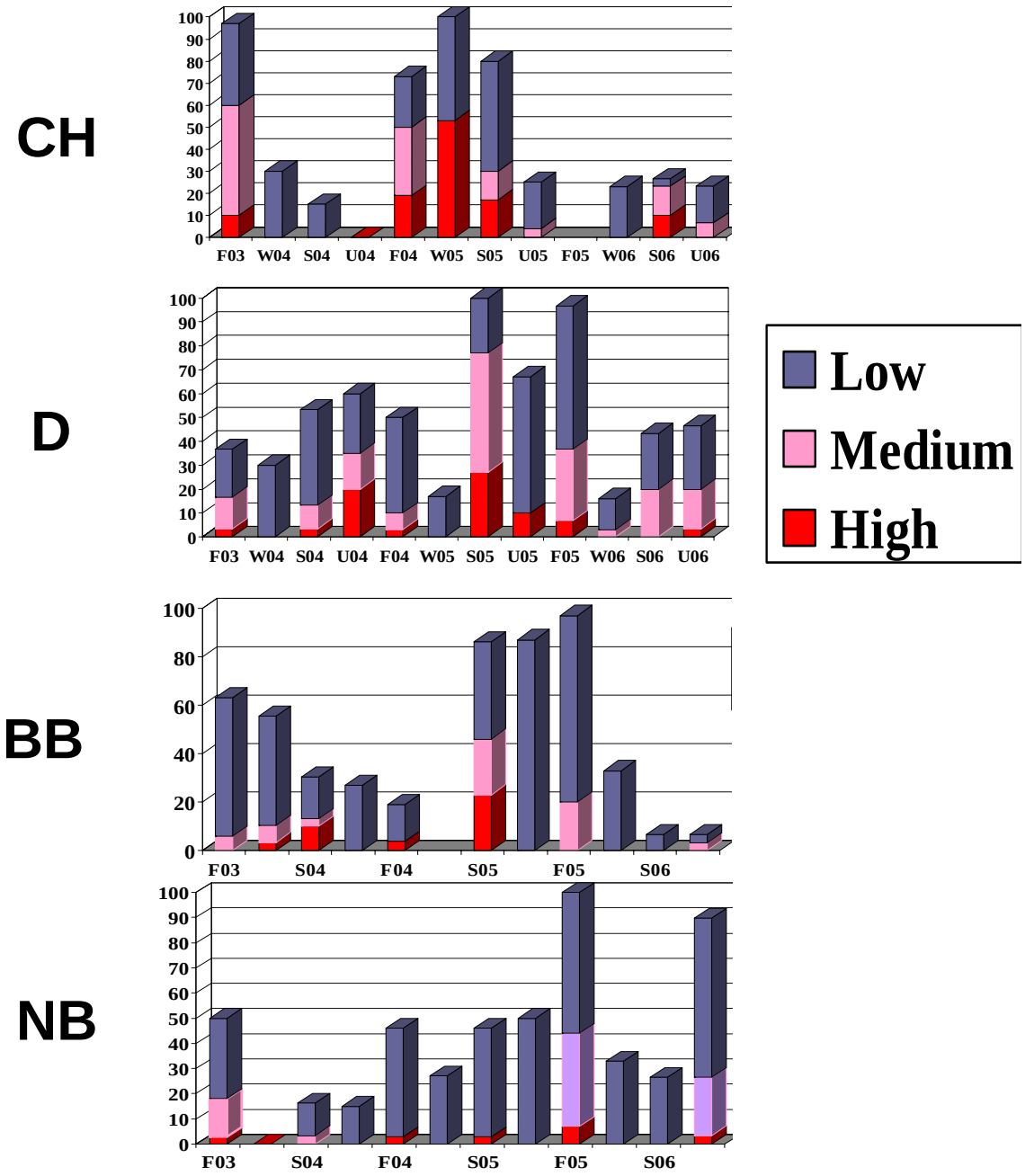

Figure 3.6. Prevalence and intensity of *Renibacterium salmoninarum* in lake whitefish by quantitative ELISA. CHE: Cheboygen, DV: Detour, BBN: Big Bay DeNoc, NB: Naubenway.

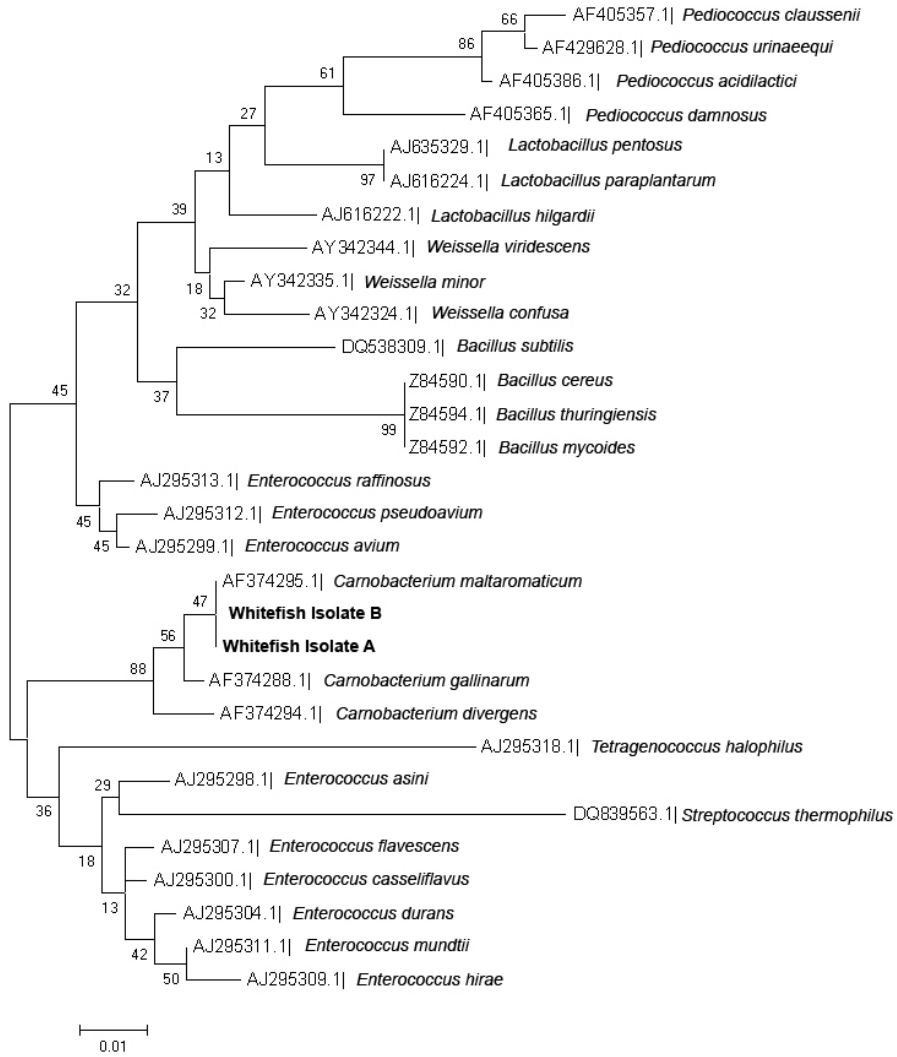

Figure 3.7. A phylogenetic tree of *C. maltaromaticum* isolates retrieved from lake whitefish based upon portions of the 16S rRNA and 23S rRNA genes. Generated sequences were analyzed using the BLASTn software from the National Center for Biotechnology Information, (NCBI, Bethesda, MD) to detect homologous sequences. Homology of the generated sequences with that of the database was assessed using the nucleotide database from NCBI. Using the CLUSTAL W program from Molecular Evolutionary Genetics Analysis (MEGA) 3.1., a phylogenetic tree was constructed using the Neighbor-Joining method as the bootstrap test of phylogeny. Accession numbers from NCBI precede scientific names.

PART 4 – INTEGRATED DATA ANALYSIS

Methods

Partitioning variation in fish health indicators

We used mixed models to examine how the total variation in fish health indicators was partitioned among spatial and temporal sources. The spatial and temporal components of variation that we quantified 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 that was due to independent seasonal or annual variation among stocks, seasons, and years. The mixed model used to partition the total variation was:

$$y_{i,j,k,l} = u + \alpha_j + \upsilon_l + \eta_{j,k} + \pi_{j,l} + \tau_{k,l} + e_{i,j,k,l} \quad (1)$$

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 in season k, $k = 1\ldots,4$, where seasons correspond to season of sampling and include spring, summer, fall, and winter, 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 $N\left(\sigma_\alpha{}^2\right)$; υ_l is a random effect for the l^{th} year, iid as $N\left(\sigma_\upsilon{}^2\right)$; $\eta_{j,k}$ is a random effect for stock j in season k, iid as $N\left(\sigma_\eta{}^2\right)$; $\pi_{j,l}$ is a random effect for stock j in year l, iid as $N\left(\sigma_\pi{}^2\right)$; $\tau_{k,l}$ is a random effect for season k in year l, iid as $N\left(\sigma_\tau{}^2\right)$; and $e_{i,j,k,l}$ is the residual variation, iid as $N\left(\sigma_e{}^2\right)$. 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 the total variability in fish health indicators, mixed models were fit that included fixed effects to explain variation that was partitioned into different spatial and temporal components. Specifically, we were interested in explaining variation among individual fish, stocks, and years. Fixed effects were examined to explain variation among individual fish, stocks, and years in fish health indicators are shown in Table 4.1. The analyses were performed using the following steps. First, variance components, estimated using restricted maximum likelihood as described above, were identified and significant parameters 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 that we used was:

$$(2)\ y_{i,j,k,l} = u + \sum_{k=1}^{3} \beta_k season + \sum_{r=0}^{R} \varphi + \sum_{f=0}^{F} \theta_{i,j,k,l} + \sum_{s=0}^{S} \lambda_i + \sum_{b=0}^{B} \xi_l + e_{i,j,k,l}$$

Where y and u are as defined above, β_k is the estimated fixed effect for season k, k=0…,3, φ is a random effect described in equation 1, 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.

Natural mortality and fish health indicators

To examine patterns between natural mortality estimates for each stock 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 fatty acids and percent lipids and water analyses that had significant variation among stocks. The prevalence and intensity of infection for fish pathogens were also plotted against natural mortality estimates. BLUPs were not plotted for pathogens because unlike other health indicators, prevalence and intensity were point estimates calculated for each stock and not modeled using mixed models. Because very little is know 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 any specific hypothesis.

Multivariate stock differentiation

We used classification and regression tree analyses (CART) to determine if select fatty acids could serve as biomarkers to differentiate among lake whitefish stocks in upper Lakes Michigan and Huron. CART is a non-parametric method that does not assume any specific distribution of the data and thus is not influenced by data transformations, nor are the results influenced by outliers. The CART procedure operates by recursive partitioning of the dataset into subsets that are most homogenous in terms of the response variable. Each split is made at a particular value of the explanatory variable (e.g., a value of a fatty acid). The terminal nodes of the tree (leaves) are the end product of classification. One of the concerns when using CART is finding good splits and knowing when to stop splitting the tree to avoid over-fitting the data. To address these concerns we examined plots of a complexity parameter, which is a result of a cross-validation procedure, to determine at which size the tree should stop splitting.

We performed CART analyses using fish sampled in the last two years of the study. Fish from the first year of sampling were excluded because of the pooling of fish that occurred. Pooled samples were not appropriate for examining how useful fatty acid profiles of individual fish were for differentiation among stocks. CART analyses were performed separately for each sex and for each season of sampling. The rationale for separate analyses was that we expected differences among male and female fish (e.g., reproductive condition would influence the fatty acid profiles of females), and during different seasons of the year. We combined fish across years, however, because we were interested in differentiating among stocks despite possible

inter-annual variability in fatty acid profiles. All of the select fatty acids and ratios we examined, from all tissue types, and both methods of reporting were included as potential predictor variables in the analysis. To assess misclassification rates (percent misclassified), a training data set was created by randomly selecting 2/3 of the fish from each sex/season data set. A CART model was then fit to the training data set. The remaining 1/3 of the fish were used as a test data set, and the stock of origin for each fish in the test data set was predicted using the CART model developed with the training data set.

Results

Partitioning variation in fish health indicators

Because we were primarily interested in quantifying stock-to-stock and year-to-year variation (specifically the random year main effect: year-to-year variation that affects all stocks in a similar manner), we focused on health indicators that had significant stock and/or year variance components. Of the 41 fish health indicators examined, 11 exhibited significant variation among stocks and 14 demonstrated significant temporal variation (Table 4.2). Indicators with significant stock and year effects included whole-fish percent water and several fatty acids from all three tissue types (muscle, eye, and liver). However, the total variation among stocks was small and ranged from 2% of the total variation for percent lipids in eye tissue to 16% of the total variation for liver ALA concentrations (Figures 4.1-4.6 show components of variation for health indices that exhibited significant stock and/or yearly variation). The proportion of the total variation attributed to annual variation ranged from 8% for the DHA/ARA ratio in muscle tissue (measured as both μg/mg dry weight of tissue extracted and μg/mg polar lipid) and palmitoleic acid in muscle tissue (measured as μg/mg polar lipid) to 52% of the total variation for DHA in muscle tissue (measured as μg/mg polar lipid). For most health indicators, residual variation comprised a majority of the total variation ranging from 34 to 85% of the total variation (Figures 4.1-4.6).

Stock effect

Plots of the best linear unbiased predictors (BLUPs) of the stock and year effects revealed both spatial and temporal patterns in fish health indicators. Percent total lipids in the eye tissue and percent water in whole-fish homogenates exhibited significant stock variation. For percent total lipids in eye tissues, Big Bay de Noc and Detour stocks tended to have higher estimates compared to Naubinway and Cheboygan (Figure 4.7). Patterns in percent water, however, suggested a lake-effect where Big Bay de Noc and Naubinway fish (Lake Michigan) tended to have lower percent water compared to Cheboygan and Detour (Lake Huron; Figure 4.7).

Plots of BLUPs for fatty acid stock effects revealed that Naubinway fish tended to differ from the other three stocks in terms of the select fatty acids we examined. This pattern was evident in all samples from all three tissue types and tissue fraction extracted (Figures 4.8-4.9). For example, Naubinway fish tended to have higher palmitoleic acid concentrations in muscle and eye tissues, a lower DHA/ARA ratio in both muscle and eye tissues, and lower ALA in liver tissues compared to the other stocks (Figures 4.8-4.9).

Year effect

Total percent lipids (for both whole-fish and tissue-specific measures) and percent water did not demonstrate significant year effects; however, year effects were evident for several fatty

acids. For example, for the fatty acids measured as µg/per mg dry weight tissue extracted, DHA (both from muscle and liver tissue), EPA, DHA/ARA ratio, and Palmitoleic acid (all from muscle tissue) demonstrated significant year effects (Figure 4.10). Mean levels of the highly unsaturated fatty acid DHA exhibited declines of 33% in muscle tissue and 19% in liver tissue over the three period of this study. It is worth noting that DHA in the eye tissue did not show a significant year effect. EPA also exhibited a substantial decline, with annual averages declining by 27%. Trends for the DHA/ARA ratio and Palmitoleic acid were less pronounced.

Fatty acids measured from the polar lipid fraction (per mg polar lipid) that demonstrated significant year effects included DHA (muscle and eye tissue), EPA (muscle tissue), the DHA/ARA ratio (muscle tissue), Palmitoleic acid (muscle and eye tissue), and ARA (eye tissue; Figure 4.11). When trends were present, fatty acid concentrations decreased over time. For example, mean DHA declined by 35% and 36% in muscle and eye tissue, respectively. The temporal pattern for EPA in muscle and eye tissue was similar to that of DHA, with mean concentrations decreasing by 45% and 30%, respectively. Mean values of the unsaturation index, which is greatly influenced by DHA, also declined over the three year study from 367 ± 1.2 in year one to 321 ± 0.94 in year three. Mean palmitoleic acid declined in the muscle and eye tissues declining 38% and 37%, respectively. Mean ARA decline in the eye tissue by 21%, while the decline in the mean DHA/ARA ratio of muscle tissue was less pronounced, declining by 15%.

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 – 77% for linoleic acid and ALA in liver tissue measured as per mg dry weight tissue extracted, respectively. The covariates that explained variation among fish, stocks, and years, and the direction of the effects varied among health indicators (Appendix 4.1).

Seasonal patterns

Seasonal patterns (i.e., a significant season main effect) were evident for whole-fish measures of percent lipids and water and for five fatty acids. The fatty acids included EPA in muscle tissue measured as both µg/mg dry weight of tissue extracted and µg/mg polar lipid, palmitoleic acid, ALA, and the DHA/ARA ratio in liver tissue, and linoleic acid in muscle tissue measured as µg/mg dry weight of tissue extracted, and ALA in muscle tissue measured as µg/mg polar lipid (Appendix 4.1). As expected, seasonal patterns of percent lipids and water were characterized by seasonal lows in percent lipid levels and correspondingly seasonal highs in percent water levels in the winter (winter percent lipids least-squares means (LSM) = 16.3% ± 0.97) and winter percent water = 75.6% ± 0.32). Highest percent lipids 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 = 74.6 ± 0.32 in summer and 74.1 ± 0.32 in fall for percent water and 17.1% ± 0.97 in summer 17.8% ± 0.98 in fall for percent lipids).

Stock covariates

Of the health indicators with significant variation among stocks (i.e., a significant stock random effect; Table 2), three were significantly correlated with either average stock intensity or prevalence of *Cystidicola* or prevalence of *R. salmoninarum* (Appendix 4.1). It is important to

keep in mind that although we are explaining variation among stocks, the total variation we are explaining is a small proportion of the total variation that exists in these fish health indicators (i.e., stock variation ranges from 2-16% of the total variation). Variation in percent water among stocks was positively correlated with *Cystidicola* intensity of infection, with Lake Michigan stocks (Big Bay de Noc and Naubinway) having lower percent water and lower *Cystidicola* intensity of infection, while Lake Huron stocks (Detour and Cheboygan) characterized by higher percent water and higher *Cystidicola* intensity of infection (Figure 4.12). Stock effects for palmitoleic acid in muscle tissue were negatively correlated with *Cystidicola* prevalence, including palmitoleic acid measured as µg/mg dry weight of tissue extracted and µg/mg polar lipid (Figure 4.13). The relationship between palmitoleic acid and *Cystidicola* prevalence also highlights the differences among lakes, with Lake Michigan stocks having higher palmitoleic acid concentrations and lower *Cystidicola* prevalence rates compared to Lake Huron stocks. Lastly, stock effects for ALA in liver samples (measured as µg/mg dry weight of tissue extracted) were negatively correlated with *R. salmoninarum* prevalence (Figure 4.14). Differences among Lakes Michigan and Huron were not evident in this relationship, with Cheboygan and Big Bay de Noc stocks having lower prevalence and higher ALA concentrations, while Detour and Naubinway stocks having higher *R. salmoninarum* prevalence and lower ALA concentrations.

Year covariates

Variation among years in four health indicators was correlated with either *Cystidicola* intensity or prevalence. Year effects for the DHA/ARA ratio in muscle tissue (measured as both µg/mg dry weight of tissue extracted and µg/mg polar lipid) were positively correlated with average annual *Cystidicola* intensity of infection (Figure 4.15). Over the three year study, the third year (fall 2005 – summer 2006) was associated with the lowest DHA/ARA ratio, on average, and the lowest *Cystidicola* intensity. Annual concentrations of the highly unsaturated fatty acids EPA and DHA demonstrated negative trends over time, and these year effects were correlated with annual *Cystidicola* prevalence (Figures 4.16 and 4.17). DHA in the muscle measured as µg/mg polar lipid and in the liver measured as µg/mg dry weight of tissue extracted demonstrated similar relationships with *Cystidicola* prevalence. DHA in muscle tissue measured as µg/mg dry weight of tissue extracted also demonstrated a negative relationship with *Cystidicola* prevalence, but was not statistically significant at $\alpha = 0.05$ ($P = 0.07$). The annual decline in EPA in both the muscle (including that measured as µg/mg dry weight of tissue extracted and µg/mg polar lipid) and eye tissues (measured as µg/mg polar lipid) showed a similar negative correlation with *Cystidicola* prevalence to that observed with DHA (Figure 4.18).

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 and versus pathogen prevalence and intensity of infection (Figures 4.19 – 4.23). The small number of stocks included in this study reduced our ability to identify any potential relationships. In addition, uncertainty in both the natural mortality estimates and BLUPs prevented any generalizations from being made.

Multivariate stock differentiation

There was moderate-to-low ability to discriminate among lake whitefish stocks using fatty acids, however the fatty acids important in discriminating among stocks varied by sex and season of the year (Figures 4.24 and 4.25). In addition, the misclassification rate varied considerably among stocks (averaged over seasons) from 31 – 77% for male fish sampled from Cheboygan and Big Bay de Noc, respectively, and from 29 – 93% for female fish sampled from Naubinway and Cheboygan, respectively. Seasonal misclassification rates (averaged across stocks) ranged from 44% for male fish sampled in the fall to 60% for male fish sampled in the summer, and from 45% for female fish sampled in the summer to 60% for female fish sampled in the spring (Table 4.3).

Table 4.1. 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[1]	*Cystidicola farionis* prevalence[1]
Sex	*Cystidicola farionis* intensity[2]	*Cystidicola farionis* intensity[2]
Weight (g)	*Renibacterium salmoninarum* prevalence[1]	*Renibacterium salmoninarum* prevalence[1]
Percent lipids (whole fish)		
Percent water (whole fish)		
Tissue-specific percent lipids		

[1]Prevalence was calculated as the number of infected fish divided by the total number of fish.
[2]Intensity was calculated as the total number of *Cystidicola* divided by the number of infected fish.

Table 4.2. P values for spatial and temporal components of variance for selected fatty acids (FAs), tissue-specific and whole fish percent lipids, whole fish percent water for four lake whitefish stocks in Lakes Huron and Michigan. 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 FAs from three tissue types including muscle, eye, and liver tissues. Muscle and eye samples are measured as μg FA/mg dry weight of tissue extracted and μg FA/mg polar lipid. Liver samples are measured as μg FA /mg dry weight of tissue extracted. Significant P values ($P < 0.10$) are shown in bold.

