# SUBLETHAL IMMUNE, ENDOCRINE, AND BIOENERGETICS RESPONSES TO SEA LAMPREY PARASITISM IN TWO LAKE TROUT MORPHOTYPES FROM LAKE SUPERIOR

By

Sara Elizabeth Smith

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Fisheries and Wildlife – Master of Science

2013

ABSTRACT

SUBLETHAL IMMUNE, ENDOCRINE, AND BIOENERGETICS RESPONSES TO SEA LAMPREY PARASITISM IN TWO LAKE TROUT MORPHOTYPES FROM LAKE SUPERIOR

By

Sara Elizabeth Smith

Sea lamprey (*Petromyzon marinus*) are an invasive species in the Great Lakes and have caused extensive mortality to native lake trout (*Salvelinus namaycush*). Past research has focused primarily on the lethal effects of parasitism and very little is known about the sublethal effects. This study examined lake trout response to sublethal sea lamprey parasitism by measuring parameters related to immune modulation, endocrine disruption, and bioenergetics changes (growth and reproduction). I also compared two Lake Superior lake trout morphotypes (lean and siscowet) to determine how life history influences parasitism responses. Leans and siscowets are partially bathymetrically isolated in Lake Superior and have genetically-based morphological differences, including higher muscle lipid concentrations in siscowets. On both morphotypes, I measured immediate responses to sea lamprey attacks through a laboratory experiment and long-term, cumulative effects through Lake Superior field studies. Sublethal sea lamprey parasitism was associated with endocrine disruption (suppressed plasma testosterone and pituitary follicle-stimulating hormone [FSH]) in the lean and siscowet morphotypes. Parasitized siscowets showed indications of immune-related modulation (hepatosomatic index increase with parasitism duration and differential regulation of immune-related genes) as an immediate response to parasitism and a trend towards decreased muscle lipid concentrations in wild populations. These results support the hypothesis that sea lamprey parasitism affects immune, endocrine, and bioenergetics related parameters, and that parasitism response is influenced by life history.

## ACKNOWLEDGEMENTS

I would like to thank my graduate adviser Cheryl Murphy for her invaluable help with this research. Thank you for being a wonderful mentor and role model. I also thank my graduate committee members, Mike Jones and Mohamed Faisal, for their support and guidance.

The Great Lakes Fishery Commission provided funding for this research. MSU Council of Graduate Students, MSU Fisheries and Wildlife Graduate Student Organization, the Society of Environmental Toxicology and Chemistry, and MSU College of Agriculture and Natural Resources provided additional travel and fellowship funding.

Thank you to Rick Goetz for conducting the RNAseq and qPCR analyses. Thank you to the Michigan Department of Natural Resources, and especially Shawn Sitar, for your help conducting the field sampling. Thank you to the laboratory technicians (Brandon Armstrong, Jakub Bednarek, Ellie Monarch) and undergraduates (Joseph Parzych, Dane Larson, Jeremy Lowell, Karishma Chopra, Andrew Bartholomew, Rachel Gardner, Erin Smigielski, Rachel Mistak) for your research help. Thank you to the Juli Wade and Weiming Li labs for their guidance on the radioimmunoassay procedure. I am also very grateful for the research space, equipment, and assistance provided by the Hammond Bay Biological Station (Nick Johnson, Roger Bergstedt, Erik Larson), MSU containment facility (Randy Shoemaker), Sea Lamprey Control Centre (Gale Bravener), Jordon River National Fish Hatchery (Roger Gordon), and USFWS.

Lastly, thank you to my friends and family for your support and for listening to me talk about sea lamprey more than you probably would have liked. There are far too many individuals who encouraged me throughout my education to list here, and for that I am truly thankful.

## TABLE OF CONTENTS

**LIST OF TABLES**…… v

**LIST OF FIGURES**…… vi

**KEY TO SYMBOLS OR ABBREVIATIONS**…… ix

**INTRODUCTION**…… 1
- **Objective 1: Evaluate immune, endocrine, and bioenergetics responses to sea lamprey parasitism**…… 2
- **Objective 2: Evaluate impact of life history differences on sublethal parasitism response**…… 6
- **Experimental design**…… 8

**METHODS**…… 10
- **Immediate parasitic response—laboratory experiment**…… 10
- **Long term parasitic response—field sampling**…… 12
- **Measurements related to sea lamprey parasitism behavior**…… 14
- **Gross measurements of immune-related modulation**…… 15
- **Physiological measurements related to endocrine disruption**…… 15
- **Physiological measurements related to bioenergetics**…… 17
- **Data analysis of immune, endocrine, and bioenergetics parameters**…… 18
- **Hepatic genetic regulation to determine immune, growth and endocrine effects**…… 21

**RESULTS**…… 23
- **Measurements related to sea lamprey parasitism behavior**…… 23
- **Gross measurements of immune-related modulation**…… 26
- **Physiological measurements related to endocrine disruption**…… 28
- **Physiological measurements related to bioenergetics**…… 31
- **Evaluation of changes in hepatic genetic regulation**…… 37

**DISCUSSION**…… 41
- **Immune, endocrine, and bioenergetics responses to parasitism in lean and siscowet lake trout**…… 41
- **Life history influence on sea lamprey parasitism behavior**…… 49
- **Overall conclusions**…… 52

**APPENDICES**…… 54
- **APPENDIX A: LIST OF ALTERNATIVE MODELS**
- **APPENDIX B: MODELS WITHIN TWO AIC VALUES OF BEST MODEL**

**REFERENCES**…… 71

## LIST OF TABLES

**Table 1.** Table of transformations for immune, endocrine, and bioenergetics response variables.…….……. 20

**Table 2.** Best models for measurements related to sea lamprey parasitism behavior….. 24

**Table 3.** Best models for gross measurements of immune-related modulation. Morphotype and parasitism status were considered fixed effects and included in all alternative models……..……... 26

**Table 4.** Best models for physiological measurements related to endocrine disruption. Morphotype and parasitism status were considered fixed effects and included in all alternative models……..... 29

**Table 5.** Best models for bioenergetics measurements related to growth. Morphotype and parasitism status were considered fixed effects and included in all alternative models ……..…….. 32

**Table 6.** Best models for bioenergetics measurements related to reproduction. Morphotype and parasitism status were considered fixed effects and included in all alternative models……..……... 35

**Table 7.** Summary of genes with the greatest regulation differences between parasitized and non-parasitized leans and siscowets from RNAseq analysis. Numbers in parentheses represents the magnitude difference in genetic regulation…..……..... 37

**Table 8.** Description of differentially expressed gene functions from qPCR analysis…. 38

**Table 9.** Predictor variables for models within two AIC values of the best model for measurements related to sea lamprey behavior……. 63

**Table 10.** Predictor variables for models within two AIC values of the best model for measurements of immune-related modulation…. 64

**Table 11.** Predictor variables for models within two AIC values of the best model for physiological measurements related to endocrine disruption…… 65

**Table 12.** Predictor variables for models within two AIC values of the best model for bioenergetics measurements related to growth……. 67

**Table 13.** Predictor variables for models within two AIC values of the best model for bioenergetics measurements related to reproduction…… 69

## LIST OF FIGURES

**Figure 1.** Photo of a sea lamprey wound penetrating the muscle with minimal healing. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis...……...…..……... 3

**Figure 2.** Schematic representation of the female fish hypothalamus-pituitary-gonadal (HPG) axis…….…… 4

**Figure 3.** The lean lake trout (a) and siscowet lake trout (b) morphotypes..…...……. 7

**Figure 4.** Initial weight distribution of sea lamprey used in the laboratory experiment.. 11

**Figure 5.** Locations of muscle lipid concentration readings taken using a non-invasive fish fatmeter (Model FM-692, Distell Inc., West Lothian, Scotland)……... 11

**Figure 6.** Sampling sites were located in Lake Superior outside Marquette, MI…...….. 13

**Figure 7.** Average egg diameter calculated using six eggs per lake trout did not significantly differ from average egg diameter calculated using 36 eggs per lake trout in a random subsample of 15 wild lake trout…… 18

**Figure 8.** (A) Sea lamprey feeding on siscowet hosts showed a statistical trend for gaining a higher percentage of body weight during parasitism (p=0.0599). Error bars represent standard errors. (B) Number of sea lamprey scars per lake trout was not significantly different ($X^2$=4.747, p=0.1905) between wild parasitized leans (n=32) and siscowets (n=25).…...…… 25

**Figure 9.** (A) The relationship between HSI and test duration was not significant for parasitized leans (R2=0.0475, p=0.2747) and non-parasitized leans (R2=0.0165, p=0.5445) from the laboratory experiment. (B) Non-parasitized siscowets from the laboratory experiment did not have a significant relationship between HSI and test duration (R2=0.0008, p=0.8807). This relationship was significant in parasitized siscowets (R2=0.1802, p=0.0173). (C) HSI marginal means were not significantly different between parasitized and non-parasitized leans (p=0.2456) or parasitized and non-parasitized siscowets (p=0.1776) in the laboratory experiment. Error bars represent the 95 percent confidence intervals of the marginal means. (D) HSI was not significantly different between wild leans and siscowets and their non-parasitized counterparts (p=0.9016). Error bars represent standard error……... 27

**Figure 10.** (A) Plasma testosterone marginal means were significantly different between parasitized leans and siscowets and their non-parasitized counterparts in the laboratory experiment (p=0.0215). (B) Parasitized and non-parasitized lake trout in the laboratory experiment did not have significantly different plasma estradiol concentrations (p=0.2285). (C) Marginal means were not significantly different between wild parasitized and non-parasitized lake trout for plasma testosterone concentrations (p=0.6746) or (D) plasma estradiol concentrations (p=0.5926). (E) Wild parasitized lake trout had significantly lower pituitary FSH levels than non-parasitized lake trout (p=0.0061). Error bars represent 95 percent confidence intervals of the marginal means (A, C, D) or standard error (B, E). * = significant difference p<0.05……..... 30

**Figure 11.** (A) Muscle lipid concentration marginal means, measured using the non-invasive fatmeter, were not significantly different between parasitized and non-parasitized lake trout in the laboratory experiment (p=0.8605). (B) Muscle lipid concentration marginal means, measured using the non-invasive fatmeter, were not significantly different between wild parasitized and non-parasitized lake trout (p=0.9580). (C) Muscle lipid concentration, measured by laboratory lipid extraction, were not significantly different between parasitized and non-parasitized leans (T=-0.2429, p=0.8107); parasitized and non-parasitized siscowets were almost significantly different (T=1.7133, p=0.0536). Error bars represent the 95 percent confidence intervals of the marginal means (A,B) or standard error (C)……...… 33

**Figure 12.** (A) Age distributions significantly differed between wild parasitized leans (n=31) and non-parasitized leans (n=48) (X2=15.68, p=0.0035). (B) Age distributions were almost significantly different between wild parasitized siscowets (n=23) and non-parasitized siscowets (n=25) (X2=8.56, p= 0.073). (C) Length-at-age relationships were significantly different (p=0.0170) between parasitized leans (R2=0.3371, p=0.0024) and non-parasitized leans (R2= 0.1246, p=0.0139). (D) Length-at-age relationships were significantly different (p=0.0106) between parasitized siscowets (R2=0.3181, p=0.0051) and non-parasitized siscowets ($R^2$=<0.0001, p=0.9872). (E) Marginal mean length was significantly different between parasitized and non-parasitized leans (p=0.0005) and siscowets (p=0.0061). Error bars represent marginal means 95 percent confidence intervals. * = significant difference p<0.05……. 34

**Figure 13.** (A) Parasitized and non-parasitized lake trout from the laboratory experiment did not significantly differ in weight-adjusted fecundity (p=0.2622), (B) GSI (p=0.1395), (C) or average egg diameter (p=0.9312). (D) Wild parasitized and non-parasitized lake trout weight-adjusted fecundity was not significantly different (p=0.5806). (E) GSI marginal means were not significantly different between wild parasitized and non-parasitized leans (p=0.5334) or between parasitized and non-parasitized siscowets (p=0.9178). (F) Wild parasitized and non-parasitized lake trout had significantly different average egg diameters (p=0.0139). Error bars represent standard error (A, B, C, D, F) or the 95 percent confidence intervals of the marginal means (E)……...…… 36

**Figure 14.** Comparison of four differentially regulated hepatic genes between parasitized (n=6) and non-parasitized (n=6) leans, as determined by qPCR analysis. Error bars represent standard error. * = significant difference $p<0.05$……. 39

**Figure 15.** Comparison of four differentially regulated hepatic genes between parasitized (n=6) and non-parasitized (n=6) siscowets, as determined by qPCR analysis. Error bars represent standard error……...….. 40

## KEY TO SYMBOLS OR ABBREVIATIONS

| | |
|---|---|
| AIC | Akaike information criterion |
| ANOVA | Analysis of variance |
| DPM | Disintegrations per minute |
| EIA | Enzyme immunoassay |
| g | Gram |
| GLM | General linear model |
| GSI | Gonadosomatic index |
| HPG | Hypothalamus-pituitary-gonadal |
| HSI | Hepatosomatic index |
| L | Liter |
| LH | Luteinizing hormone |
| mm | Millimeter |
| MS-222 | Tricaine methanesulfonate |
| ng | Nanogram |
| PCR | Polymerase chain reaction |
| qPCR | Real-time polymerase chain reaction |
| RIA | Radioimmunoassay |
| RNA | Ribonucleic acid |
| RPM | Revolutions per minute |
| SSBP | Sex steroid binding protein |
| SSBR | Spawning stock biomass per recruit |
| wt/w | Wet weight |

# INTRODUCTION

Since the invasion of the sea lamprey (*Petromyzon marinus*) into the Great Lakes Basin there has been much research into the impacts of parasitic sea lamprey on native Great Lakes species. The Welland Canal construction in the late 1800's allowed sea lamprey access to Lake Erie and by the 1950's sea lamprey had established themselves throughout the Great Lakes (Dymond 1922; Smith and Tibbles 1980; Sullivan et al. 2003). Sea lamprey are external parasites which feed on host tissue fluids and often kill hosts after a parasitic event. Sea lamprey parasitize a variety of Great Lakes species, including native lake trout (*Salvelinus namaycush*). It is thought that sea lamprey parasitism, combined with over-harvesting, contributed to the decline of self-sustaining, naturally reproducing lake trout in the Upper Great Lakes (Coble et al. 1990; Eshenroder 1992; Jensen 1994).

Past research on interactions between sea lamprey and lake trout has focused primarily on the lethal effects. Sea lamprey scars on surviving hosts are used to estimate parasitism mortality (Bence et al. 2003). These mortality estimates are incorporated into lake trout population models and are used to establish an optimal level of sea lamprey control (Sitar et al. 1999; Bence et al. 2003; Irwin et al. 2012). However, not all lake trout are killed by a sea lamprey attack. In fact, it is estimated that 45 to 75 percent of lake trout survive a parasitism event (Swink 2003; Madenjian et al. 2008). Little is known about these parasitism survivors and, due to a lack of research suggesting otherwise, they are assumed to grow and reproduce the same as non-wounded lake trout do, and are modeled as such.

The purpose of this thesis was to identify and quantify sublethal lake trout response to sea lamprey parasitism. For my first research objective I evaluated responses to parasitism on lake trout immediately following a parasitism event and after the sea lamprey wound had healed. Results from this study will identify which physiological systems are most affected by a sea

lamprey attack. This will aid evaluation of the full impact of sea lamprey parasitism on lake trout beyond the lethal effects, provide connections to population-level responses, and give direction for future research. As a second research objective I compared responses to sublethal sea lamprey parasitism between two morphotypes of Lake Superior lake trout—the lean and siscowet—to determine whether life history influences lake trout reaction to parasitism.

**Objective 1: Evaluate immune, endocrine, and bioenergetics responses to sea lamprey parasitism**

I predicted that parasitized lake trout would have altered immune and endocrine-related parameters as immediate responses to a parasitism event, followed by bioenergetics changes (e.g. reproduction and growth) as a long-term response. These predictions follow the general physiological model of stress response, proposed by Selye (1950) and first observed in fish by Mazeaud et al. (1977). These general stages of stress response have been observed for a variety of fish species and stressor types, although the details of these responses are species- and stressor-dependent (Barton and Iwama 1991; Schreck et al. 2001). In this thesis I identify and describe the stages of lake trout stress response when parasitized by sea lamprey.

Suppression of immune-related parameters is a commonly observed response immediately following acute stress (Ortuño et al. 2001; Cheng et al. 2004). Immune-related modulation has been previously observed in lamprey-wounded rainbow trout (*Oncorhynchus mykiss*) (Kinnunen and Johnson 1985). Kinnunen and Johnson (1985) found declines in lymphocytes in rainbow trout hosts during sea lamprey parasitism. After the parasitism event, rainbow trout were observed to have increased lymphocyte levels, indicating recovery and coping. Sea lamprey parasitism causes extensive tissue damage and exposes muscle to the environment (Figure 1). It would be expected that sea lamprey hosts would cope with the effects

of a parasitic event by altering immune-related parameters, which may reduce the likelihood of developing secondary infections.

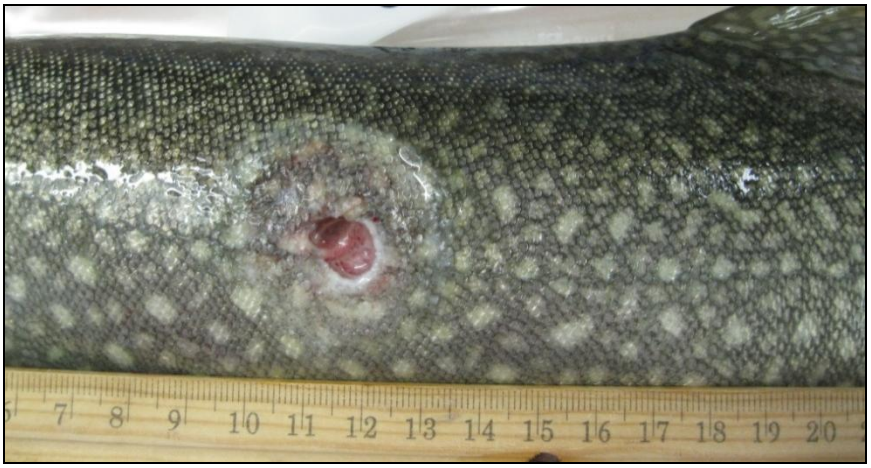


**Figure 1.** Photo of a sea lamprey wound penetrating the muscle with minimal healing. For interpretation of the references to color in this and all other figures, the reader is referred to the electronic version of this thesis.

Following immune-related modulations I predicted that sublethal sea lamprey parasitism would affect endocrine function. Endocrine disruption can occur in a variety of systems, but it is usually used to indicate reproductive impairment and typically involves measurements of an altered hypothalamus-pituitary-gonadal (HPG) axis which can translate into population-relevant endpoints, such as reproductive output (Kramer et al. 2011). The HPG axis represents the physiological cascade of events initiated by environmental stimuli and resulting in maturation and reproduction. The HPG axis regulates reproduction and in female fish is initiated when environmental cues trigger the release of gonadotropin-releasing hormones (GnRH), which facilitates the release of gonadotropins (follicle-stimulating hormone [FSH] and luteinizing hormone [LH]) from the anterior pituitary gland. After release, the gonadotropins stimulate testosterone production in thecal cells in the ovary, which is converted to estradiol through aromatase in the granulosa cells. Estradiol is released into the bloodstream and travels to the liver to bind to the estrogen receptor, stimulating vitellogenin production (Figure 2). Vitellogenin is

the egg yolk precursor protein and a good indicator of fecundity and reproductive success (Miller et al. 2007).