	Variance component				
Response variable	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
% lipids (eye)	0.034	0.5	0.15	0.5	<0.0001
% lipids (liver)	0.16	0.5	**<0.0001**	0.5	**<0.0001**
% water (whole fish)	**0.038**	0.5	**<0.0001**	**<0.0001**	**<0.0001**
Per mg dry weight of tissue extracted					
DHA eye	0.16	0.5	**<0.0001**	0.5	**<0.0001**
DHA muscle	0.5	**<0.0001**	**<0.0001**	**0.001**	**<0.0001**
DHA liver	0.35	**0.07**	**<0.0001**	**0.0005**	**<0.0001**
EPA eye	0.27	0.34	**<0.0001**	**0.001**	**<0.0001**
EPA muscle	0.49	**0.016**	**<0.0001**	**0.0005**	**0.0028**
EPA liver	0.17	0.12	**<0.0001**	0.31	**0.0001**
DHA/ARA eye	**0.085**	0.5	**<0.0001**	0.5	**<0.0001**
DHA/ARA muscle	**0.028**	**0.089**	**<0.0001**	**<0.0001**	**<0.0001**
DHA/ARA liver	0.50	0.30	**<0.0001**	**<0.0001**	**<0.0001**
Palmitoleic acid eye	**0.011**	0.5	**0.0004**	**0.073**	**<0.0001**
Palmitoleic acid muscle	**0.003**	**0.009**	**<0.0001**	**0.024**	**0.027**
Palmitoleic acid liver	0.14	0.5	**<0.0001**	**0.002**	**<0.0001**
ARA eye	0.27	0.5	**<0.0001**	**0.004**	**<0.0001**
ARA muscle	0.18	0.23	**<0.0001**	**<0.0001**	**<0.0001**
ARA liver	0.33	0.13	**<0.0001**	**<0.0001**	**<0.0001**
Linoleic acid eye	0.46	0.5	**<0.0001**	0.45	**<0.0001**
Linoleic acid muscle	**0.09**	0.5	**<0.0001**	**<0.0001**	**0.0028**
Linoleic acid liver	0.23	0.39	**<0.0001**	**0.03**	**<0.0001**
ALA eye	0.24	0.29	**0.003**	**0.005**	**<0.0001**
ALA muscle	0.5	0.5	**<0.0001**	**0.003**	**<0.0001**
ALA liver	**0.02**	0.42	**<0.0001**	**0.018**	**<0.0001**
Per mg polar lipid					
Unsaturation index	**0.01**	**0.0002**	**0.008**	**0.048**	**<0.0001**
DHA eye	0.12	**0.001**	**<0.0001**	0.12	**<0.0001**
DHA muscle	0.38	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
EPA eye	0.43	**0.018**	**<0.0001**	**<0.0001**	**<0.0001**
EPA muscle	0.5	**0.0009**	**<0.0001**	**0.001**	0.082

DHA/ARA eye	**0.074**	0.5	**<0.0001**	0.5	**<0.0001**
DHA/ARA muscle	**0.028**	**0.09**	**<0.0001**	**<0.0001**	**<0.0001**
Palmitoleic acid eye	0.11	**0.049**	**<0.0001**	**<0.0001**	**<0.0001**
Palmitoleic acid muscle	**0.005**	**0.003**	**<0.0001**	**0.009**	**0.035**
ARA eye	0.42	**0.084**	0.46	**0.005**	**<0.0001**
ARA muscle	0.45	0.5	**<0.0001**	**0.004**	**<0.0001**
Linoleic acid eye	0.5	0.13	**<0.0001**	0.5	**<0.0001**
Linoleic acid muscle	0.16	0.4	**<0.0001**	**0.0009**	**<0.0001**
ALA eye	0.48	0.38	**<0.0001**	**<0.0001**	**<0.0001**
ALA muscle	0.24	0.17	**<0.0001**	**<0.0001**	**<0.0001**

Table 4.3. Misclassification rates (percent misclassified) from classification and regression tree analyses (CARTs). Separate CARTs were fit for male and female lake whitefish and for each season. Lake whitefish were from four stocks, two from upper Lake Michigan (Big Bay de Noc and Naubinway) and two from upper Lake Huron (Cheboygan and Detour). Fish used in the analysis were sampled from fall 2004 – summer 2005. A training data set was created by randomly selecting 2/3 of the fish from each sex/season data set, while the remaining 1/3 of the fish were used as a test data set to generate misclassification rates.

	Season				
	Fall	Winter	Spring	Summer	
Male					Stock average
Big Bay de Noc	73	100	33	100	77
Naubinway	50	38	64	42	48
Cheboygan	25	0	33	67	31
Detour	30	75	55	30	47
Season average	44	53	46	60	
Female					
Big Bay de Noc	43	50	44	0	34
Naubinway	20	21	50	25	29
Cheboygan	100	100	70	100	93
Detour	38	38	75	53	51
Season average	50	52	60	45	

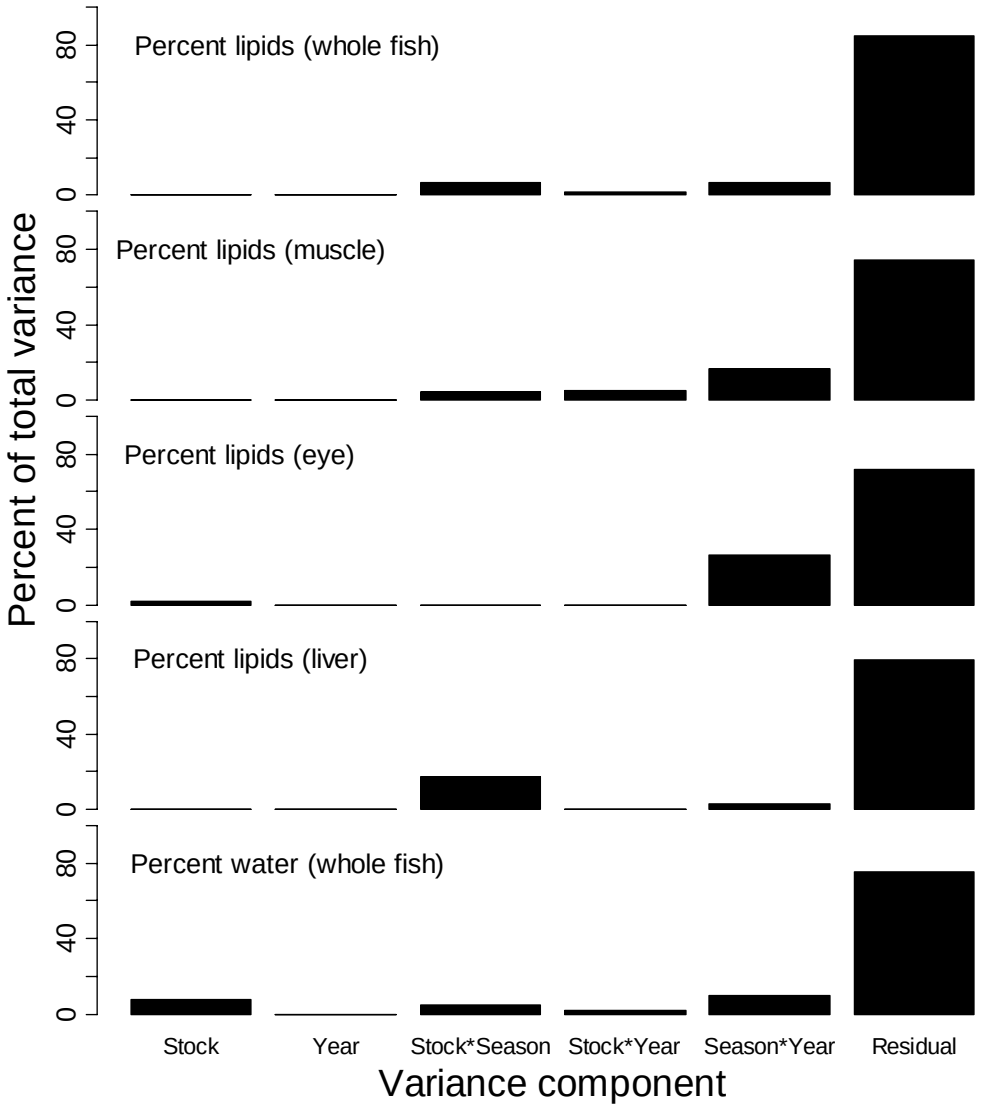

Figure 4.1. The proportion of the total variance in whitefish health indicators due to spatial and temporal factors. Variances were estimated using linear mixed models.

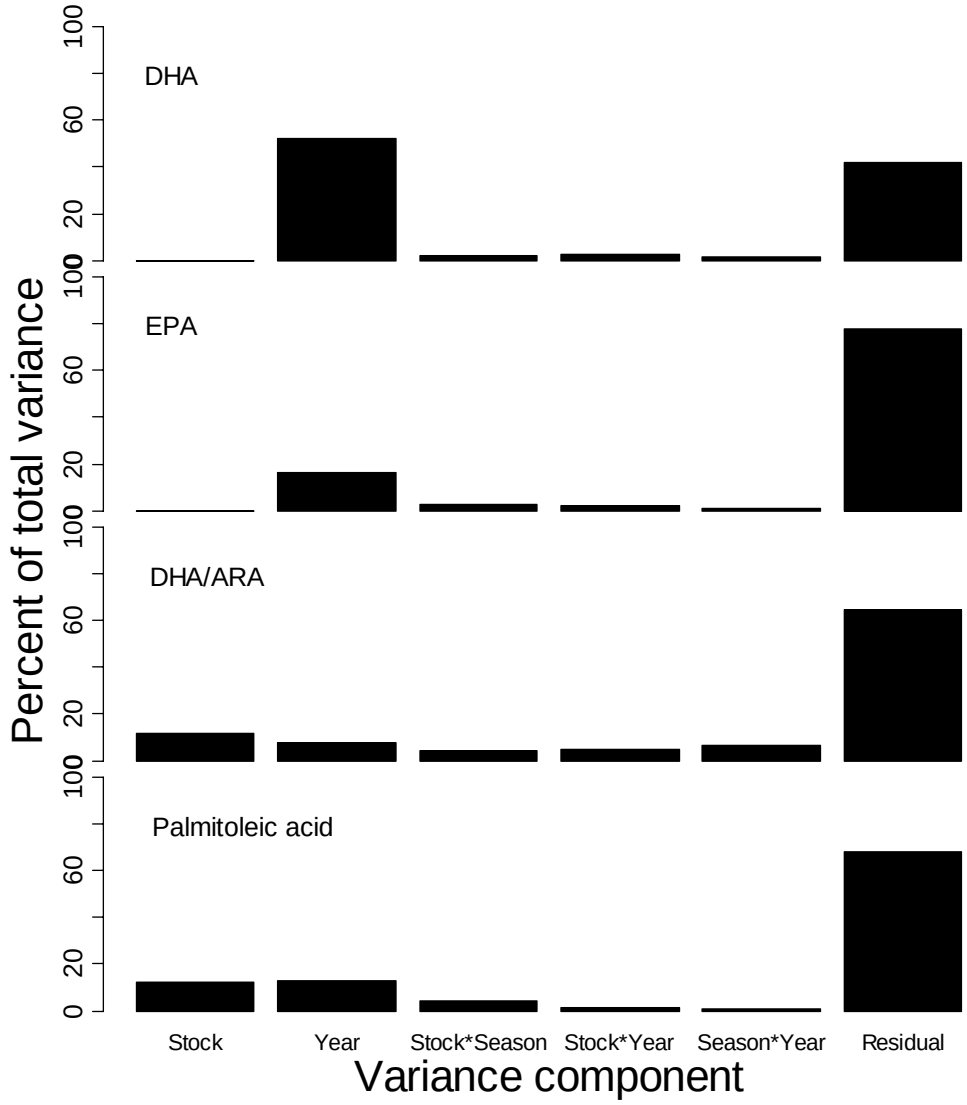

Figure 4.2. The proportion of the total variance in whitefish health indicators due to spatial and temporal factors. Figures are for fatty acids with significant stock and/or year effects. Fatty acids are for muscle samples measured as µg fatty acid/mg polar lipid. Variances were estimated using linear mixed models.

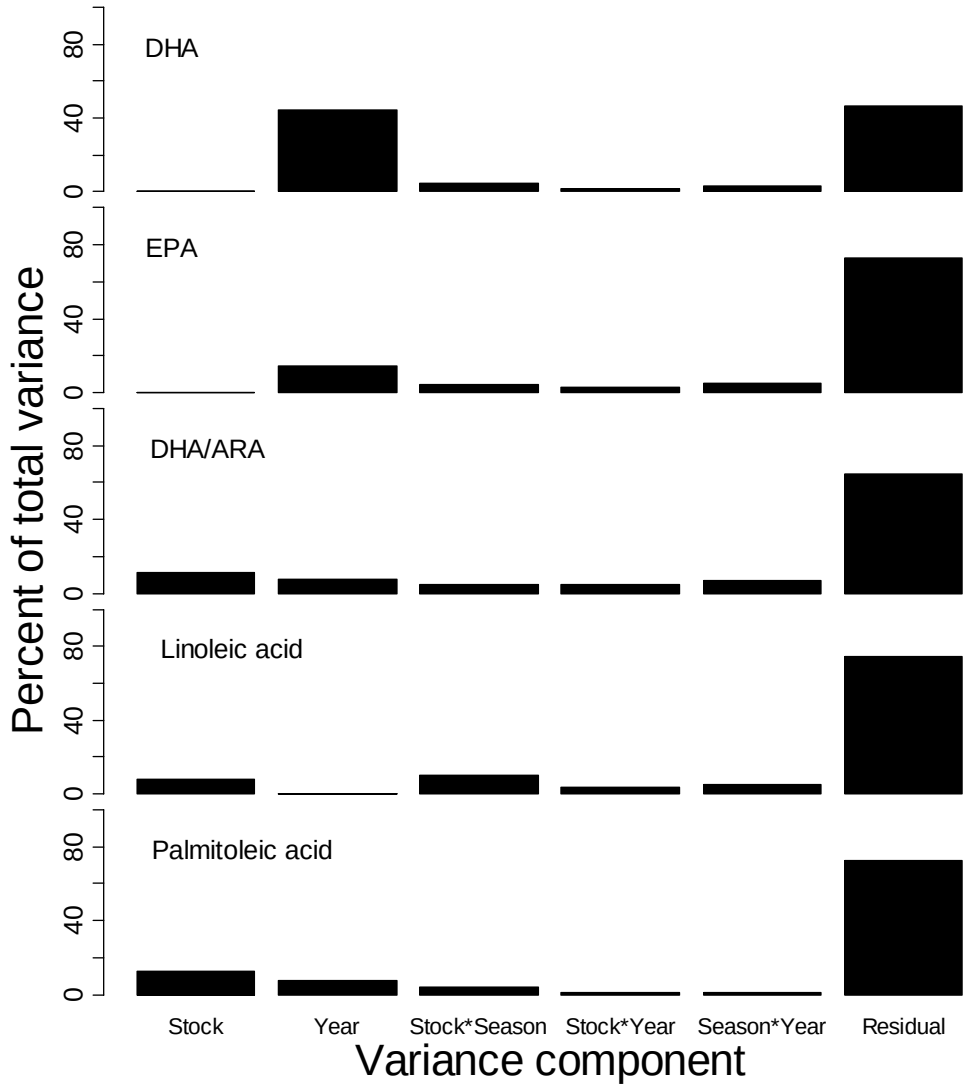

Figure 4.3. The proportion of the total variance in whitefish health indicators due to spatial and temporal factors. Figures are for fatty acids with significant stock and/or year effects. Fatty acids are for muscle samples measured as µg fatty acid/mg dry weight of tissue extracted. Variances were estimated using linear mixed models.

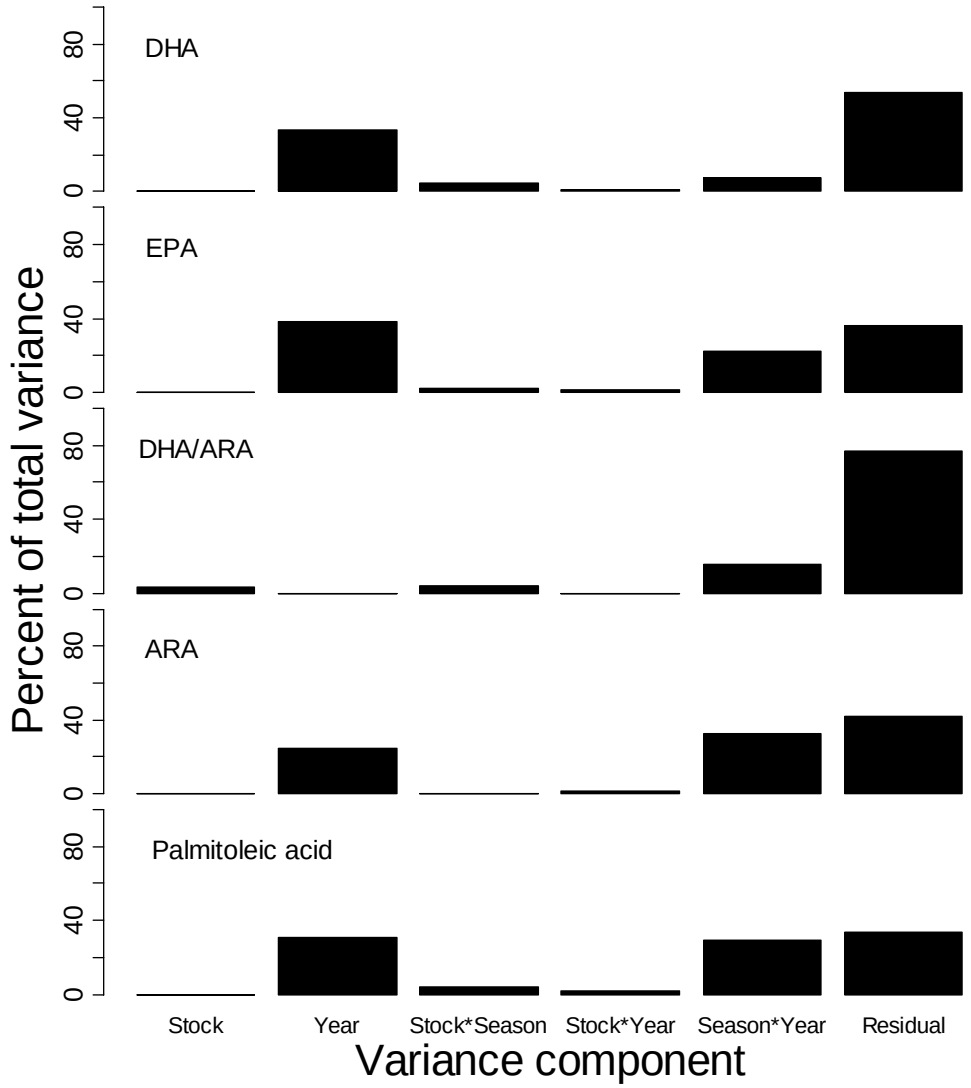

Figure 4.4. The proportion of the total variance in whitefish health indicators due to spatial and temporal factors. Figures are for fatty acids with significant stock and/or year effects. Fatty acids are for eye samples measured as µg fatty acid/mg polar lipid. Variances were estimated using linear mixed models.

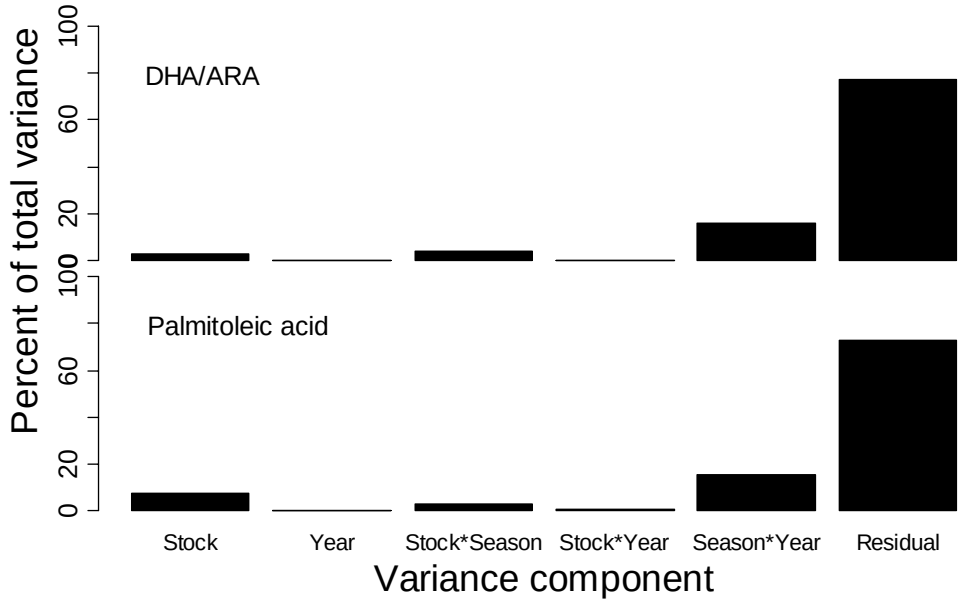

Figure 4.5. The proportion of the total variance in whitefish health indicators due to spatial and temporal factors. Figures are for fatty acids with significant stock and/or year effects. Fatty acids are for eye samples measured as µg fatty acid/mg dry weight of tissue extracted. Variances were estimated using linear mixed models.

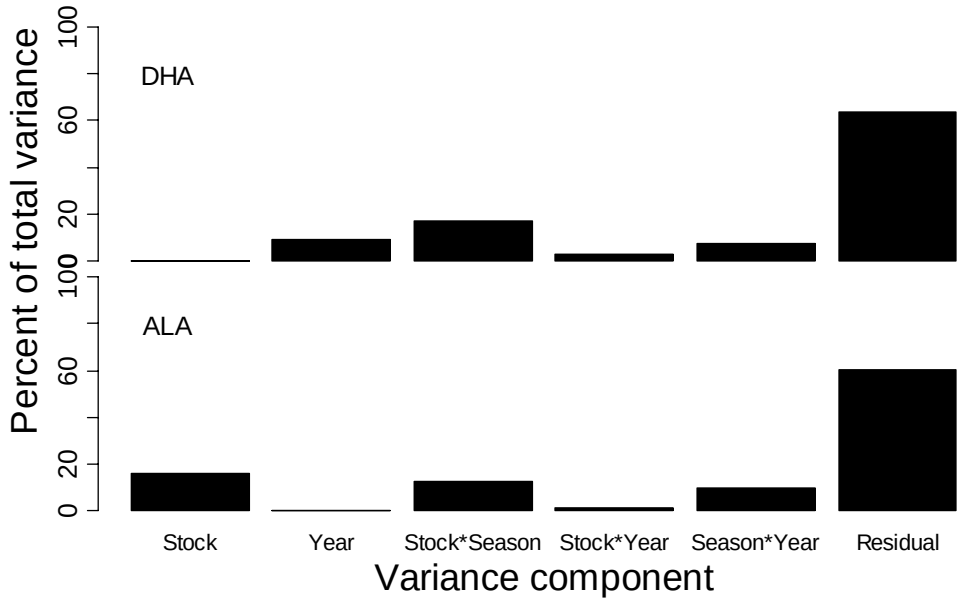

Figure 4.6. The proportion of the total variance in whitefish health indicators due to spatial and temporal factors. Figures are for fatty acids with significant stock and/or year effects. Fatty acids are for liver samples measured as µg fatty acid/mg dry weight of tissue extracted. Variances were estimated using linear mixed models.

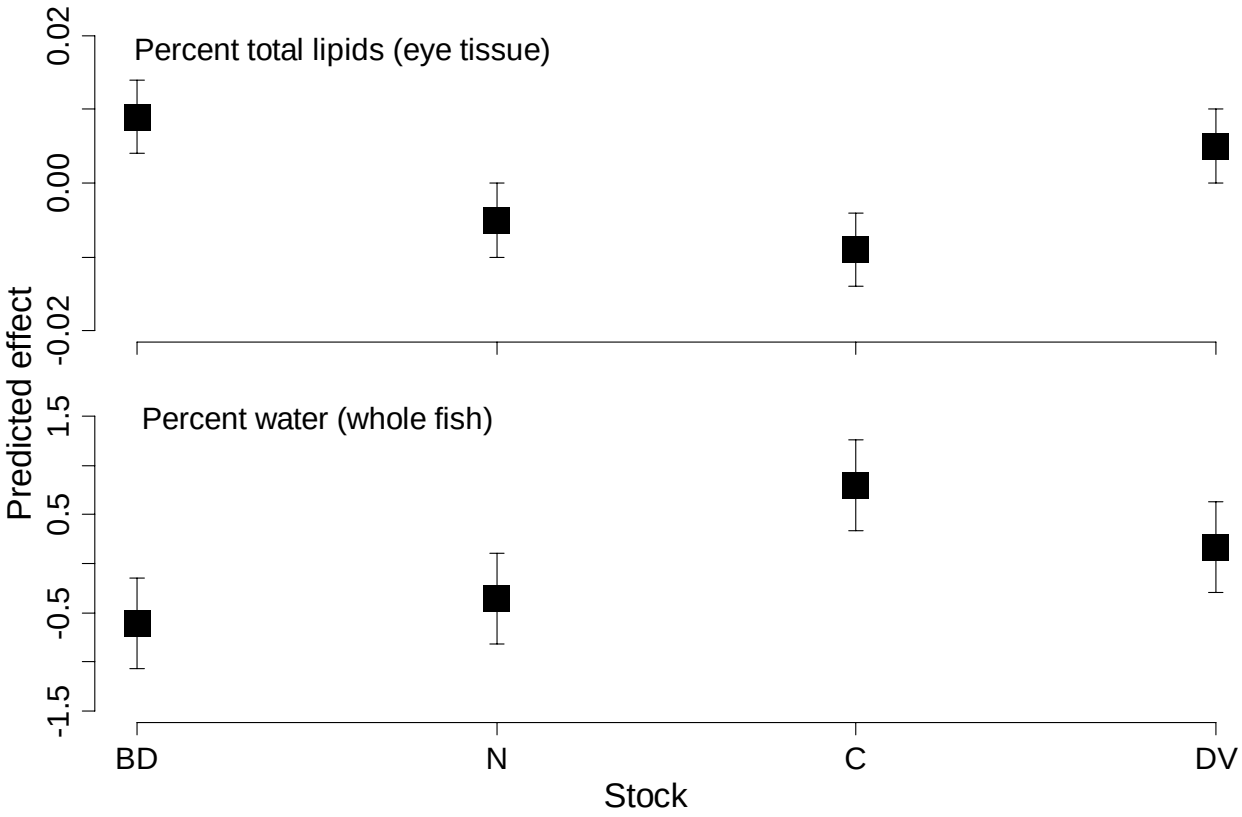

Figure 4.7. Predicted stock effects (± SE) for percent lipids measured in eye tissue and percent water of whole fish for four lake whitefish stocks. Stocks are defined as BD = Big Bay de Noc, N = Naubinway, C = Cheboygan, and DV = Detour. The BD and N stocks are located in northern Lake Michigan and C and DV are located in northern Lake Huron. Predicted effects are best linear unbiased predictors for significant stock effects from linear mixed models.

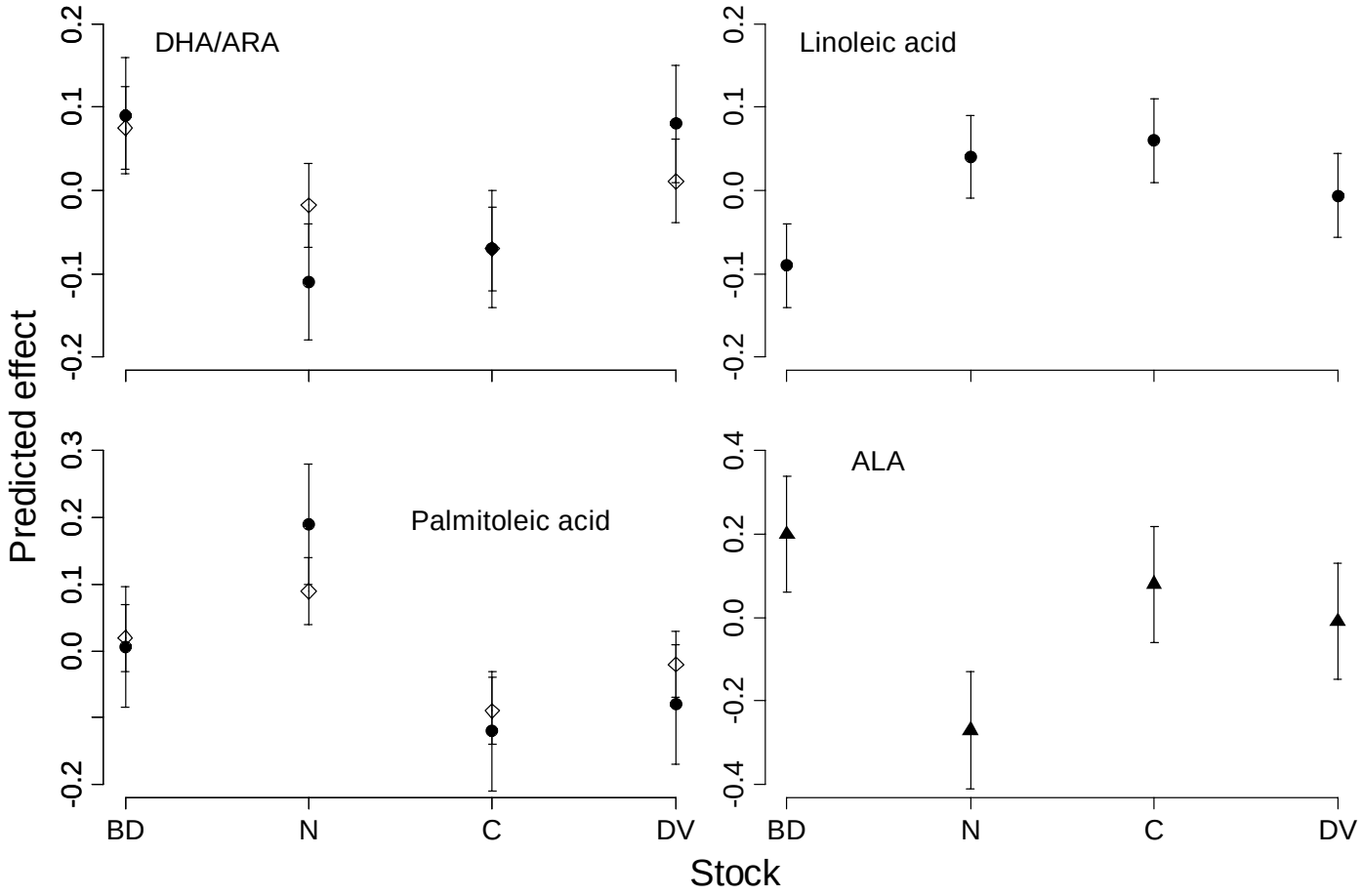

Figure 4.8. Predicted stock effects (± SE) for select fatty acids measured in muscle (●), eye (◊) and liver (▲) tissue samples for four lake whitefish stocks. Fatty acids were measured as µg fatty acid/mg dry weight of tissue extracted. Stocks are defined as BD = Big Bay de Noc, N = Naubinway, C = Cheboygan, and DV = Detour. The BD and N stocks are located in northern Lake Michigan and C and DV are located in northern Lake Huron. Predicted effects are best linear unbiased predictors for significant stock effects from linear mixed models.

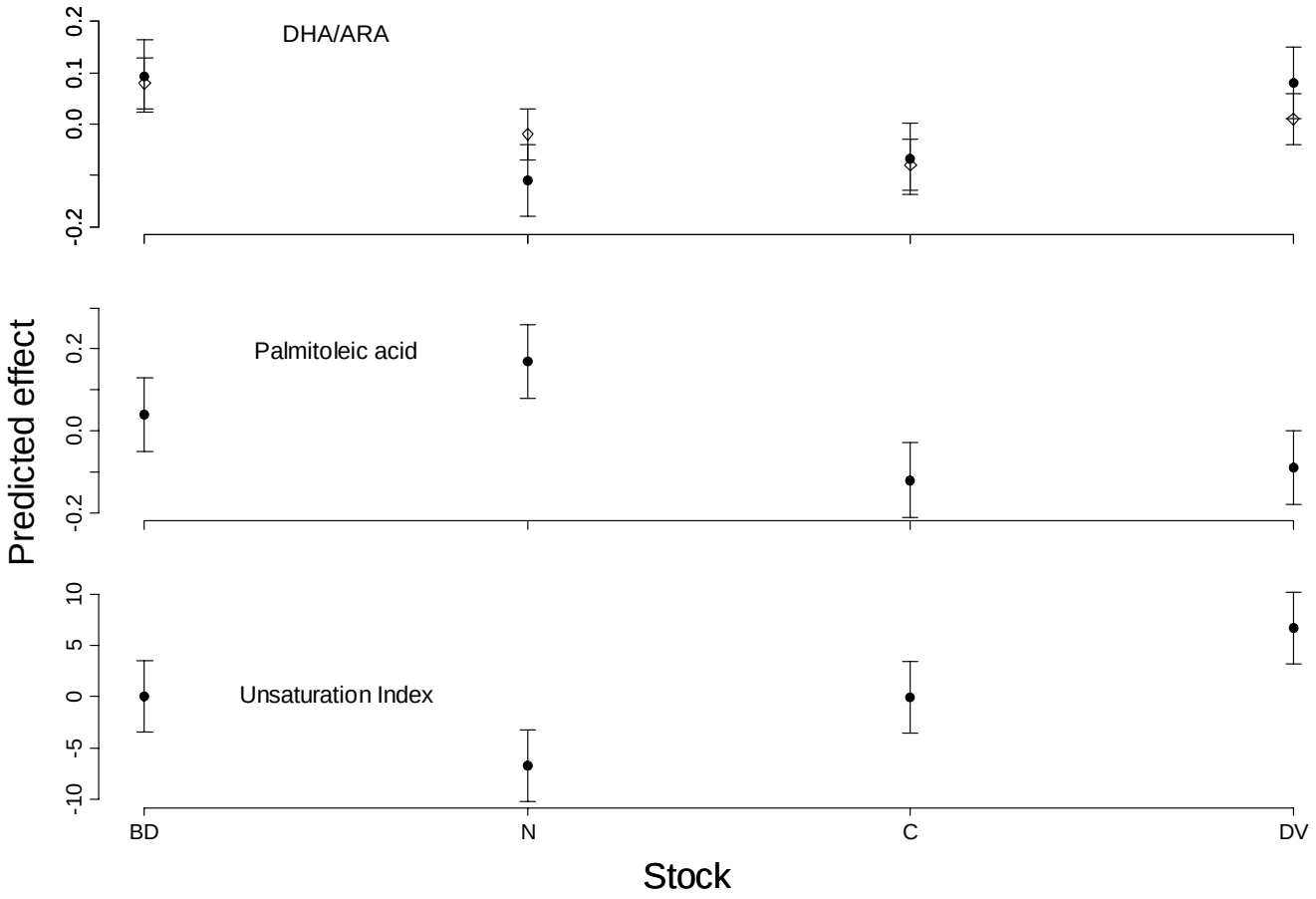

Figure 4.9. Predicted stock effects (± SE) for select fatty acids measured in muscle (●) and eye (◊) tissue samples for four lake whitefish stocks. Fatty acids were measured as µg fatty acid/mg polar lipid. Stocks are defined as BD = Big Bay de Noc, N = Naubinway, C = Cheboygan, and DV = Detour. The BD and N stocks are located in northern Lake Michigan and C and DV are located in northern Lake Huron. Predicted effects are best linear unbiased predictors for significant stock effects from linear mixed models.

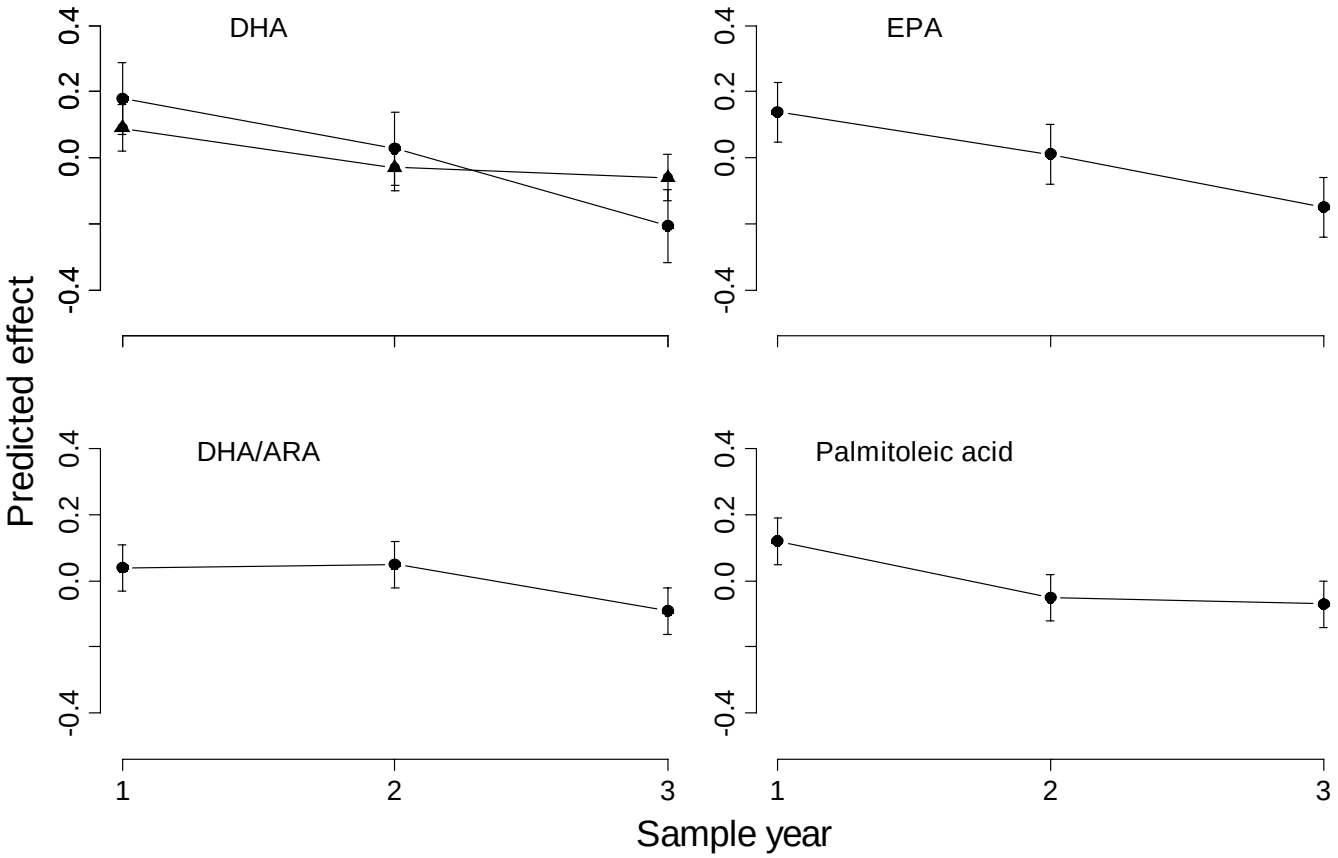

Figure 4.10. Predicted year effects (± SE) for select fatty acids measured in muscle (●) and liver (▲) tissue samples for four lake whitefish stocks. Fatty acids were measured as µg fatty acid/mg dry weight of tissue extracted. Predicted effects are best linear unbiased predictors for significant year effects from linear mixed models. Year of sampling is indicated by numbers 1 – 3. Sample year one was from fall 2003 – summer 2004, sample year two was from fall 2004 – summer 2005, and sample year three was from fall 2005 – summer 2006.

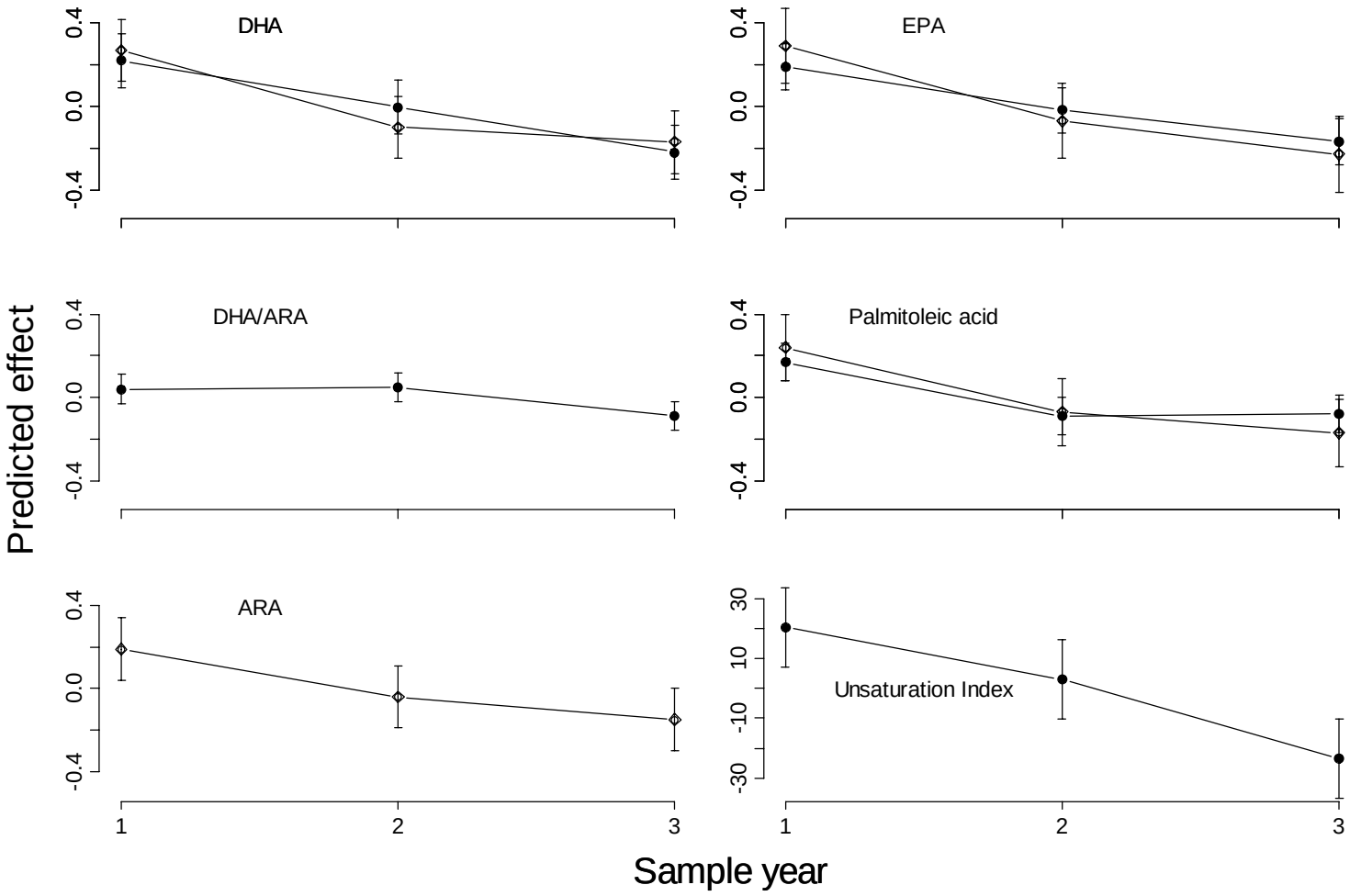

Figure 4.11. Predicted year effects (± SE) for select fatty acids measured in muscle (●) and eye (◊) tissue samples for four lake whitefish stocks. Fatty acids were measured as µg fatty acid/mg polar lipid. Predicted effects are best linear unbiased predictors for significant year effects from linear mixed models. Year of sampling is indicated by numbers 1 – 3. Sample year one was from fall 2003 – summer 2004, sample year two was from fall 2004 – summer 2005, and sample year three was from fall 2005 – summer 2006.

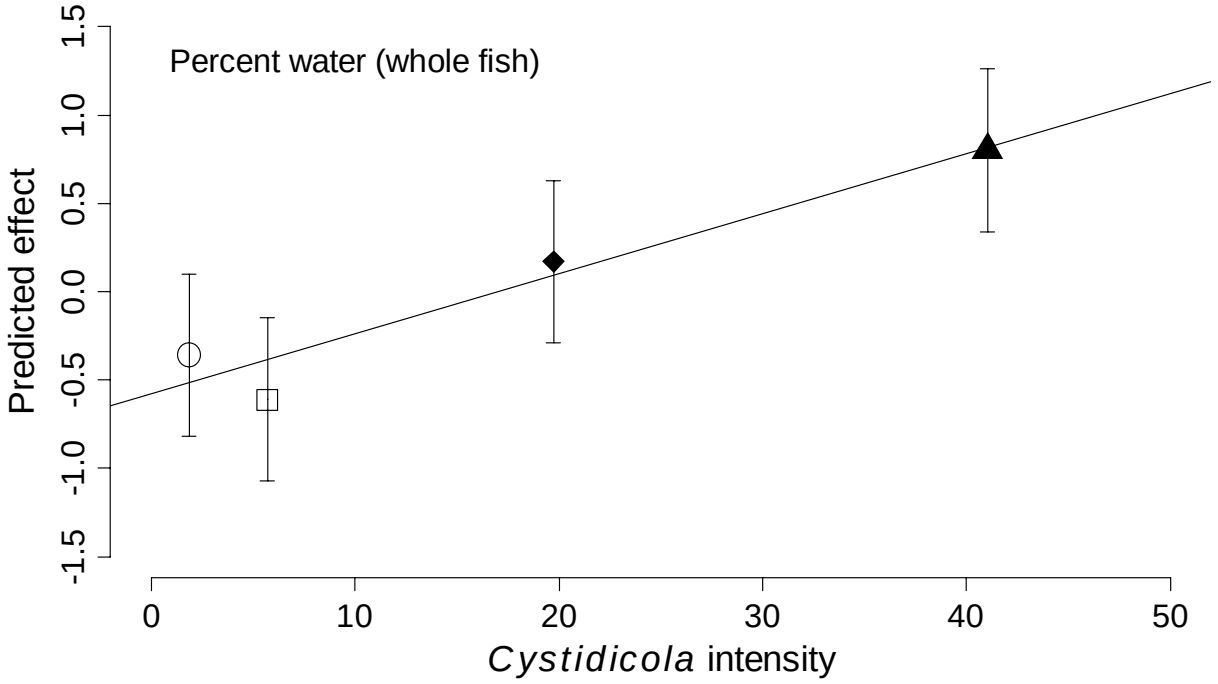

Figure 4.12. Relationship between predicted stock effects (± SE) for percent water sampled from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦), and average *Cystidicola* intensity of infection. Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model.