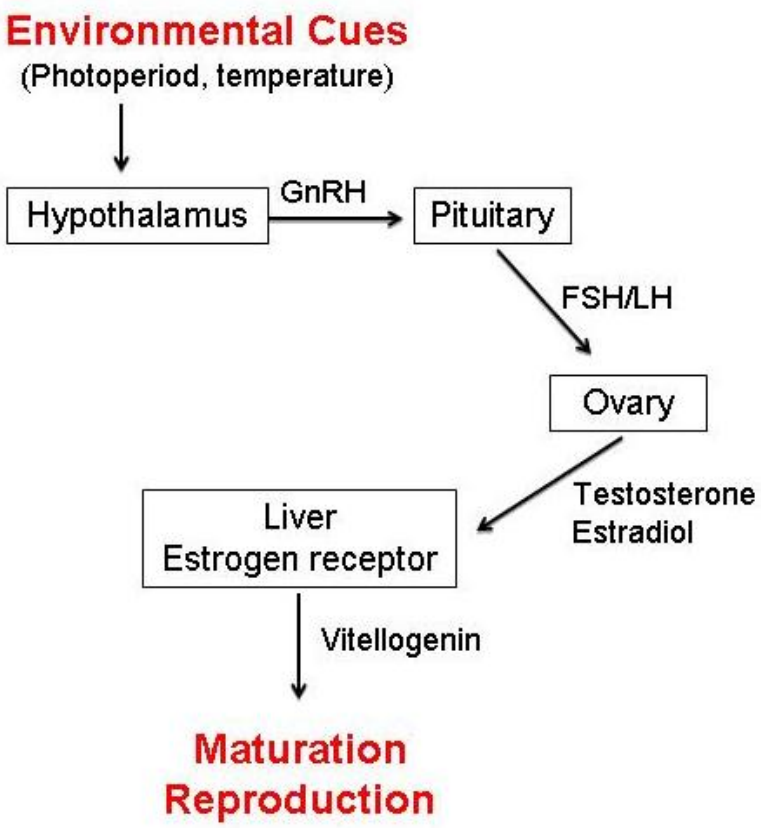


**Figure 2.** Schematic representation of the female fish hypothalamus-pituitary-gonadal (HPG) axis.

The connection between endocrine disruption of the reproductive system and general stress is still unclear. Most studies agree that stress causes decreased levels of plasma testosterone and, less clearly, estradiol (Foo and Lam 1993; Pankhurst and Van Der Kraak 2000). The effect of stress on gonadotropin is less well understood. Acute stress has been found to both decrease (Carragher et al. 1989) and have no effect on (Pankhurst and Van Der Kraak 2000) gonadotropin levels. This ambiguity in the literature may be due to studies using different test species, or due to different experimental designs with varying stressor characteristics (e.g. stressor type, stress duration and severity).

I measured components of the HPG axis (FSH, plasma testosterone and estradiol) in mature female lake trout to identify and quantify reproductive-related endocrine disruption associated with sea lamprey parasitism. Determining if sea lamprey parasitism causes endocrine disruption is important because it will provide insight into how reproductive processes are being altered, which may potentially be linked to egg production in future studies to determine population-level responses (Kramer et al. 2011).

The immediate responses to sea lamprey parasitism (e.g. immune-related modulation and endocrine disruption) can affect lake trout bioenergetics by causing hosts to alter energetic allocation towards growth and reproductive processes. Organisms invest their limited energetic resources into reproduction and somatic growth to maximize lifetime reproductive success (Roff 1983). The tradeoff between reproductive success and growth is influenced by life history characteristics and environmental conditions, including stressful events such as parasitism (Stark et al. 2004; Spromberg and Birge 2005). To evaluate this trade-off in response to sea lamprey parasitism I measured bioenergetics parameters linked with growth and reproduction.

In addition to growth measurements such as length-at-age and weight I used muscle lipid concentration as an indicator of energetic stores. Parasitism can cause changes in host growth dynamics, including decreasing lipid concentrations (Lemly and Esch 1984). In wild populations muscle lipid concentration represents the cumulative energetic cost of sublethal sea lamprey parasitism, which may include both direct (e.g. healing tissue and replacing lost blood) and indirect (e.g. decreased foraging success and secondary infections) energetic sinks. Muscle lipid measurement is a practical bioenergetics indicator and has direct management implications. Use of a non-invasive microwave fish fatmeter (Distell Inc., West Lothian, Scotland) provides a quick and non-lethal method for determining muscle lipid content. The fatmeter operates by

determining the water content of fish muscle, which may be converted to percent muscle lipid content. Lipid content is correlated with lake trout growth and caloric density, which are components of existing lake trout bioenergetics models (Stewart et al. 1983; Madenjian and O'Connor 1999; Madenjian et al. 2000). My research will indicate whether lipid content is affected by parasitism and whether adjusting existing lake trout bioenergetics models to accommodate changes due to parasitism is a worthwhile effort for future research.

Parasitized lake trout may also cope with the energetic demands of parasitism by diverting energy away from reproductive processes. This would cause reductions in reproductive success, likely through reduced fecundity or egg size. Whether an acute stress event results in reduced fecundity or egg size appears to be at least partially dependent on what maturation stage the fish was in during the stress event (Campbell et al. 1992; Contreras-Sánchez et al. 1998). Decreased fecundity has direct impacts on reproductive success since it represents a reduction in overall offspring production. Spawning stock biomass per recruit (SSBR) estimates assume constant fecundity, so a reduction in fecundity due to sea lamprey parasitism may affect the accuracy of this assumption. Decreased egg size also lowers reproductive success by decreasing offspring fitness and survival (Einum and Fleming 1999). Understanding how sublethal sea lamprey parasitism affects lake trout reproduction is important because it may affect population-level lake trout reproductive success.

**Objective 2: Evaluate impact of life history differences on sublethal parasitism response**

An additional objective of this study was to compare responses to sublethal sea lamprey parasitism between two Lake Superior lake trout morphotypes—the lean and siscowet (Figure 3) to determine if life history may play a role in mediating response to parasitism. Leans occupy shallow (less than 100m) nearshore waters and are found throughout the Great Lakes. Siscowets

are unique to Lake Superior and occupy deeper regions (greater than 100m) (Bronte et al. 2003; Sitar et al. 2008). Siscowets have shorter snouts, wider caudal peduncles, and higher muscle lipid concentrations than leans (Eschmeyer and Phillips 1965; Moore and Bronte 2001). Lean and siscowet phenotypic differences are largely genetically based, with some phenotypic plasticity. Siscowets and leans raised under common conditions retain their morphological differences, but to a somewhat lesser degree than in the wild (Goetz et al. 2010).

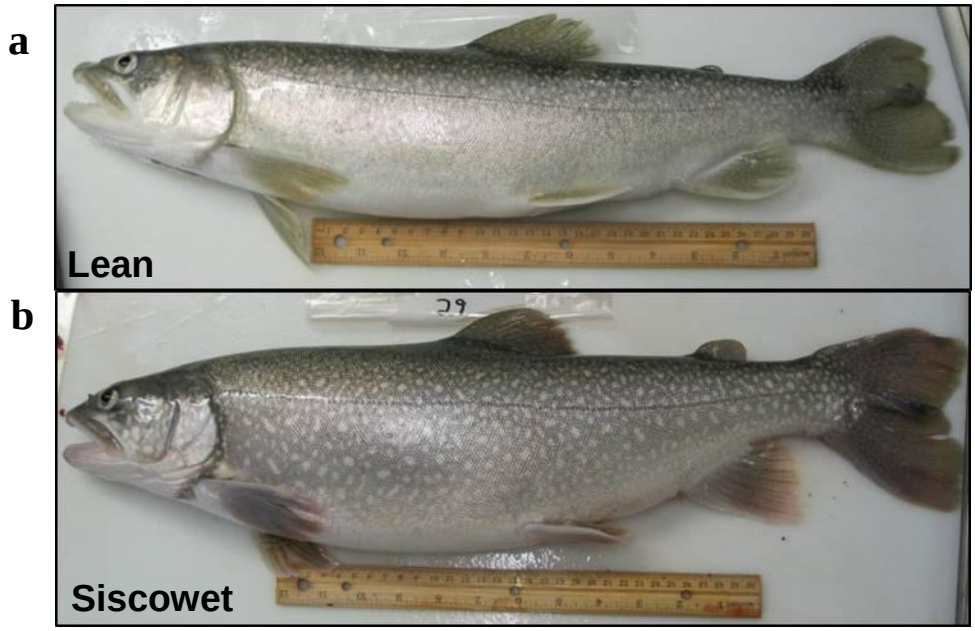


**Figure 3.** The lean lake trout (a) and siscowet lake trout (b) morphotypes.

Most research on sea lamprey parasitism has focused on the commercially important lean morphotype, but siscowets are also important sea lamprey hosts. Siscowets are currently the most abundant predator in Lake Superior and have significantly greater numbers of sea lamprey scars per fish than leans (Bronte et al. 2003; Sitar et al. 2008). Siscowets may experience lower parasitism mortality than leans, which would explain why wild siscowets have greater numbers of sea lamprey scars (Sitar et al. 2008). I tested the hypothesis that siscowets are less sensitive to sea lamprey parasitism than leans, which suggests an increase in survival rate after a parasitism event and may explain the differential scarring patterns between the morphotypes. Siscowets

have higher muscle lipid concentrations than leans, which may allow them to better cope with the energetic demands of parasitism (Eschmeyer and Phillips 1965). I predicted parasitized siscowets would have less severe immune-related modulation, endocrine disruption, and bioenergetics alteration responses to sublethal sea lamprey parasitism. In addition, I compared sea lamprey parasitism behavior between lake trout morphotype hosts to better understand whether potential morphotype-specific responses (e.g. immune, endocrine, bioenergetics) to parasitism could be due to differential sea lamprey feeding behavior in addition to host physiological differences.

Better understanding of the parasite-host relationship between siscowets and sea lamprey has important management implications. With their large population size siscowets may be serving as a buffer against sea lamprey parasitism of leans. This has previously occurred in Lake Erie when lake trout were observed to be acting as a buffer against sea lamprey parasitism of burbot (*Lota lota*) (Stapanian and Madenjian 2007). If siscowets serve as a buffer against parasitism of leans, then siscowet population abundance may potentially be manipulated with the goal of decreasing parasitism on leans. This would likely require continued sea lamprey control efforts to ensure siscowets do not maintain or increase sea lamprey population sizes by serving as additional hosts. Consideration of siscowets as a potential management avenue is particularly relevant given the interest in both harvesting siscowets for their omega-3 fatty acids and re-establishing siscowets in their historical lower Great Lake ranges (Wang et al. 1990; Janssen et al. 2007).

**Experimental design**

In this thesis, lake trout immune, endocrine, and bioenergetics responses to sea lamprey parasitism were measured and compared through a laboratory experiment and a field study. The

observational field study focused on long-term sublethal parasitism effects on individuals. The laboratory experiment measured the immediate effects of parasitism while controlling for environmental variation (e.g. water temperature, foraging success) which may affect parasitism outcomes. Additionally, the responses of the lean and siscowet lake trout morphotypes to parasitism were compared in the laboratory experiment and field study to determine how life history affects sea lamprey parasitism response.

In addition to measuring immune, endocrine, and bioenergetics parameters, I also evaluated changes in hepatic genetic regulation by transcriptome analysis using next-generation sequencing technology. Next-generation sequencing does not require probing for selected genes which is necessary for techniques such as microarrays. This approach is a useful tool in non-model species, including the lake trout, and allows comparison of a large quantity of potential genetic biomarkers of sea lamprey parasitism. Changes in genetic regulation provides additional insight for how immune-related modulation, endocrine disruption, and bioenergetics changes are associated with sea lamprey parasitism. Furthermore, evaluating changes in genetic regulation can also identify additional physiological systems that could be important components of the lake trout parasitism response.

# METHODS

## Immediate parasitic response—laboratory experiment

### *Fish*

Siscowet and lean lake trout gametes from Marquette strain Lake Superior stock were obtained in fall 2006 and raised to the time of the experiments in common laboratory conditions at the Great Lakes Water Institute, Milwaukee, WI. Laboratory experiments took place October through December 2010 and 2011. The lake trout were immature in 2010 and were sexually mature in 2011.

Sea lamprey used in the experiments were obtained from commercial fishermen in the Hammond Bay, Michigan and Blind River, Ontario areas. All sea lamprey were parasitizing a host at the time of capture to ensure that the sea lamprey used in this experiment were in the parasitic phase. Captured sea lamprey ranged in weight distribution (Figure 4). Criteria for sea lamprey selection was based on previous work which suggested that sea lamprey above 85g consume a similar percentage of their initial body weight during parasitism, while those under 85g have been observed to consume less blood (Farmer et al. 1975). In my experiments most of the sea lamprey were above 85g except for two cases (68g and 70g), which I used because of the large number of sea lamprey needed to complete my experiments. I evaluated my statistical analyses of response variables to ensure lake trout which were parasitized by these smaller sea lamprey did not influence overall statistical conclusions. Statistical conclusions which were affected by these data points were noted.

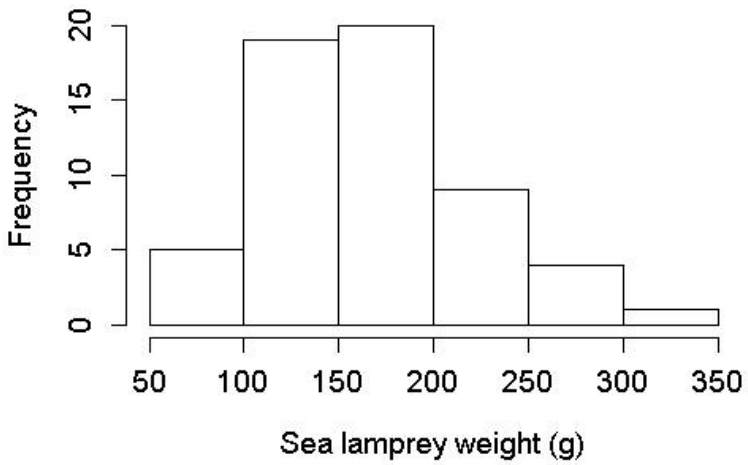


**Figure 4.** Initial weight distribution of sea lamprey used in the laboratory experiments.

***Experiment***

Prior to the start of an experiment that was conducted either in 2010 or 2011, lake trout were anesthetized individually in 2-phenoxyethanol (Sigma-Aldrich, St. Louis, MO) until immobile. Lake trout were then weighed and muscle lipid concentration was measured using a non-invasive fatmeter (Model FM-692, Distell Inc., West Lothian, Scotland) at four locations on the right external side of the body (Figure 5). Fatmeter measurements were converted to percent muscle lipid content using a previously determined relationship for lean and siscowet lake trout (Claus 2011).

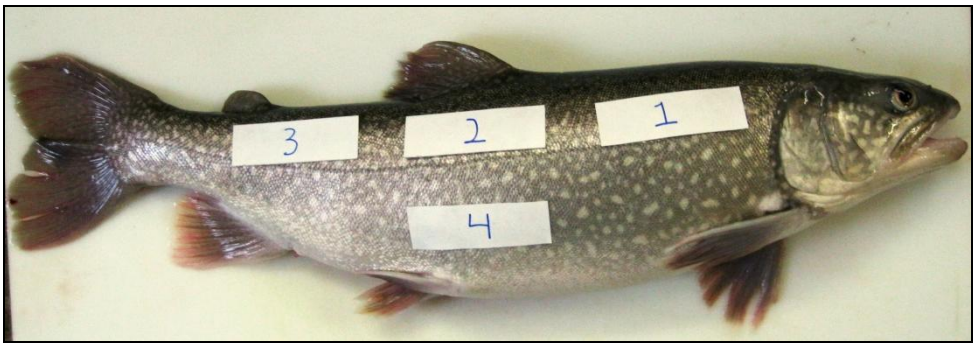


**Figure 5**. Locations of muscle lipid concentration readings taken using a non-invasive fish fatmeter (Model FM-692, Distell Inc., West Lothian, Scotland).

Lake trout were placed in individual covered tanks (265L) for experimental trials. Each test lake trout was randomly paired with a control lake trout which remained in its individual

tank for the same duration of time but was not parasitized. Test and control lake trout were usually of the same sex, although errors in sex identification did occasionally occur. Four sea lamprey were randomly chosen, weighed, identified by fin clips, and placed in each test lake trout tank. After the addition of sea lamprey to the test tanks, test and control lake trout were checked three times per day. Once a sea lamprey attached to a lake trout, the other non-attached lamprey were removed from the tank. Sea lamprey were allowed to feed for one to five days or until detachment. I estimated sea lamprey feeding duration to be from when the sea lamprey was first noted to be attached to when the sea lamprey was first noticed to have detached or was physically detached from the test lake trout.

After the parasitism trial I recorded sea lamprey final weights. After removing the sea lamprey, test and control lake trout were euthanized using an overdose of tricaine methanesulfonate (MS-222) (Sigma-Aldrich, St. Louis, MO). Lake trout weight and length were measured, as well as muscle lipid concentration using a non-invasive fatmeter (Figure 5). The number and type of sea lamprey wounds on the lake trout were characterized according to Ebener et al. (2006) by wound depth. Blood samples were taken by caudal vein puncture using a heparinized syringe and centrifuged. Plasma was extracted and stored at -80°C. Gonads and livers were weighed and sexual maturity was noted. The pituitary and an approximately 1.5g liver subsection were dissected and stored at -80°C.

**Long term parasitic response—field sampling**

Lean and siscowet lake trout were sampled during fall 2010 and fall 2011 outside Marquette, Michigan by gillnet (Figure 6). Sampling locations were chosen based on previous lake trout capture success by the Michigan Department of Natural Resources (DNR) lake trout surveys. Gillnets targeting leans were set at less than 50m; nets targeting siscowets were set at

greater than 100m. Nets were allowed to set overnight. Gillnet mesh sizes (11.4, 12.7, 14.0, and 15.2 cm) were chosen to target lake trout larger than 550mm, above which a majority of lake trout have been observed to be mature.

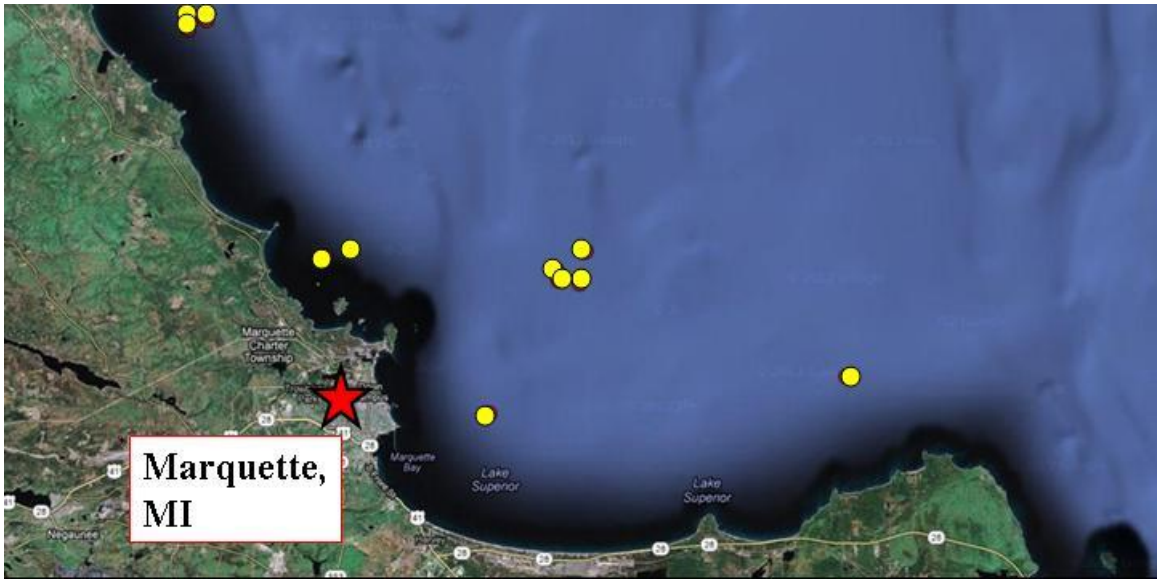


**Figure 6.** Sampling sites were located in Lake Superior outside Marquette, MI.

Morphotype differentiation between siscowets and leans was based on morphological differences in fin size, eye position and size, and body form (Figure 3). Morphotypes which were not clearly distinguished as either form were omitted. On board, after pulling the gill nets, weight and length were measured. Muscle lipid concentration was measured using a non-invasive fish fatmeter at four locations along the external right side of the body (Figure 5). Pituitaries and an approximately 1.5g liver subsample were immediately stored on dry ice in cryovials. Otoliths were extracted and stored for aging at a later date. Number and type of sea lamprey scars were characterized according to Ebener et al. (2006) by wound depth and healing progress. Sea lamprey scar types A1 through A4 and B1 through B4, according to Ebener et al. (2006), were included in my study. Blood samples were taken by caudal vein puncture using a heparinized syringe and stored on ice for a maximum of eight hours before processing.