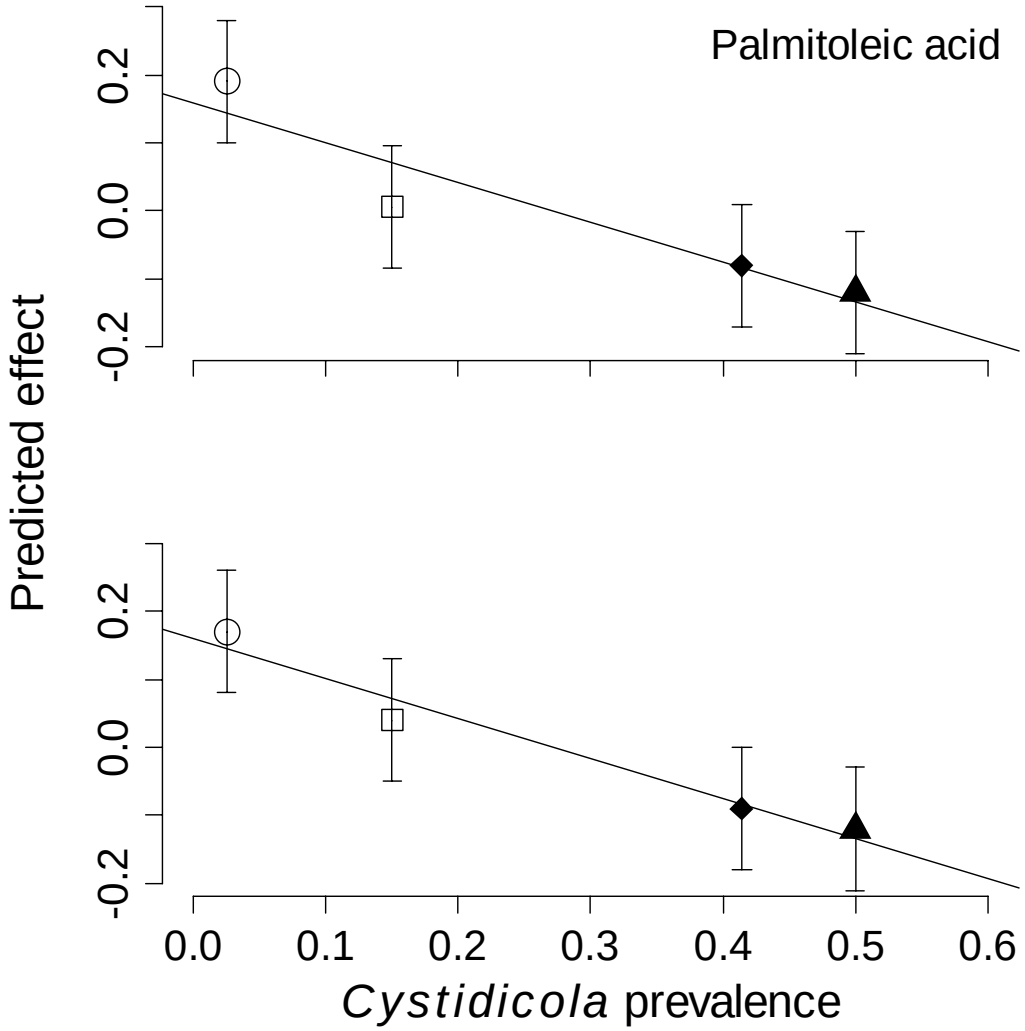

Figure 4.13. Relationship between predicted stock effects (± SE) for palmitoleic acid in muscle tissue sampled from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦), and average *Cystidicola* prevalence. Palmitoleic acid was measured as µg/mg dry weight of tissue extracted (A), as µg/mg polar lipid (B). Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model.

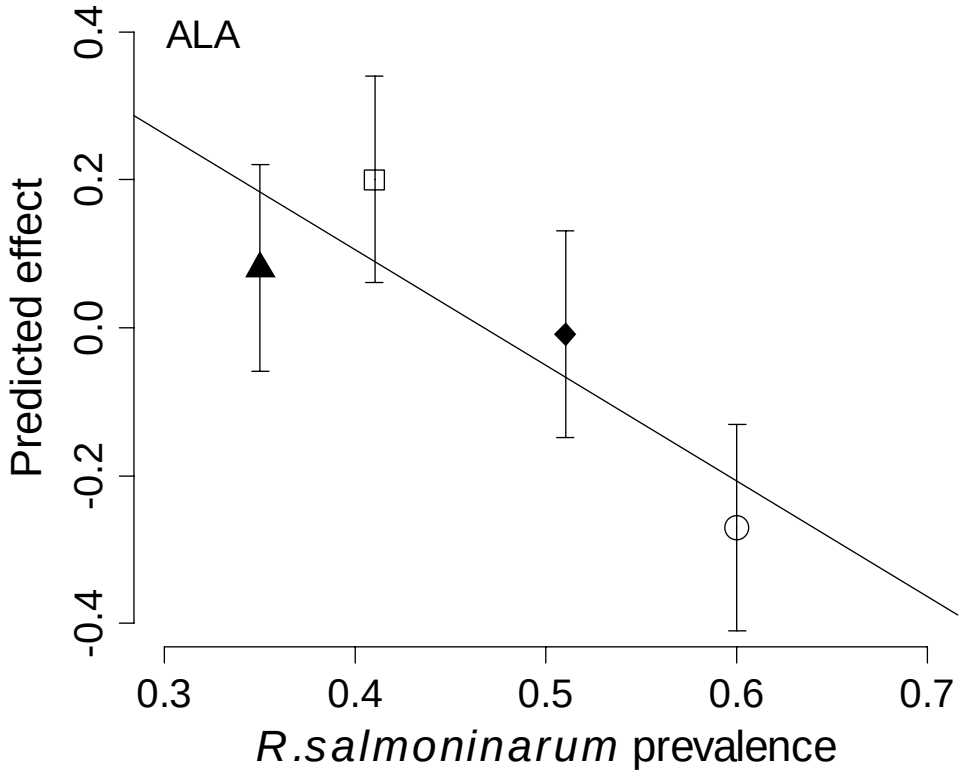

Figure 4.14. Relationship between predicted stock effects (± SE) for ALA in liver tissue sampled from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦), and average *R. salmoninarum* prevalence. ALA was measured as µg/mg dry weight of tissue extracted. Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model.

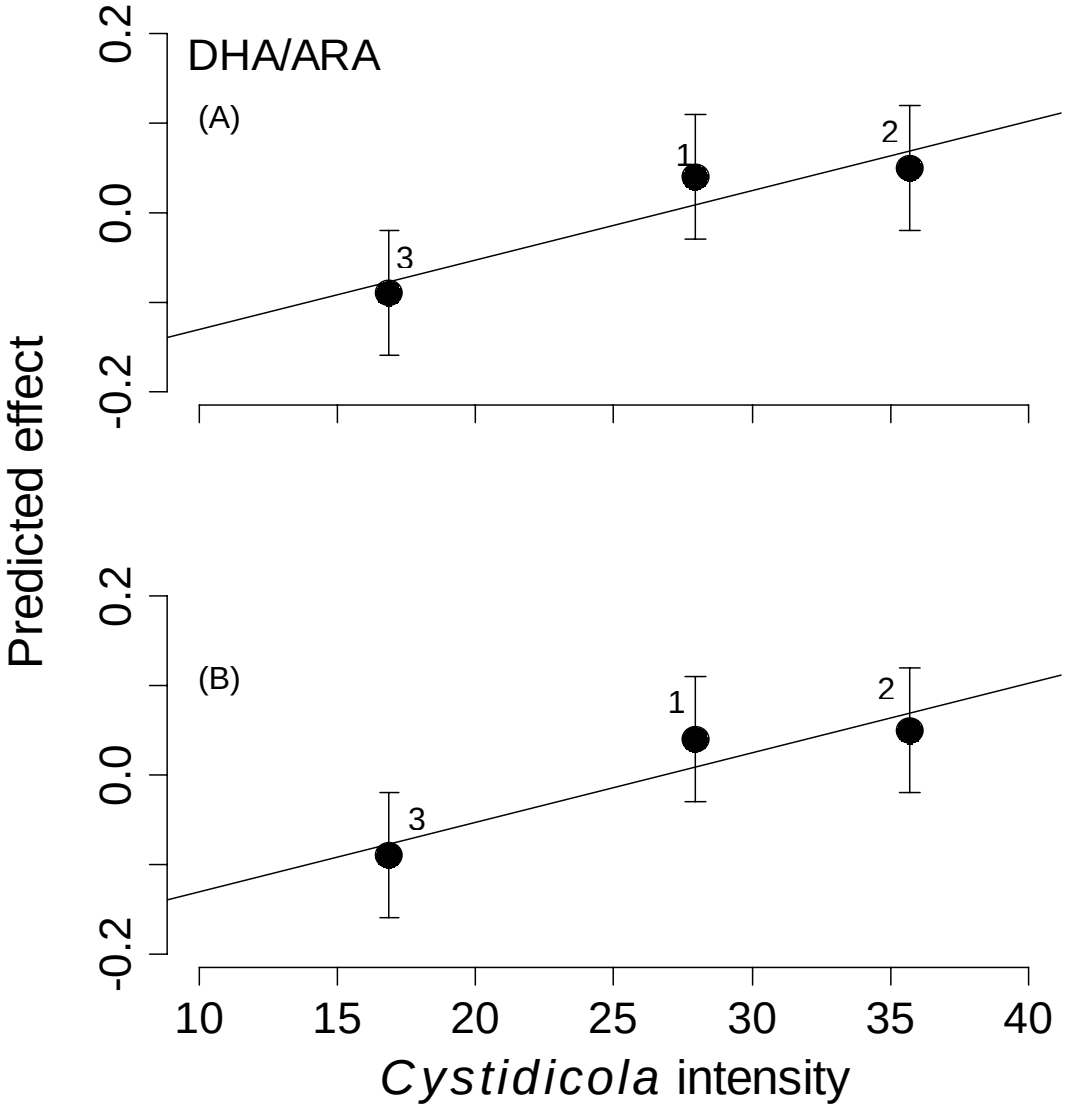

Figure 4.15. Relationship between predicted year effects (± SE) for the DHA/ARA ratio in muscle tissue sampled from four lake whitefish stocks and average annual *Cystidicola* intensity of infection. The DHA/ARA ratio was measured as µg/mg dry weight of tissue extracted (A), as µg/mg polar lipid (B). 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 – summer 2004, sample year two was from fall 2004 – summer 2005, and sample year three was from fall 2005 – summer 2006.

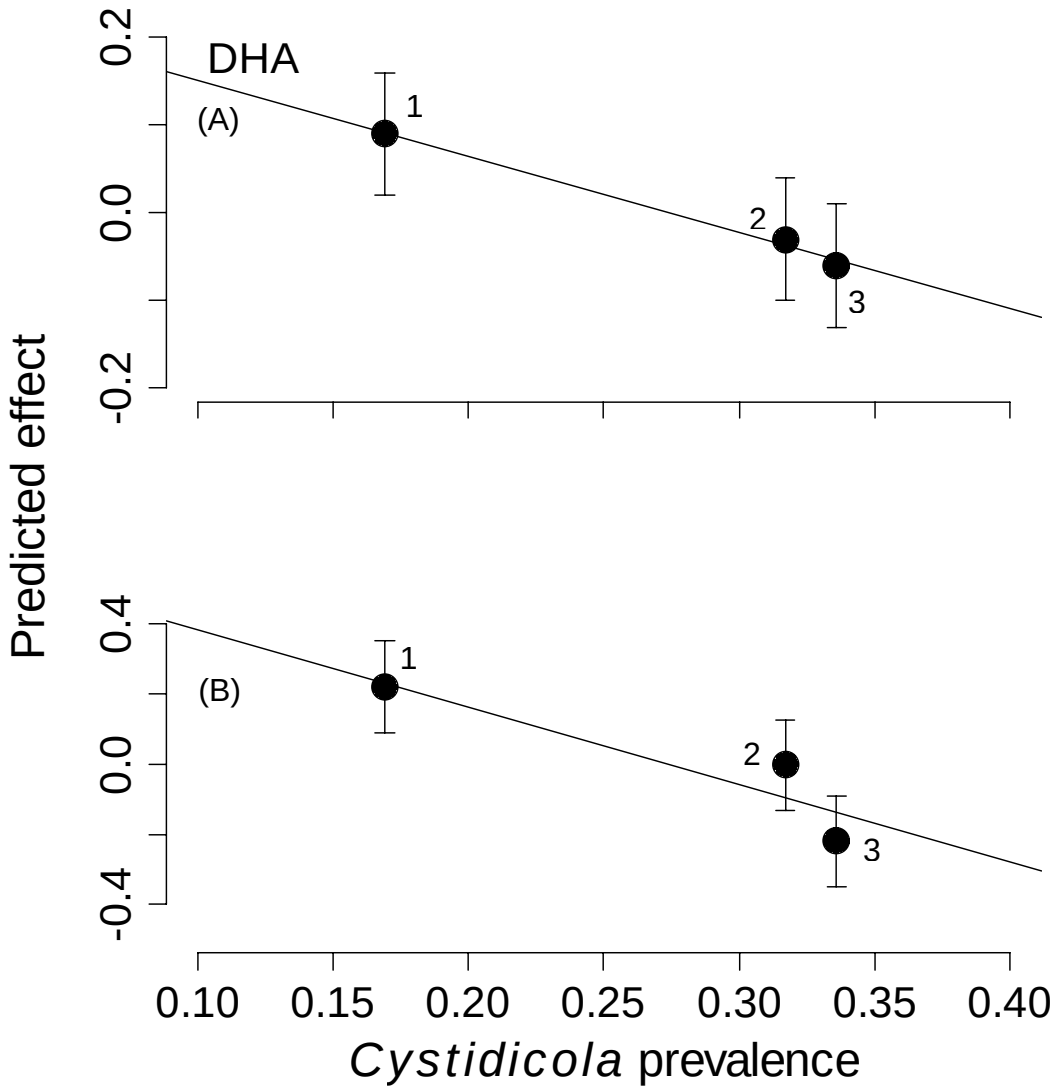

Figure 4.16. Relationship between predicted year effects (± SE) for DHA in liver (A) and muscle (B) tissues from four lake whitefish stocks and average annual *Cystidicola* prevalence. DHA was measured as µg/mg dry weight of tissue extracted for liver samples, and as µg/mg polar lipid for muscle samples. 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 – summer 2004, sample year two was from fall 2004 – summer 2005, and sample year three was from fall 2005 – summer 2006.

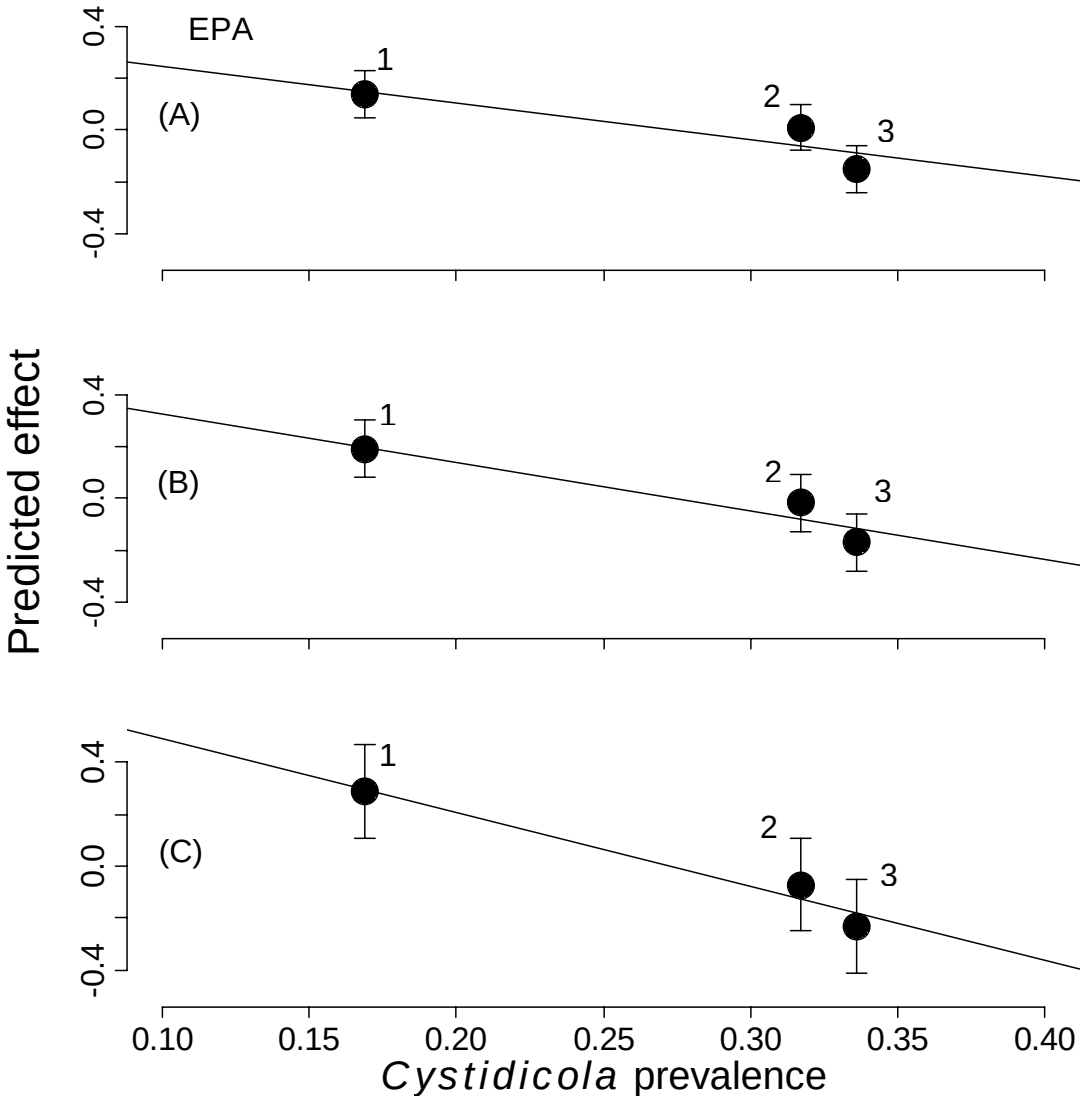

Figure 4.17. Relationship between predicted year effects (± SE) for EPA in muscle (A and B) and eye (C) tissues from four lake whitefish stocks and average annual *Cystidicola* prevalence. EPA was measured as µg/mg dry weight of tissue extracted for muscle samples in (A), and as µg/mg polar lipid for muscle and eye samples (B and C). 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 – summer 2004, sample year two was from fall 2004 – summer 2005, and sample year three was from fall 2005 – summer 2006.

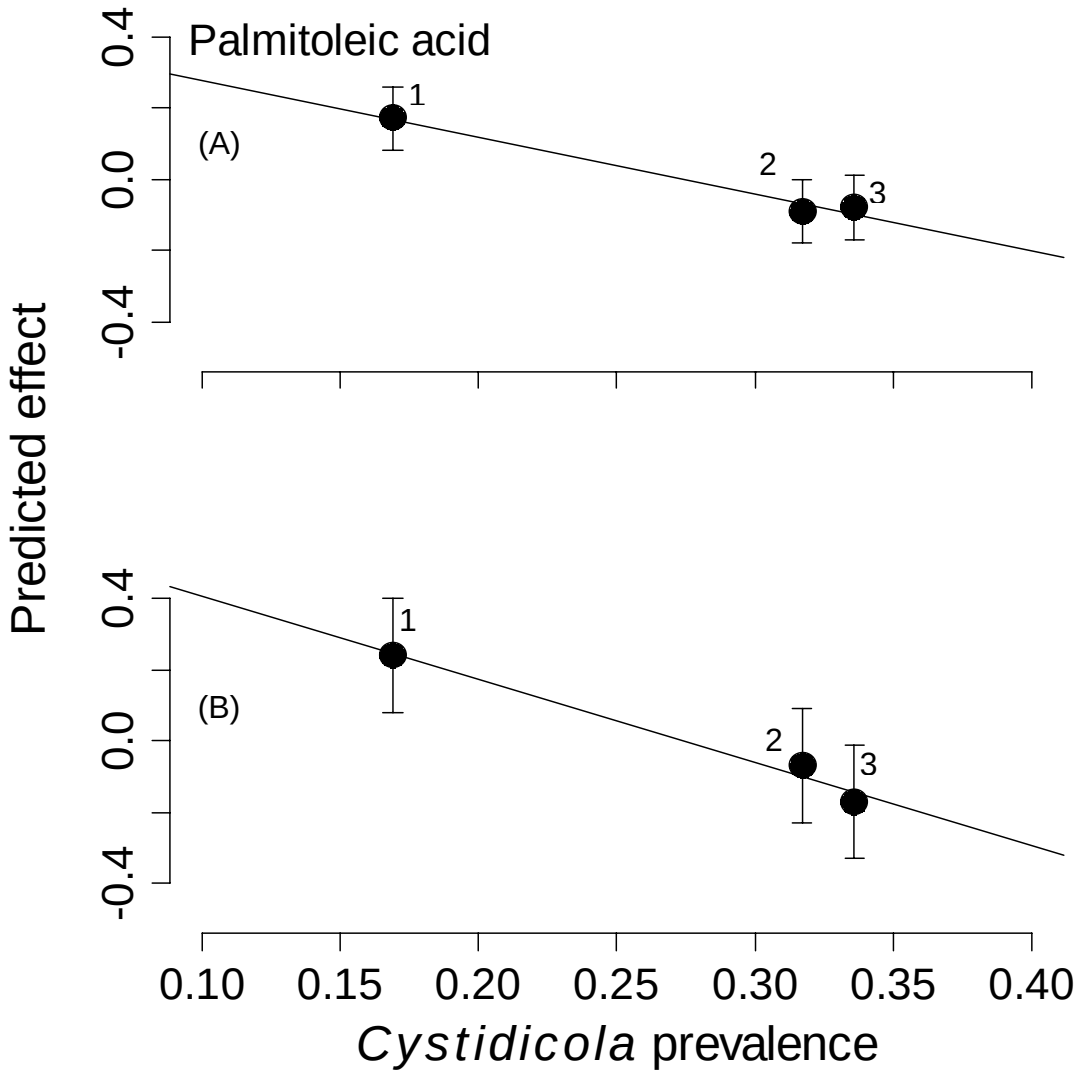

Figure 4.18. Relationship between predicted year effects (± SE) for palmitoleic acid in muscle (A) and eye (B) tissues from four lake whitefish stocks and average annual *Cystidicola* prevalence. Palmitoleic acid was measured as µg/mg polar lipid. Predicted effects are best linear unbiased predictors 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 – summer 2004, sample year two was from fall 2004 – summer 2005, and sample year three was from fall 2005 – summer 2006.

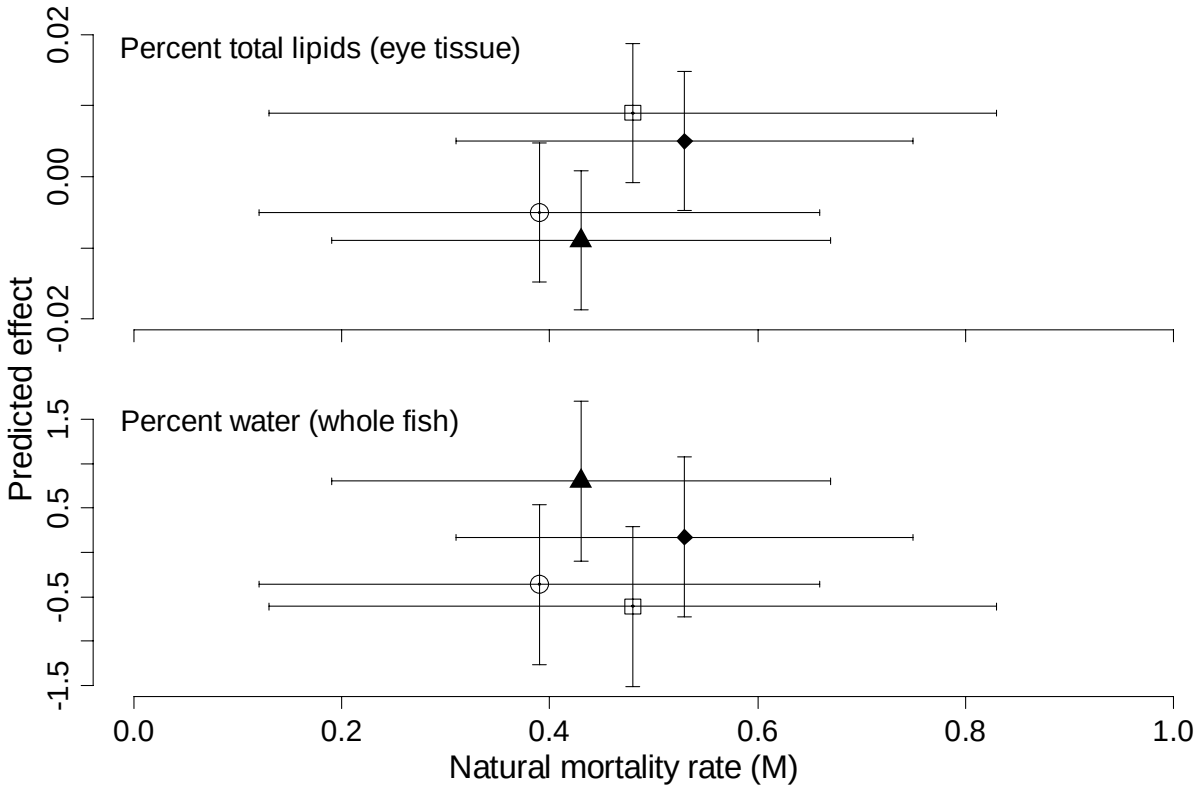

Figure 4.19. Relationship between predicted stock effects for percent total lipids in eye tissue and percent water from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦), and natural mortality rates. Error bars are 95% confidence intervals. Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model.

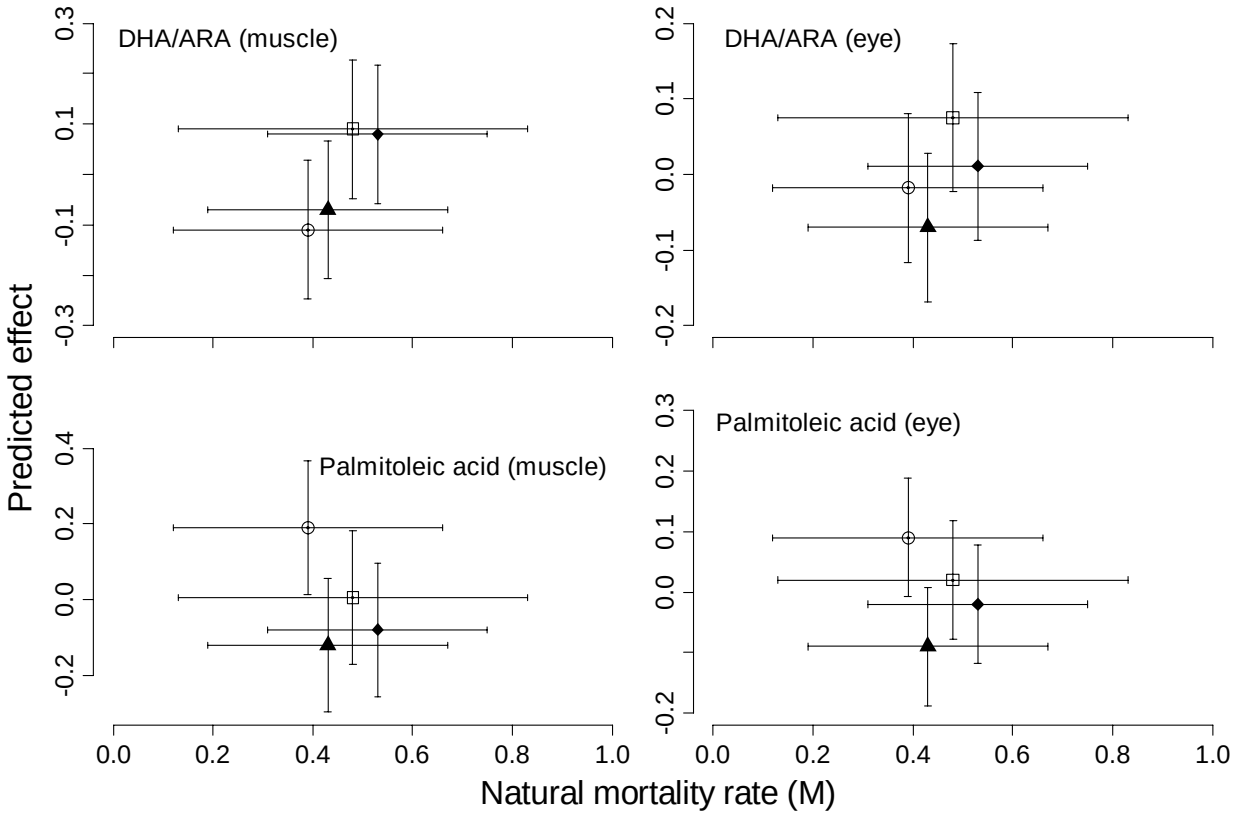

Figure 4.20. Relationship between predicted stock effects for DHA/ARA ratio and palmitoleic acid in muscle and eye tissue sampled from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦), and natural mortality rates. Error bars are 95% confidence intervals. ALA was measured as µg/mg dry weight of tissue extracted. Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model.

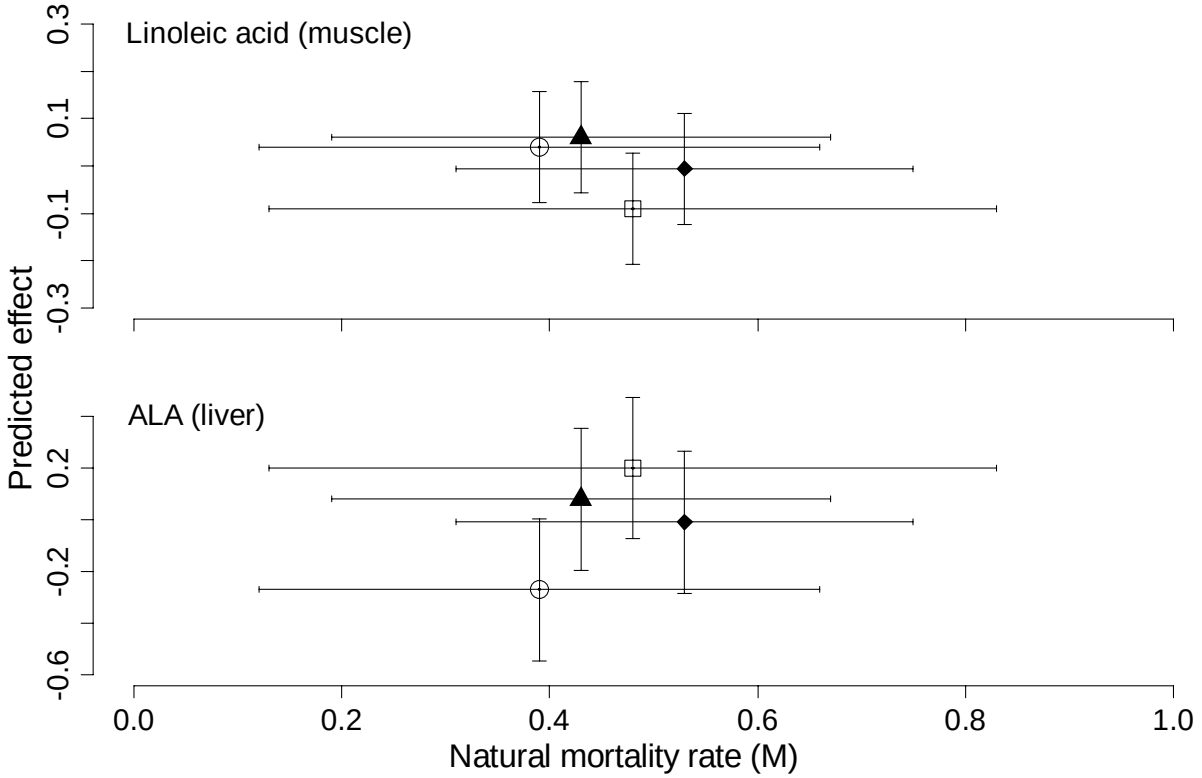

Figure 4.21. Relationship between predicted stock effects for linoleic acid and ALA in muscle and liver tissue sampled from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦), and natural mortality rates. Error bars are 95% confidence intervals. ALA was measured as µg/mg dry weight of tissue extracted. Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model.

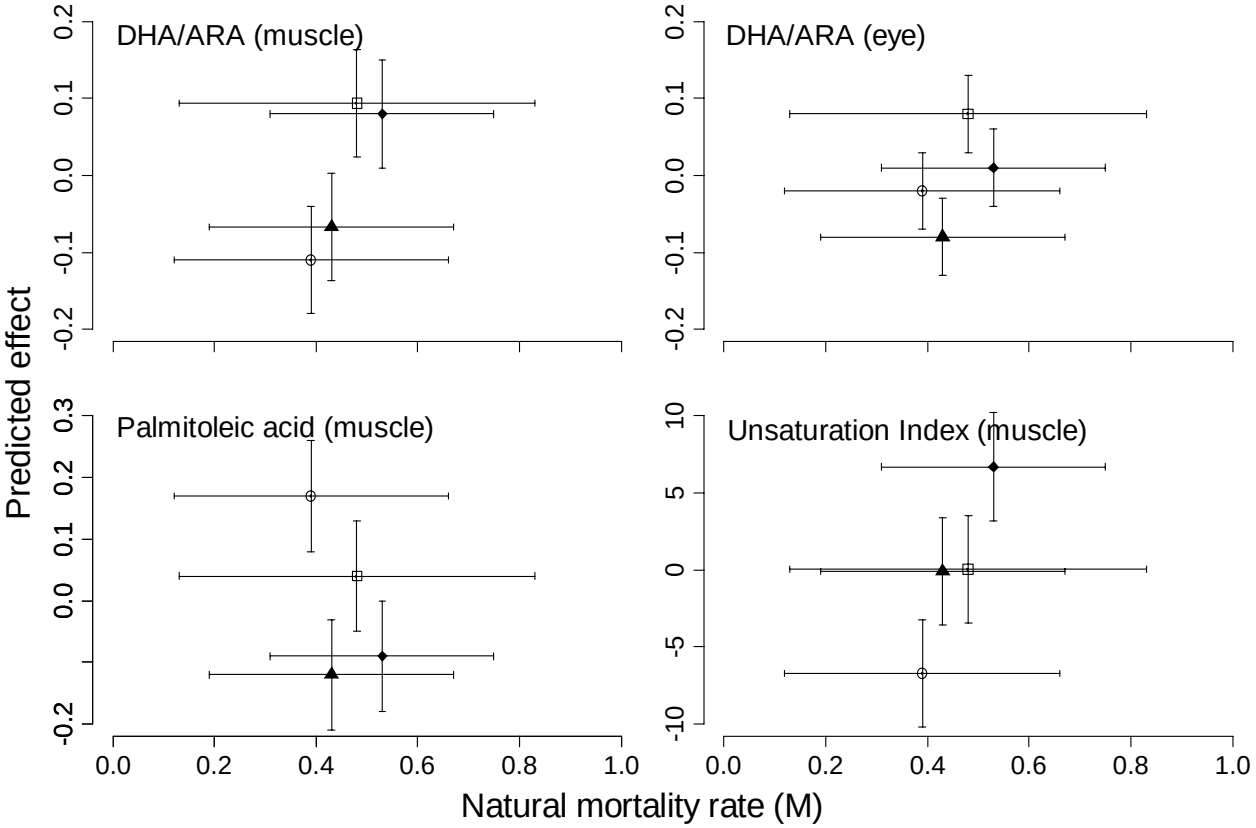

Figure 4.22. Relationship between predicted stock effects for DHA/ARA ratio, palmitoleic acid and the unsaturation index in muscle and eye tissue sampled from four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦), and natural mortality rates. Error bars are 95% confidence intervals. ALA was measured as µg/mg polar lipid. Predicted effects are best linear unbiased predictors for significant stock effects from a linear mixed model.

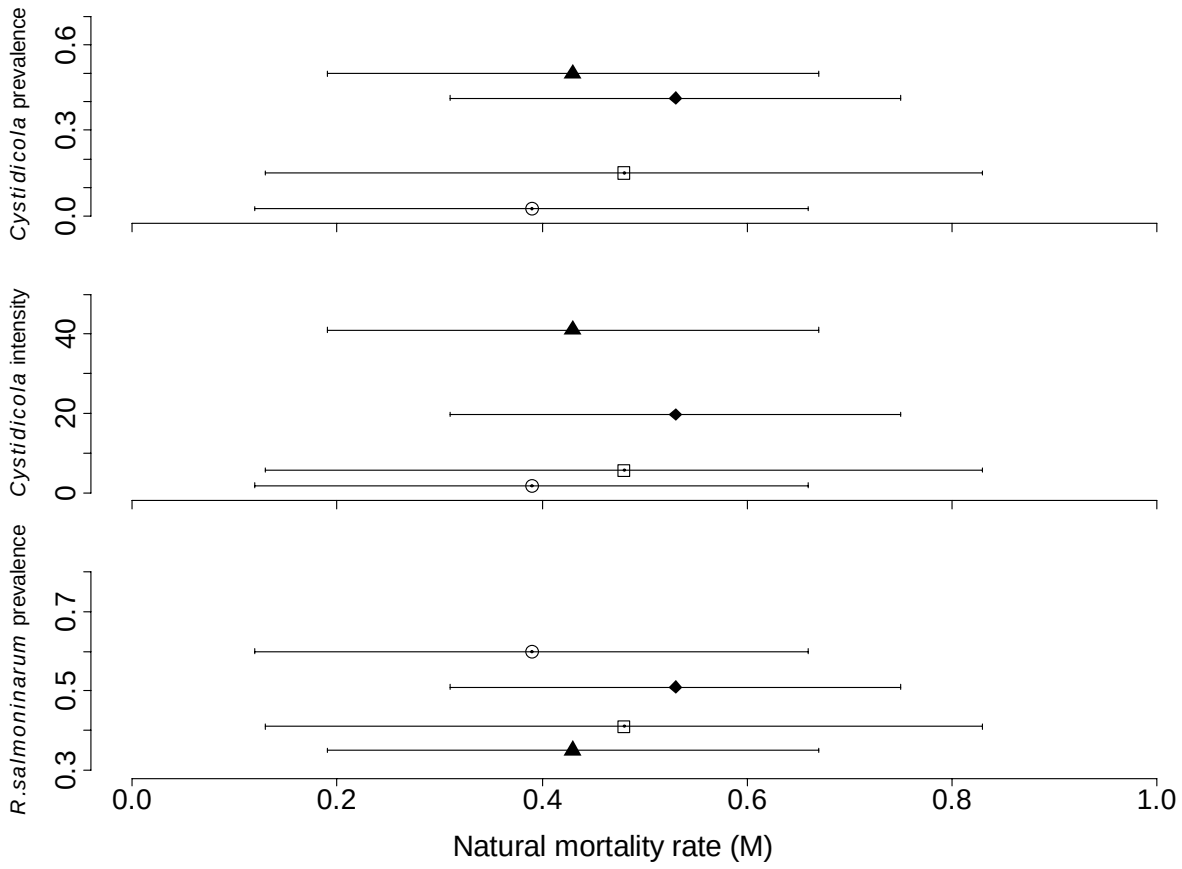

Figure 4.23. Relationship between *Cystidicola* spp. prevalence and intensity of infection and *Renibacterium salmoninarum* prevalence in four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦), and natural mortality rates. Error bars for natural mortality estimates are 95% confidence intervals. Prevalence and intensity of infection are point estimates for each stock and do not have confidence intervals.

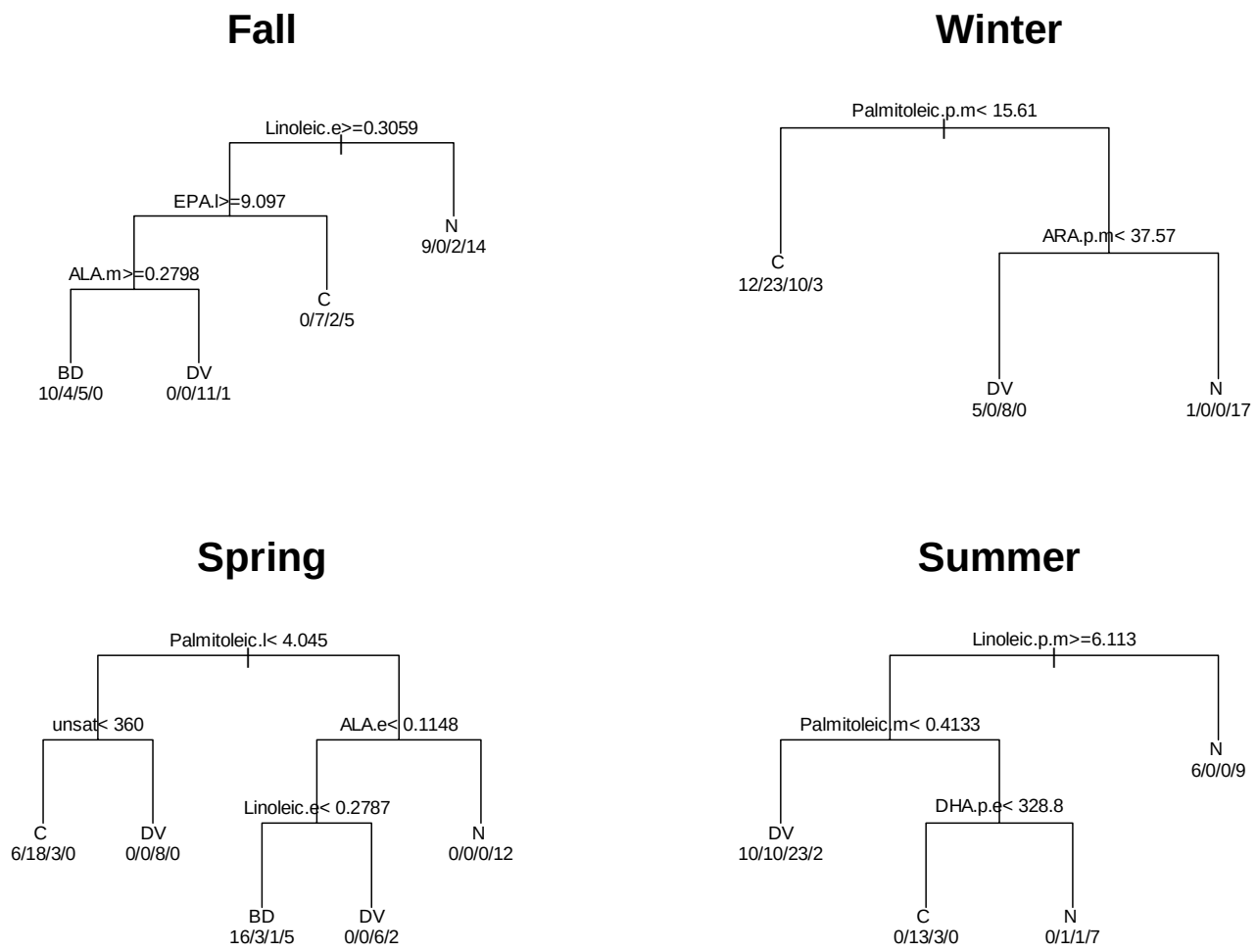

Figure 4.24. Classification trees for male lake whitefish sampled in upper Lakes Michigan and Huron. Fish were sampled from fall 2004 –summer 2006. Stock codes are listed at the terminal end of the trees: Lake Michigan stocks, BD = Big Bay de Noc, N = Naubinway; Lake Huron stocks, C = Cheboygan, and DV = Detour. Names of fatty acids are followed by a period and then a letter designating tissue type and fraction. l = liver, m = muscle, e = eye, unsat = unsaturation index. p = polar lipid fraction measured as µg FA/mg polar lipid, otherwise fatty acids measured as µg FA/mg dry weight of tissue extracted. Numbers at the terminal ends of the trees correspond to the number of fish classified as either BD/C/DV/N. The bars above the numbers provide a graphical representation of classification distributions. Each CART model is read from top to bottom: the most important split is the first one, and subsequent splits explain further variation in the data.