On shore blood was centrifuged and plasma was extracted and stored at -80°C. Gonads and livers were weighed. An extra 1.5g was added to total liver weights to account for the approximately 1.5g liver subsample which was previously taken onboard. Ovaries were stored at 4°C for a maximum of one week for fecundity and egg diameter measurements.

**Measurements related to sea lamprey parasitism behavior**

***Laboratory experiment***

Sea lamprey weight gain during the laboratory experiment was used as a measurement of sea lamprey feeding rate. All weight gained during parasitism was assumed to be due to the ingestion of host tissue fluids. This assumption was based on previous research which found that host tissues consisted of less than two percent of the total matter consumed by sea lamprey during parasitism (Farmer et al. 1975). Three sea lamprey lost weight during feeding, likely due to minimal or no feeding. These sea lamprey were modeled as having no change in weight to reflect their likely lack of feeding. Since sea lamprey initial weight may affect its feeding rate I divided each sea lamprey's weight change during parasitism by its initial weight to calculate the percent weight consumed during parasitism. A square root transformation was applied to this parameter for normality. Sea lamprey percent body weight consumption was analyzed using general linear models (GLM) and I compared alterative models by Akaike Information Criterion (AIC) values (Appendix A). Additive models with all possible combinations of morphotype, host beginning weight, host muscle lipid concentration, and test duration as explanatory variables were compared using R. The model with the lowest AIC value was considered the best model and was examined for normality of residuals, homoscedasicity, and assessed for highly influential data points, including sea lamprey weighing less than 85g. Models within two AIC

values of the best model were also examined for differences in overall conclusions (Appendix B).

***Field study***

Sea lamprey scarring patterns were compared between wild leans and siscowets. Distributions of the number of sea lamprey scars per lake trout were compared between parasitized leans and siscowets by a chi-squared test of independence. An additive binomial model was used to identify significant predictors of the probability of sea lamprey scarring. Morphotype, age, length, sample date, and lake trout weight were included as possible explanatory variables (Appendix A).The model with the lowest AIC value was considered the best model. Models within two AIC values of the best model were also examined for differences in overall conclusions (Appendix B).

**Gross measurements of immune-related modulation**

Hepatosomatic index (HSI) was calculated by dividing liver weight (g) by total body weight (g). Increase in HSI is associated with immune-related modulation (Tahir et al. 1993; Secombes et al. 1995).

**Physiological measurements related to endocrine disruption**

A radioimmunoassay (RIA) procedure was used to analyze plasma samples from the laboratory experiment for total estradiol and testosterone concentrations, and to analyze plasma samples from the field study for total estradiol concentrations. A pooled plasma sample from six lake trout (three leans and three siscowets) was used to verify an antigen-antibody dose response relationship (e.g. parallelism). Hormones were extracted from plasma twice in diethyl ether, incubated at 35°C for two hours, dried, then reconstituted in a phosphate buffered saline solution. Sample recovery was determined by comparing tritium-labeled estradiol or testosterone added

before (100 DPM) and after extraction. A standard curve was made by serial dilution of estradiol or testosterone in phosphate buffered saline solution. Duplicate samples were incubated overnight at 4°C with tritium-labeled estradiol or testosterone (2000 DPM) and estradiol or testosterone polyclonal antibody (AbD Serotec, Oxford, UK). Unbound hormone was removed by incubating with dextran-coated charcoal for 15 minutes at 4°C. Samples were centrifuged at 2500 RPM for 12 minutes at 4°C. After decanting the supernatant Ultima Gold scintillation fluid was added and beta radioactivity was measured using a scintillation counter (Beckman Coulter LS 6500). Mean RIA extraction percent recovery was 79.5 percent. Mean inter-assay variation was 3.4 percent. An enzyme immunoassay (EIA) kit (Cayman Chemicals, product # 582701) was used to measure plasma testosterone concentrations in field samples. Methods followed kit instructions. Extraction efficiency was not determined for these samples.

Follicle-stimulating hormone (FSH) was analyzed for wild lake trout from the field study. FSH was not measured in the laboratory experiment due to experimental timing. Pituitary FSH peaks in August and declines throughout final maturation (Goetz et al. 2011). Because the laboratory experiment was conducted in the late stages of lake trout maturation (October through December), I did not predict FSH to be significantly affected by parasitism. FSH was measured in wild lake trout indirectly by quantifying pituitary mRNA beta subunit levels according to Goetz et al. (2011). Briefly, pituitary total RNA was isolated using the illustra RNAspin 96 RNA isolation kit (GE Heathcare). Complementary DNA (cDNA) was produced by reverse-transcription PCR using degenerate primers (GenBank accession number HM057170). Raw data was analyzed using Real-Time PCR Miner and expression was quantified according to Zhao and Fernald (2005). FSH mRNA transcript levels were expressed as a proportion of total pituitary mRNA transcripts.

**Physiological measurements related to bioenergetics**

Measurements taken using the non-invasive fatmeter (Figure 5) were converted to percent muscle lipid concentration using a previously determined relationship calibrated for siscowet and lean lake trout (Claus 2011):

$$Muscle\ lipid\ concentration = e^{(1.9436*(\ln(fatmeter\ reading))-9.05022)} * 100$$

Fatmeter results were verified by analyzing a subsample of Lake Superior lake trout muscle tissues by laboratory analysis. Wild lean and siscowet fillets were taken from between the dorsal and adipose fin and stored at -20°C. An approximately 40g section of thawed muscle tissue was blended until homogenized, separated into three approximately 3g subsamples, and dried at 120°C. Subsamples were weighed before and after drying to determine muscle water content. Dried subsamples were re-homogenized and reconstituted in water. Hydrated subsamples were then extracted twice with chloroform according to Bligh and Dyer (1959) and dried. Age was determined for field samples by counting sagittal otolith annuli (North Shore Environmental Services).

Ovaries were analyzed to determine GSI, fecundity, and average egg diameter. GSI was calculated by dividing ovary weight (g) by total body weight (g). Fecundity was determined for mature pre-ovulatory females from the field study and laboratory experiment. Two samples were taken from each ovary lobe weighing approximately 10 to 14g. Samples were weighed, counted, and total egg number was determined. Total body weight was used to calculate weight-adjusted fecundity, represented as eggs per gram body weight (Quince et al. 2008). Egg diameter was measured in mature females. Eggs were randomly chosen and photographed. Diameters were measured on six axes per egg. For the fall 2010 samples 36 eggs were measured per lake trout. For a subsample of 15 lake trout, average egg diameter was calculated by averaging diameter

measurements from six randomly selected eggs. I compared average egg diameters calculated from 36 eggs with average egg diameters calculated from six eggs by a two sample t-test. Calculating egg diameters from six eggs minimally differed from calculating egg diameters from 36 eggs (T=0.0676, p=0.9466) (Figure 7). I simplified my protocol based on this observation, and in the fall 2011 I only measured diameters for six eggs.

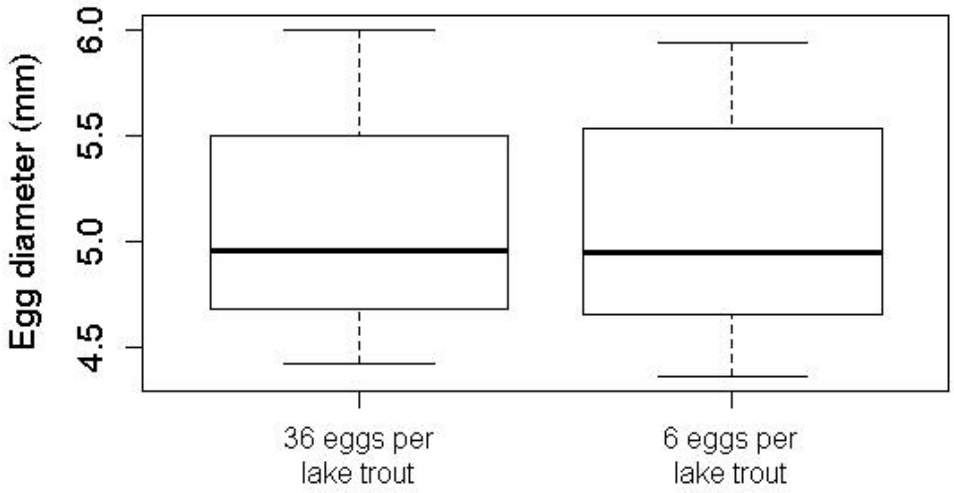


**Figure 7.** Average egg diameter calculated using six eggs per lake trout did not significantly differ from average egg diameter calculated using 36 eggs per lake trout in a random subsample of 15 wild lake trout.

**Data analysis of immune, endocrine, and bioenergetics parameters**

Parameters measuring immunity (HSI), endocrine disruption (plasma sex steroid concentrations and pituitary FSH), and bioenergetics (muscle lipid concentration, fork length, fecundity, egg diameter, GSI), from the laboratory experiment and field study were analyzed by a model fitting procedure. All statistical analyses on field study parameters used data from mature females only. Statistical analyses on laboratory experiment parameters related to reproduction (plasma sex steroids, fecundity, egg diameter, GSI) used data from mature females only. Immature and mature males and females were included in statistical analyses of HSI and muscle lipid concentration for the laboratory experiment.

Response variables were first examined for normality and transformed accordingly before analysis (Table 1). Response variables were evaluated by GLM with parasitism status and morphotype as explanatory variables. Alternative models containing additional explanatory variables, including covariates and meaningful interaction terms (procedure described below) were compared using R (Appendix A). Alternative models were compared by AIC values, and the model with the lowest AIC value was considered the best model. Because the primary interest of my study was the relationship between morphotype, parasitism status, and the response variables I considered morphotype and parasitism status as fixed effects, and they were included in all alternative models. Models within two AIC values of the best model were also examined for differences in overall conclusions (Appendix B).

**Table 1.** Table of transformations for immune, endocrine, and bioenergetics response variables.

| **Response variable** | **Response variable transformation** |
|---|---|
| Laboratory experiment | |
| Plasma estradiol (ng/mL) | Reciprocal |
| Plasma testosterone (ng/mL) | Square root |
| Average egg diameter (mm) | Square root |
| Weight-adjusted fecundity (total eggs per g body weight) | Square root |
| Gonadosomatic index (GSI) | None |
| Hepatosomatic index (HSI) | Lognormal |
| Muscle lipid concentration (% wt/w), by fatmeter | None |
| Field study | |
| Plasma estradiol (ng/mL) | Lognormal |
| Plasma testosterone (ng/mL) | Lognormal |
| Average egg diameter (mm) | Lognormal |
| Weight-adjusted fecundity (total eggs per g body weight) | Lognormal |
| Gonadosomatic index (GSI) | None |
| Follicle-stimulated hormone (FSH) | Square root |
| Hepatosomatic index (HSI) | Lognormal |
| Muscle lipid concentration (% wt/w) by fatmeter | Lognormal |
| Muscle lipid concentration (% wt/w) by laboratory analysis | Square root |
| Fork length (mm) | Lognormal |
| Age (years) | Lognormal |

For response variables from the laboratory experiment test duration and processing date were included as possible covariates. If the lake trout was a test fish then test duration was defined as the number of hours which parasitism occurred. If the lake trout was a control fish

then test duration was defined as the number of hours which parasitism occurred in its paired test fish. Maturity was also included as a potential model parameter – in 2010 the fish were immature, and in 2011 the fish were reproductively mature. In 2011 some lake trout had skin fungal infections so the presence of fungal infection was noted and included as a possible model parameter. Morphotype and parasitism interaction was included as a potential model parameter to detect significant inverse responses to parasitism between the morphotypes. Interactions between morphotype, parasitism status, and test duration were tested to accommodate different relationships between the response variable and test duration according to morphotype and parasitism status.

Lake trout age and sample date were included as possible covariates in models of response variables from the field study. Interaction between morphotype and parasitism status and between morphotype, parasitism status, and age were included as possible model parameters.

Final models were examined for normality of residuals, homoscedasicity, and assessed for highly influential data points in the model. Marginal means were calculated for models that included a significant covariate (e.g. age). If the model was fit using transformed response data then the mean and standard error were back-transformed accordingly. For covariate models fit using transformed data the predicted marginal means and 95 percent confidence intervals were also adjusted for transformation bias with the bias correction factor ($\sigma^2/2$).

Age distributions were compared between wild parasitized and non-parasitized leans and between parasitized and non-parasitized siscowets using a chi-squared test of independence.

**Hepatic genetic regulation to determine immune, growth and endocrine effects**

Hepatic transcriptome analysis was performed on a subsample of lake trout from the laboratory experiment. Parasitized and non-parasitized leans and siscowets with their paired controls were chosen (n=6 per group) based on ideal experimental conditions. Ideal experimental

condition was classified as continuous sea lamprey attachment to test lake trout resulting in a lamprey wound classified as A-1 according to Ebener et al. (2006), no early sea lamprey detachment, no premature death, and no noted fungal infections in either the control or test fish. Equal numbers of males and females were included in each group. Initial sea lamprey weight ranged from 111g to 256g and sea lamprey weight gain during the parasitic event ranged from 22g to 64g. Sea lamprey which parasitized lean hosts had an average initial weight of 182.7g and gained an average of 32.8g during the parasitic event. Sea lamprey which parasitized siscowet hosts had an average initial weight of 167.5g and gained an average of 42g during the parasitic event. Only immature lake trout were analyzed using RNAseq because the stress response would have been masked by reproductive processes occurring in the mature female fish as she underwent vitellogenesis.

Liver subsamples were pooled within groups (e.g. parasitized leans, non-parasitized leans, parasitized siscowets, and non-parasitized siscowets) and extracted according to Goetz et al. (2010). RNA pools were constructed from livers taken from parasitized and non-parasitized leans and siscowets and sequenced on the Illumina platform. Sequences were mapped to existing lean and siscowet 454 sequenced datasets derived from liver and muscle transcriptome analysis (Goetz et al. 2010). Mapped reads were then analyzed by RNAseq (CLC Genomics). Genes which were differentially expressed in parasitized lake trout were then analyzed by qPCR by individual lake trout according to Goetz et al. (2004) and Roberts et al. (2009).

# RESULTS

## Measurements related to sea lamprey parasitism behavior

### *Laboratory experiment*

The best model for sea lamprey percent body weight consumption from the laboratory experiment contained morphotype and host beginning weight as predictor variables. Sea lamprey had greater percent body weight consumption when feeding on siscowet hosts, although the difference was not statistically significant (Figure 8A). This trend remained whether I removed data collected using sea lamprey weighing less than 85g (p=0.1209), and whether the host size was kept relatively similar (using siscowets and leans with overlapping weight ranges; p=0.1157). Average length of time for sea lamprey attachment was similar between the morphotypes (67.3 hours on lean hosts and 69.1 hours on siscowet hosts). Test duration was not included in the final model and was not a significant predictor of percent body weight change of sea lamprey that parasitized lean hosts ($R^2$=0.0375, p=0.3535) or siscowet hosts ($R^2$=0.0263, p=0.3834).

### *Field study*

The distributions of the number of sea lamprey scars per lake trout was not significantly different between wild parasitized leans and parasitized siscowets ($X^2$=4.747, p=0.1905) (Figure 8B). The best binomial model for the probability of sea lamprey scarring on wild lake trout contained age and fork length as explanatory variables. Morphotype was not statistically significant in any of the compared binomial models. Best models of measurements related to sea lamprey parasitism behavior are listed in Table 2.

**Table 2.** Best models for measurements related to sea lamprey parasitism behavior.

| | Response variable | Predictor variable<br>p-values | AIC value |
|---|---|---|---|
| **Laboratory experiment** | Sea lamprey percent body weight consumption | Host morphotype: 0.0599<br>Host beginning weight: 0.0592 | -30.6 |
| **Field study** | Probability of sea lamprey scarring in lake trout | Host age: 0.0006<br>Host length: <0.0001 | 139.5 |

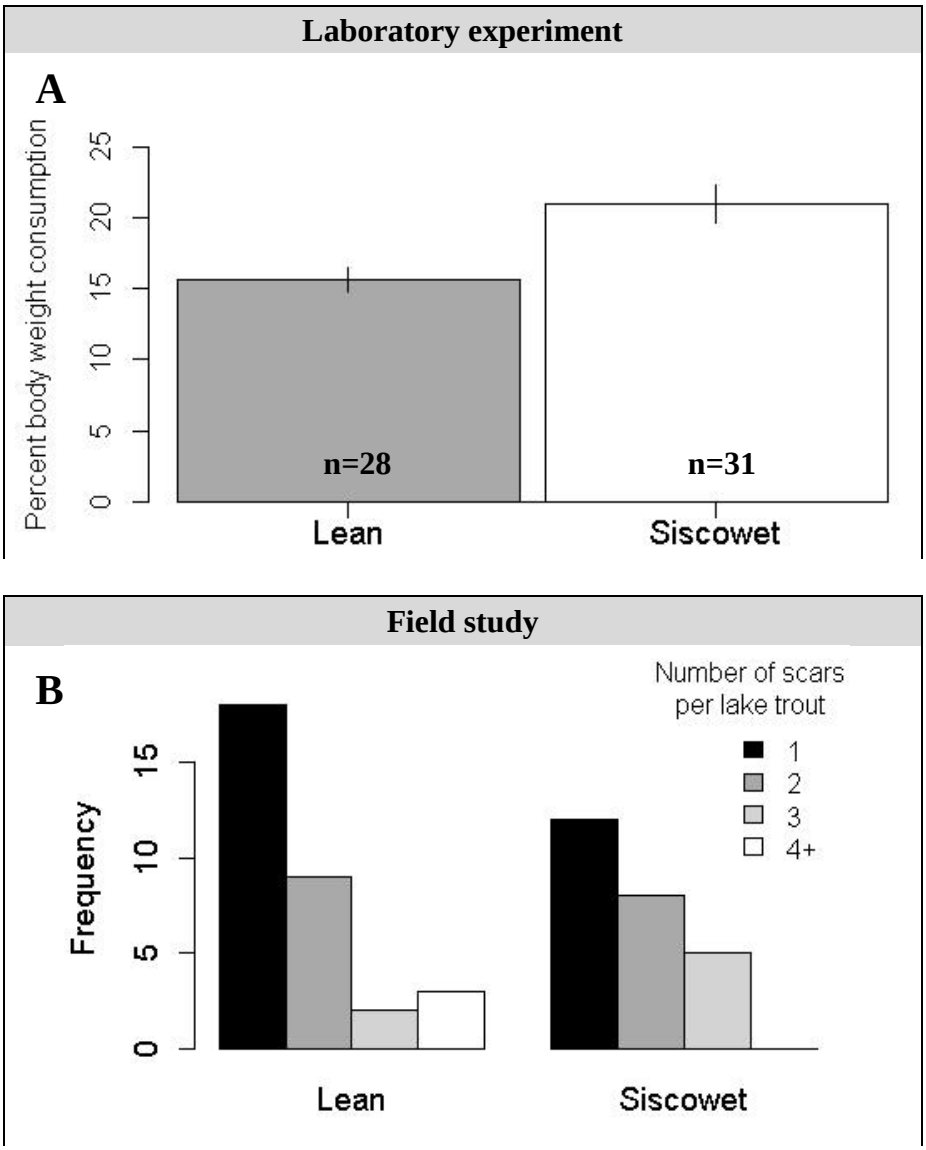


**Figure 8.** (A) Sea lamprey feeding on siscowet hosts showed a statistical trend for gaining a higher percentage of body weight during parasitism (p=0.0599). Error bars represent standard errors. (B) Number of sea lamprey scars per lake trout was not significantly different ($X^2$=4.747, p=0.1905) between wild parasitized leans (n=32) and siscowets (n=25).

## Gross measurements of immune-related modulation

### *Laboratory experiment*

Test duration was significant in the best model for HSI. Parasitized and non-parasitized leans did not have significant relationships between HSI and test duration (Figure 9A). Parasitized siscowets had a significant increase in HSI with test duration, and this relationship was not observed in non-parasitized siscowets (Figure 9B). One lake trout with extensive skin fungal infections was a significant HSI outlier and removed from analysis. Removal of the outlier did not change statistical significance. HSI marginal means were not significantly different between parasitized and non-parasitized leans, or between parasitized and non-parasitized siscowets (Figure 9C).

### *Field study*

HSI marginal means were not significantly different between parasitized and non-parasitized leans, or between parasitized and non-parasitized siscowets (Figure 9D). Best HSI models are displayed in Table 3.

**Table 3.** Best models for gross measurements of immune-related modulation. Morphotype and parasitism status were considered fixed effects and included in all alternative models.

| | Response variable | Predictor variable p-values | AIC value |
|---|---|---|---|
| **Laboratory experiment** | Hepatosomatic index (HSI) | Morphotype: 0.0001<br>Parasitism: 0.0515<br>Test duration: 0.0114<br>Sample date: 0.0001<br>Parasitism * Test duration: 0.1444 | -140.8 |
| **Field study** | Hepatosomatic index (HSI) | Morphotype: 0.0107<br>Parasitism: 0.9016<br>Sample date: 0.0001 | -107.0 |

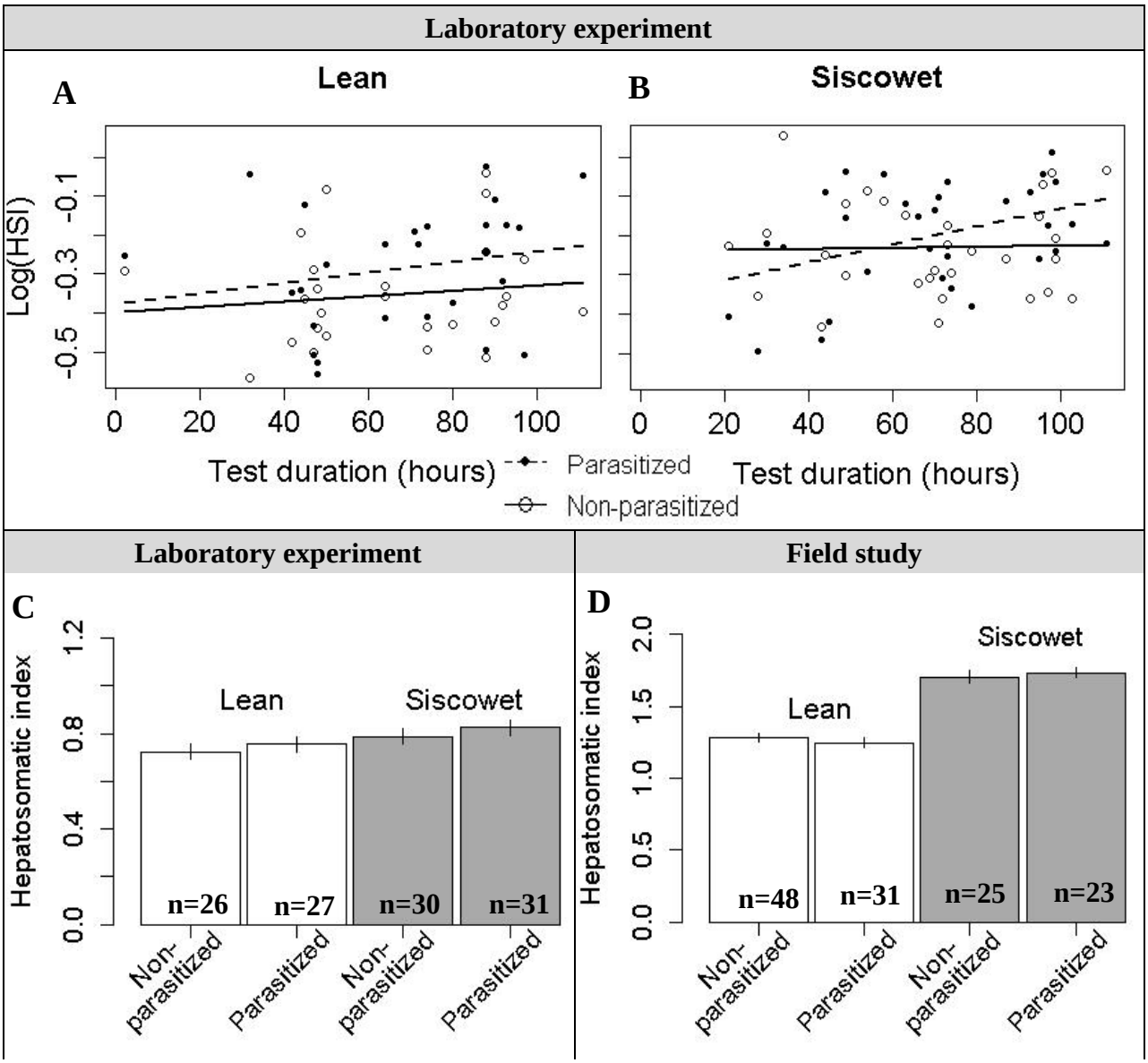


**Figure 9.** (A) The relationship between HSI and test duration was not significant for parasitized leans ($R^2$=0.0475, p=0.2747) and non-parasitized leans ($R^2$=0.0165, p=0.5445) from the laboratory experiment. (B) Non-parasitized siscowets from the laboratory experiment did not have a significant relationship between HSI and test duration ($R^2$=0.0008, p=0.8807). This relationship was significant in parasitized siscowets ($R^2$=0.1802, p=0.0173). (C) HSI marginal means were not significantly different between parasitized and non-parasitized leans (p=0.2456) or parasitized and non-parasitized siscowets (p=0.1776) in the laboratory experiment. Error bars represent the 95 percent confidence intervals of the marginal means. (D) HSI was not significantly different between wild leans and siscowets and their non-parasitized counterparts (p=0.9016). Error bars represent standard error.

## Physiological measurements related to endocrine disruption

### *Laboratory experiment*

Parasitized leans and siscowets had significantly lower plasma testosterone concentrations than their non-parasitized counterparts in the laboratory experiment (Figure 10A). No significant differences between parasitized and non-parasitized lake trout were observed for plasma estradiol within each morphotype (Figure 10B).

### *Field study*

Wild parasitized lake trout did not significantly differ in plasma testosterone (Figure 10C) or estradiol (Figure 10D) concentrations when compared with non-parasitized lake trout within each morphotype. Pituitary FSH levels were significantly lower in parasitized lake trout when compared with non-parasitized lake trout (Figure 10E). One outlier was removed from the pituitary FSH model to normalize residuals and reduce heteroscedasticity. Removal of this outlier did not change overall statistical conclusions. Best models for parameters measuring endocrine disruption are displayed in Table 4.

**Table 4.** Best models for physiological measurements related to endocrine disruption. Morphotype and parasitism status were considered fixed effects and included in all alternative models.

| | Response variable | Predictor variable p-values | AIC value |
|---|---|---|---|
| **Laboratory experiment** | Plasma testosterone (ng/mL) | Morphotype: 0.0215<br>Parasitism: 0.0045<br>Test duration: 0.8376<br>Morphotype * Test duration: 0.0591 | 78.1 |
| | Plasma estradiol (ng/mL) | Morphotype: 0.0024<br>Parasitism: 0.2285 | 49.6 |
| **Field study** | Plasma testosterone (ng/mL) | Morphotype: 0.9443<br>Parasitism: 0.2627<br>Age: 0.2864<br>Sample date: 0.0034 | 339.5 |
| | Plasma estradiol (ng/mL) | Morphotype: 0.9566<br>Parasitism: 0.2116<br>Age: 0.4271<br>Sample date: <0.0001 | 377.4 |
| | Pituitary follicle-stimulating hormone (FSH) | Morphotype: 0.9229<br>Parasitism: 0.0061 | -166.4 |

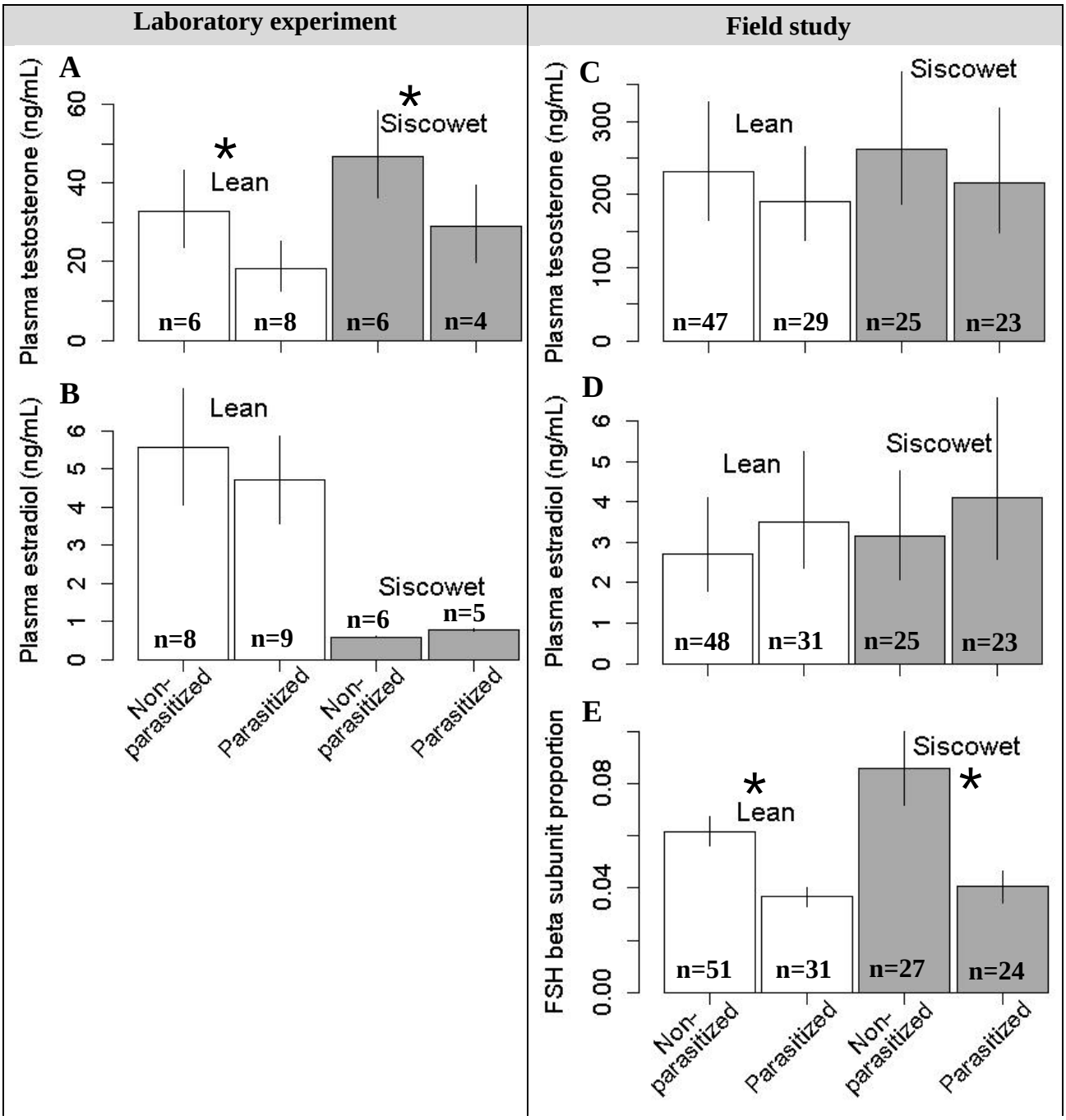


**Figure 10.** (A) Plasma testosterone marginal means were significantly different between parasitized leans and siscowets and their non-parasitized counterparts in the laboratory experiment (p=0.0215). (B) Parasitized and non-parasitized lake trout in the laboratory experiment did not have significantly different plasma estradiol concentrations (p=0.2285). (C) Marginal means were not significantly different between wild parasitized and non-parasitized lake trout for plasma testosterone concentrations (p=0.6746) or (D) plasma estradiol concentrations (p=0.5926). (E) Wild parasitized lake trout had significantly lower pituitary FSH levels than non-parasitized lake trout (p=0.0061). Error bars represent 95 percent confidence intervals of the marginal means (A, C, D) or standard error (B, E). * = significant difference $p<0.05$

## Physiological measurements related to bioenergetics

### *Bioenergetics parameters measuring growth*

*Laboratory experiment*

Muscle lipid concentrations measured using the non-invasive fatmeter were not significantly different between parasitized and non-parasitized leans or between parasitized and non-parasitized siscowets (Figure 11A).

*Field study*

Muscle lipid concentrations measured using the non-invasive fatmeter were not significantly different between parasitized and non-parasitized leans or between parasitized and non-parasitized siscowets (Figure 11B). The best model for muscle lipid concentration determined by laboratory lipid extraction contained the interaction between morphotype and parasitism, so leans and siscowets were evaluated separately. Lean muscle lipid concentrations were evaluated by a two-tailed t-test. A one-tailed t-test was performed between parasitized and non-parasitized siscowets due to clear directional differences. There was a non-significant trend for lowered muscle lipid concentrations in wild parasitized siscowets when compared with non-parasitized siscowets (Figure 11C).

Wild parasitized and non-parasitized leans had significantly different age distributions (Figure 12A). Parasitized and non-parasitized siscowets had almost significantly different age distributions (Figure 12B). Wild parasitized leans and siscowets were significantly longer than their non-parasitized counterparts (Figure 12E). Parasitized and non-parasitized leans had significantly different length-at-age relationships (Figure 12C), as did parasitized and non-parasitized siscowets (Figure 12D). Best models for bioenergetics parameters related to growth are listed in Table 5.

**Table 5.** Best models for bioenergetics measurements related to growth. Morphotype and parasitism status were considered fixed effects and included in all alternative models.

| | Response variable | Predictor variable p-values | AIC value |
|---|---|---|---|
| **Laboratory experiment** | Muscle lipid concentration (% wt/w), by fatmeter | Morphotype: <0.0001<br>Parasitism: 0.4333<br>Sample date: 0.0946 | 634.8 |
| **Field study** | Fork length (mm) | Morphotype: <0.0001<br>Parasitism: <0.0001<br>Age: <0.0001<br>Parasitism * Age: 0.0289 | -232.3 |
| | Muscle lipid concentration (% wt/w), by fatmeter | Morphotype: <0.0001<br>Parasitism: 0.6149<br>Age: 0.1992 | 152.2 |
| | Muscle lipid concentration (% wt/w), by laboratory analysis | Morphotype: <0.0001<br>Parasitism: 0.4905<br>Parasitism * Morphotype: 0.1455 | -48.9 |

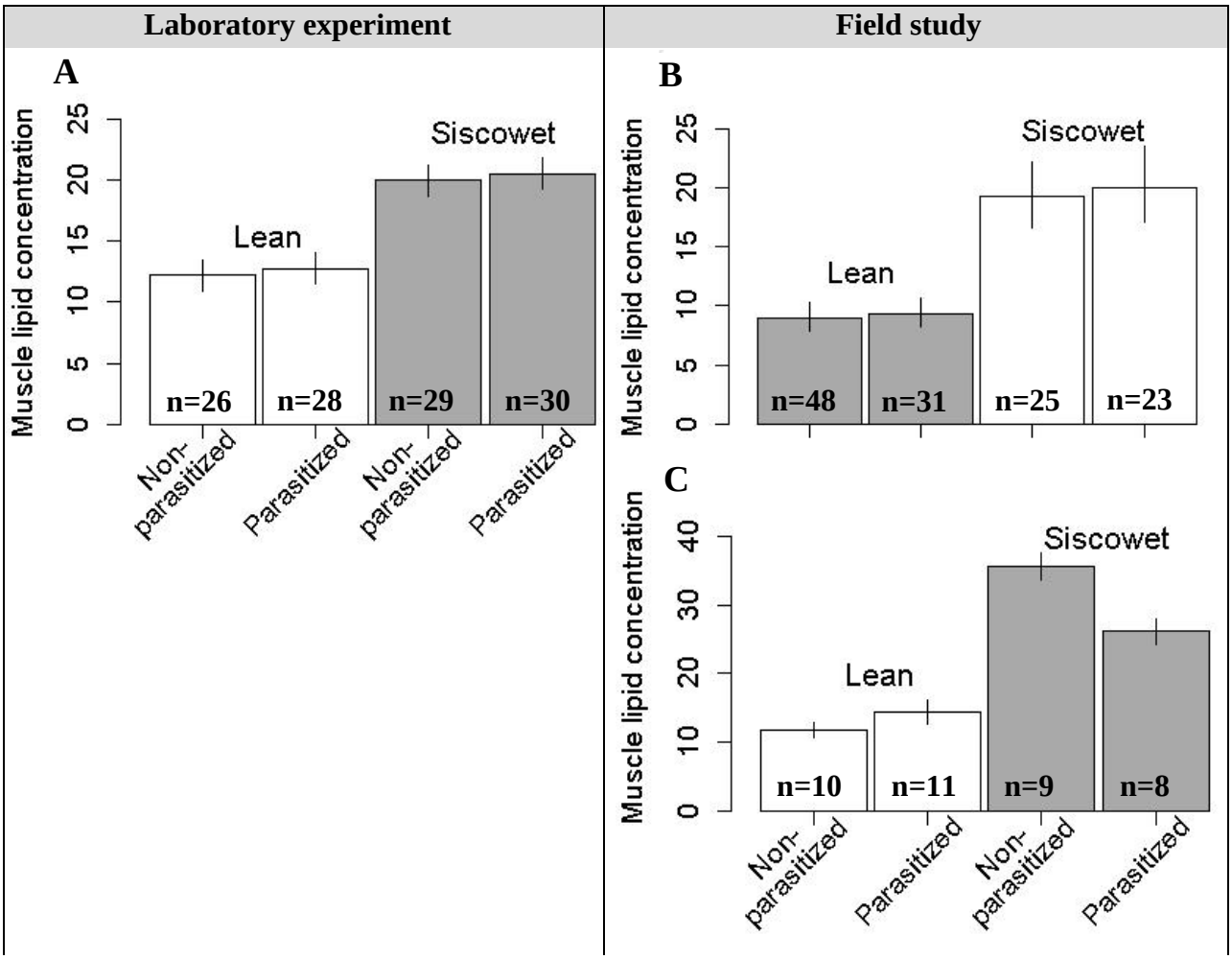


**Figure 11.** (A) Muscle lipid concentration marginal means, measured using the non-invasive fatmeter, were not significantly different between parasitized and non-parasitized lake trout in the laboratory experiment (p=0.8605). (B) Muscle lipid concentration marginal means, measured using the non-invasive fatmeter, were not significantly different between wild parasitized and non-parasitized lake trout (p=0.9580). (C) Muscle lipid concentration, measured by laboratory lipid extraction, were not significantly different between parasitized and non-parasitized leans (T=-0.2429, p=0.8107); parasitized and non-parasitized siscowets were almost significantly different (T=1.7133, p=0.0536). Error bars represent the 95 percent confidence intervals of the marginal means (A,B) or standard error (C).