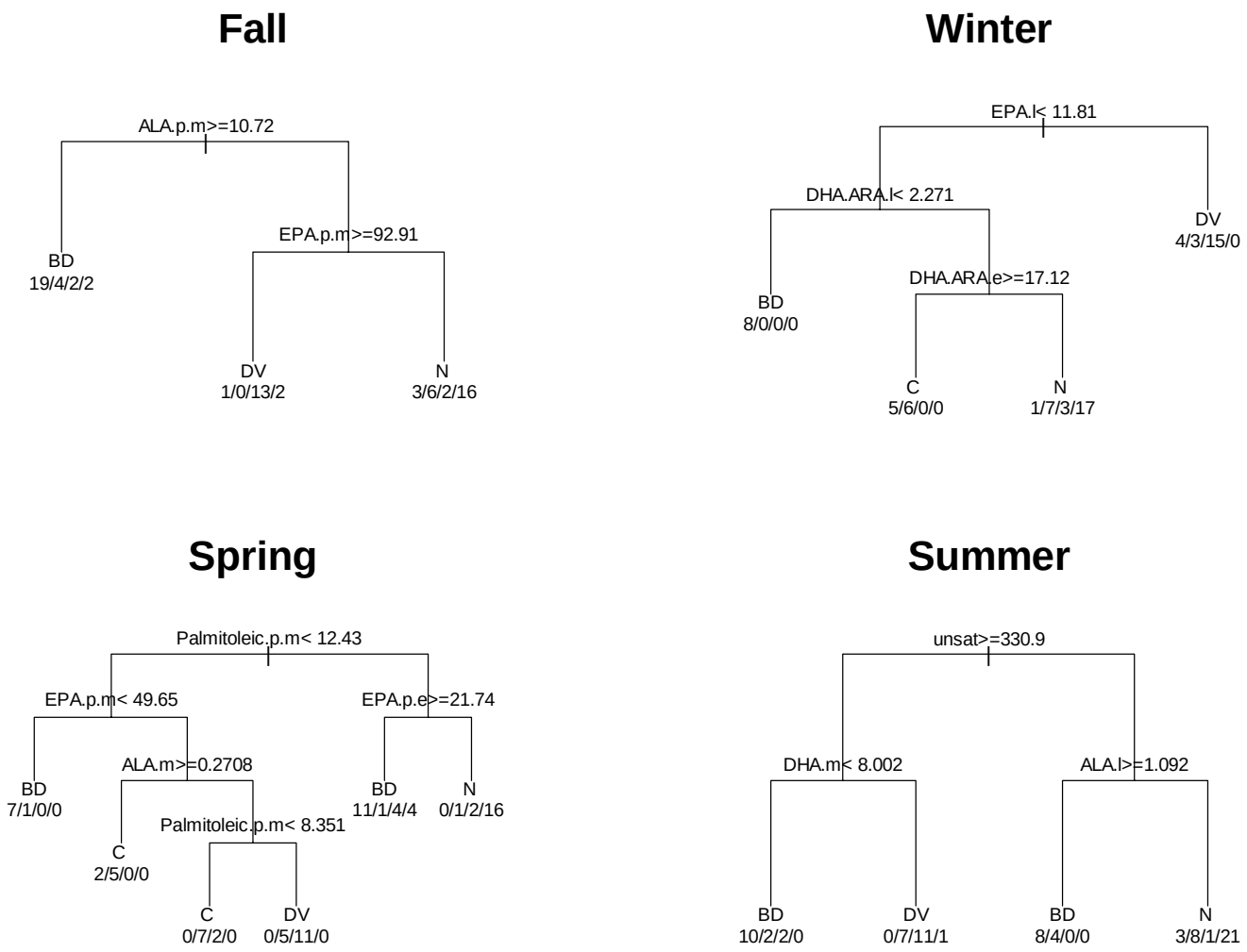

Figure 4.25. Classification trees for female lake whitefish sampled in upper Lakes Michigan and Huron. Fish were sampled from fall 2004 – summer 2006. Stock codes are listed at the terminal end of the trees: Lake Michigan stocks, BD = Big Bay de Noc, N = Naubinway; Lake Huron stocks, C = Cheboygan, and DV = Detour. Names of fatty acids are followed by a period and then a letter designating tissue type and fraction. l = liver, m = muscle, e = eye, unsat = unsaturation index. p = polar lipid fraction measured as µg FA/mg polar lipid, otherwise fatty acids measured as µg FA/mg dry weight of tissue extracted. Numbers at the terminal ends of the trees correspond to the number of fish classified as either BD/C/DV/N. The bars above the numbers provide a graphical representation of classification distributions. Each CART model is read from top to bottom: the most important split is the first one, and subsequent splits explain further variation in the data.

Appendix 4.1. Parameter estimates followed by standard errors in parentheses for mixed models for selected fatty acids (FAs) and percent lipids and water for four lake whitefish stocks in lakes Huron and Michigan. Analyses were performed on natural log-transformed FAs from three tissue types including muscle, eye, and liver tissues. Muscle and eye samples were measured as µg FA/mg dry weight of tissue extracted and µg FA/mg polar lipid. Liver samples were measured as µg FA /mg dry weight of tissue extracted. 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 for selection process for random effects, and see Table 4.1 for complete description of covariates. Cys (s in) = *Cystidicola* intensity, a stock-level covariate; Cys (s) = *Cystidicola* prevalence, a stock-level covariate; Cys (y) = *Cystidicola* prevalence, a year covariate; Cys (y in) = *Cystidicola* 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.

Response variable	Fixed effects							Random effects					
% lipids (whole fish)	Intercept	Weight	Spring	Summer	Fall			Stock	Year	Stock×season	Stock×year	Season×year	Residual
(10.8%)	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)	Intercept	Weight	Percent lipids (whole fish)	Female									
(24.1%)	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)
% lipids (eye)	Intercept	Female	Percent lipids (whole fish)										
(6.5%)	0.50 (0.01)	0.01 (0.004)	0.002 (0.0003)					0.00007 (0.00006)	--	--	--	0.001 (0.0005)	0.003 (0.0001)
% lipids (liver)	Intercept	Female	Percent lipids (whole fish)										
(6.3%)	0.45 (0.009)	-0.008 (0.003)	0.002 (0.0003)					--	--	0.0005 (0.0002)	--	0.0001 (0.00007)	0.003 (0.0001)
% water (whole fish)	Intercept	Spring	Summer	Fall	Female	Weight	Cys (s in)						

(27.5%)	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**	--	0.12 (0.06)	0.06 (0.05)	0.16 (0.09)	4.6 (0.16)
Per mg dry weight of tissue extracted													
DHA eye (4.6%)	Intercept	Percent total lipids (eye)											
	3.8 (0.10)	-0.005 (0.002)						--	--	0.018 (0.009)	--	0.06 (0.03)	0.19 (0.008)
DHA muscle (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)
DHA liver (14.8%)	Intercept	Female	Percent lipids (liver)	Cys (y)									
	4.0 (0.13)	-0.04 (0.02)	-0.01 (0.001)	-1.27 (0.42)				--	0.0**	0.02 (0.007)	0.003 (0.002)	0.006 (0.004)	0.059 (0.003)
EPA eye (1.6%)	Intercept	Length											
	1.5 (0.12)	-0.0005 (0.0002)						--	--	0.005 (0.003)	0.003 (0.002)	0.02 (0.009)	0.09 (0.004)
EPA muscle (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)
EPA liver (5.3%)	Intercept	Percent lipids (liver)						--	--	0.009 (0.004)	0.0007 (0.001)	0.01 (0.007)	0.09 (0.004)
	2.7 (0.06)	-0.01 (0.002)											
DHA/ARA eye (4.0%)	Intercept	Percent water											
	3.5 (0.40)	-0.01 (0.005)						0.003 (0.004)	--	0.006 (0.004)	--	0.02 (0.01)	0.14 (0.006)
DHA/ARA	Intercept	Female	Percent	Cys (y									

muscle (14.6%)			lipids (whole fish)	in)								
	1.6 (0.13)	-0.08 (0.2)	0.004 (0.001)	0.01 (0.004)			0.009 (0.009)	0.0**	0.005 (0.003)	0.005 (0.003)	0.007 (0.004)	0.07 (0.003)
DHA/ARA liver (12.0%)	Intercept	Spring	Summer	Fall	Weight							
	1.1 (0.06)	0.29 (0.08)	0.09 (0.08)	0.20 (0.08)	0.0001 (0.00003)		--	--	0.007 (0.003)	0.006 (0.003)	0.003 (0.002)	0.06 (0.003)
Palmitoleic acid eye (10.5%)	Intercept	Weight	Cys									
	0.29 (0.04)	0.0002 (0.00003)	-0.42 (0.08)				0.0**	--	0.002 (0.001)	0.0004 (0.0005)	0.01 (0.006)	0.06 (0.003)
Palmitoleic acid muscle (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**	0.01 (0.01)	0.004 (0.003)	0.005 (0.003)	0.005 (0.003)	0.11 (0.005)
Palmitoleic acid liver (41.9%	Intercept	Spring	Summer	Fall	Percent lipids (liver)	Weight						
	0.75 (0.12)	-0.18 (0.12)	-0.34 (0.12)	-0.45 (0.12)	0.04 (0.002)	0.0003 (0.00004)	0.01 (0.01)	0.0006 (0.007)	0.007 (0.004)	0.01 (0.009)	0.01 (0.008)	0.10 (0.005)
ARA eye (5.8%)	Intercept	Percent lipids (eye)										
	1.2 (0.07)	-0.003 (0.001)					--	--	0.004 (0.002)	0.002 (0.001)	0.04 (0.02)	0.06 (0.003)
ARA muscle (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)
ARA liver (2.8%)	Intercept	Weight	Percent lipids (whole									

			fish)										
	2.2 (0.05)	-0.0001 (0.00004)	-0.006 (0.002)				--	--	0.006 (0.003)	0.007 (0.005)	0.004 (0.003)	0.075 (0.003)	
Linoleic acid eye (3.5%)	Intercept	Weight	Percent lipids (whole fish)										
	-1.1 (0.06)	-0.0002 (0.00004)	-0.005 (0.002)				--	--	--	0.02 (0.008)	0.01 (0.007)	0.09 (0.004)	
Linoleic acid muscle (9.6%)	Intercept	Spring	Summer	Fall	Percent water	Weight							
	-1.8 (0.29)	-0.12 (0.07)	-0.25 (0.07)	-0.80 (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)	
Linoleic acid liver (1.1%)	Intercept	Female	Weight	Percent water									
	-0.79 (0.35)	-0.06 (0.02)	-0.0002 (0.00004)	0.017 (0.005)			--	--	0.01 (0.006)	0.002 (0.002)	0.009 (0.005)	0.09 (0.004)	
ALA eye (1.4%)	Intercept	Weight											
	-2.3 (0.13)	-0.0003 (0.0001)					--	--	0.03 (0.02)	0.02 (0.02)	0.15 (0.07)	0.74 (0.03)	
ALA muscle (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)	
ALA liver (76.7%)	Intercept	Female	Weight	Percent lipids (liver)	Rs (s)								
	1.0 (0.31)	-0.14 (0.03)	-0.0001 (0.00006)	-0.01 (0.003)	-1.9 (0.63)		0.003 (0.01)	--	0.04 (0.02)	0.005 (0.005)	0.03 (0.01)	0.18 (0.008)	
Per mg polar lipid	Intercept												
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 eye (35.7%)	Intercept	Cys (y)										
							--	0.0**	0.01 (0.004)	--	0.01 (0.005)	0.10 (0.004)
DHA muscle (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 eye (45.6%)	Intercept	Weight	Percent lipids (eye)	Cys (y)								
	4.2 (0.23)	-0.00008 (0.00004)	0.003 (0.001)	-3.3 (0.78)			--	0.0**	0.003 (0.002)	0.004 (0.002)	0.03 (0.02)	0.08 (0.003)
EPA muscle (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 eye (3.9%)	Intercept	Percent water										
	3.5 (0.41)	-0.01 (0.005)					0.003 (0.004)	--	0.006 (0.004)	--	0.02 (0.01)	0.14 (0.006)
DHA/ARA muscle (14.6%)	Intercept	Female	Percent lipids (muscle)	Cys (y in)			0.009 (0.008)	0.0**	0.005 (0.003)	0.005 (0.003)	0.007 (0.004)	0.07 (0.003)
Palmitoleic acid eye (39.0%)	Intercept	Weight	Percent lipids (eye)	Cys (y)								
	3.1 (0.24)	0.0002 (0.00003)	0.004 (0.001)	-2.9 (0.83)			--	0.0**	0.008 (0.005)	0.004 (0.003)	0.04 (0.01)	0.06 (0.003)
Palmitoleic acid muscle (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**	0.0**	0.006 (0.003)	0.003 (0.002)	0.002 (0.002)	0.11 (0.005)
ARA eye (33.2%)	Intercept	Female	Percent lipids (whole fish)	Cys (y)									
	4.2 (0.24)	-0.04 (0.02)	-0.005 (0.001)	-2.6 (0.84)				--	0.0**	--	0.002 (0.002)	0.04 (0.02)	0.07 (0.003)
ARA muscle (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)
Linoleic acid eye (4.5%)	Intercept	Percent lipids (whole fish)	Female	Weight									
	1.1 (0.10)	-0.006 (0.002)	-0.06 (0.02)	0.0001 (0.00005)				--	--	0.03 (0.01)	--	0.08 (0.03)	0.12 (0.006)
Linoleic acid muscle (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 eye (9.7%)	Intercept	Female	Percent lipids (eye)										
	-0.21 (0.16)	-0.17 (0.03)	0.01 (0.003)					--	--	0.03 (0.01)	0.02 (0.01)	0.18 (0.08)	0.28 (0.01)
ALA muscle (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)

**Variance estimated to be zero after accounting for stock or year covariate.

PART 5 – SUMMARY AND RECOMMENDATIONS

Natural Mortality Rates

The tagging study that we conducted as part of this project suggested that overall there were little differences in estimates of *M* among the four lake whitefish stocks. Ninety-five percent confidence intervals for estimates of *M* for the stocks encompassed the *M*s for all other stocks. Compared to the natural mortality rates predicted for the stocks from the Pauly (1980) equation, the estimates from this study were greater then what was predicted (Figure 5.1). For the Big Bay de Noc stock, the estimate of M from the tagging study ($M = 0.48$) was only slightly greater then that predicted from the Pauly (1980) equation ($M = 0.42$). There was a slightly greater difference in the *M* estimates for the Naubinway stock (tagging study: M = 0.39; Pauly equation: M = 0.25). For Cheboygan, the *M* estimated from the tagging study was 0.43, which was nearly double the *M* predicted from the Pauly (1980) equation ($M = 0.22$). For the Detour stock, the *M* estimated from this study ($M = 0.53$) was more then twice that predicted from the Pauly (1980) equation ($M = 0.20$). The deviation of our estimates of *M* from those predicted by the Pauly (1980) equation is not surprising given that equation only provides an average natural mortality prediction for a given population characteristic (Miranda and Bettoli 2007). However, our finding that all the *M* estimates were larger than those predicted from the Pauly (1980) equation is informative and suggests that there may be something occurring with these stocks that is causing natural mortality rates to deviate from this empirical relationship. One factor that in particular may explain why natural mortality in the lake whitefish stocks is greater then expected is the occurrence of sea lamprey parasitism on lake whitefish. Sea lamprey first invaded the Great Lakes in the early 1900s and are believed to have played a strong role in reducing several native Great Lakes fish stocks, including the lake whitefish. Although control efforts have led to a 90% reduction in sea lamprey populations in the Great Lakes, sea lamprey parasitism on lake whitefish still occurs and some stocks are believed to be more heavily impacted then others. During lake whitefish tagging, a higher incidence of sea lamprey marks were observed on fish from the Cheboygan and Detour stocks, which also had the largest deviations in *M* estimates when compared to the predictions from the Pauly (1980) equation. This finding indeed suggests that at least for some of the stocks the deviation from the Pauly equation may be partly due to sea-lamprey caused mortality in lake whitefish.

Sea lamprey-caused mortality is considered an additive mortality component in some of the assessment models that are used to set harvest policies for lake whitefish in the Great Lakes. Sea lamprey-caused mortality is calculated based on the number of A1 and A2 marks that are observed on lake whitefish during assessment surveys using the relationship developed by Ebener et al. (2005). As a result, sea lamprey-caused mortality differs by year and size of fish. For the Cheboygan and Detour stocks, estimates of sea lamprey-caused mortality in the assessment have generally ranged from 0.01 to 0.12 in recent years. Using the same Ebener et al. (2005) relationship to estimate sea-lamprey caused mortality on tagged fish for this study, we estimated sea-lamprey caused mortality to be as high as 0.19 and 0.29 for the Cheboygan and Detour stocks. This further suggests that the large differences in M estimates from this study relative to the predictions from the Pauly (1980) equation was due to sea lamprey mortality acting on tagged fish.

It should be pointed out that there are several factors that may have caused our estimates of natural mortality rates from the tagging study to be inflated, which may also explain some of the differences observed between our estimates and the predictions from the Pauly (1980)

equation. First, based on simulation study (see Supplement) that we conducted based on the tagging protocol used in this research (i.e., 2,000 fish tagged per year; recoveries occurring over a 4-yr period), we found that estimates of *M* were on average positively biased by around 20% relative to actual mortality. At a target tagging level of 10,000 fish, this bias was reduced to around 5%. Based on this evaluation, it is likely that our estimates of *M* from the tagging study may be positively biased, and that actual *M* may be closer to the order of 0.33 to 0.45. With the exception of the Big Bay de Noc stock, these corrected estimates of *M* would still be larger then the predictions from the Pauly (1980) equation. Another factor that may have caused our estimates of *M* to be inflated pertained to the manner by which tag reporting rates were calculated for the tagging study. When tag reporting rates are calculated using onboard or dockside observers, it is assumed that 100% of the tags from the inspected catches are reported. If this assumption is violated, then the estimate of the tag reporting rates for the un-inspected catch will be positively biased (Pollock et al. 2001, 2002), which, according to our simulation study, will result in positively biased estimates of natural mortality.

Despite the possibility that the natural mortality rate estimates from this research were inflated, we believe it to be likely that actual natural mortality rates for the lake whitefish stocks are somewhat greater then those that are currently used in the assessment models for the stocks. One of the consequences of assuming lower estimates of *M* in the assessment models is that enacted harvest policies may be overly conservative because the assessment models predict that there are fewer fish in the population. If the actual natural mortality rates are greater then what is assumed in the assessment models, then the stocks may be capable of supporting larger catches that what currently are allowed. It would be prudent to conduct additional evaluations of natural mortality rates in lake whitefish stocks before allowing greater harvest of lake whitefish in order to prevent overharvest of the stocks.

Fish health indicators

A large proportion of the total variation in health indicators could not be attributed to spatial or temporal sources; rather it was due to 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 could not explain much of the variation among individual fish using the covariates we measured, suggesting that differences among individuals, such as differences in diet, behavior, or physiology, that were not captured by the covariates we measured, likely contributed to the observed among fish variability.

Because lake whitefish from the different stocks have similar abilities for fatty acid synthesis and modifications, any observed differences in fatty acid signatures will reflect differences in foraging patterns (Thiemann et al. 2008). Although, on average, fish from the Naubinway stock tended to differ in the concentrations of several fatty acids compared to fish from other stocks, overall we observed low variation among stocks. 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 during sampling resulting in a weak stock ‘signature’ when using health indicators. Although, our tagging study indicated some site fidelity during the spawning season, there was movement of fish throughout the rest of the year, likely contributing to our low ability to discriminate among stocks.

Variation among years in health indicators was more common and of larger magnitude compared to among-stock differences. We observed temporal trends in several fatty acids, and these trends were common to all four stocks (i.e., a significant year main-effect). For fish health indicators with relatively large annual variation, it was often due to a linear decrease over the three year study period. Although our study period only spanned three years, declines in highly unsaturated fatty acids may have important implications, from a biochemical perspective, for the health and condition of lake whitefish. For example, the decreasing trend observed for the unsaturation index may have important implications for the health of lake whitefish as the degree of unsaturation of membrane lipids (phospholipids) has long been associated with increased membrane "fluidity"; a vital adaptive response to cold temperature challenge (Arts and Kohler 2008). In addition, long-chain highly unsaturated fatty acids (HUFA), such as DHA and EPA, are required for the normal development and reproduction in fish (Sargent 1999; Tocher 2003). HUFAs are also involved in maintaining the structural integrity of cell membranes, including the maintenance of membrane fluidity under low temperature conditions (Arts and Kohler 2008), and are important for neural development and as precursors for eicosanoids (specifically ARA and EPA; Tocher 2003): biochemicals involved in a wide-range of physiological processes, including egg production, spawning and hatching, schooling behavior, and involvement in immunological responses (Brett et al. 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 (Benítez-Santana et al. 2007) leading to a 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 macroinvertebrates rich in HUFAs such as *Diporeia* spp., *Mysis* spp., and Chironomidae (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 dreissenids and gastropods (Pothoven and Madenjian 2008). 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 our study was not designed to elucidate the effects of *Diporeia* abundances on lake whitefish fatty acid composition, the temporal decreases in HUFAs, in addition to the recent declines in HUFA-rich prey in the Great Lakes, suggests that a better understanding of lake whitefish – prey – 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 rates. Identifying 'critical' levels of important fatty acids, below (or above) which survival may be reduced, will greatly improve the interpretability of studies using fatty acids as health indicators.

The spatial and temporal trends in 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 vulnerability to pathogens (Eya and Lovell 1998; Lim and Klesius 2003; Ai et al. 2006), and that certain pathogens can induce mortality in fishes; however, quantifying these interactions is difficult. Of the 11 health indicators with significant variation among stocks, three were significantly correlated with either average stock intensity or prevalence of *Cystidicola* spp. or prevalence of *R. salmoninarum.* In addition, variation among years in four health indicators was also correlated with either

Cystidicola intensity or prevalence. It is impossible to determine cause-and-effect relationships between fish pathogen prevalence and intensity of infection and fatty acid concentrations, however, if lake whitefish immune systems become compromised due to HUFA deficiencies, then their susceptibility to pathogens may increase.

Some of the difficulty in quantifying the interactions between fish nutrition and diseases are due to uncertainties related to the causes of pathogen outbreaks, the factors that lead to susceptible hosts, and the rate and intensity of infection that causes mortality. For example, adults of the swimbladder parasite *Cystidicola* spp. are relatively long-lived, living up to several years in the swimbladder (up to 10 years in charr; Black and Lankester 1980), with no apparent movement of adults out of the swimbladder. The long life-span and lack of movement out of the swimbladder provides the opportunity for large numbers of parasites to accumulate over a fish's lifetime and to have potentially lethal effects on the host. Although there is evidence to suggest that macroparasites (such as *Cystidicola*) can cause mortality in fish populations, for example, Knudsen et al. (2002) provided indirect evidence that *Cystidicola farionis* caused mortality in an Arctic charr (*Salvelinus alpinus*) population in Norway, the intensity of infection that impairs feeding and reproduction and thus affects individual survival and ultimately the population remains unknown.

Uncertainties with regards to the life-cycle of some pathogens also reduce our abilities to predict the effects on host populations. For example, for parasites such as *Cystidicola* spp., a better understanding of the parasite's life-cycle is needed in order to predict the potential affects of a changing benthic food web on infection rates in lake whitefish. Benthic invertebrates, including *Diporeia*, amphipods, and crustaceans such as *Mysis* spp. are potential intermediate host for *Cystidicola* spp. in the Great Lakes (Black 1984; Miscampbell et al. 2004), and the potential effects of a changing benthic food web (including high abundances of dreissenids and decreased abundance of *Diporeia*) on intermediate host dynamics and subsequent transmission to lake whitefish remains unknown. In addition, we hypothesize that interactions between pathogens, such as *Cystidicola* and *R. salmoninarum*, may be important. For example, fatty acid deficiencies may compromise the immune response of lake whitefish leading to infection with both *Cystidicola* and *R. salmoninarum*, the combination of which might be associated with mortality. This remains speculative however, and detailed laboratory experiments, coupled with field investigations and modeling, will greatly improve our ability to make inferences regarding the effects of altered benthic food webs and pathogen interactions on Great Lake whitefish populations. In addition, statistically valid fish health surveys and the integration of fish health surveys with stock assessment will assist in understanding the dynamics and effects of pathogens such as *Cystidicola* and *R. salmoninarum* on lake whitefish populations (Fenichel et al. in review).