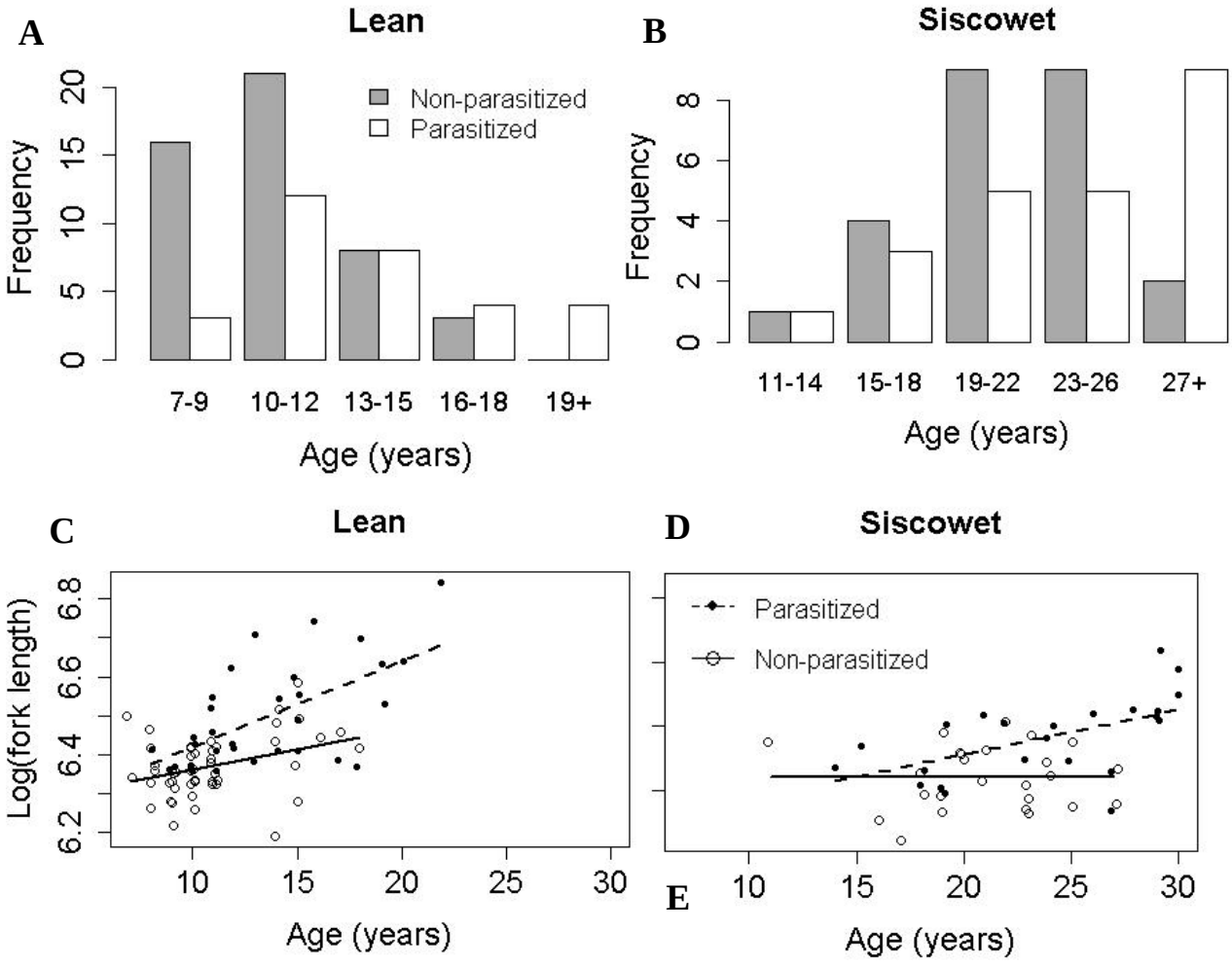


**Figure 12.** (A) Age distributions significantly differed between wild parasitized leans (n=31) and non-parasitized leans (n=48) ($X^2$=15.68, p=0.0035). (B) Age distributions were almost significantly different between wild parasitized siscowets (n=23) and non-parasitized siscowets (n=25) ($X^2$=8.56, p= 0.073). (C) Length-at-age relationships were significantly different (p=0.0170) between parasitized leans ($R^2$=0.3371, p=0.0024) and non-parasitized leans ($R^2$= 0.1246, p=0.0139). (D) Length-at-age relationships were significantly different (p=0.0106) between parasitized siscowets ($R^2$=0.3181, p=0.0051) and non-parasitized siscowets ($R^2$=<0.0001, p=0.9872). (E) Marginal mean length was significantly different between parasitized and non-parasitized leans (p=0.0005) and siscowets (p=0.0061). Error bars represent marginal means 95 percent confidence intervals. * = significant difference p<0.05

***Bioenergetics measurements measuring reproduction***

*Laboratory experiment*

Parasitized lake trout did not significantly differ from non-parasitized lake trout in weight-adjusted fecundity (Figure 13A), GSI (Figure 13B), or average egg diameter (Figure 13C).

*Field study*

Parasitized lake trout did not significantly differ from non-parasitized lake trout in weight-adjusted fecundity (Figure 13D) or GSI (Figure 13E). Wild parasitized leans tended to have higher egg diameters than non-parasitized leans, although not significantly (Figure 13F). However, when age was included in the best model for average egg diameter this trend was no longer apparent (p=0.3043). Best models are displayed in Table 6.

**Table 6.** Best models for bioenergetics measurements related to reproduction. Morphotype and parasitism status were considered fixed effects and included in all alternative models.

| | Response variable | Predictor variable p-values | AIC value |
|---|---|---|---|
| **Laboratory experiment** | Fecundity (total eggs per g body weight) | Morphotype: 0.5268<br>Parasitism: 0.2622 | -10.7 |
| | Gonadosomatic index (GSI) | Morphotype: 0.6041<br>Parasitism: 0.1395 | 94.3 |
| | Egg diameter (mm) | Morphotype: 0.9425<br>Parasitism: 0.9312 | -72.1 |
| **Field study** | Fecundity (total eggs per g body weight) | Morphotype: <0.0001<br>Parasitism: 0.5806<br>Sample date: 0.0577 | 23.9 |
| | Gonadosomatic index (GSI) | Morphotype: 0.0096<br>Parasitism: 0.2335<br>Age: 0.0076<br>Sample date: 0.0003<br>Morphotype * Parasitism * Age: 0.0396 | 640.1 |
| | Egg diameter (mm) | Morphotype: <0.0001<br>Parasitism: 0.0139<br>Sample date: 0.0001 | -318.5 |

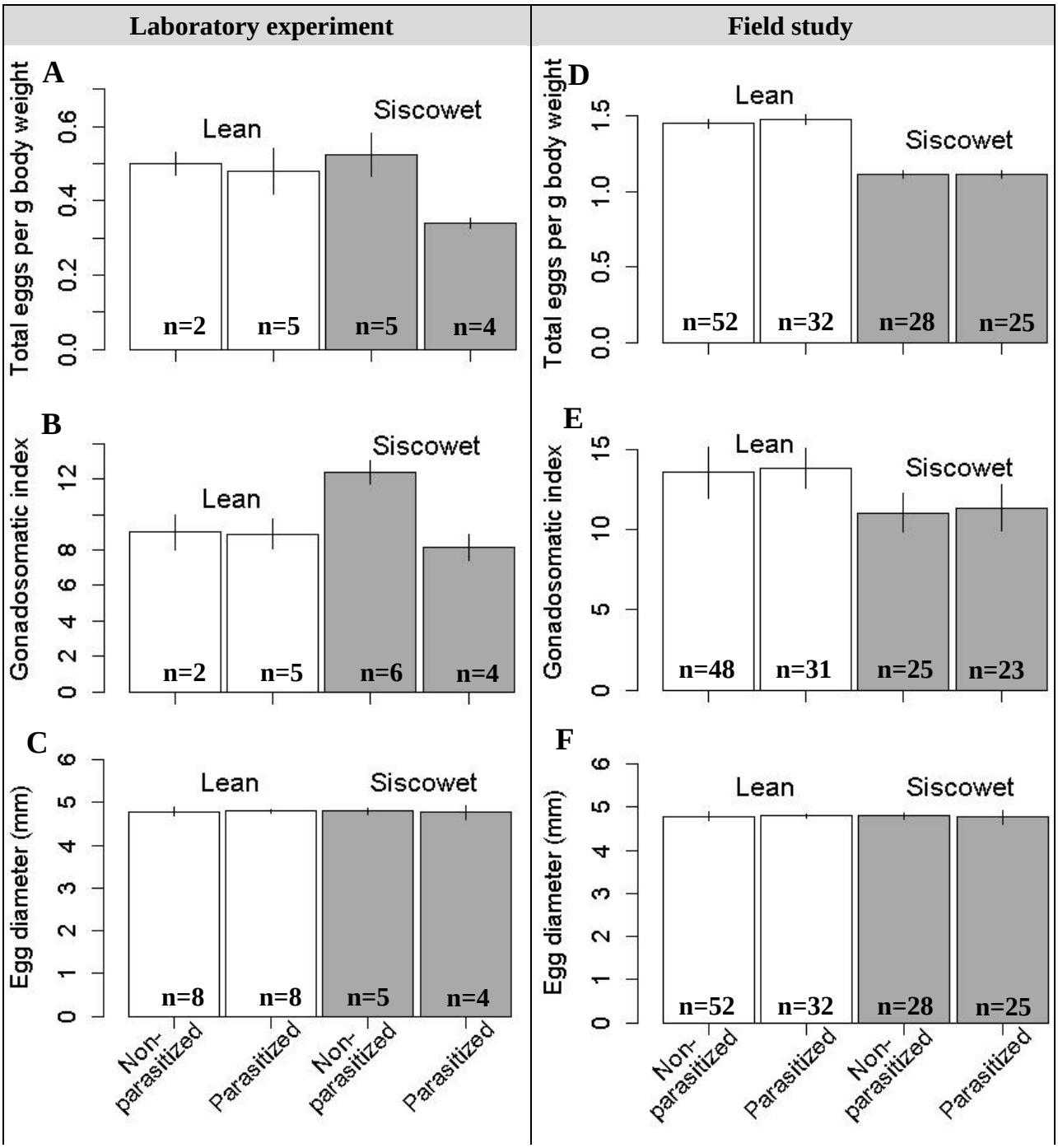


**Figure 13.** (A) Parasitized and non-parasitized lake trout from the laboratory experiment did not significantly differ in weight-adjusted fecundity (p=0.2622), (B) GSI (p=0.1395), (C) or average egg diameter (p=0.9312). (D) Wild parasitized and non-parasitized lake trout weight-adjusted fecundity was not significantly different (p=0.5806). (E) GSI marginal means were not significantly different between wild parasitized and non-parasitized leans (p=0.5334) or between parasitized and non-parasitized siscowets (p=0.9178). (F) Wild parasitized and non-parasitized lake trout had significantly different average egg diameters (p=0.0139). Error bars represent standard error (A, B, C, D, F) or the 95 percent confidence intervals of the marginal means (E).

**Evaluation of changes in hepatic genetic regulation**

Parasitized lean and siscowet transcriptome analyses were compared with results from their non-parasitized counterparts for reference. Differentially regulated genes refer to the difference between parasitized leans when compared with non-parasitized leans, and between parasitized siscowets compared with non-parasitized siscowets. Genes with the greatest regulation differences between parasitized and non-parasitized lake trout from RNAseq transcriptome analysis were not shared between parasitized leans and parasitized siscowets (Table 7). Parasitized leans had 424 genes which were differentially regulated with at least a 10-fold difference compared with non-parasitized leans; parasitized siscowets had 208 differentially regulated genes compared with non-parasitized siscowets. Of these differentially regulated genes 24 were shared between parasitized leans and siscowets.

**Table 7.** Summary of genes with the greatest regulation differences between parasitized and non-parasitized leans and siscowets from RNAseq analysis. Numbers in parentheses represents the magnitude difference in genetic regulation.

| | **Up-regulated in parasitized** | **Down-regulated in parasitized** |
|---|---|---|
| Lean | Cytochrome b (234x) | Chain A, Met-Trout Iv Hemoglobin (70x) |
| Siscowet | Fucolectin-4 precursor (33x) | Angptl3 protein (72x) |

The two greatest differentially up- and down-regulated annotated genes from qPCR analysis also showed varying genetic changes in response to parasitism between the morphotypes. Parasitized leans up-regulated genes related to energetics and stress responses and down-regulated genes related to hemoglobin and immune modulation (Figure 14 and Table 8). Parasitized siscowets up-regulated a gene related to immune modulation and down-regulated genes regulating bile acid production and steroid binding proteins (Figure 15 and Table 8).

**Table 8.** Description of differentially expressed gene functions from qPCR analysis.

| | |
|---|---|
| Fucolectin | A fructose-binding lectin which recognizes bacterial liposaccharides. |
| Hepcidin | Regulates plasma iron levels and mediates anemic response to systemic infections. |
| Hemoglobin | Iron-containing component of red blood cells which facilitates oxygen transport. |
| Albumin | The main protein component of plasma. Serum albumin binds to a variety of molecules, including fatty acids, hormones, cations, and water. It controls blood osomotic pressure and facilitates transport of hydrophobic molecules. |
| Cytochrome P450 7A1 | Enzyme which facilitates the production of bile from cholesterol. |
| Cytochrome b | A component of the mitochondrion respiratory chain complex III, which is involved in the electron transport chain and couples the electron transfer in the generation of ATP. |
| Growth arrest and DNA damage (GADD45) | A stress sensor mediating DNA repair and apoptosis. |
| Liver antimicrobial peptide | Protein effective against gram negative bacterial pathogens. |

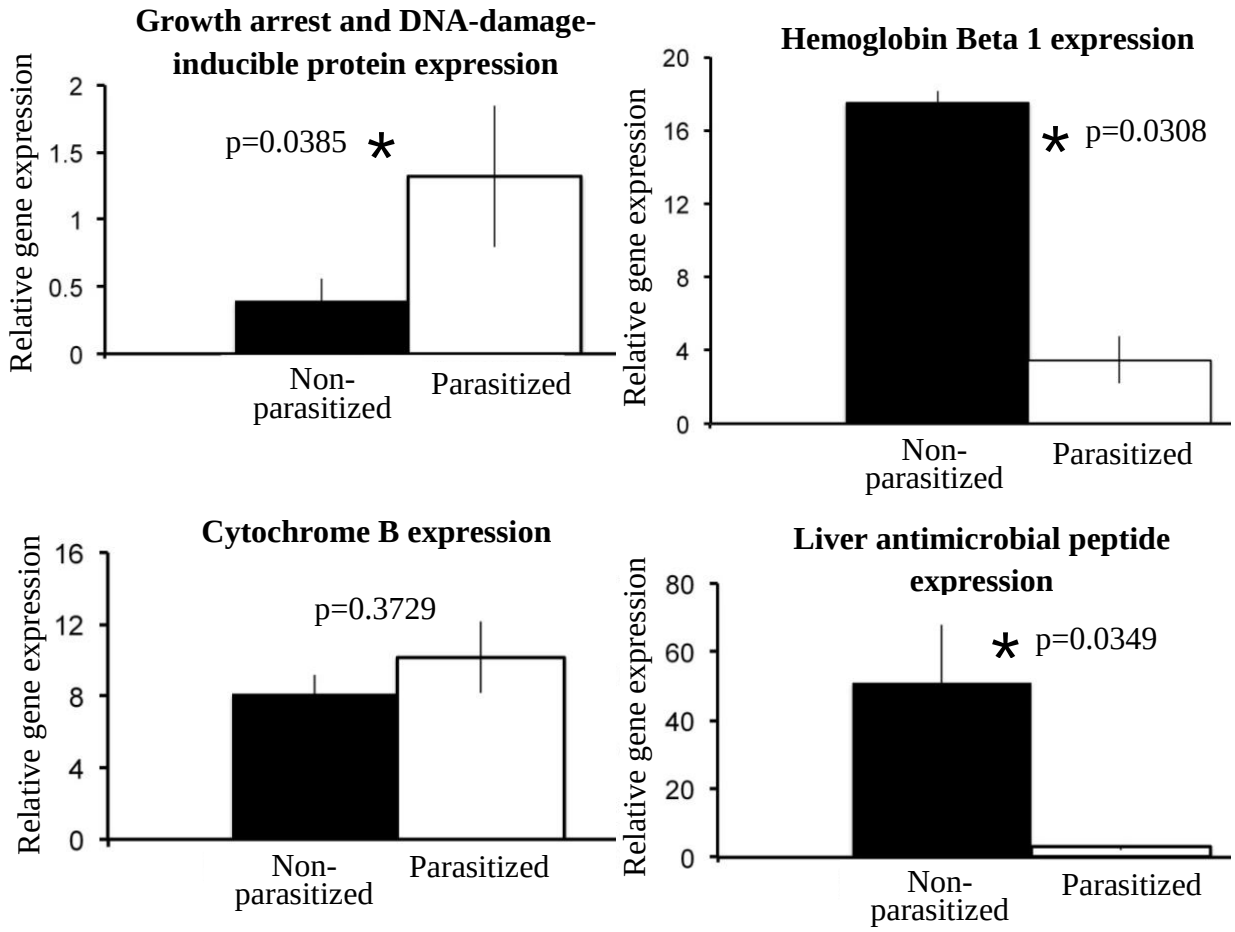


**Figure 14.** Comparison of four differentially regulated hepatic genes between parasitized (n=6) and non-parasitized (n=6) leans, as determined by qPCR analysis. Error bars represent standard error. * = significant difference $p<0.05$

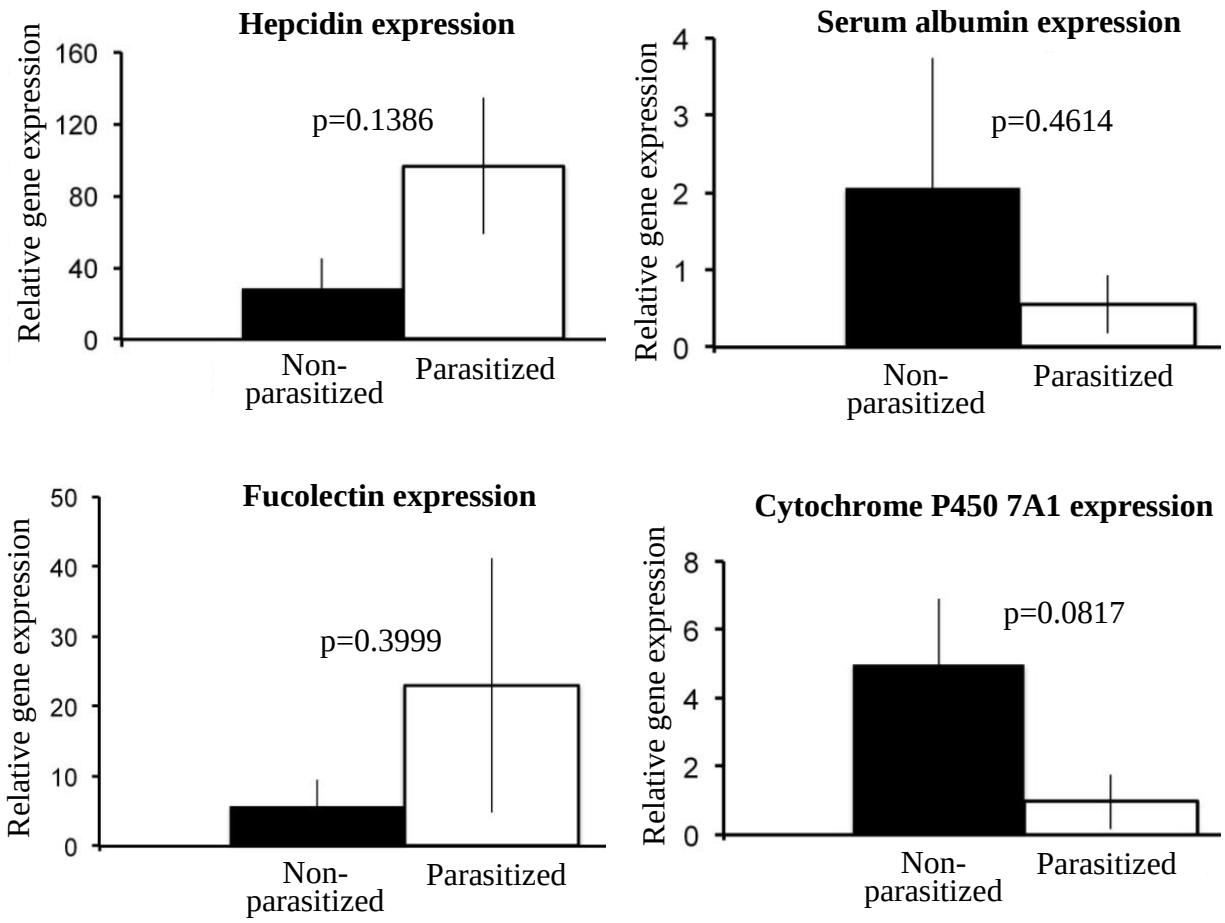


**Figure 15.** Comparison of four differentially regulated hepatic genes between parasitized (n=6) and non-parasitized (n=6) siscowets, as determined by qPCR analysis. Error bars represent standard error.

# DISCUSSION

## Immune, endocrine, and bioenergetics responses to parasitism in lean and siscowet lake trout

The results from this study support the hypothesis that sea lamprey parasitism imposes sublethal effects on lake trout. These effects could lead to substantial impacts on lake trout populations that have previously been ignored. My results suggest that a sea lamprey attack evokes immune-related modulation, endocrine disruption, and bioenergetics alterations. I also observed that life history may play a role in mediating the response to parasitism. This was supported by the differences in how morphotypes modulated immune-related parameters as an immediate response to parasitism and bioenergetics alterations as a longer-term response.

### *Gross measurements of immune-related modulation and stress responses*

The immediate response to a sea lamprey attack manifested as changes in genetic regulation in siscowets and leans, and in HSI in siscowets. Parasitized siscowets had a significant positive relationship between test duration and HSI (Figure 9B), which is a general indicator of immune-related modulation, although it cannot be exclusively linked to immune modulation (Tahir et al. 1993; Secombes et al. 1995). Parasitized siscowets also differentially regulated two immune-related genes (Figure 15). Fucolectin, which was up-regulated in parasitized siscowets, is a fructose-binding lectin, which is a group of proteins enabling recognition and agglutination of non-self cells (Honda et al. 2000; Bianchet et al. 2002). Parasitized siscowets also up-regulated hepcidin, a protein involved in mediating iron homeostasis. Hepcidin is down-regulated in response to anemia, which increases plasma iron concentrations. An increase in hepcidin, as observed in parasitized siscowets, plays a role in the immune response to systemic infections. Increased hepcidin production causes a decrease in iron availability, which is thought

to be a host defense mechanism to decrease iron availability for pathogens (Jurado 1997; Nemeth et al. 2004; Nemeth and Ganz 2006).

Changes in genetic regulation in parasitized leans suggested increased energetic mobilization and immune-related modulation that was different than siscowets. Parasitized leans differentially regulated genes related to ATP production (cytochrome b), stress response (GADD45), and circulatory compensation (hemoglobin β1) (Figure 14). In addition, parasitized leans down-regulated an immune-related gene (liver antimicrobial peptide; Figure 14). The changes in genetic regulation observed in parasitized leans correspond well with the initial stages of the general stress response model, which include energetic mobilization and immune suppression (Selye 1950; Mazeaud et al. 1977). This suggests that the lean lake trout parasitized in my laboratory experiment were still experiencing an initial acute stress response at the time of sampling and maintained this acute stress response throughout the duration of the experiment. It is possible that the immediate acute stress response, as indicated by the gene expression pattern in leans which have experienced a parasitism event, may eventually manifest as changes in bioenergetics (e.g. reproductive success and growth), although additional research is necessary to better understand this connection. Future research on immune-related modulation may include identifying changes in genetic regulation in other immune-related systems, such as the spleen or head kidney.

Parasitized siscowets did not show indications of energetic mobilization, stress response, or suppression of immune-related parameters, suggesting that siscowets had transitioned beyond the initial acute stress response at the time of sampling. Or, siscowets may be less sensitive to sea lamprey parasitism and may not be experiencing severe enough stress to result in immune suppression. The results from my laboratory experiment support the hypothesis that siscowets

have a less severe immediate response to sea lamprey parasitism than leans, and this may lead to differential population responses. Future studies that measure more physiologically-based immune and energetic related parameters and that compare between the morphotypes may provide additional support for the hypothesis that life history mediates the stress response. The use of RNA-sequencing, as demonstrated here, may be a useful tool to explore these questions and to identify key genes that differ between morphotypes.

***Measurements of endocrine disruption***

Endocrine disruption as a result of sea lamprey parasitism was also observed in this study through decreased testosterone levels in the laboratory experiment (Figure 10A) and FSH suppression in the field study (Figure 10E). The decreased plasma testosterone concentrations observed in parasitized lake trout from the laboratory experiment may be because of suppressed steroidogenesis or increased metabolism and clearance of the steroid. Stress may reduce steroid levels due to increased production of cortisol, a main mediator hormone of the stress response. Pankhurst and Van Der Kraak (2000) observed a decrease in plasma testosterone concentrations directly following an injection of cortisol in rainbow trout. Plasma estradiol concentrations did not decline until the rainbow trout were allowed to recover for three hours. Estradiol is produced through the aromatization of testosterone by cytochrome p450 in the theca cells (Nagahama 1994), and a decline in estradiol could be due to testosterone substrate availability (Pankhurst and Van Der Kraak 2000). My results suggest a subtle decrease in plasma estradiol concentrations in parasitized leans, although it was not significant (Figure 10B, $p=0.2285$). Had my study allowed for a recovery period after parasitism, a significant decline in plasma estradiol may have been observed.

It is also possible that parasitized lake trout have lowered testosterone levels due to increased steroid metabolism and degradation rates. Steroid-binding globulins, such as sex steroid binding protein (SSBP) and albumin, transport and normally protect sex steroids from degradation (Dunn et al. 1981; Petra et al. 1983). There is some evidence supporting that SSBP binding affinity is lowered by cortisol (Hobby et al. 2000a). If SSBP affinity was lowered enough to cause competition for binding sites then testosterone would be expected to bind less than estradiol because its binding affinity is 33.8 percent of estradiol (Hobby et al. 2000b), thereby increasing the concentration of free testosterone that is subject to degradation. Furthermore, in my study qPCR results showed a down-regulation of albumin in parasitized siscowets (Figure 15). Although albumin has a low binding affinity (compared to SSBP), it is usually present in large concentrations in the serum and therefore binds to a large quantity of plasma sex steroids (Dunn et al. 1981; Södergard et al. 1982; Miller et al. 1983). In humans, decreased albumin concentrations caused increased unbound testosterone levels, thereby increasing metabolism of the steroid (Dunn et al. 1981). Therefore, it is possible that the down-regulation of albumin in parasitized siscowets is linked to the lowered testosterone concentrations, but this will need to be explored in future studies.

An additional observation of my endocrine analysis was the differences in plasma testosterone and estradiol concentrations between my field study and laboratory experiment (Figures 10A - D). This may be due to the artificial conditions of the laboratory experiment or differences in sampling dates between the laboratory and field study. Fish maturation is sensitive to environmental cues, including water temperature and photoperiod, and it is difficult to replicate natural conditions in the laboratory (Davies et al. 1999; Bromage et al. 2001). Artificial experimental settings may be an explanation for the lower plasma sex steroid concentrations

observed in lake trout from my laboratory experiment. In addition, plasma samples were taken over a longer time period in my laboratory experiment (periodically October through December) compared with my field study (four sample dates total, taken in September and October). Plasma sex steroid concentrations change during final maturation, which may have resulted in different average testosterone and estradiol concentrations between the laboratory and field studies (Taranger et al. 1998; Goetz et al. 2011).

Lower pituitary FSH levels in wild parasitized lake trout may also be due to endocrine suppression (Figure 10E). FSH's primary role is in early maturation when it induces oocyte uptake of vitellogenin and oocyte recruitment (Tyler et al. 1991; Tyler et al. 1997). In lake trout FSH peaks in August and September followed by a decline in October. However, detectable levels of FSH are still present in October (Goetz et al. 2011). Little is known about the function of FSH in the latter stages of maturation. In male mammals FSH stimulates LH receptors in late maturation, increasing gonad weight and sensitivity to LH (Odell et al. 1973; Chase 1983; Kerr and Sharpe 1985). However, I know of no comparable research for fish.

It is also possible that FSH has little or no function in late maturation and that the slow decline in FSH concentrations in the fall represents down-regulation through negative feedback or degradation. This is true of vitellogenin, which is present throughout rainbow trout maturation. The presence of vitellogenin in late maturation, long after vitellogenesis is complete, is thought to be due to its slow degradation rates (Bon et al. 1997). If the presence of FSH in later stages of maturation is merely an artifact of its gradual degradation and down-regulation, then the lower levels of FSH in parasitized lake trout could also indicate an earlier maturation schedule or suppressed FSH production in early maturation. If this gradual degradation of FSH is true, perhaps evidence can be found by close examination of plasma estradiol dynamics.

Plasma estradiol levels tend to decrease throughout final maturation (Norberg et al. 1989; Goetz et al. 2011). However, wild parasitized lake trout in my study had a non-significant trend for increased estradiol levels (Figure 10D). This suggests that parasitized lake trout were not in later stages of maturation than non-parasitized trout at the time of sampling. Therefore, it is most likely the suppressed pituitary FSH levels observed in parasitized lake trout is due to decreased production.

Additional research is necessary to determine whether FSH production was actively being suppressed at the time of sampling or if the lower FSH levels were an artifact of decreased production in earlier maturation. This would require measuring pituitary FSH earlier in maturation. An additional limitation of my FSH analysis is that I was not able to measure this parameter in my laboratory experiment. But, the suppressed pituitary FSH levels I observed in my field study indicate that FSH is an important component of the lake trout endocrine response to sea lamprey parasitism which should be considered for future study (Goetz et al. 2011). Additional research may also include measurement of LH. LH, the gonadotropin involved in final maturation (Prat et al. 1996; Breton et al. 1998; Figure 2), was also not measured in my study because it peaks sharply directly before ovulation making it difficult to capture in field studies (Goetz et al. 2011). It is very possible that LH may be affected by sublethal sea lamprey parasitism as well.

***Measurements of bioenergetics alterations***

The endocrine response shown in both the short-term laboratory experiment and field study suggests that sea lamprey parasitism affects lake trout endocrine function well beyond the short-term stress response stage, and likely results in reduced reproductive success. However, future research is necessary to understand this connection. Wild parasitized lake trout showed

little indication of altered reproductive success (e.g. GSI, fecundity, egg diameter; Figure 13D, E, F). The difference in length-at-age between wild parasitized and non-parasitized leans and siscowets (Figure 12C, D) was a confounding factor for measurements of reproductive success. These measurements tend to change with age, making interpretation difficult. Given that the parasitized lake trout were larger and older (Figure 12A, B, E), and thus should have higher fecundities (Goetz et al. 2011), it is possible that parasitized lake trout are experiencing impairments in reproductive success but that these declines are undetectable in this dataset.

Understanding how sea lamprey parasitism affects measurements of reproductive success may be more suited to a laboratory experiment that can control for potential confounding factors, such as differences in length and age compositions. The short time frame of my laboratory experiment was designed to measure the immediate endocrine disruption and immune-related modulation in response to parasitism, but this also limited my analysis of reproduction measurements, including fecundity and egg diameter. The mature lake trout used in my laboratory experiment conducted in the fall were likely at or near completion of vitellogenesis, which would mean that females would have already made their energetic investment into gonadal growth and therefore were unlikely to show any changes due to parasitism.

Laboratory experiments in which lake trout are parasitized prior to maturation, given time to heal sea lamprey wounds, and then assessed for reproductive alteration after maturation would provide a clearer link between sea lamprey parasitism, endocrine disruption, and reproductive success. This experimental design would enable sea lamprey parasitism to be linked with changes in reproduction, such as fecundity and egg diameter. Additionally, future research on reproductive alteration may also include the development of a lake trout physiological model similar to the model developed by Murphy et al. (2005). This model connects HPG axis

components, including gonadotropin and plasma sex steroids, and estimates cumulative vitellogenin production. Development of a similar model for lake trout would allow endocrine disruption due to parasitism to be scaled to a measurement of reproductive success.

The results from my study also support that lake trout alter growth-related bioenergetics parameters in response to sea lamprey parasitism. Muscle lipid concentration measurements determined by the laboratory lipid extraction method showed a non-significant trend for decreased lipid levels in parasitized wild siscowets when compared with non-parasitized wild siscowets (Figure 11C). This suggests that siscowets use muscle lipid energetic reserves to cope with the energetic demands of sea lamprey parasitism. The decreased muscle lipid concentration in wild parasitized siscowets was not observed in measurements taken using the non-invasive fatmeter (Figure 11B). This is likely due to lower precision of the fatmeter. When operating the fatmeter, I observed that muscle lipid level readings fluctuated with minor position changes. The fatmeter can distinguish between the morphotypes well, but it is not precise enough to detect the smaller changes due to sea lamprey parasitism. This would explain why a trend was observed when measuring muscle lipid concentrations by laboratory lipid extraction analysis but not by fatmeter measurement.

The differing results between muscle lipid concentration measured by laboratory lipid extraction and by fatmeter measurement made analysis of this parameter difficult. I measured muscle lipid concentration by laboratory lipid extraction for only a subsample of wild lake trout, in order to verify fatmeter measurement results, so small sample size likely limited the statistical power of this analysis. In addition, the short duration of my laboratory experiment made it unlikely that I would observe changes in muscle lipid concentration in response to sea lamprey

parasitism. Measurements of more immediate energetic demands, such as plasma triglycerides or liver glycogen, may be better measurements of short-term bioenergetics alterations.

Wild parasitized lake trout were observed to have significantly greater length-at-age relationships compared with non-parasitized lake trout (Figure 12C, D). There are a number of different reasons why this could occur. One, sea lamprey parasitism could alter lake trout growth trajectories, resulting in greater energetic allocation towards somatic growth. This explanation has obvious management implications for the accuracy of lake trout bioenergetics models. Additionally, greater lake trout size may cause increased sea lamprey parasitism. In addition to being preferred hosts, larger lake trout are also more likely to survive parasitism (Swink 1990; Swink 1991). This may result in a skewed sea lamprey scarring distribution towards older and larger individuals. Since reproductive success tends to increase with age and size, sea lamprey parasitism could be hindering reproduction of the population's most productive individuals (Berkeley et al. 2004; Olin et al. 2012). This effect may be similar to the reproductive pressures observed in fish populations where fishing industries select for the largest and oldest females (Birkeland and Dayton 2005; Olsen et al. 2005).

**Life history influence on sea lamprey parasitism behavior**

A second objective of my study was to compare the immune, endocrine, and bioenergetics responses to sea lamprey parasitism between the lean and siscowet morphotypes. As previously discussed, morphotype life history adaptations seemed to influence the immediate and long-term responses to parasitism, seen through differential immune-related modulation and bioenergetics alteration. Parasitized siscowets in the laboratory experiment mounted an immune response (Figure 9B, 15) and wild parasitized siscowets seemed to sacrifice muscle lipid

concentration (although not significant) (Figure 11C); these responses were not observed in parasitized leans.

In addition to comparing immune, endocrine, and bioenergetics responses to parasitism between the morphotypes I also evaluated measurements of sea lamprey parasitism behavior to test whether morphotype differences could be due to parasite behavior, not just host response differences. Results from my laboratory experiment suggest that sea lamprey have different feeding rates depending on lake trout host morphotype (Figure 8A). Sea lamprey had a tendency to consume a greater body weight percentage when parasitizing siscowet hosts. Sea lamprey have been previously observed to alter feeding rates depending on host species, but this has been attributed to differences in initial host weight (Swink and Hanson 1986). Host morphotype and host weight were correlated in my experiment ($\rho$=0.4340), making it difficult to interpret these effects individually. However, when I re-fit the sea lamprey feeding model using data from leans and siscowets with overlapping weight ranges only there was still a statistical trend for greater sea lamprey percent body weight consumption when parasitizing siscowet hosts (p=0.1157).

My results suggest that sea lamprey alter their feeding rates depending on lake trout host morphotype, and that this difference may be independent of initial host weight. Alteration of sea lamprey feeding rate between the morphotypes may be due to physiological or behavioral differences between lake trout host morphotypes. Host physiological differences which may increase sea lamprey feeding rate include increased blood flow or greater overall blood volume. Additionally, host behavior may affect sea lamprey feeding rate. Although not measured in my experiment I did observe that siscowets were more docile than leans when being parasitized. Sea lamprey parasitizing siscowet hosts may experience less stress than those parasitizing lean hosts, which may increase blood consumption.

In addition to measuring sea lamprey feeding rate in the laboratory experiment, I also measured sea lamprey scarring patterns on wild lake trout to assess sea lamprey behavior at the population level. My results showed no indications of differential survival or parasitism selectivity between wild leans and siscowets when differences in length and age compositions were accounted for (Table 2, Figure 8B). Differences in length-at-age are important to consider when comparing sea lamprey parasitism rate between the morphotypes because both greater size and older ages are associated with an increased probability of sea lamprey parasitism having occurred. Siscowets have lower length-at-age than leans (Miller and Schram 2000). At a given age, when compared with siscowets, leans are preferred hosts due to sea lamprey selectivity for larger lake trout (Swink 1991). At a given length siscowets will be significantly older than leans, which also increases the probability of parasite-host contact having occurred. Since fisheries sampling often targets fish of a particular length, sampling will be biased towards older siscowets and younger leans which could lead to misinterpretation of the scarring data if differences in length-at-age are not considered.

My research results may be further evaluated in future studies by comparing sea lamprey scarring patterns between lake trout morphotypes in a greater number of age cohorts. Increased sample size may also decrease the influence of potentially misidentified scars, including fully healed scars which are no longer visible or scars from other sources, such as ulcers, with similar appearances to sea lamprey scars.