Natural Mortality and Fish Health Indicators

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 fatty acid profiles. If the lake whitefish health indicators we examined were sensitive indicators of natural mortality, then we would 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 our estimates of natural mortality rates. The best-performing model (based on QAIC) was a model that assumed a constant natural mortality rate among stocks. Although the second best-performing model estimated separate natural mortality rates for each stock, model averaged stock-specific estimates only ranged from 0.39 – 0.53. In addition, there were no obvious relationships between natural mortality rates and stock-specific health indicator BLUPs, suggesting that average stock health status was not related to the variation in observed natural mortality.

The extensive movement exhibited by lake whitefish during this study likely is a major reason why the stocks do not have larger differences in natural mortality rates. While fish from the individual stocks are highly segregated during the spawning season, which lasts through the fall and early winter, during the remainder of the year the stocks may become largely intermixed. The fish thus experience similar levels of resource availability, sea lamprey encounters, and other stressors, which results in similar levels of natural mortality for the stocks. When managing mixed-stock fisheries, it is important to consider the effects of harvest on each stock in order to protect the overall abundance, productivity, and genetic diversity of the species (Hallerman 2003).

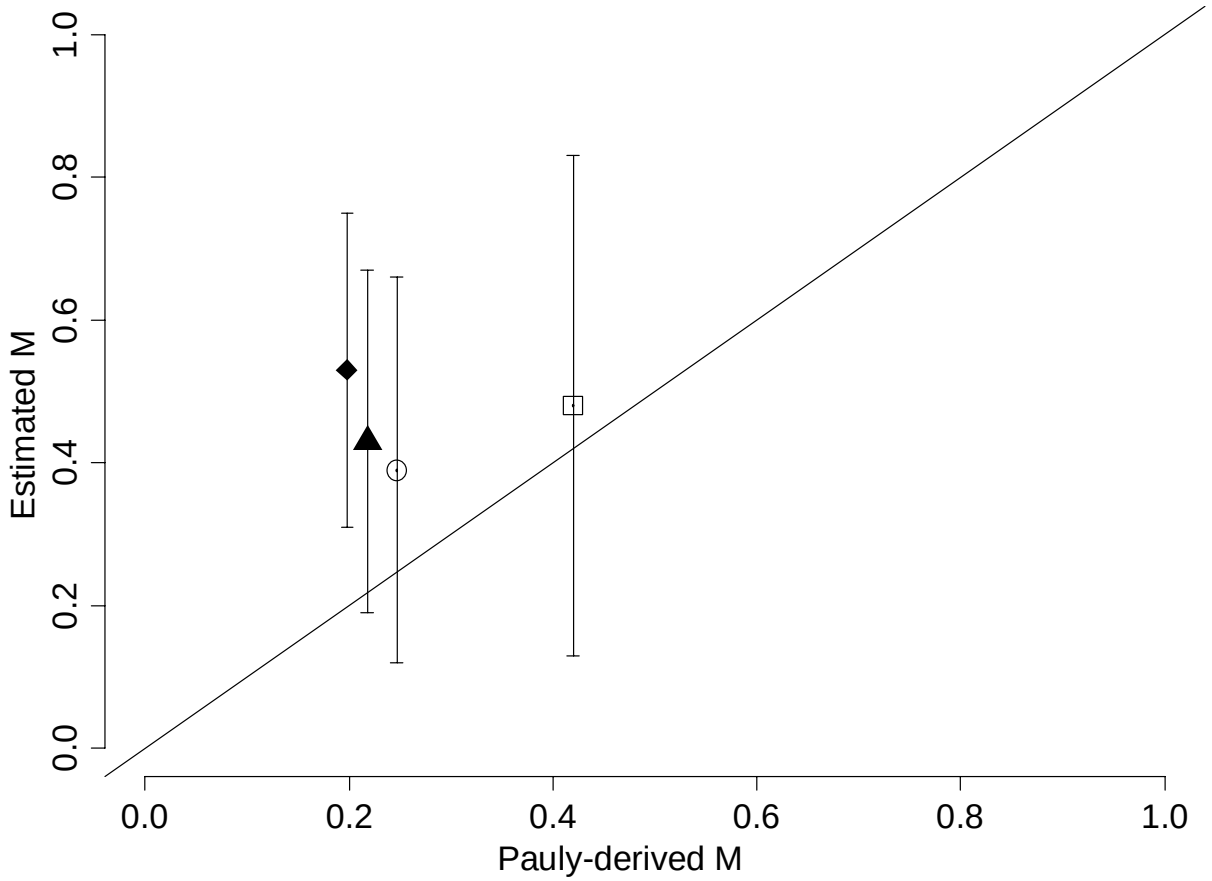

Figure 5.1. Relationship between natural mortality rates estimated in the current study (Estimated M) and natural mortality rates derived from the Pauly (1980) equation (Pauly-derived M) for four lake whitefish stocks, including Big Bay de Noc (□), Naubinway (○), Cheboygan (▲), and Detour (♦). Error bars for 'Estimated M' natural mortality estimates are 95% confidence intervals and solid line represents the one-to-one line.

SUPPLEMENT – SENSITIVITY OF TAG-RECOVERY MORTALITY ESTIMATES TO TAG SHEDDING, HANDLING MORTALITY, AND REPORTING RATE INACCURACIES

INTRODUCTION

Tag-recovery models (Brownie et al. 1985) are widely used to estimate mortality of fish in both marine and freshwater systems. Several factors, including tag shedding, handling mortality, and tag reporting, are known to affect the recovery and reporting of tags and consequently can affect tag-recovery mortality estimates. While it is possible to estimate at least some of these rates as part of fitting a tag-recovery model, accurate estimation has been found to require many years of data (Hoenig et al. 1998). As a result, the ability to accurately estimate mortality at least partly depends on the collection of auxiliary data pertaining to these confounding factors. Each of these can be measured in a variety of ways: tag shedding can be estimated by double tagging or supplemental marking of fish (Pierce and Tomcko 1993, Fabrizio et al. 1999, Latour et al. 2001, Miranda et al. 2002, Livings et al. 2007); handling mortality can be estimated by withholding samples of tagged fish in tanks, pens, or cages (Pierce and Tomcko 1993, Latour et al. 2001, Miranda et al. 2002, Taylor et al. 2006); tag reporting can be estimated through the use of high-reward tags (Pollock et al. 2001, Pollock et al. 2002, Taylor et al. 2006), planted tags (Hearn et al. 2003), or creel or port surveys (Hearn et al. 1999, Pollock et al. 2002).

Even when data concerning tag shedding, handling mortality, and tag reporting rates are collected as part of a tagging study, biased mortality estimates may still occur if measurements of these rates are not accurate (Miranda et al. 2002). Inaccurate measurements of these factors can arise in a number of situations. For example, overestimation of handling mortality may result if fish withheld in nets or pens become overly stressed due to biofouling of the enclosure material (Ahlgren 1998, Udomkusonsri and Noga 2005). Alternatively, handling mortality may be underestimated if favorable conditions in aquaculture tanks promote the recovery of tagged specimens. In either case, mortality estimates would be biased because of the handling mortality inaccuracies. Knowing how such inaccuracies may affect mortality estimates can be beneficial when designing a tag-recovery study as it allows more resources to be devoted to measuring those factors that most strongly influence the mortality component that researchers are particularly interested in.

Our interest in how tag shedding, handling mortality, and reporting rate inaccuracies can affect mortality estimation stemmed from our involvement in a project meant to clarify the relationship between fish health and natural mortality in four lake whitefish (*Coregonus clupeaformis* Mitchill) stocks in northern lakes Huron and Michigan. For the lake whitefish study, fish were tagged with individually numbered t-bar anchor tags, and the recovery and reporting of tags by commercial fishermen were used to estimate fishing and natural mortality rates for the lake whitefish stocks. Data pertaining to tag shedding, handling mortality, and tag reporting were collected as part of the study; however, there was concern that some of these measurements were inaccurate. For example, short-term (< 14 days) survival and tag retention were monitored by withholding tagged lake whitefish at an on-shore holding facility. While at this facility, many tagged fish developed fungal infections and died. It was believed that these infections were caused by the transport and holding of fish at the onshore facility rather then by the tagging process, but it nevertheless let us to question whether our estimates of handling mortality were accurate, and, if not, how our mortality might be affected.

Another factor that concerned us with the lake whitefish study was how possible spatial differences in tag reporting rates might affect mortality estimation. For the study, tag reporting rates by lake whitefish commercial fishermen were measured through the use of onboard observers (Pollock et al. 2002). However, we were only able to collect suitable data to estimate yearly tag reporting rates for the stocks. Given the sizes of the systems that we were studying, we believed tag reporting rates likely varied depending on where in lakes Huron and Michigan tags were recovered. This also led us to question how our lake whitefish mortality estimates of might be affected by using a single tag reporting rate to summarize spatially varying tag reporting.

The purpose of this research was to evaluate the sensitivity of tag-recovery mortality estimates to inaccuracies in tag shedding, handling mortality, and tag reporting rates. We additionally were interested in determining how level of tagging effort affected the sensitivity of mortality estimates to inaccuracies in these factors. We hoped that this research would provide useful information regarding the collection of auxiliary data to ensure accurate mortality estimation for those interested in using a tagging study to estimate mortality of Great Lakes fish.

METHODS

We used Monte Carlo simulations to explore the sensitivity of tag-recovery mortality estimates. Our simulations consisted of both a data generating model that generated tag recoveries, and an estimation model that used the number of recovered and reported tags to estimate instantaneous fishing and natural mortality rates under various assumptions concerning tag shedding, handling mortality, and tag reporting. To provide a sense of realism to our research, we based our simulations on the tagging protocol and spatial framework of the aforementioned lake whitefish study. For our data generating model, fish were tagged once a year for four years, with tag recoveries occurring over a four-year period that began with the initial tagging event. Fish were assumed to be tagged at a single site in northern Lake Michigan, with recovery of tags occurring at various points within the lake (Figure S.1). We considered two levels of tagging effort in the simulations: 2,000 and 10,000 tagged fish per year. The actual number of tagged fish in any given year for the simulations was determined by random draw from a normal distribution with a mean equal to the target tagging effort and a standard deviation equal to 5% of the mean (rounded to the nearest whole integer). Immediately after tagging, fish were assumed to disperse to various parts of Lake Michigan, with dispersal solely a function of distance from the tagging site. The fraction of tagged fish dispersing to areas within 25 km, from 25 to 50 km, from 50 to 100 km, from 100 to 200 km, and beyond 200 km of the tagging site for the simulations was determined by random draw from a multinomial distribution with expected cell probabilities of 50, 25, 15, 8, and 2%, respectively. Dispersal of fish to individual 10-minute grids within these distances of the tagging site was assumed to be purely random.

Tag recoveries for the data generating model were determined using the Hoenig et al. (1998) instantaneous mortality formulation of a tag-recovery model for an assumed Type-II fishery. Instantaneous fishing (F) and natural mortality (M) rates for the data generating model were set equal to 0.15 and 0.40, respectively. To mimic the lake whitefish study, we divided the year into three seasons that differed in both length of year and amount of harvest. The fraction of the year for the seasons was 0.417 (season 1), 0.333 (season 2), and 0.25 (season 3). The fraction of the harvest for the seasons was 0.20 (season 1), 0.30 (season 2), and 0.50 (season 3). For simplicity, we assumed that fishing and natural mortality were constant throughout the lake and for each year of the study. We also assumed that the fraction of the harvest that occurred in the seasons was constant.

Handling mortality and short-term tag shedding rates were each assumed to equal 10% for the data generating model. Long-term tag shedding was assumed to be a sigmoidal function of months since tagging and was modeled with the equation

$$TS = \frac{\alpha}{1+\exp(\beta - x)} = \frac{0.36}{1+\exp(9.7 - x)}, \quad (1)$$

where TS was the long-tem tag shedding rate, x was the number of months since initial tagging, and α and β were model parameters describing the maximum long term tag shedding rate and the point of inflection for the tag loss model, respectively. With this function, long-term tag shedding was near zero for this first 6 months after tagging, and then progressively increased during the next several months before finally stabilizing at a tag loss rate of approximately 36% at around 14 months after tagging. This pattern in long-term tag loss was similar to what we observed in the lake whitefish study.

Tag-reporting rates for Lake Michigan 10-minute grids in the data generating model were a function of distance from the tagging site. Grid reporting rates were calculated with the equation

$$RR_i = 0.5 \cdot \exp(-0.015 \cdot y_i) + 0.25, \quad (2)$$

where RR_i was the reporting rate for grid i and y_i was the distance in kilometers of the centroid of grid i from the tagging site. This equation resulted in tag reporting rates ranging from 25 to 60% for the study area (Figure S.2). These simulated reporting rates were close to what we calculated for the lake whitefish study, and are similar to reporting rates that have been reported elsewhere (Jenkins et al. 2000, Polachek et al. 2006).

Like the data generating model, our estimation model for the simulations was based on the Hoenig et al. (1998) instantaneous mortality formulation of a tag-recovery model for an assumed Type-II fishery. With the estimation model, however, F and M were model unknowns that were estimated based on the number of recovered tags from the data generating model. With the estimation model, we assumed several different rates and functions for tag shedding, handling mortality, and tag reporting so that sensitivity of mortality rate estimates to inaccuracies in these factors could be evaluated. For handling mortality and short-term tag shedding, we evaluated rates of 0, 5, 10, 15, and 20%. For long-term tag shedding, we considered five functions that related long-term tag loss to number of months since tagging (Figure S.3). Function 1 was the same equation used in the data generating model, and thus represented the case where long-term tag shedding rates were precisely known. Functions 2 and 3 were similar in form to Function 1, but differed with respect to the inflection point (β). For Function 2, the inflection point (β) was set equal to 15, while for Function 3 it was set equal to 5. Thus, Functions 2 and 3 corresponded to situations where it was (wrongly) assumed that tags were retained for longer and briefer periods of time, respectively (Figure S.3). For Function 4, we simply assumed long-term tag loss was equal to 20% at all time periods. For Function 5, long-term tag loss was assumed to be asymptotically related to the number of months since tagging, thus the probability of tag loss rapidly increased during the first few months after tagging before stabilizing at around 12 months after tagging.

For tag reporting rates, we considered four scenarios. For the first scenario, we assumed that tag reporting rates were exactly known for all grid cells. For the other scenarios, we assumed that tag reporting rates equaled either 25%, 40%, or 60%. These rates were equivalent to the minimum, median, and maximum tag reporting that were used in the data generating model for the Lake Michigan 10-minute grids.

We used the macro capabilities of SAS Version 9.1 (SAS Institute, Inc. 2003) to conduct our simulations. One hundred simulations were conducted for each combination of target tagging effort, assumed handling mortality rate, assumed short-tern tag shedding rate, assumed long-term tag shedding rate, and tag reporting rates. Maximum likelihood estimates of *F* and *M* for the data generating model were obtained using the NLP procedure (SAS Institute 2007) in SAS. Yearly estimates of *F* and a constant estimate of *M* were obtained for each simulation run. The objective function for the estimation model, which consisted of the summed multinomial negative log-likelihoods for the four tagged cohorts in the simulations, was minimized using quasi-Newton optimization (SAS Institute, Inc. 2007).

The sensitivity of the mortality estimates to tag shedding, handling mortality, and reporting rates inaccuracies was determined using linear mixed models. The mixed models consisted of the *F* and *M* estimates from the simulations as the response variables, with target tagging level as a fixed effect and short- and long-term tag shedding, handling mortality, and tag reporting rates and functions nested within the fixed tagging level effect as random effects. We chose to consider tagging level as a fixed effect since this to some extent is under control of those implementing the study, while the other factors are not. Intercepts were not included in the mixed models. Sensitivity was evaluated by partitioning the observed variation in mortality rate estimates to the tag shedding, handling mortality, and reporting rate random effects. Additionally, we calculated the empirical best linear unbiased estimates (EBLUEs) and empirical best linear unbiased predictors (EBLUPs) for the fixed and random effect components to determine the relative effect of the different tag shedding, handling mortality, and reporting rate levels on the mortality estimates. We conducted the mixed model analyses in SAS used the MIXED procedure (SAS Institute, Inc. 2004).

RESULTS

Altogether, 1,000 variable combinations were explored in our simulations (2 tagging levels × 4 tag reporting rates × 5 long-term tag shedding functions × 5 short-term tag shedding rates × 5 handling mortality rates). Model convergence was achieved in each of the simulations. Yearly estimates of *F* from the simulations ranged from 0.07 to 0.56. Estimates of *M* from the simulations ranged from 0.16 to 0.91.

Based on our mixed model analyses of the mortality estimates from our simulation results, we found that inaccuracies in tag reporting rates had the largest effect on the yearly estimates of *F*. The variance estimates for the tag reporting effect ranged from 15 to 35 times greater then the residual variance estimates for *F* (Figure S.4). In comparison, the variance estimates for the tag shedding and reporting rate effects generally were between 1 to 5 times that of the residual variance estimate.

The EBLUEs and EBLUPs for the fixed and random effects from our linear mixed model analyses of the yearly estimates of *F* were very similar for the two target tagging levels (Table S.1), indicating that the lower tagging effort would suffice for estimating *F*. When tag shedding, handling mortality, and reporting rates for the estimation model were the same as those in the data-generating model, the EBLUPs for the yearly estimates of *F* equaled 0.16 at a target tagging level of 2,000 fish. In comparison, the EBLUPs for the yearly estimates of *F* equaled 0.15 at a target tagging level of 10,000 fish. The sensitivity of the *F* estimates to tag reporting rate inaccuracies were evident from the EBLUPs associated with the individual tag reporting rate levels. The EBLUP for an assumed tag reporting rate of 25% was +0.11, indicating that *F* generally was overestimated with this assumed tag reporting rate. Conversely, the EBLUP for an

assumed tag reporting rate of 60% was -0.07, indicating that *F* generally was underestimated with this assumed level of tag reporting. The EBLUP for an assumed tag reporting rate of 40% was -0.01, indicating that an accurate estimate of *F* could be obtained by assuming this level of tag reporting even when though tag reporting was known to vary spatially.

The EBLUPs for the long-term tag shedding functions ranged in value from -0.04 to +0.03 for the yearly estimates of *F*, while the EBLUPs for the short term tag shedding and handling mortality rates ranged from -0.02 to +0.02. Thus, the error that would result from assuming these different rates and function would generally be less then 20% of the actual values of *F*.

Similar to what we found for estimates of *F*, inaccuracies in tag reporting rates had the largest effect on the estimation of *M.* The variance estimate for the tag reporting effect was approximately 7 times greater then the residual variance estimate (Figure S.4). Long term tag shedding also appeared to have a large impact on *M*; the variance estimate for this effect was approximately 6 times greater then the residual variance estimate (Figure S.4). Conversely, the variance estimates for the handling mortality and short-term tag loss effects were approximately 30% that of the residual variance estimate, suggesting that these effects did not strongly influence estimates of *M*.

When tag shedding, handling mortality, and reporting rates for the estimation model were the same as those in the data generating model, the EBLUP of *M* equaled 0.48 at a target tagging level of 2,000 fish, indicating that estimates of *M* were inherently biased at this level of tagging effort. This bias was reduced at the higher tagging effort level. The EBLUP of *M* was 0.42 with a target tagging level of 10,000 fish. The EBLUPs for the assumed tag reporting rate levels ranged from -0.10 to +0.08 and -0.11 to +0.07 for target tagging levels of 2,000 and 10,000 fish, respectively. The EBLUP for an assumed tag reporting rate of 25% was -0.10 (2,000 tagged fish) and -0.11 (10,000 tagged fish), indicating that *M* generally was underestimated with this assumed level of tag reporting. Conversely, the EBLUP for an assumed tag reporting rate of 60% was +0.08 (2,000 tagged fish) and +0.07 (10,000 tagged), indicates that *M* generally was overestimated with this assumed level of tag reporting. With an assumed tag reporting rate of 40%, the estimate of *M* at a target tagging level of 10,000 fish was predicted to be 0.42 if tag shedding and handling mortality rates were accurately measured. As with the yearly estimates of *F*, this suggests that an accurate estimate of *M* can still be obtained using a single tag reporting rate even when tag reporting is known to spatially vary.

The EBLUPS for the long-term tag shedding functions that we evaluated ranged from -0.06 to +0.12 for both levels of tagging effort. The largest bias in *M* would result from assuming that long-term tag shedding was a constant rate of 0.20 (Table S.1), assuming this rate would cause overestimation of *M.* Modeling long-term tag shedding with an incorrect model inflection point or with an exponential decay function had somewhat smaller effects on estimates of *M.* The EBLUPS for these different functions ranged from -0.04 to +0.01, which in each case would still result in overestimation of *M* because of the natural bias in estimating this mortality components.

The EBLUPs for the incorrect short term tag shedding and handling mortality rates ranged from -0.02 to +0.02 for both target tagging levels when estimating *M*. Thus, the error that would result from assuming these different rates and function would generally be less then 5% of the actual values of *M.*

DISCUSSION

When designing a tagging study, a number of decisions must be made, such as how many fish will be tagged, how much of a reward will be offered for the return of tags, and how much effort will be devoted to collected information concerning tag loss, handling mortality, and tag reporting (Guy *et al.* 1996). Because of budgetary restrictions, the answers to almost all of these questions are interrelated. The reward offered for tag returns will depend on how may specimens were tagged and expected return rates. The employment of observers to measure tag reporting rates may limit how many tags initially can be purchased. When designing a tagging study, one strives to optimally allocate resources so that mortality estimates are as accurate and precise as possible. Finding that optimal allocation of resources may prove difficult however because of the range of conditions that one may encounter with a tagging study. As a result, deciding how much resources should be devoted to any particular aspect of a tagging study will require substantial examination of the individual species and system that the researchers are studying. The intent of our research was to evaluate the sensitivity of tag-recovery mortality estimates to inaccuracies in tag shedding, handling mortality, and reporting rate in order to provide beneficial information to those planning on conducting their own tagging study. We chose to base our simulations on a lake whitefish tagging study conducted in northern lakes Huron and Michigan as tagging studies conducted in the Great Lakes have many issues that can arise that can substantially affect mortality estimation (e.g., potential for substantial movement of tagged specimens, likeliness of spatially varying tag reporting rates). Even though such issues are not unique to the Great Lakes, these issues are magnified by the size of the systems relative to other inland systems.