Genetic adaptation to differing environmental pressures may explain why lake trout host morphotype influenced sea lamprey feeding rate in the laboratory experiment (Figure 8A), but was not a significant predictor of sea lamprey scarring patterns in wild populations (Table 2, Figure 8B). The lean and siscowet morphotypes live in different bathymetric regions of Lake

Superior and have adapted genetically to their respective environments (Goetz et al. 2010). While life history appears to influence immune-related modulation, and possibly bioenergetics, responses to sea lamprey parasitism this could represent an adaptive response to differing environmental pressures experienced by leans and siscowets. This would result in life history differences in parasitism response, but similar overall survival in wild populations. It is possible that the colder water temperatures occupied by siscowets may slow the healing of sea lamprey wounds, leaving muscle tissue exposed for longer. This could explain the greater modulation of immune-related parameters in parasitized siscowets. The trend for decreased muscle lipid levels in wild siscowets may also be a result of occupying a less productive environment than leans where replenishing muscle lipid reserves may take longer or come at a greater energetic cost.

Lean and siscowet differences in length-at-age may also affect population-level responses to sea lamprey parasitism. Although wild leans and siscowets did not have differential sea lamprey scarring rates it is possible that younger lake trout are parasitized differently depending on morphotype. The difference in growth dynamics between the morphotypes could mean that leans and siscowets become preferred sea lamprey hosts at different ages. Due to their slower growth siscowets have more years between reproductive maturity and when they are larger and more preferred lamprey hosts (Swink 1991; Sitar and He 2006; Mata 2009). With more years to reproduce under lower sea lamprey parasitism pressures siscowets may experience less reproductive impact at the population level. This difference would likely only be apparent by studying a larger range of lake trout cohorts or through population modeling.

**Overall conclusions**

In this thesis I presented observations which support that lake trout respond to sea lamprey parasitism by altering immune, endocrine, and bioenergetics parameters. Additionally,

life history adaptations may affect short-term sea lamprey parasitism responses in lake trout and life history characteristics, such as age and size at maturity, may influence susceptibility to an attack. My study is a first step in identifying key physiological systems affected by sea lamprey parasitism. From these results it is apparent that sea lamprey affect lake trout populations beyond the direct mortality they inflict. The 45 to 75 percent of lake trout which survive a sea lamprey parasitism event undergo a stress response, immune-related modulation, and endocrine disruption (Swink 2003; Madenjian et al. 2008). These changes likely affect population-level growth and reproductive success, which should be considered when evaluating the effects of sea lamprey parasitism on lake trout populations.

# APPENDICES

## APPENDIX A: LIST OF ALTERNATIVE MODELS

The following is a list of all alternative models compared for each response variable. Alternative models were compared by AIC value and the model with the lowest AIC value out of those listed below was considered the best model.

**Response variable:** Sea lamprey percent body weight consumption

Morphotype + Weight + Sample date + Test duration + Muscle lipid
Morphotype + Weight + Sample date + Test duration
Morphotype + Weight + Sample date + Muscle lipid
Morphotype + Weight + Test duration + Muscle lipid
Morphotype + Sample date + Test duration + Muscle lipid
Weight + Sample date + Test duration + Muscle lipid
Morphotype + Weight + Sample date
Morphotype + Weight + Muscle lipid
Morphotype + Test duration + Muscle lipid
Sample date + Test duration + Muscle lipid
Morphotype + Weight + Test duration
Morphotype + Sample date + Muscle lipid
Weight + Test duration + Muscle lipid
Morphotype + Sample date + Test duration
Weight + Sample date + Muscle lipid
Weight + Sample date + Test duration
Test duration + Muscle lipid
Sample date + Test duration
Weight + Sample date
Morphotype + Weight
Sample date + Muscle lipid
Weight + Test duration
Morphotype + Sample date
Weight + Muscle lipid
Morphotype + Test duration
Muscle lipid
Test duration

**Response variable (cont'd):** Sea lamprey percent body weight consumption

Sample date
Weight
Morphotype

**Response variables:** HSI, muscle lipid concentration from laboratory experiment

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Parasitism status + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Parasitism status + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Parasitism status + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Parasitism status

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Sample date

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration

**Response variables (cont'd):** HSI, muscle lipid concentration from laboratory experiment

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Sample date

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Parasitism status + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Parasitism status + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Parasitism status + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

**Response variables (cont'd):** HSI, muscle lipid concentration from laboratory experiment

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Parasitism status + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Parasitism status

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Fungus

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status

Morphotype + Parasitism status + Test duration + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration

Morphotype + Parasitism status + Fungus + Sample date + Morphotype * Parasitism status

Morphotype + Parasitism status + Fungus + Sample date

Morphotype + Parasitism status + Sample date + Morphotype * Parasitism status

**Response variables (cont'd):** HSI, muscle lipid concentration from laboratory experiment

Morphotype + Parasitism status + Sample date
Morphotype + Parasitism status + Fungus + Morphotype * Parasitism status
Morphotype + Parasitism status + Fungus
Morphotype + Parasitism status + Morphotype * Parasitism status
Morphotype + Parasitism status

**Response variables**: Plasma testosterone, plasma estradiol, egg diameter, fecundity, GSI from laboratory experiment

Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Morphotype * Test duration + Morphotype * Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Morphotype * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Test duration + Morphotype * Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status + Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date + Morphotype * Parasitism status * Test duration
Morphotype + Parasitism status + Test duration + Sample date
Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

**Response variables (cont'd)**: Plasma testosterone, plasma estradiol, egg diameter, fecundity, GSI from laboratory experiment

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Morphotype * Test duration + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Parasitism status * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status

Morphotype + Parasitism status + Test duration + Morphotype * Test duration

Morphotype + Parasitism status + Test duration + Parasitism status * Test duration

Morphotype + Parasitism status + Test duration + Morphotype * Parasitism status * Test duration

Morphotype + Parasitism status + Test duration

Morphotype + Parasitism status + Sample date + Morphotype * Parasitism status

Morphotype + Parasitism status + Sample date

Morphotype + Parasitism status + Morphotype * Parasitism status

Morphotype + Parasitism status

**Response variable:** Probability of sea lamprey scarring in lake trout

Morphotype + Age + Length + Weight + Sample date

Morphotype + Age + Length + Weight

Morphotype + Age + Length + Sample date

Morphotype + Age + Weight + Sample date

Morphotype + Length + Weight + Sample date

Age + Length + Weight + Sample date

Morphotype + Age + Length

**Response variable (cont'd):** Probability of sea lamprey scarring in lake trout

Morphotype + Age + Sample date
Morphotype + Weight + Sample date
Length + Weight + Sample date
Morphotype + Age + Weight
Morphotype + Length + Sample date
Age + Weight + Sample date
Morphotype + Age
Morphotype + Sample date
Weight + Sample date
Morphotype + Length
Age + Sample date
Morphotype + Length
Length + Sample date
Length + Weight
Age + Length
Morphotype
Age
Length
Weight
Sample date

**Response variables:** HSI, muscle lipid concentration (by fatmeter), muscle lipid concentration (by laboratory lipid extraction), plasma testosterone, plasma estradiol, FSH, egg diameter, fecundity, GSI from field study

Parasitism status + Morphotype + Age + Sample date + Parasitism status:Morphotype + Parasitism status * Morphotype * Age
Parasitism status + Morphotype + Age + Sample date + Parasitism status:Morphotype
Parasitism status + Morphotype + Age + Parasitism status:Morphotype + Parasitism status * Morphotype * Age
Parasitism status + Morphotype + Parasitism status:Morphotype + Age
Parasitism status + Morphotype + Parasitism status:Morphotype + Sample date
Parasitism status + Morphotype + Parasitism status:Morphotype
Parasitism status + Morphotype + Age + Sample date + Parasitism status * Morphotype * Age
Parasitism status + Morphotype + Age + Sample date
Parasitism status + Morphotype + Age + Parasitism status * Morphotype * Age

**Response variables (cont'd):** HSI, muscle lipid concentration (by fatmeter), muscle lipid concentration (by laboratory lipid extraction), plasma testosterone, plasma estradiol, FSH, egg diameter, fecundity, GSI from field study

Parasitism status + Morphotype + Age

Parasitism status + Morphotype + Sample date

Parasitism status + Morphotype

## APPENDIX B: MODELS WITHIN TWO AIC VALUES OF BEST MODEL

**Table 9.** Predictor variables for models within two AIC values of the best model for measurements related to sea lamprey behavior.

| | Response variable | Predictor variable p-values | AIC values |
|---|---|---|---|
| **Laboratory experiment** | Sea lamprey percent body weight consumption | Morphotype: 0.2695 | -29.3 |
| | | Host beginning weight: 0.2654 | -28.7 |
| | | Sample date: 0.2208 | -29.1 |
| | | Morphotype: 0.0593<br>Host beginning weight: 0.0583<br>Test duration: 0.7328 | -28.8 |
| | | Morphotype: 0.0944<br>Host beginning weight: 0.0832<br>Sample date: 0.4278 | -28.8 |
| **Field study** | Probability of sea lamprey scarring in lake trout | Morphotype: 0.3338<br>Host age: 0.2419<br>Host length: <0.0001 | 140.5 |

**Table 10.** Predictor variables for models within two AIC values of the best model for measurements of immune-related modulation.

| | Response variable | Predictor variable p-values | AIC values |
|---|---|---|---|
| **Laboratory experiment** | Hepatosomatic index (HSI) | Morphotype: 0.0001<br>Parasitism: 0.0526<br>Test duration: 0.0118<br>Mature: 0.9618<br>Sample date: 0.0002<br>Parasitism * Test duration: 0.1462 | -138.8 |
| | | Morphotype: 0.0001<br>Parasitism: 0.05182<br>Test duration: 0.0125<br>Sample date: 0.0001<br>Morphotype * Parasitism: 0.4143<br>Parasitism * Test duration: 0.1328 | -139.5 |
| | | Morphotype: 0.0011<br>Parasitism: 0.0532<br>Test duration: 0.0130<br>Sample date: 0.0001<br>Morphotype * Parasitism: 0.4697 | -139.1 |
| | | Morphotype: 0.0012<br>Parasitism: 0.0513<br>Test duration: 0.0121<br>Sample date: 0.0001<br>Morphotype * Test duration: 0.6091 | -138.8 |
| | | Morphotype: 0.0009<br>Parasitism: 0.0514<br>Test duration: 0.0114<br>Sample date: 0.0001<br>Parasitism * Test duration: 0.1444 | -140.8 |
| | | Morphotype: 0.0011<br>Parasitism: 0.0527<br>Test duration: 0.0119<br>Sample date: 0.0001 | -140.6 |
| **Field study** | Hepatosomatic index (HSI) | Parasitism: 0.9018<br>Morphotype: 0.0192<br>Sample date: 0.0001<br>Parasitism * Morphotype: 0.6125 | -105.2 |

**Table 11.** Predictor variables for models within two AIC values of the best model for physiological measurements related to endocrine disruption.

| | Response variable | Predictor variable p-values | AIC values |
|---|---|---|---|
| **Laboratory experiment** | Plasma testosterone (ng/mL) | Morphotype: 0.0115<br>Parasitism: 0.0068 | 80.0 |
| | | Morphotype: 0.0253<br>Parasitism: 0.0058<br>Test duration: 0.8407<br>Morphotype * Parasitism: 0.9909<br>Morphotype * Test duration: 0.0685 | 80.1 |
| | | Morphotype: 0.0244<br>Parasitism: 0.0086<br>Test duration: 0.8975<br>Sample date: 0.7238<br>Morphotype * Test duration: 0.0632 | 78.8 |
| | Plasma estradiol (ng/mL) | Morphotype: 0.0029<br>Parasitism: 0.1734<br>Sample date: 0.0821 | 50.0 |
| | | Morphotype: 0.0036<br>Parasitism: 0.1826<br>Sample date: 0.0896<br>Morphotype * Parasitism: 0.8998 | 50.0 |
| | | Morphotype: 0.0049<br>Parasitism: 0.1843<br>Sample date: 0.1202<br>Test duration: 0.9094 | 48.0 |
| **Field study** | Plasma testosterone (ng/mL) | Parasitism: 0.3637<br>Morphotype: 0.6356<br>Age: 0.1210<br>Sample date: 0.0071<br>Parasitism * Morphotype * Age: 0.1722 | 341.6 |
| | | Parasitism: 0.3673<br>Morphotype: 0.6381<br>Age: 0.1291<br>Sample date: 0.0046<br>Parasitism * Morphotype: 0.2551 | 340.1 |

**Table 11 (cont'd)**

| | | | |
|---|---|---|---|
| **Field study** | Plasma testosterone (ng/mL) | Parasitism: 0.3648<br>Morphotype: 0.6364<br>Age: 0.1272<br>Sample date: 0.0101<br>Parasitism * Morphotype: 0.2527<br>Parasitism * Morphotype * Age: 0.2462 | 340.1 |
| | Plasma estradiol (ng/mL) | Parasitism: 0.2128<br>Morphotype: 0.9564<br>Age: 0.4356<br>Sample date: <0.0001<br>Parasitism * Morphotype: 0.5386 | 379.0 |
| | Pituitary follicle-stimulating hormone (FSH) | Parasitism: 0.0034<br>Morphotype: 0.6448<br>Sample date: 0.3222 | -165.8 |
| | | Parasitism: 0.0063<br>Morphotype: 09232<br>Parasitism * Morphotype: 0.9718 | -164.4 |

**Table 12.** Predictor variables for models within two AIC values of the best model for bioenergetics measurements related to growth.

| | Response variable | Predictor variable p-values | AIC values |
|---|---|---|---|
| **Laboratory experiment** | Muscle lipid concentration (% wt/w), by fatmeter | Morphotype: <0.0001<br>Parasitism: 0.4706 | 635.7 |
| | | Morphotype: <0.0001<br>Parasitism: 0.4351<br>Sample date: 0.0962<br>Morphotype * Parasitism: 0.7138 | 636.6 |
| | | Morphotype: <0.0001<br>Parasitism: 0.4276<br>Fungus: 0.6886<br>Sample date: 0.1181 | 636.6 |
| | | Morphotype: <0.0001<br>Parasitism: 0.4332<br>Test duration: 0.7437<br>Sample date: 0.0974 | 636.7 |
| | | Morphotype: <0.0001<br>Parasitism: 0.4015<br>Test duration: 0.7410<br>Sample date: 0.0753<br>Morphotype * Test duration: 0.1204 | 636.1 |
| **Field study** | Fork length (mm) | Parasitism: <0.0001<br>Morphotype: <0.0001<br>Age: <0.0001<br>Sample date: 0.0818<br>Parasitism * Morphotype * Age: 0.0234 | -233.6 |
| | | Parasitism: <0.0001<br>Morphotype: <0.0001<br>Age: <0.0001<br>Sample date: 0.0818<br>Parasitism * Morphotype: 0.7858<br>Parasitism * Morphotype * Age: 0.0188 | -232.3 |
| | Muscle lipid concentration (% wt/w), by fatmeter | Parasitism: 0.6121<br>Morphotype: <0.0001<br>Age: 0.1956<br>Parasitism * Morphotype * Age: 0.1769 | 154.1 |

**Table 12 (cont'd)**

| | | | |
|---|---|---|---|
| **Field study** | Muscle lipid concentration (% wt/w), by fatmeter | Parasitism: 0.7541<br>Morphotype: <0.0001<br>Age: 0.1747<br>Sample date: 0.1746 | 153.0 |
| | | Parasitism: 0.6163<br>Morphotype: <0.0001<br>Age: 0.2017<br>Parasitism * Morphotype: 0.7847 | 153.0 |
| | Muscle lipid concentration (% wt/w), by laboratory analysis | Parasitism: 0.4386<br>Morphotype: <0.0001<br>Parasitism * Morphotype: 0.4801 | -47.5 |

**Table 13.** Predictor variables for models within two AIC values of the best model for bioenergetics measurements related to reproduction.

| | Response variable | Predictor variable p-values | AIC values |
|---|---|---|---|
| Laboratory experiment | Fecundity (total eggs per g body weight) | Morphotype: 0.5390<br>Parasitism: 0.2766<br>Morphotype * Parasitism: 0.5955 | -8.7 |
| | | Morphotype: 0.5168<br>Parasitism: 0.2570<br>Sample date: 0.6304 | -9.0 |
| | | Morphotype: 0.5708<br>Parasitism: 0.2884<br>Test duration: 0.9707 | -9.0 |
| | Gonadosomatic index (GSI) | Morphotype: 0.5997<br>Parasitism: 0.1361<br>Morphotype * Parasitism: 0.2578 | 94.5 |
| | | Morphotype: 0.7245<br>Parasitism: 0.1387<br>Sample date: 0.2414 | 94.4 |
| | | Morphotype: 0.7243<br>Parasitism: 0.1397<br>Sample date: 0.3023<br>Morphotype * Parasitism: 0.3223 | 95.0 |
| | | Morphotype: 0.4832<br>Parasitism: 0.1280<br>Test duration: 0.3295 | 95.0 |
| | | Morphotype: 0.4919<br>Parasitism: 0.1041<br>Test duration: 0.3394<br>Morphotype * Test duration: 0.4759 | 96.2 |
| | | Morphotype: 0.4902<br>Parasitism: 0.1349<br>Test duration: 0.5897<br>Morphotype * Parasitism: 0.4395 | 96.1 |
| | | Morphotype: 0.6369<br>Parasitism: 0.1430<br>Test duration: 0.6094<br>Sample date: 0.4204 | 96.0 |

**Table 13 (cont'd)**

| | | | |
|---|---|---|---|
| **Laboratory experiment** | Egg diameter (mm) | Morphotype: 0.9439<br>Parasitism: 0.9328<br>Morphotype * Parasitism: 0.8704 | -70.2 |
| | | Morphotype: 0.9542<br>Parasitism: 0.9709<br>Sample date: 0.7048 | -70.3 |
| | | Morphotype: 0.9406<br>Parasitism: 0.9341<br>Test duration: 0.7738 | -70.2 |
| **Field study** | Fecundity (total eggs per g body weight) | Parasitism: 0.6689<br>Morphotype: <0.0001 | 23.9 |
| | | Parasitism: 0.5819<br>Morphotype: 0.0006<br>Sample date: 0.0594<br>Parasitism * Morphotype: 0.7570 | 25.8 |
| | Gonadosomatic index (GSI) | Parasitism: 0.2324<br>Morphotype: 0.0094<br>Age: 0.0077<br>Sample date: 0.0002<br>Parasitism * Morphotype: 0.0947<br>Parasitism * Morphotype * Age: 0.0671 | 640.4 |
| | Egg diameter (mm) | Parasitism: 0.0143<br>Morphotype: 0.0004<br>Sample date: 0.0001<br>Parasitism * Morphotype: 0.9888 | -316.5 |

# REFERENCES

## REFERENCES


Barton, B.A. and Iwama, G.K. 1991. Physiological changes in fish from stress in aquaculture with emphasis on the response and effects of corticosteroids. *Annual Review of Fish Diseases* 1(6): 3–26.

Bence, J.R., Bergstedt, R.A., Christie, G.C., Cochran, P.A., Ebener, M.P., Koonce, J.F., Rutter, M.A., and Swink, W.D. 2003. Sea lamprey (*Petromyzon marinus*) parasite-host interactions in the Great Lakes. *Journal of Great Lakes Research* 29(Sup. 1): 253–282.

Berkeley, S.A., Chapman, C., and Sogard, S.M. 2004. Maternal age as a determinant of larval growth and survival in a marine fish, *Sebastes melanops*. *Ecology* 85(5): 1258–1264.

Bianchet, M.A., Odom, E.W., Vasta, G.R., and Amzel, L.M. 2002. A novel fucose recognition fold involved in innate immunity. *Nature Structural Biology* 9(8): 628–634.

Birkeland, C. and Dayton, P.K. 2005. The importance in fishery management of leaving the big ones. *Trends in Ecology & Evolution* 20(7): 356–358.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology* 37: 911–917.

Bon, E., Barbe, U., Nuñez Rodriguez, J., Cuisset, B., Pelissero, C., Sumpter, J.P., and Le Menn, F. 1997. Plasma vitellogenin levels during the annual reproductive cycle of the female rainbow trout (*Oncorhynchus mykiss*): establishment and validation of an ELISA. *Comparative Biochemistry and Physiology* 117B(1): 75–84.

Breton, B., Govoroun, M., and Mikolajczyk, T. 1998. GTH I and GTH II secretion profiles during the reproductive cycle in female rainbow trout: relationship with pituitary responsiveness to GnRH-A stimulation. *General and Comparative Endocrinology* 111(1): 38–50.

Bromage, N., Porter, M., and Randall, C. 2001. The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* 197: 63–98.

Bronte, C.R., Ebener, M.P., Schreiner, D.R., DeVault, D.S., Petzold, M.M., Jensen, D.A., Richards, C., and Lozano, S.J. 2003. Fish community change in Lake Superior, 1970-2000. *Canadian Journal of Fisheries and Aquatic Sciences* 60: 1552–1574.

Campbell, P.M., Pottinger, T.G., and Sumpter, J.P. 1992. Stress reduces the quality of gametes produced by rainbow trout. *Biology of Reproduction* 47(6): 1140–1150.

Carragher, J.F., Sumpter, J.P., Pottinger, T.G., and Pickering, A.D. 1989. The deleterious effects of cortisol implantation on reproductive function in two species of trout, *Salmo trutta* L. and *Salmo gairdneri* Richardson. *General and Comparative Endocrinology* 76: 310–321.

Chase, D.J. 1983. Modification of acute testosterone responsiveness to luteinizing hormone by follicle-stimulating hormone and luteinizing hormone in the domestic cockerel. *Biology of Reproduction* 29: 143–150.

Cheng, W., Hsiao, I.S., Hsu, C.H., and Chen, J.C. 2004. Change in water temperature on the immune response of Taiwan abalone *Haliotis diversicolor supertexta* and its susceptibility to *Vibrio parahaemolyticus. Fish & Shellfish Immunology* 17: 235–243.

Claus, L.C. 2011. Influence of life history traits on accumulation of polybrominated diphenyl ethers and polychlorinated biphenyls in three lake trout populations from Lake Superior. Master's thesis: Michigan State University, Department of Fisheries and Wildlife.

Coble, D.W., Bruesewitz, R.E., Fratt, T.W., and Scheirer, J.W. 1990. Lake trout, sea lampreys, and overfishing in the upper Great Lakes: a review and analysis. *Transactions of the American Fisheries Society* 119: 985–995.

Contreras-Sánchez, W.M., Schreck, C.B., Fitzpatrick, M.S., and Pereira, C.B. 1998. Effects of stress on the reproductive performance of rainbow trout (*Oncorhynchus mykiss*). *Biology of Reproduction* 58(2): 439–447.

Davies, B., Bromage, N., and Swanson, P. 1999. The brain-pituitary-gonadal axis of female rainbow trout *Oncorhynchus mykiss*: effects of photoperiod manipulation. *General and Comparative Endocrinology* 115(1): 155–166.

Dunn, J.F., Nisula, B.C., and Rodbard, D. 1981. Transport of steroid hormones: binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *Journal of Clinical Endocrinology and Metabolism* 53: 58–68.

Dymond, J.R. 1922. A provisional list of the fishes of Lake Erie. *Publications of the Ontario Fisheries Research Laboratory.* The Department of Biology, University of Toronto.

Ebener, M.P., King, E.L., and Edsall, T.A. 2006. Application of a dichotomous key to the classification of sea lamprey marks on Great Lakes fish. Great Lakes Fishery Commission Miscellaneous Publication 2006-02.

Einum, S. and Fleming, I.A. 1999. Maternal effects of egg size in brown trout (*Salmo trutta*): norms of reaction to environmental quality. *Proceedings of the Royal Society B: Biological Sciences* 266: 2095–2100.

Eschmeyer, P.H. and Phillips Jr, A.M. 1965. Fat content of the flesh of siscowets and lake trout from Lake Superior. *Transactions of the American Fisheries Society* 94(1): 62–74.

Eshenroder, R.L. 1992. Decline of lake trout in Lake Huron. *Transactions of the American Fisheries Society* 121(4): 548–554.

Farmer, G.J., Beamish, F.W., and Robinson, G.A. 1975. Food consumption of the adult landlocked sea lamprey, *Petromyzon marinus*, L. *Comparative Biochemistry and Physiology* 50(4): 753–757.

Foo, J.T.W. and Lam, T.J. 1993. Retardation of ovarian growth and depression of serum steroid levels in the tilapia, *Oreochromis mossambicus*, by cortisol implantation. *Aquaculture* 115: 133–143.

Goetz, F., Sitar, S., Rosauer, D., Swanson, P., Bronte, C.R., Dickey, J., and Simchick, C. 2011. The reproductive biology of siscowet and lean lake trout in southern Lake Superior. *Transactions of the American Fisheries Society* 140(6): 1472–1491.

Goetz, F., Rosauer, D., Sitar, S., Goetz, G., Simchick, C., Roberts, S., Johnson, R., Murphy, C., Bronte, C.R., and Mackenzie, S. 2010. A genetic basis for the phenotypic differentiation between siscowet and lean lake trout (*Salvelinus namaycush*). *Molecular Ecology* 19(Sup. 1): 176–196.

Goetz, F.W., Iliev, D.B., McCauley, L.A.R., Liarte, C.Q., Tort, L.B., Planas, J.V., and Mackenzie, S. 2004. Analysis of genes isolated from lipopolysaccharide-stimulated rainbow trout (*Oncorhynchus mykiss*) macrophages. *Molecular Immunology* 41(12): 1199–1210.

Hobby, A.C., Geraghty, D.P., and Pankhurst, N.W. 2000a. Differences in binding characteristics of sex steroid binding protein in reproductive and nonreproductive female rainbow trout (*Oncorhynchus mykiss*), black bream (*Acanthopagrus butcheri*), and greenback flounder (*Rhombosolea tapirina*). *General and Comparative Endocrinology* 120: 249–259.

Hobby, A.C., Pankhurst, N.W., and Haddy, J.A. 2000b. The effect of short term confinement stress on binding characteristics of sex steroid binding protein (SBP) in female black bream (*Acanthopagrus butcheri*) and rainbow trout (*Oncorhynchus mykiss*). *Comparative Biochemistry and Physiology* 125: 85–94.

Honda, S., Kashiwagi, M., Miyamoto, K., Takei, Y., and Hirose, S. 2000. Multiplicity, structures, and endocrine and exocrine natures of eel fucose-binding lectins. *The Journal of Biological Chemistry* 275(42): 33151–33157.

Irwin, B. J., Liu, W., Bence, J. R., and Jones, M. L. 2012. Defining economic injury levels for sea lamprey control in the Great Lakes Basin. *North American Journal of Fisheries Management* 32(4): 760–771.

Janssen, J., Marsden, J.E., Bronte, C.R., Jude, D.J., Sitar, S.P., and Goetz, F.W. 2007. Challenges to deep-water reproduction by lake trout: pertinence to restoration in Lake Michigan. *Journal of Great Lakes Research* 33(Supp. 1): 59–74.

Jensen, A.L. 1994. Larkin's predation mode of lake trout (*Salvenlinus namaycush*) extinction with harvesting and sea lamprey (*Petromyzon marinus*) predation: a qualitative analysis. *Canadian Journal of Fisheries and Aquatic Sciences* 51: 942–945.

Jurado, R.L. 1997. Iron, infections, and anemia of inflammation. *Clinical Infectious Diseases* 25: 888–895.

Kerr, J.B. and Sharpe, R.M. 1985. Follicle-stimulating hormone induction of Leydig cell maturation. *Endocrinology* 116(6): 2592–2604.

Kinnunen, R.E. and Johnson, H.E. 1985. Impact of sea lamprey parasitism on the blood features and hemopoietic tissues of rainbow trout. Great Lakes Fishery Commission Technical Report No. 46.

Kramer, V.J., Etterson, M.A., Hecker, M., Murphy, C.A., Roesijadi, G., Spade, D.J., Spromberg, J.A., Wang, M., Ankley, G.T. 2011. Adverse outcome pathways and ecological risk assessment: bridging to population-level effects. *Environmental Toxicology and Chemistry* 30(1): 64–76.

Lemly, A. and Esch, G.W. 1984. Effects of the trematode *Uvulifer ambloplitis* on juvenile bluegill sunfish, *Lepomis macrochirus*: ecological implications. *The Journal of Parasitology* 70: 475–492.

Madenjian, C.P., Chipman, B.D., and Marsden, J.E. 2008. New estimates of lethality of sea lamprey (*Petromyzon marinus*) attacks on lake trout (*Salvelinus namaycush*): implications for fisheries management. *Canadian Journal of Fisheries and Aquatic Sciences* 65(3): 535–542.

Madenjian, C.P., Elliott, R.F., DeSorcie, T.J., Stedman, R.M., O'Connor, D.V., and Rottiers, D.V. 2000. Lipid concentrations in Lake Michigan fishes: seasonal, spatial, ontogenetic, and long-term trends. *Journal of Great Lakes Research* 26: 427–444.

Madenjian, C.P. and O'Connor, D.V. 1999. Laboratory evaluation of a lake trout bioenergetics model. *Transactions of the American Fisheries Society* 128: 802–814.

Mata, M.T. 2009. Temporal and spatial trends in siscowet (*Salvelinus namaycush*) abundance and biology for Michigan waters of Lake Superior. Master's thesis: Michigan State University, Department of Fisheries and Wildlife.

Mazeaud, M.M., Mazeaud, F., and Donaldson, E.M. 1977. Primary and secondary effects of stress in fish: some new data with a general review. *Transactions of the American Fisheries Society* 106(3): 201–212.

Miller, D.H., Jensen, K.M., Villeneuve, D.L., Kahl, M.D., Makynen, E.A., Durhan, E.J., and Ankley, G.T. 2007. Linkage of biochemical responses to population-level effects: a case

study with vitellogenin in the fathead minnow (*Pimephales promelas*). *Environmental Toxicology and Chemistry* 26(3): 521–527.

Miller, M.A. and Schram, S.T. 2000. Growth and contaminant dynamics of Lake Superior lake trout. *Journal of Great Lakes Research* 26(1): 102–111.

Miller III, W.R., Hendricks, A.C., and Cairns Jr., J. 1983. Normal ranges for diagnostically important hematological and blood chemistry characteristics of rainbow trout (*Salmo gairdneri*). *Canadian Journal of Fisheries and Aquatic Sciences* 40: 420–425.

Moore, S.A. and Bronte, C.R. 2001. Delineation of sympatric morphotypes of lake trout in Lake Superior. *Transactions of the American Fisheries Society* 130(6): 1233–1240.

Murphy, C.A., Rose, K.A., and Thomas, P. 2005. Modeling vitellogenesis in female fish exposed to environmental stressors: predicting the effects of endocrine disturbance due to exposure to a PCB mixture and cadmium. *Reproductive Toxicology* 19: 395–409.

Nagahama, Y. 1994. Endocrine regulation of gametogenesis in fish. *International Journal of Developmental Biology* 38(2): 217–229.

Nemeth, E. and Ganz, T. 2006. Regulation of iron metabolism by hepcidin. *Annual Review of Nutrition* 26: 323–342.

Nemeth, E., Rivera, S., Gabayan, V., Keller, C., Taudorf, S., Pedersen, B.K., and Ganz, T. 2004. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. *The Journal of Clinical Investigation* 113(9): 1271–1276.

Norberg, B., Bjornsson, B.T., Brown, C.L., Wichardt, U.F., Deftos, L.J., and Haux, C. 1989. Changes in plasma vitellogenin, sex steroids, calcitonin, and thyroid hormones related to sexual maturation in female brown trout (*Salvo trutta*). *General and Comparative Endocrinology* 75: 316–326.

Odell, W.D., Swerdloff, R.S., Jacobs, H.S., and Hescox, M.A. 1973. FSH induction of sensitivity to LH: one cause of sexual maturation in the male rat. *Endocrinology* 92(1): 160–165.

Olin, M., Jutila, J., Lehtonen, H., Vinni, M., Ruuhijärvi, J., Estlander, S., Rask, M., Kuparinen, A., and Lappalainen, J. 2012. Importance of maternal size on the reproductive success of perch, *Perca fluviatilis*, in small forest lakes: implications for fisheries management. *Fisheries Management and Ecology* 19: 363–374.

Olsen, E.M., Lilly, G.R., Heino, M., Morgan, M.J., Brattey, J., and Dieckman, U. 2005. Assessing changes in age and size at maturation in collapsing populations of Atlantic cod (*Gadus morhua*). *Canadian Journal of Fisheries and Aquatic Sciences* 62: 811–823.

Ortuño, J., Esteban, M.A., and Meseguer, J. 2001. Effects of short-term crowding stress on the gilthead seabream (*Sparus aurata* L.) innate immune response. *Fish & Shellfish Immunology* 11: 187–197.

Pankhurst, N.W., and Van Der Kraak, G. 2000. Evidence that acute stress inhibits ovarian steroidogenesis in rainbow trout in vivo, through the action of cortisol. *General and Comparative Endocrinology* 117(2): 225–237.

Petra, P.H., Stanczyk, F.Z., Senear, D.F., Namkung, P.C., Novy, M.J., Ross, J.B.A., Turner, E., Brown, J.A. 1983. Current status of the molecular structure and function of the plasma sex steroid-binding protein (SBP). *Journal of Steroid Biochemistry* 19(1): 699–706.

Prat, F., Sumpter, J.P., and Tyler, C.R. 1996. Validation of radioimmunoassays for two salmon gonadotropins (GTH I and GTH II) and their plasma concentrations throughout the reproductive cycle in male and female rainbow trout (*Oncorhynchus mykiss*). *Biology of Reproduction* 54: 1375–1382.

Quince, C., Abrams, P.A, Shuter, B.J., and Lester, N.P. 2008. Biphasic growth in fish II: empirical assessment. *Journal of Theoretical Biology* 254(2): 197–206.

Roberts, S., Goetz, G., White, S., and Goetz, F. 2009. Analysis of genes isolated from plated hemocytes of the Pacific oyster, *Crassostreas gigas*. *Marine Biotechnology* 11: 24–44.

Roff, D.A. 1983. An allocation model of growth and reproduction in fish. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 1395–1404.

Schreck, C.B., Contreras-Sanchez, W., and Fitzpatrick, M.S. 2001. Effects of stress on fish reproduction, gamete quality, and progeny. *Aquaculture* 197: 3–24.

Secombes, C., White, A., and Fletcher, T. 1995. Immune parameters of plaice, *Pleuronectes platessa*, L. along a sewage sludge gradient in the Firth of Clyde, Scotland. *Ecotoxicology* 340: 329–340.

Selye, H. 1950. Stress and the general adaptation syndrome. *British Medical Journal* June(4667): 1383–1392.

Sitar, S.P., Morales, H.M., Mata, M.T., Bastar, B.B., Dupras, D.D., Kleaver, G.D., and Rathbun, K.D. 2008. Survey of siscowet lake trout at their maximum depth in Lake Superior. *Journal of Great Lakes Research* 34(2): 276–286.

Sitar, S.P. and He, J.X. 2006. Growth and maturity of hatchery and wild lean lake trout during population recovery in Michigan waters of Lake Superior. *Transactions of the American Fisheries Society* 135(4): 915–923.

Sitar, S.P., Bence, J.R., Johnson, J.E., Ebener, M.P., and William, W.W. 1999. Lake trout mortality and abundance in southern Lake Huron. *North American Journal of Fisheries Management* 19(4): 881–900.

Smith, B.R. and Tibbles, J.J. 1980. Sea lamprey (*Petromyzon marinus*) in Lakes Huron, Michigan, and Superior: history of invasion and control, 1936-1978. *Canadian Journal of Fisheries and Aquatic Sciences* 37: 1780–1801.

Södergard, R., Bäckström, T., Shanbhag, V., and Carstensen, H. 1982. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *Journal of Steroid Biochemistry* 16: 801–810.

Spromberg, J.A. and Birge, W.J. 2005. Modeling the effects of chronic toxicity on fish populations: the influence of life-history strategies. *Environmental Toxicology and Chemistry* 24: 1532–1540.

Stapanian, M.A. and Madenjian, C.P. 2007. Evidence that lake trout served as a buffer against sea lamprey predation on burbot in Lake Erie. *North American Journal of Fisheries Management* 27(1): 238–245.

Stark, J.D., Banks, J.E., and Vargas, R. 2004. How risky is risk assessment: the role that life history strategies play in susceptibility of species to stress. *Proceedings of the National Academy of Sciences of the United States of America* 101: 732–736.

Stewart, D.J., Weininger, D., Rottiers, D.V., and Edsall, T.A. 1983. An energetics model for lake trout, *Salvelinus namaycush*: application to the Lake Michigan population. *Canadian Journal of Fisheries and Aquatic Sciences* 40: 681–698.

Sullivan, W.P., Christie, G.C., Cornelius, F.C., Fodale, M.F., Johnson, D.A., Koonce, J.F., Larson, G.L., McDonald, R.B., Mullett, K.M., Murray, C.K., and Ryan, P.A. 2003. The sea lamprey in Lake Erie: a case history. *Journal of Great Lakes Research* 29(Supp. 1): 615–636.

Swink, W.D. 2003. Host selection and lethality of attacks by sea lampreys (*Petromyzon marinus*) in laboratory studies. *Journal of Great Lakes Research* 29(Supp. 1): 307–319.

Swink, W.D. 1991. Host-size selection by parasitic sea lampreys. *Transactions of the American Fisheries Society* 120(5): 637–643.

Swink, W.D. 1990. Effect of lake trout size on survival after a single sea lamprey attack. *Transactions of the American Fisheries Society* 119(6): 996–1002.

Swink, W.D. and Hanson, L.H. 1986. Survival from sea lamprey (*Petromyzon marinus*) predation by two strains of lake trout (*Salvenlinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences* 43: 2528–2531.

Tahir, A., Fletcher, T., Houlihan, D., and Secombes, C. 1993. Effect of short-term exposure to oil-contaminated sediments on the immune response of dab, *Limanda limanda*(L.). *Aquatic Toxicology* 27 71–82.

Taranger, G.L., Haux, C., Stefansson, S. O., Thrandur, B., Walther, B., and Hansen, T. 1998. Abrupt changes in photoperiod affect age at maturity, timing of ovulation and plasma testosterone and oestradiol-17 [beta] profiles in Atlantic salmon, *Salmo salar*. *Aquaculture* 162: 85–98.

Tyler, C.R., Pottinger, T.G., Coward, K., Prat, F., Beresford, N., and Maddix, S. 1997. Salmonid follicle-stimulating hormone (GtH I) mediates vitellogenic development of oocytes in the rainbow trout, *Oncorhynchus mykiss*. *Biology of Reproduction* 57: 1238–1244.

Tyler, C.R., Sumpter, J.P., Kawauchi, H., and Swanson, P. 1991. Involvement of gonadotropin in the uptake of vitellogenin into vitellogenic oocytes of the rainbow trout, *Oncorhynchus mykiss*. *General and Comparative Endocrinology* 299: 291–299.

Wang, Y.J., Miller, L.A., Perren, M., and Addis, P.B. 1990. Omega-3 fatty acids in Lake Superior fish. *Journal of Food Science* 55(1): 71–76.

Zhao, S. and Fernald, R.D. 2005. Comprehensive algorithm for quantitative real-time polymerase chain reaction. *Journal of Computational Biology* 12(8): 1047–1064.