Based on the results of this research, we suggest that when designing a tagging study some initial consideration should be given to which mortality component researchers are most interested. This will help in selecting the target tagging level as well as how much resources should be devoted to measuring factors that may confound mortality estimates. We found only small differences in yearly estimates of F for the target tagging levels that we included in our simulations. Consequently, using the higher target tagging level may simply increase cost of the project without necessarily resulting in more accurate mortality estimates. Conversely, we found that estimates of M were more strongly affected by the target tagging level. At a target tagging level of 2,000 fish per year, estimates of M were inherently upwardly biased. Thus, if the intent of the tagging project is to estimate natural mortality, then a greater level of tagging effort may be needed to ensure that estimates of M are not biased

Regardless of which mortality component researchers are interested in, accurate measurement of tag reporting rates is an important consideration for estimating fish mortality rates. In our research, we found that assumptions concerning tag reporting led to the greatest amount of uncertainty in both F and M estimates. Tag reporting rate is considered one of the most difficult variables to measure for a tagging study (Denson et al. 2002, Miranda et al. 2002), and when combined with the fact that both spatially and temporally varying tag reporting rates are frequently reported (Jenkins et al. 2000, Pollock et al. 2002, Polacheck et al. 2006, Taylor et al. 2006), should serve as a warning to those designing a tagging study that significant resources may need to be devoted to measured tag reporting. A number of factors are likely to affect tag reporting by both recreational anglers and commercial fishermen, including publicity of the tagging program, prior acquaintanceship with those conducting the study, preconceived notions as to how the tagging information will be used, and general indifference to the tagging. program. Because of the dynamic nature of these factors, it may be extremely difficult to develop a complete picture of tag reporting rates for the system being studied. At the very least, researchers should attempt to develop a central tendency measure of tag reporting by measuring reporting

rates in areas or fishery components were reporting is expected to be both high and low. Based on our research, a central tendency measure of tag reporting can yield accurate mortality estimates even when reporting rates vary spatially. Measuring reporting rates only in areas where reporting is expected or believed to be high should be avoided as this can impart significant biases on estimates of *F* and *M*.

Accurate measurement of long-term tag shedding will also be beneficial when a tagging study is conducted primarily for the purpose of estimating *M*. Compared to tag reporting, measurement of long-term tag shedding is relatively easy, and can be accomplished by either double tagging or supplementally marking tagged fish. The major question that needs to be answered is how many double tagged or supplementally marked fish should be released. This question is particularly important for studies where tag recoveries occur over a several year period. With too few double tagged or supplementally marked fish, tag shedding rates at longer time periods may only be calculated from one or two recovered individuals, which can make fitting a long-term tag shedding model difficult. While a variety of models can be used to represent long-term tag shedding (Fabrizio et al. 1996), slight deviations in the model form or fitted equations may not have a strong impact on mortality estimates based on our findings. Rather, it may be more important to simply capture the general trend of tag loss with time.

Despite our finding that short-term tag loss and handling mortality had relatively minor effects on the accuracy of mortality estimates, we do not recommend completely ignoring these factors when designing and conducting a tagging study. Rather, we believe our results suggest that it may not be necessary to devote substantial resources to measure these rates, particularly if that means devoting resources away from measuring more important factors, such as tag reporting rates.

Admittedly, the results of our study were driven by the different rates and functions that were assumed in the estimation model for tag shedding, handling mortality, and tag reporting. In designing our simulations, we intentionally limited our assumptions about tag shedding, handling mortality, and tag reporting to what was most likely given what was used in the data generating model. For example, we did not consider 90% short-term tag shedding and handling mortality rates in our estimation model as we felt it was highly unlikely that a measurement error of this magnitude would occur when actual short-term tag shedding was only 10%. Additionally, we attempted to incorporate rates and functions that were within the realm of acceptability based on published findings. For example, our assumption that tag reporting rates varied between 25 and 60% matched the range of rates that have been reported by Hearn et al. (1999), Pollock et al. (2001), Polacheck et al. (2006), and Taylor et al. (2006). Similarly, our assumed levels of tag shedding was within the range reported by Ebener and Copes (1982), Muoneke (1992), Buzby and Deegan (1999), and Miranda et al. (2002), and our assumed levels of handling mortality were similar to those reported in Pierce and Tomcko (1993) and Miranda et al. (2002). Thus, although we only considered a limited range of possibilities, we feel that the different rates and functions that were included in our simulations were appropriate and that our results accurately reflect the uncertainty associated with mortality estimation.

As stated previously, our interest in this research stemmed from our involvement in a lake whitefish tagging study where the was to gain better understanding between measures of fish health and natural mortality rates for four stocks in northern lakes Huron and Michigan. If we had completed our computer simulations prior to conducting the lake whitefish study, we likely would have tried to change several aspects of the tagging protocol employed (e.g., greater number of tagged fish, greater effort to measure tag reporting rates) in order to increase our

ability to detect a relationship between natural mortality and measures of lake whitefish health. As pointed out by Pollock et al. (2001), these types computer simulations are very useful for evaluating proposed tagging study designs in light of various assumptions concerning tag reporting, handling mortality, and tag shedding. We highly encourage those considering a tagging study for the purpose of estimating fish mortality to employ similar computer simulations to help in choosing an appropriate tagging protocol.

Table S.1. Empirical best linear unbiased estimates (EBLUEs) and predictors (EBLUPs) of the fixed and random effect levels from our linear mixed model analyses of the mortality estimates from our simulations used to evaluate the sensitivity of mortality estimates to inaccuracies in tag shedding (LT = long term shedding; ST = short term shedding), handling mortality (HM), and tag reporting rates (RR). Predicted mortality estimates for the different combinations of tagging effort, tag shedding, handling mortality, and tag reporting rates can be calculated by addition (EBLUE+EBLUP).

	Tagging Effort = 2,000 fish					Tagging Effort = 10,000 fish				
	F_1	F_2	F_3	F_4	M	F_1	F_2	F_3	F_4	M
Fixed effect	0.20	0.20	0.20	0.20	0.53	0.21	0.20	0.20	0.20	0.45
RR = known	-0.03	-0.03	-0.03	-0.03	+0.01	-0.03	-0.03	-0.03	-0.03	+0.03
RR = 25%	+0.11	+0.11	+0.11	+0.11	-0.10	+0.11	+0.11	+0.11	+0.11	-0.11
RR = 40%	-0.01	-0.01	-0.01	-0.01	+0.01	-0.01	-0.01	-0.01	-0.01	+0.01
RR = 60%	-0.07	-0.07	-0.07	-0.07	+0.08	-0.07	-0.07	-0.07	-0.07	+0.07
LT Fn. 1	-0.03	-0.02	-0.02	-0.02	-0.06	-0.03	-0.02	-0.02	-0.02	-0.06
LT Fn. 2	-0.04	-0.03	-0.03	-0.03	-0.03	-0.04	-0.04	-0.03	-0.03	-0.03
LT Fn. 3	+0.01	+0.01	+0.01	+0.00	-0.04	+0.01	+0.01	+0.01	+0.00	-0.04
LT Fn. 4	+0.02	+0.02	+0.02	+0.03	+0.12	+0.02	+0.02	+0.02	+0.02	+0.12

6 Table S.1. Cont.

	Tagging Effort = 2,000 fish					Tagging Effort = 10,000 fish				
	F_1	F_2	F_3	F_4	M	F_1	F_2	F_3	F_4	M
LT Fn. 5	+0.03	+0.03	+0.03	+0.03	+0.01	+0.03	+0.03	+0.03	+0.03	+0.01
ST = 0%	-0.02	-0.02	-0.02	-0.02	+0.02	-0.02	-0.02	-0.02	-0.02	+0.02
ST = 5%	-0.01	-0.01	-0.01	-0.01	+0.01	-0.01	-0.01	-0.01	-0.01	+0.01
ST = 10%	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00
ST = 15%	+0.01	+0.01	+0.01	+0.01	-0.01	+0.01	+0.01	+0.01	+0.01	-0.01
ST = 20%	+0.02	+0.02	+0.02	+0.02	-0.02	+0.02	+0.02	+0.02	+0.02	-0.02
HM = 0%	-0.02	-0.02	-0.02	-0.02	+0.02	-0.02	-0.02	-0.02	-0.02	+0.02
HM = 5%	-0.01	-0.01	-0.01	-0.01	+0.01	-0.01	-0.01	-0.01	-0.01	+0.01
HM = 10%	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00	+0.00
HM = 15%	+0.01	+0.01	+0.01	+0.01	-0.01	+0.01	+0.01	+0.01	+0.01	-0.01
HM = 20%	+0.02	+0.02	+0.02	+0.02	-0.02	+0.02	+0.02	+0.02	+0.02	-0.02

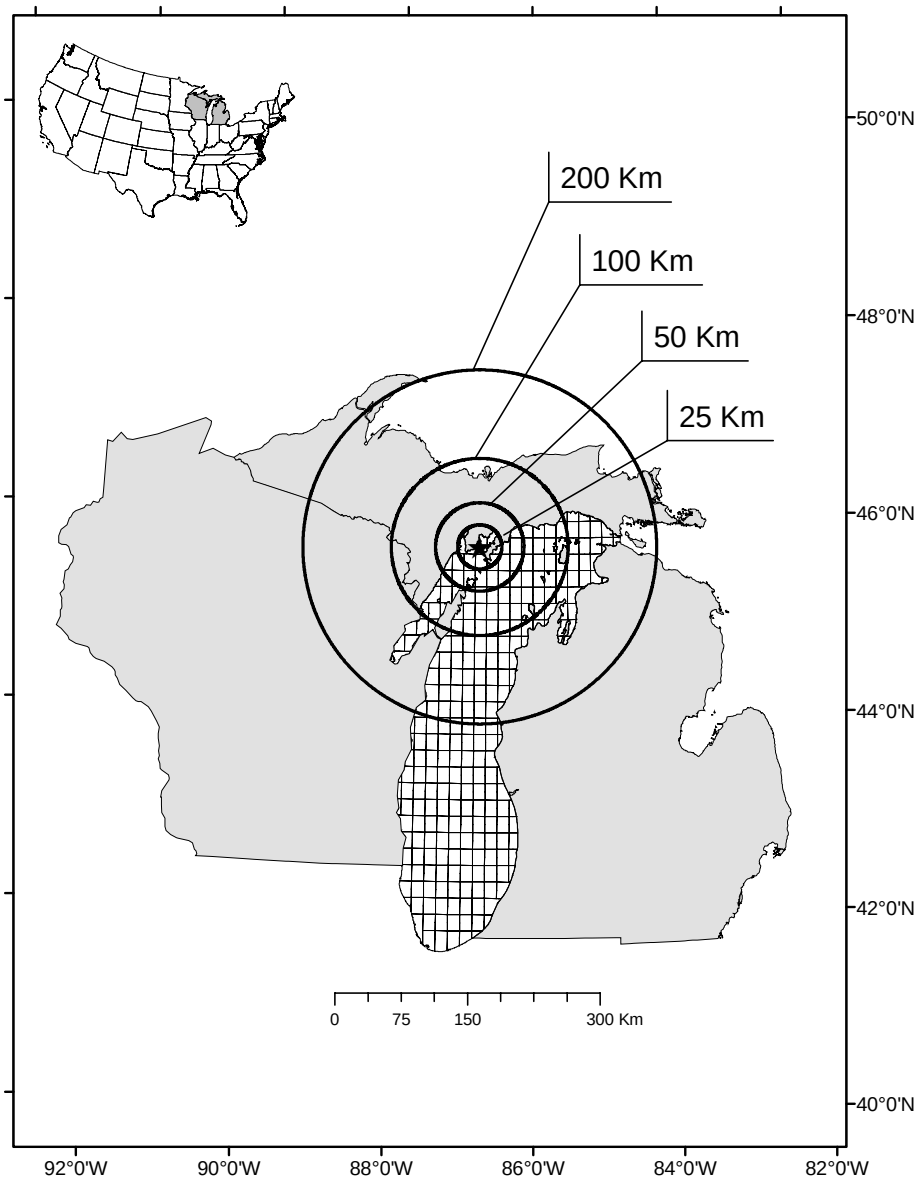

Figure S.1. The assumed spatial framework used in our simulations to evaluate the sensitivity of tag-recovery mortality estimates to tag shedding, handling mortality, and reporting rate inaccuracies. Fish were assumed to be tagged at a single site in northern Lake Michigan(★), whereupon fish dispersed to various Lake Michigan 10-minute grids. The concentric circles located around the tagging site indicate different distances from the site.

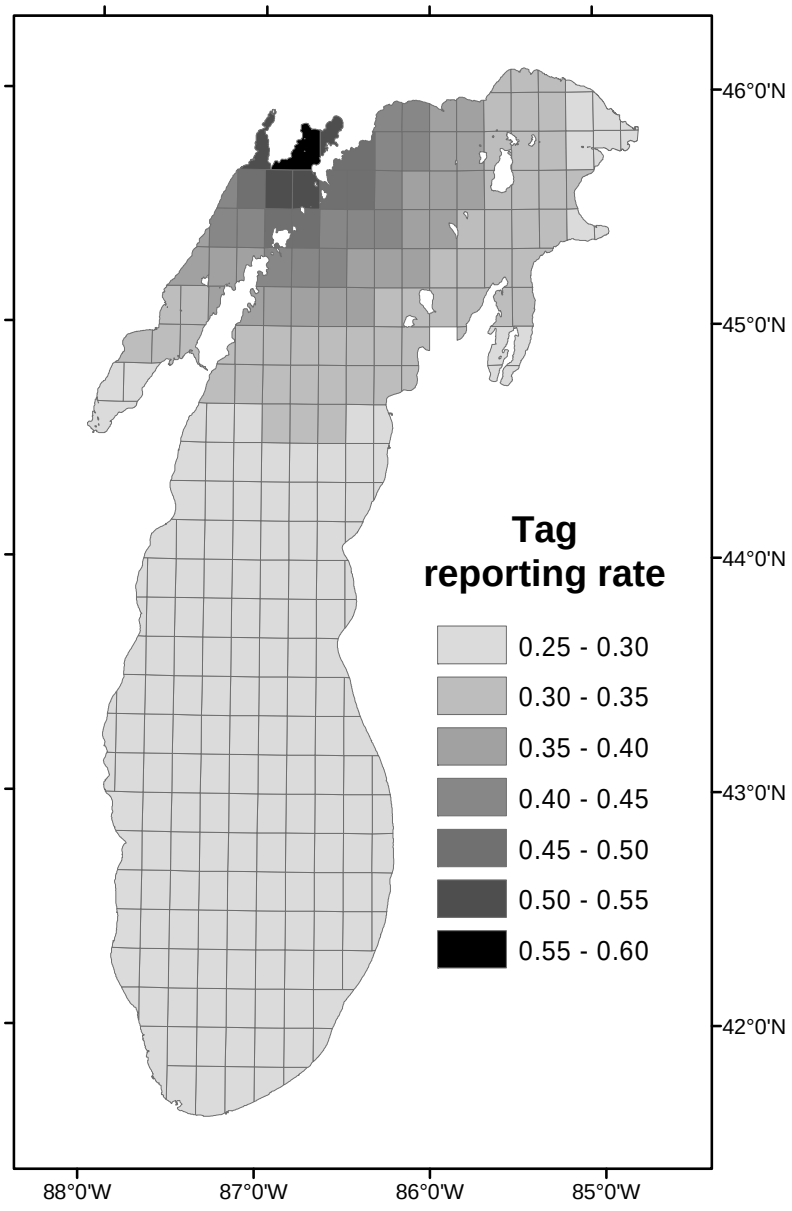

Figure S.2. Tag reporting rates for Lake Michigan 10-minute grids based on the equation assumed for the data generating model.

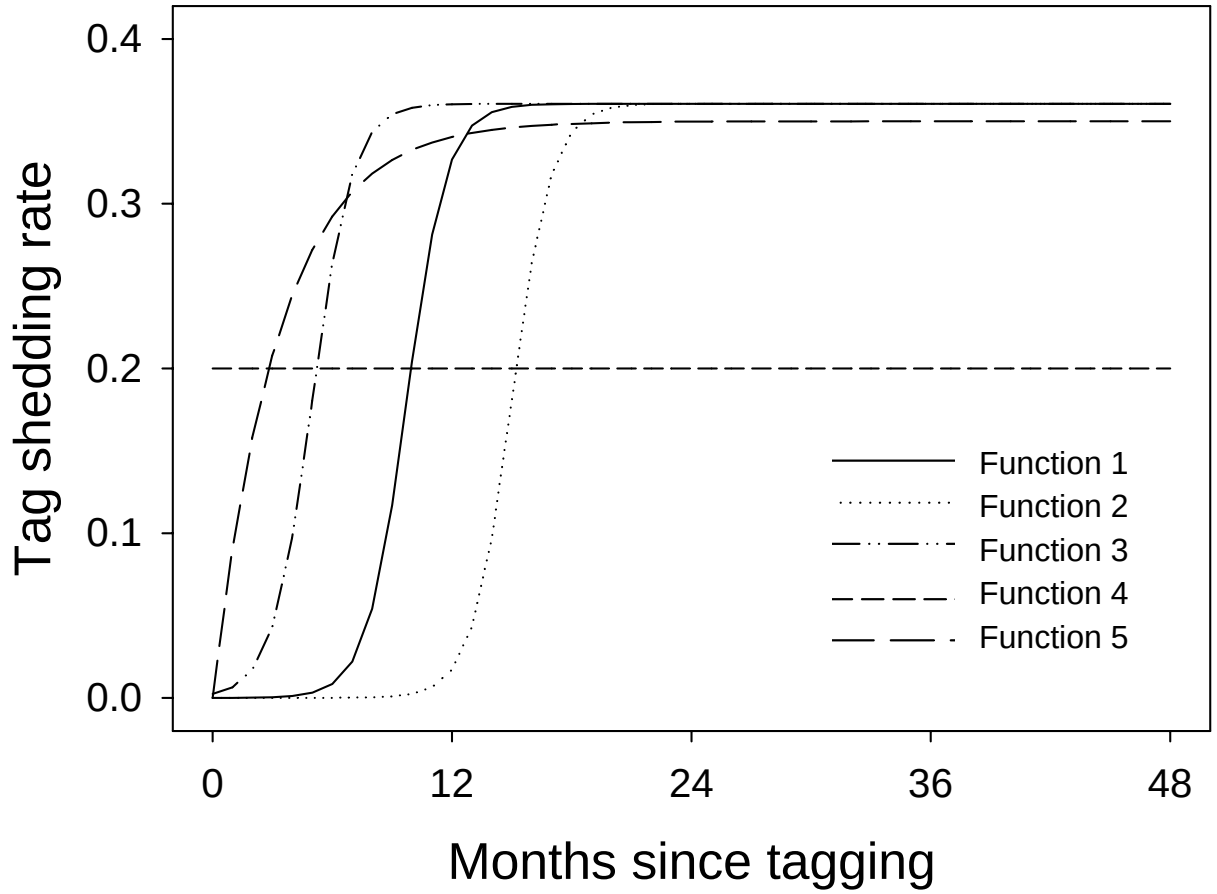

Figure S.3. Long term tag shedding functions that were used in the estimation model to evaluate sensitivity of tag-recovery mortality estimates to tag shedding, handling mortality, and tag reporting rates. Function 1 was the same long term tag shedding function incorporated in the data generating model, and thus represents the case where tag shedding was exactly known.

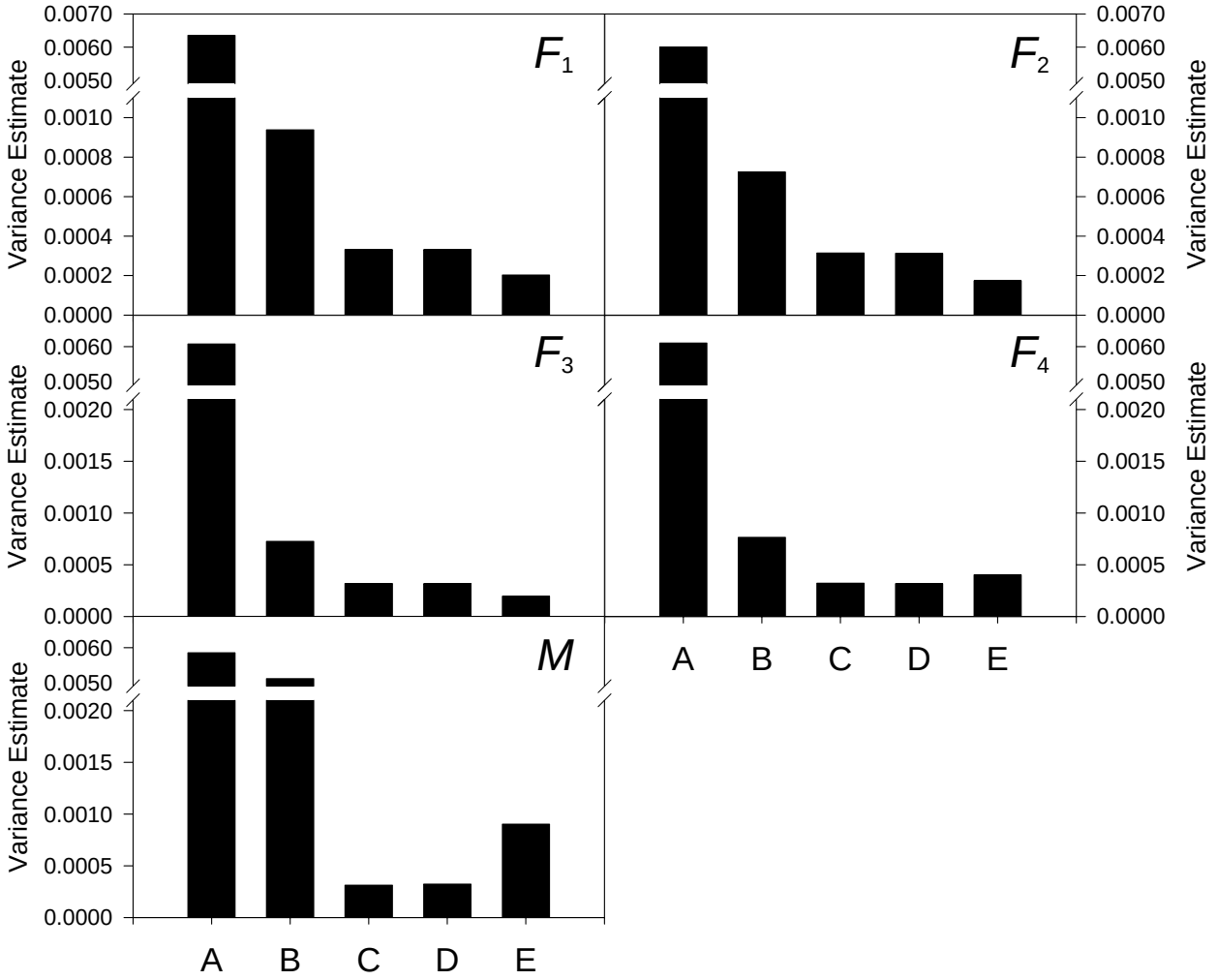

Figure S.4. Estimated variances for the random effects included in the linear mixed model analyses of the estimates of instantaneous fishing and natural mortality: A = Reporting effects; B = Long term tag retention; C = Short term tag retention; D = Handling mortality; E = Residual);

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